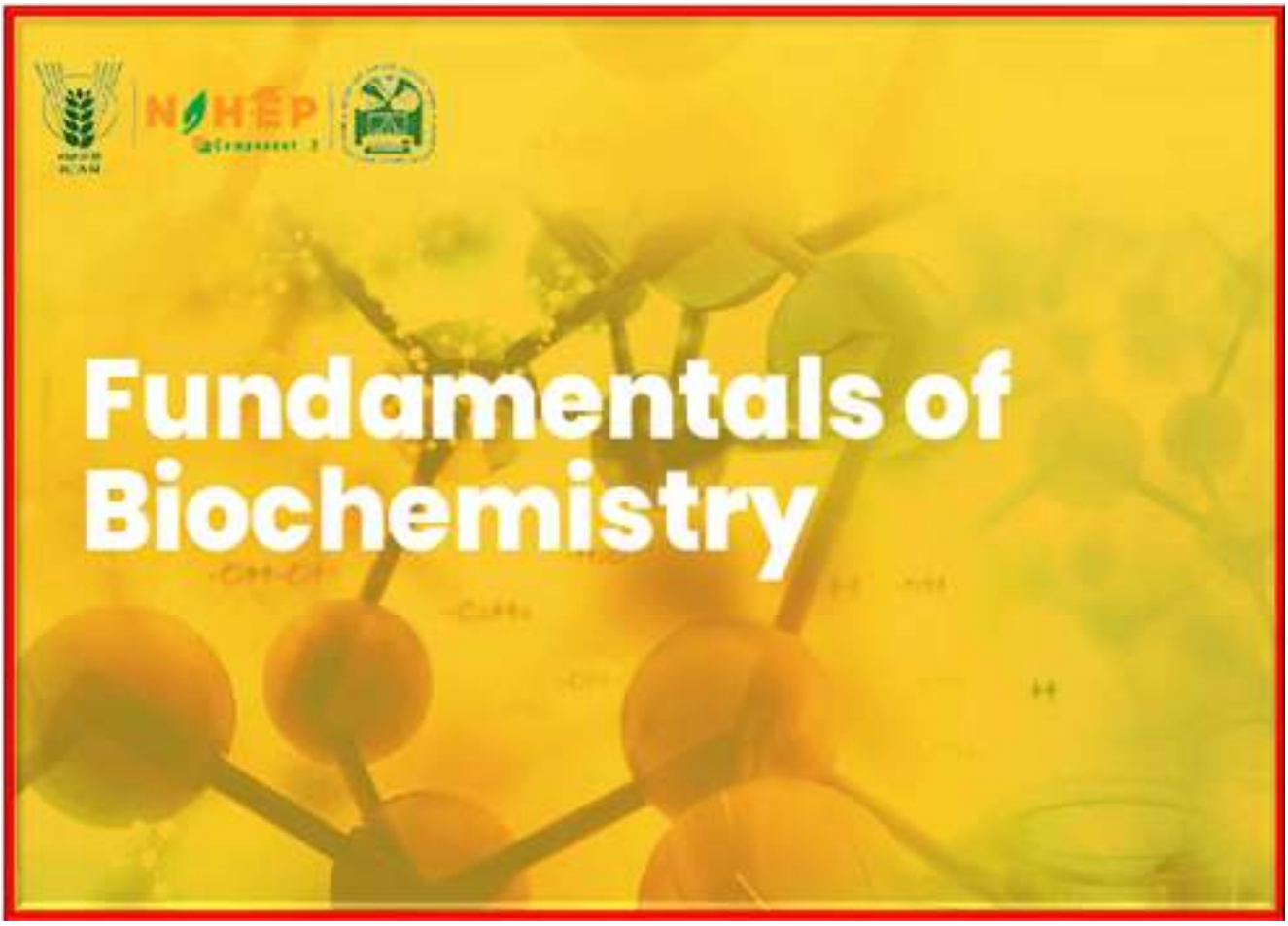
Fundamentals of Biochemistry



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Lesson No.	Lesson Title
Lesson 1	A brief introduction to developments in biochemistry and its transformation to molecular biology. Cell structure, water
Lesson 2	Carbohydrate chemistry: Structure, classification, functions (mono, di and polysaccharides) isomerism and mutarotation
Lesson 3	Metabolism of carbohydrates: glycolysis, gluconeogenesis, glycogenolysis, glycogenesis, TCA cycle, central role of TCA cycle in metabolism
Lesson 4	Protein chemistry: classifications and functions. Classification, structure, function and properties of amino acids. Essential and non-essential amino acids
Lesson 5	Primary, secondary, tertiary and quaternary structure of proteins. Amphoteric property. Biuret reaction and xanthoproteic reaction. Digestion and absorption of proteins
Lesson 6	Classification, structure, functions and properties of lipids. Essential fatty acids and phospholipids
Lesson 7	Digestion and absorption of lipids. Lipid autooxidation. Significance of Omega-3 and Omega-6 fatty acids
Lesson 8	Enzymes: nomenclature; classification; specificity; mechanism of enzyme action; kinetics and regulation of enzyme activity
Lesson 9	Steroid and peptide hormones- chemistry and function. Structure and functions of fat and water soluble vitamins. Vitamins – classification- functions. Minerals – classification –

	functions
Lesson 10	Nucleic acids: Structure function and importance of genetic code. Transcription and translation. Protein synthesis. Energy changes in chemical reactions, reversible and irreversible reactions in metabolism

Love the land, because it can give you everything.

Course Name	Fundamentals of Biochemistry
Lesson 1	A brief introduction to developments in biochemistry and its transformation to molecular biology. Cell Structure, Water
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Lesson-1

A brief introduction to developments in biochemistry and its transformation to molecular biology. Cell Structure, Water.

Objectives:

a. To understand the subject of Biochemistry and know course outline
b. To know the major milestones in the history of Biochemistry
c. To know the structure and functions of plant cell
d. To understand the structure and properties of water

Glossary

a. **Biochemistry:** Study of cellular logic at molecular level.
b. **Pectin:** A major constituent of cell wall of plants; a mixture of polymers from sugar acids such as D-galacturonic acid connected by (α1→4) glycosidic links.
c. **Tonoplast:** A membrane which bounds the chief vacuole of a plant cell.
d. **Crista:** Each of the partial partitions in a mitochondrion formed by infolding of the inner membrane.
e. **Thylakoids:** Membrane-bound compartments inside chloroplasts. They are site of light-dependent reactions of photosynthesis. They consist of a thylakoid membrane surrounding a thylakoid lumen.
f. **Microbody:** A microbody is a type of organelle that is found in the cells of plants, protozoa, and animals. Organelles in the microbody family include peroxisomes, glyoxysomes, glycosomes and hydrogenosomes.
g. **Heat capacity:** It is a physical property of matter, defined as the amount of heat to be supplied to a given mass of a material to produce a unit change in its temperature.
h. **Heat of vaporization**: It is the amount of energy that must be added to a liquid substance to transform a quantity of that substance into a gas.

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Course outline (Lessons)

a. A brief introduction to developments in biochemistry and its transformation to molecular biology. Cell structure, water.
b. **Carbohydrate chemistry:** Structure, classification, functions (mono, di and polysaccharides) isomerism and mutarotation.
c. **Metabolism of carbohydrates:** glycolysis, gluconeogenesis, glycogenolysis, glycogenesis, TCA cycle, central role of TCA cycle in metabolism.
d. **Protein chemistry:** classifications and functions. Classification, structure, function and properties of amino acids. Essential and non essential amino acids.
e. Primary, secondary, tertiary and quaternary structure of proteins. Amphoteric property. Biuret reaction and xanthoproteic reaction. Digestion and absorption of proteins.
f. Classification, structure, functions and properties of lipids. Essential fatty acids and phospholipids.
g. Digestion and absorption of lipids. Lipid autooxidation. Significance of Omega-3 and Omega-6 fatty acids.
h. **Enzymes:** nomenclature; classification; specificity; mechanism of enzyme action; kinetics and regulation of enzyme activity.
i. **Steroid and peptide hormones-** chemistry and function. Structure and functions of fat- and water-soluble vitamins. Vitamins: classification and functions. Minerals: classification and functions.
j. **Nucleic acids:** Structure function and importance of genetic code. Transcription and translation. Protein synthesis. Energy changes in chemical reactions, reversible and irreversible reactions in metabolism

1.1 Biochemistry: Definition

a. The term 'Biochemistry' was first introduced by the German Chemist Carl Alexander Neuberg in 1903.
b. He was an early pioneer in biochemistry, and referred as the 'Father of Biochemistry'.
c. Biochemistry, as the name implies, is the chemistry of living organisms. Living organisms, whether they are microorganisms, plants or animals are basically made up of the same chemical components. Biochemistry is the study of the way in which these components are synthesized and utilized by the organisms in their life processes.
d. It bridges the gap between the conventional chemistry and biology. In other words, life is nothing but thousands of ordered chemical reactions or chemistry is the logic of all biological phenomena.

1.2 History of Biochemistry and transformation to Molecular Biology

1854-1864	Louis Pasteur	Proved that fermentation is caused by microorganisms
1877-78	Kuhne	Proposed the term 'Enzyme'
1894	Emil Fischer	Demonstrated specificity of enzymes and lock and key relationship between enzyme and substrate
1897	Buckner	Discovered alcoholic fermentation in cell-free yeast extract
1902	Emil Fischer	Demonstrated that proteins are polypeptides
1903	Neuberg	First used the term 'biochemistry'
1913	Michaelis and Menten	Developed kinetic theory of enzyme action
1926	Sumner	First crystallized an enzyme, urease and proved it to be a protein

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1933	Embden Meyerhof and Parnas	Demonstrated crucial intermediates in the chemical pathway of glycolysis and fermentation
1937	Krebs	Discovered citric acid cycle
1940	Lipmann	Role of ATP in biological systems
1950	Pauling and Corey	Proposed the α-helix structure for keratins
1950-1953	Chargaff	Discovered the base composition of DNA
1953	Sanger and Thompson	Determined the complete amino acid sequence of insulin
1953	Watson and Crick	Proposed the double-helical model for DNA structure
1958	Meselson and Stahl	Confirmed the Watson-Crick model of semi conservative replication of DNA
1961	Jacob & Monod	Proposed the operon hypothesis and postulated the function of messenger RNA
1999	Ingo Potrykus	Golden rice- rich in β-carotene
2003	Scientists across the world	Human Genome Mapping
2004	Aaron Ciechanover, Avram Hershko and Irwin Rose	Discovery of ubiquitin-mediated protein degradation
2005	Barry J. Marshall and J. Robin Warren	Discovery of the bacterium Helicobacter pylori and its role in gastritis and peptic ulcer disease
2006	Andrew Z. Fire and Craig C. Mello	Discovery of RNA interference - gene silencing by double-stranded RNA

2006	Roger D. Kornberg	Studies of the molecular basis of eukaryotic transcription
2008	Osamu Shimomura, Martin Chalfie and Roger Y. Tsien	Discovery and development of the green fluorescent protein, GFP
2009	V Ramakrishnan, Thomas A. Steitz & Ada E. Yonath	Detailed studies of the structure and function of the ribosome
2012	John B. Gurdon and Shinya Yamanaka	Mature cells can be reprogrammed to become pluripotent.
2016	Yoshinori Ohsumi	Discoveries of mechanisms for autophagy.
2018	James P. Allison and Tasuku Honjo	Discovery of cancer therapy by inhibition of negative immune regulation.
2020	Emmanuelle Charpentier and Jennifer Doudna	Discovery and development of CRISPR-Cas9 genome editing

1.3 Importance of Biochemistry in Agriculture

a. Agriculture can be better managed with better varieties and practices.

b. Transgenic plants which are high yielding, nutritionally more desirable and self fertilizing can be synthesized i.e. designer plants and animals.

c. Pesticides which are more specific, biodegradable and non toxic to animals can be formulated.

d. Knowledge of biochemistry helps to evaluate nutritive value of cereals, pulses, poultry and cattle feed.

e. Knowledge of biochemistry helps in removal and inactivation of toxic or anti-nutritional factors present in food grains in general and grain legumes in particular by breeding and chemical treatments. e.g. β-N-oxalyl-amino-L-alanine in Kesari dal, Trypsin inhibitors of soybean, Aflatoxins of groundnut.

f. Biochemistry helps in development of Food preservation and processing technology vis-à-vis their nutritional quality.

g. Biochemistry helps in study of disease and pest resistance.

h. Biochemistry helps in study of drought resistance (proline and hydroxyproline imparts drought resistance).

i. Biochemistry helps in formulation of balanced diet.

j. Biochemistry helps in the use of nonconventional sources of protein foods viz., single cell proteins, fish protein concentrates, mushrooms and leaf proteins.

1.4 Plant Cell Structure

a. The word cell was coined by Robert Hooke. It is the basic unit of life.

b. A plant cell has three distinct region:

I. Cell wall (non living)
II. Vacuole (non living)
III. Protoplasm (living) - consists of cytoplasm and nucleus

c. **Cytoplasm:** Contains Mitochondria, Chloroplast, Ribosomes, Endoplasmic Reticulum, Golgi Bodies, lysosome, plastid etc.

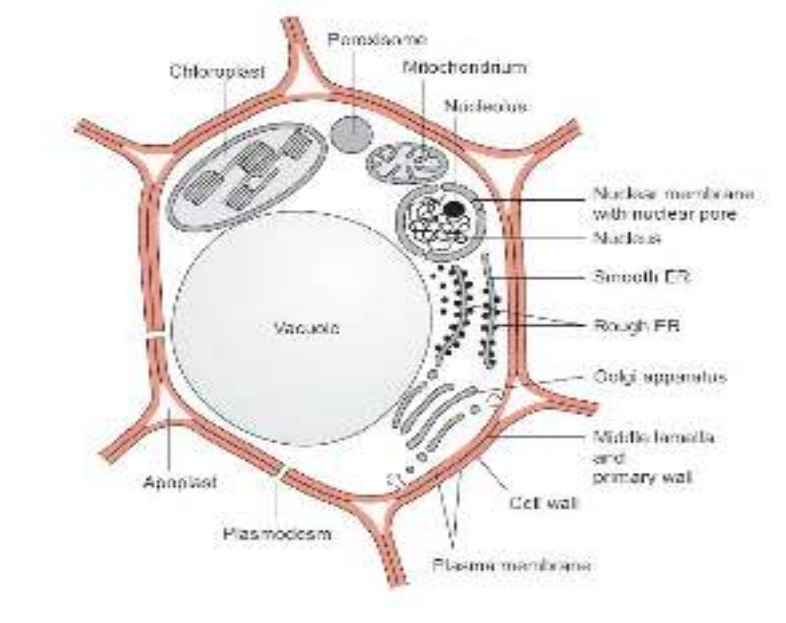

Fig. 1: A Plant Cell

1.4.1 Cell wall

a. Plant cells are surrounded by a rigid, semi-permeable cell wall.
b. The cell wall is comprised of mainly polysaccharides with some proteins and lipids.
c. The three main polysaccharide components of the cell wall are cellulose, hemicelluloses and pectin.
d. Two types of proteins like expansin and extensin are present predominantly.
e. Cell wall : Three different regions
   1. Middle Lamella
   2. Primary cell wall (1-3 µm thick and elastic)
   3. Secondary cell wall (5-10 µm thick and rigid)

Middle lamella

a. This is the first layer formed during cell division.
b. It makes up the outer wall of the cell. It is composed mainly of pectic compounds and proteins.

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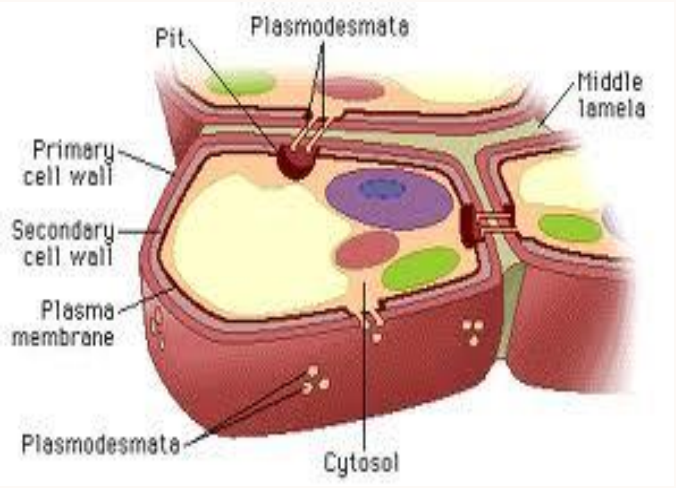

Fig. 2: Cell Wall

Primary wall

a. It is deposited by cells before and during active growth.
b. It is rich in polysaccharides.
c. Young cells have a very thin cell wall.

Secondary cell wall

a. It is formed after cell enlargement is completed.

b. It is much thicker than the primary walls and consist of 40-45% cellulose, 15-35% hemicellulose, 15-30% lignin and negligible amounts of pectic polysaccharides.

Functions of cell wall

a. It protects the inner contents of the cell.
b. It gives definite shape to the cell.
c. It provides mechanical support to the tissues and act as a skeletal framework of plants.
d. It helps in transport of substances between two cells.
e. It acts as a permeable structure during absorption of minerals and solutes.
f. It resists internal turgor pressure of cell.
g. It protects against pathogens.
h. It participates in early recognition of symbiotic nitrogen fixing bacteria.

Structure of cellulose

a. Main carbohydrate constituent is cellulose - an unbranched polymer consisting of D-glucose molecules, which are connected to each other by glycosidic (β1-4) linkages.

b. Each glucose unit is rotated by 180° from its neighbour, so that very long straight chains can be formed with a chain length of 2,000 to 25,000 glucose residues.

c. About 36 cellulose chains are associated by inter-chain hydrogen bonds and form a crystalline structure known as micro fibril.

d. These crystalline regions are impermeable to water.

e. The micro fibrils have an unusually high tensile strength, are very resistant to chemical and biological degradations, and are so stable that they are very difficult to hydrolyze.

f. However, many bacteria and fungi have cellulose-hydrolyzing enzymes called cellulases.

g. These bacteria can be found in the digestive tract of some animals (e.g., ruminants), thus enabling them to digest grass and straw.

h. Cellulose is the most abundant organic substance on earth, representing about half of the total organically bound carbon.

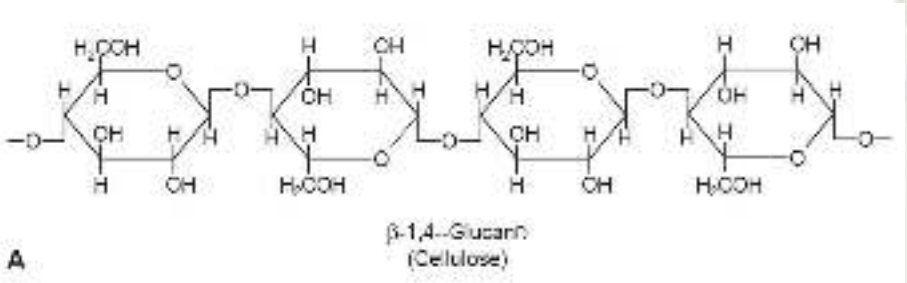

Fig. 3: Structure of Cellulose

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Structure of Hemicelluloses: another important constituent of cell wall

i. Hemicelluloses consist of a variety of polysaccharides that contain, in addition to D-glucose, other carbohydrates such as hexoses D-mannose, D-galactose, D-fucose, and the pentoses D-xylose and L-arabinose.

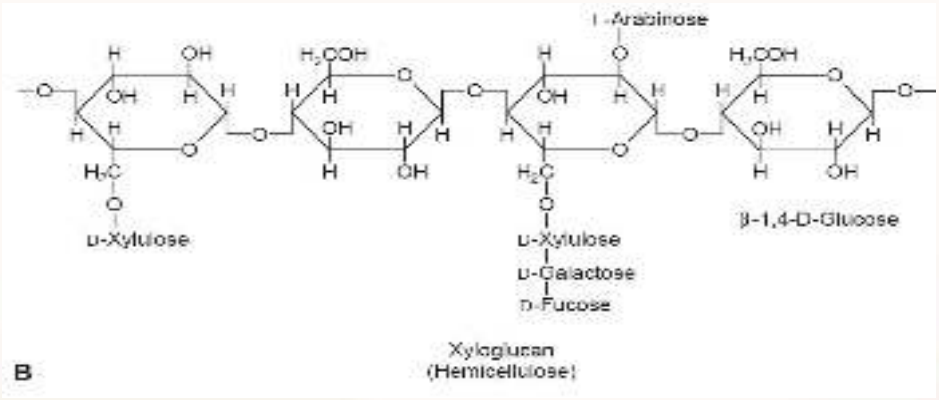

Fig. 4: Structure of Hemicellulose

Structure of Pectin

a. Another major constituent of the cell wall is a mixture of polymers from sugar acids, such as D-galacturonic acid, which are connected by (α1-4) glycosidic links.
b. Some of the carboxyl groups are esterified by methyl groups.
c. The free carboxyl groups of adjacent chains are linked by Ca^{+2} and Mg^{+2} ions.
d. When Mg^{+2} and Ca^{+2} ions are absent, pectin is a soluble compound.
e. The Ca^{+2}/Mg^{+2} salt of pectin forms an amorphous deformable gel that is able to swell.
f. The food industry makes use of this property of pectin when preparing jellies and jams.

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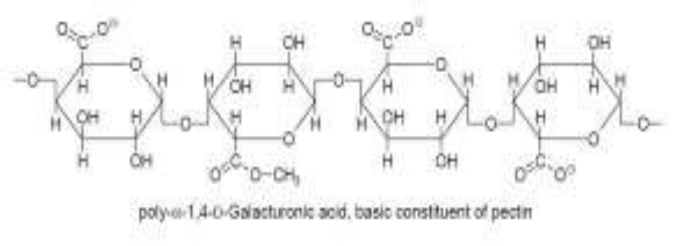

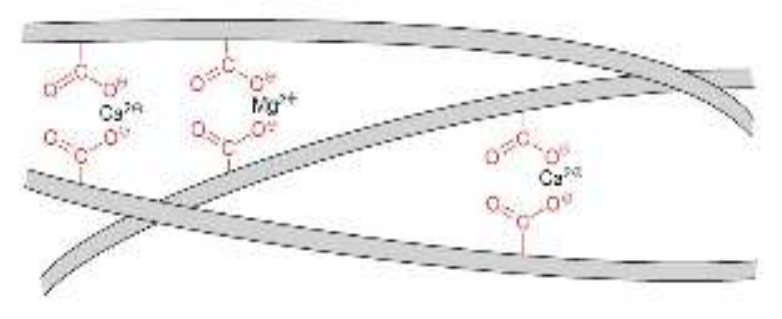

Fig. 5: Structure of Pectin

1.4.2 Cell membrane

a. All cells are enclosed by a thin, membrane called plasma membrane or plasma lemma.

b. The plasma membrane and sub-cellular membrane are collectively called biological membrane.

c. Cell membrane consists of proteins, lipids and other substances.

Proteins: The proteins present in the membranes can be categorized into two types

1. **Intrinsic proteins or integral proteins**

   a. Which are embedded or buried in the lipid layer.

   b. These proteins associate with hydrophobic interactions to the tails or fatty acid chains of the lipid layer.

   c. In addition to the hydrophobic associations, integral proteins also possess hydrophilic amino acid residues which are exposed at the surface of the membrane.

   d. These proteins cannot be removed easily.

2. Extrinsic proteins or peripheral proteins

a. They are attached to the membrane surface by weak ionic interactions.

b. These proteins are not much involved in the architecture of membranes.

Lipids

a. The cell membrane consists of phospholipids and glycolipids.

b. The fatty acid chains in phospholipids and glycolipids usually contain 16-20 even numbered carbon atoms.

c. Fatty acids may be saturated or unsaturated.

Other substances like polysaccharide, salicylic acid etc. are found attached to the proteins or lipids on the membrane.

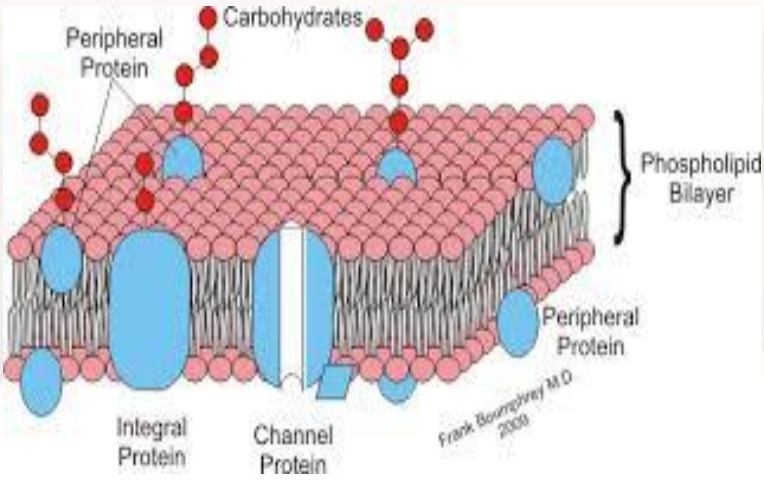

Fig. 6: Cell Membrane

Functions of cell membrane

a. The cell membrane consists of phospholipids and glycolipids.

b. The fatty acid chains in phospholipids and glycolipids usually contain 16-20 even numbered carbon atoms.

c. It is differentially permeable and able to regulate the transport across the membrane.

Table 1: Subcellular compartments in a mesophyll cell and some of their functions

Compartment	Percent of the total cell volume	Functions
Vacuole	79	Maintenance of cell turgor, store and waste deposition
Chloroplasts	16	Photosynthesis, synthesis of starch and lipids
Cytosol	3	General metabolic compartment, synthesis of sucrose
Mitochondria	0.5	Cell respiration
Nucleus	0.3	Contains the genome of the cell. Reaction site of replication and transcription
Peroxisomes		Reaction site for processes in which toxic intermediates are formed
Endoplasmic Reticulum		Storage of Ca^{+2}, participation in the export of proteins from the cell and in the transport of proteins into vacuole
Oil Bodies (Oleosomes)		Storage of triacylglycerols
Golgi bodies		Processing and sorting of proteins destined for export from the cells or transport into the vacuole

1.4.3 Nucleus

a. It is oval or spherical in shape and is generally larger in active cells than in resting cells.
b. Three main parts *viz*. nuclear envelope, nucleolus and chromatin.
c. Nucleus is separated from cytoplasm by a double membrane called the nuclear envelope.
d. Nuclear pores provide direct connection between nucleus and cytoplasm.
e. Nucleolus is a spherical, colloidal body in nucleus and is the place where DNA replication and RNA synthesis occur.
f. Chromatin is the basic unit of chromosome and contains genes which play important role in the inheritance of characters to offspring from parents.

Functions of nucleus

a. It regulates growth and reproduction of cells.
b. The nuclear envelope allows the nucleus to control its contents, and separate them from the rest of the cytoplasm.
c. DNA replication, transcription and post-transcriptional modifications occur in nucleus.

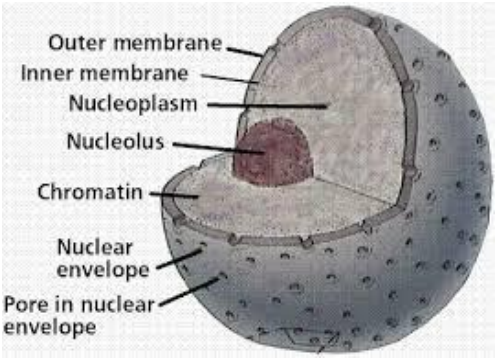

Fig. 7: Nucleus

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1.4.4 Mitochondria

a. Mitochondria are rod shaped cytoplasmic organelles, which are main sites of cellular respiration. Hence, they are referred to as power house of the cell.
b. A mitochondrion is enclosed by two concentric unit membranes comprising of an outer mitochondrial membrane (OMM) and an inner mitochondrial membrane (IMM).
c. The space between two membranes is called peri-mitochondrial space or inter membrane space (IMS).
d. The inner membrane has a series of infoldings known as cristae.
e. The fluid material in mitochondria (mt) is known as matrix.
f. The matrix is generally homogeneous, but may rarely show finely filamentous or fibrous structures. The matrix contains several copies of round or circular DNA molecules.

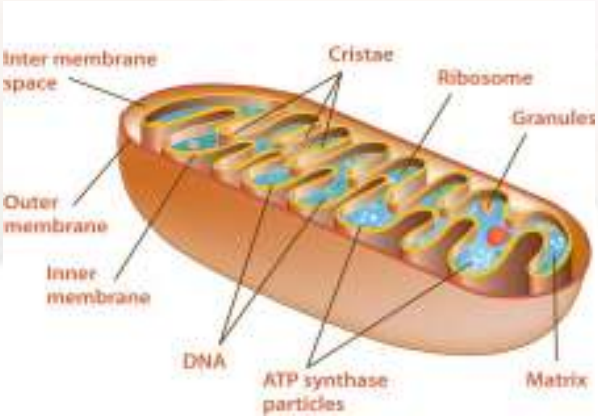

Fig. 8: Mitochondria

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Functions of Mitochondria

a. ATP, the readily available form of energy is produced in mitochondria.
b. Krebs cycle takes place in the matrix of mitochondria.
c. The enzymes of electron transport chain are found in the inner membrane or cristae of mitochondria.
d. Heme synthesis occurs in mitochondria.
e. Mitochondria are major producers of cellular reactive oxygen species.
f. Mitochondrial events trigger apoptosis (programmed cell death).
g. Mitochondrion along with chloroplast participates in photorespiration (a light-dependent CO_2 evolution and O_2 uptake).

1.4.5 Chloroplast

a. Chloroplasts are organelles found in plant cells and other eukaryotic organisms that perform photosynthesis because of the presence of green pigment, chlorophyll.
b. They are flattened discs usually 2-10 µm in diameter and 1 µm thick.
c. The chloroplast is surrounded by double layered membrane.
d. The space between these two layers is called intermembrane space.
e. Stroma is the aqueous fluid found inside the chloroplast.
f. The stroma contains the machinery required for carbon fixation, circular DNA, ribosomes etc.
g. Within the stroma the thylakoids are arranged as stacks called grana.
h. A thylakoid has a flattened disc shape and has a lumen or thylakoid space.
i. The light reactions occur on the thylakoid membrane.

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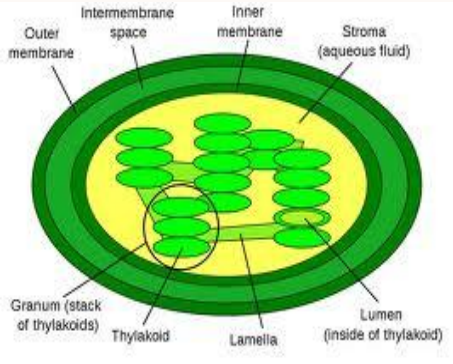

Fig. 9: Chloroplast

Functions of Chloroplast

a. The important processes of photosynthesis i.e, light and dark reactions occur within the chloroplast.
b. The granum is the site of NADP reduction forming NADPH + H^+ and photophosphorylation i.e., formation of ATP in presence of light. Thus, light reaction of photosynthesis takes place in the granum region.
c. The stroma is the main site for the dark reaction of photosynthesis.
d. The chloroplast has its own genetic system and is self replicating.

1.4.6 Endoplasmic reticulum (ER) and Golgi apparatus/body/complex (GB): A network for the distribution of biosynthesis products

a. Endoplasmic reticulum arises from the outer membrane of the nucleus.
b. Two structural types of ER can be differentiated: the rough (RER) and the smooth (SER) forms.
c. The RER consists of flattened sacs, outer side of the membranes is occupied by ribosomes.
d. The SER consists primarily of branched tubes without ribosomes.
e. Despite these morphological differences, RER and the SER are constituents of a continuous membrane system.
f. Presence of ribosomes on outer surface of ER is temporary.
g. Ribosomes are attached to the ER membrane only when the protein that they form is destined for the ER itself, for the vacuoles, or for export from the cell.

h. These proteins contain an amino acid sequence called signal sequence that causes the peptide chain during its synthesis to enter the lumen of ER.
i. Membranes of ER are also the site of membrane lipid synthesis, where the necessary fatty acids are provided by the plastids.
j. In seeds and other tissues, oil bodies (oleosomes) are present, which are derived from the ER membrane.
k. The oil bodies store triglycerides and are of great economic importance since they are the storage site of oil plants, such as rapeseed or olives.
l. The oil bodies are enclosed by a half biomembrane only, of which the hydrophobic fatty acid residues of the membrane lipids project into the oil and the hydrophilic heads project into the cytosol.
m. Proteins channelled into ER lumen are transferred to the cis side of the Golgi apparatus by membrane vesicles budding off from the ER.
n. In Golgi apparatus, proteins are selected either to be removed from the cell by exocytosis (secretion) or to be transferred to lytic vacuoles or to storage vacuoles.

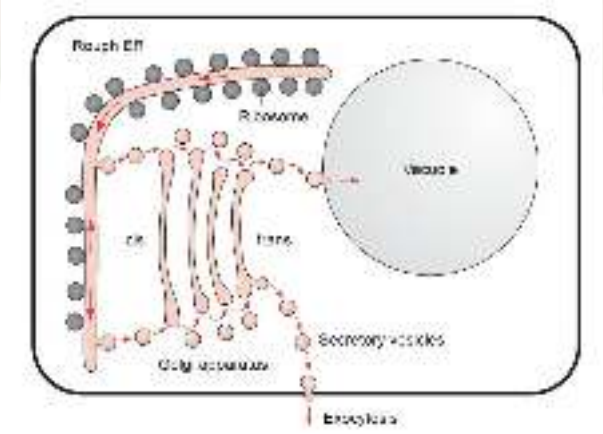

Fig. 10: Scheme of interplay between ER and Golgi apparatus in the transfer of proteins from ER to the vacuoles and in the secretion of proteins from the cell.

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1.4.7 Ribosome

a. Chemically, ribosomes are ribonucleoprotein complexes.
b. Ribosomes of prokaryotes have sedimentation coefficient of 70S and consist of two subunits of unequal sizes 50S and 30S subunits.
c. Ribosomes of eukaryotes have 80S sedimentation coefficient (40S & 60S).
d. The two or more ribosomes become connected by a single mRNA and then may be called polyribosome.

Functions:

a) They provide the platform and machinery for protein synthesis.

Fig. 11: Ribosome

1.4.8 Vacuole

a. Vacuole is enclosed by a membrane called tonoplast.
b. The number and size of the vacuoles in different plant cells vary to a great extent.
c. Young cells contain a larger number of smaller vacuoles and taken as a whole, occupy only a minor part of the cell volume.
d. When cells mature, the individual vacuoles amalgamate to form a central vacuole.
e. In cells of storage tissues, the vacuole often takes up almost the entire cellular space.

Functions

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I. **Vacuole maintain cell turgor**

a. An important function of vacuole is to maintain cell turgor. For this purpose, salts, mainly from inorganic and organic acids, are accumulated in the vacuole.
b. Accumulation of these osmotically active substances draws water into the vacuole, which, in turn, causes the tonoplast to press the protoplasm of the cell against the surrounding cell wall.
c. Plant turgor is responsible for the rigidity of non-woody plant parts.
d. The plant wilts when the turgor decreases due to lack of water.

II. **Vacuoles recycle cellular constituents**

a. Vacuoles have an important function in recycling those cellular constituents that are defective or no longer required.
b. Vacuoles contain hydrolytic enzymes for degrading various macromolecules such as proteins, nucleic acids, and many polysaccharides.
c. Structures, such as mitochondria, can be transferred by endocytosis to the vacuole and are digested there (lytic vacuoles).
d. The resulting degradation products, such as amino acids and carbohydrates are made available to the cell.

III. **Vacuoles have a storage function**

a. Many plants use the vacuole to store reserves of nitrate and phosphate.
b. Some plants [crassulacean acid metabolism (CAM)] store malic acid temporarily in the vacuoles in a diurnal cycle.
c. Vacuoles of storage tissues contain carbohydrates and storage proteins.
d. Many plant cells contain different types of vacuoles (e.g., lytic vacuoles and protein storage vacuoles beside each other).

IV. **Vacuoles function as waste deposits**

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a. With the exception of gaseous substances, leaves are unable to rid themselves of waste products or xenobiotics such as herbicides.
b. These are ultimately deposited in the vacuole.Vacuoles of storage tissues contain carbohydrates and storage proteins.

1.4.9 Microbodies

1) They are mostly spherical and have a diameter ranging from 0.2 - 1.5 µm.
   a. Two types - peroxisomes and glyoxysomes.
   b. These organelles differ in their distribution and enzyme composition, although both have the capacity to transform non-carbohydrate material into carbohydrate.
   c. Peroxisomes are found in leaves of higher plants.
   d. Glyoxysomes are temporary microbodies in that they occur during transient periods in the life cycle of a plant such as in certain beans and nuts which store fats in their seeds as energy reserves.
   e. Glyoxysomes appear in the first few days after seed germination in endosperm cells and associate closely with lipid bodies.

Functions:

a. Peroxisomes act in parallel with chloroplast and mitochondria in higher plants and participate in photorespiration.
b. Glyoxysomes are involved in the formation of sugars by the breakdown of fatty acids in germinating seeds.
c. They disappear after the storage fats are broken down and converted into carbohydrate.

1.4.10 Cytoskeleton

1) The cytoskeleton is scaffold contained within the cytoplasm and is made out of protein.
2) There are three main kinds of cytoskeleton filaments
   a. **Microfilament:-** They are composed of actin subunits.
   b. **In termediary filaments:-** They function in maintenance of cell shape by bearing tension. They also participate in cell-cell and cell matrix junctions.
   c. **Mic rotubules:-** They are like hollow cylinders mostly comprising of 13 protofilaments which in turn are alpha and beta tubulin. They are commonly organized by the centrosome.

Functions:

a. Provides mechanical support.
b. Anchors organelles.
c. Helps to move substances intracellularly.
d. Provides the cell with structure and shape.

1.5 Water

Introduction

a) Life on earth is often described as a carbon-based phenomenon but it would be equally correct to refer to it as a water-based phenomenon.
b) Life probably originated in water more than three billion years ago and all living cells still depend on water for their existence.
c) Water is the most abundant molecule in most cells accounting for 60-90% of the mass of the cell.
d) The exceptions are cells from which water is expelled such as those in seeds and spores.
e) Seeds and spores can lie dormant for long periods of time until they are revived by the reintroduction of water.
f) Life spread from the oceans to the continents about 500 million years ago. This major transition in the history of life required special adaptations to enable terrestrial life to survive in an environment where water was less plentiful.

1.5.1 Water Molecule is Polar

a. A water molecule (H_2O) is V-shaped and the H—O—H bond angle in free water molecules is 104.5° but if the electron orbitals were really pointing to the four corners of a tetrahedron, the angle would be 109.5°. The usual explanation for this difference is that there is strong repulsion between the lone electron pairs and this repulsion pushes the covalent bond orbitals closer together, reducing the angle from 109.5° to 104.5°.

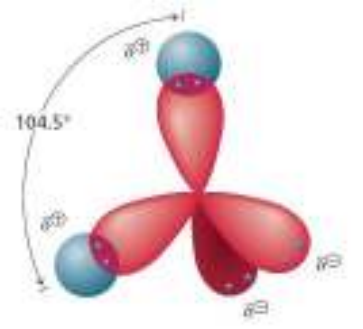

Fig. 12 Structure of water

b. Polarity of a molecule depends both on the polarity of its covalent bonds and its geometry.

c. The angled arrangement of the polar O—H bonds of H_2O creates a permanent dipole for the molecule as a whole.

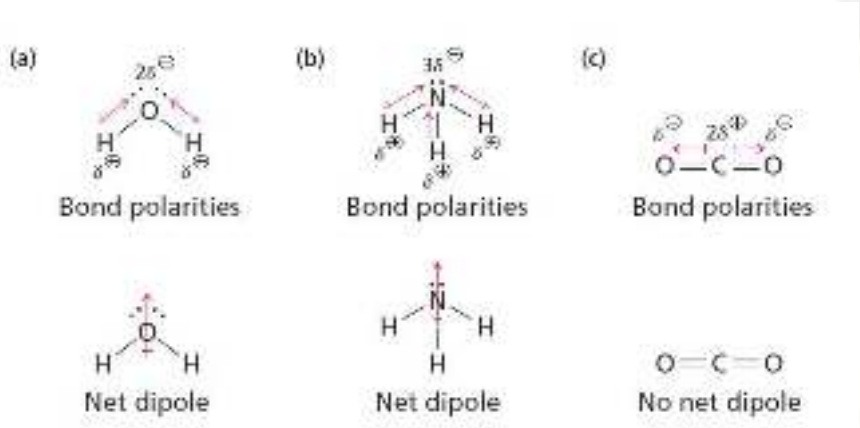

Fig. 13: Bond polarity and dipole for different molecules

d. A molecule of NH_3 also contains a permanent dipole.

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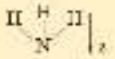
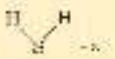
e. Thus, even though water and gaseous NH_3 are electrically neutral, both molecules are polar.
f. High solubility of polar ammonia molecules in water is facilitated by strong interactions with the polar water molecules. So, the solubility of ammonia in water demonstrates the principle that "like dissolves like."
g. Not all molecules are polar; for example, CO_2 also contains polar covalent bonds but the bonds are aligned with each other and oppositely oriented so the polarities cancel each other. As a result, CO_2 has no net dipole and is much less soluble in water than ammonia.
h. Some organisms have water-soluble carrier proteins (examples are hemoglobin and myoglobin) that facilitate the transport of O_2.
i. CO_2 forms carbonic acid in aqueous solution and is transported as the bicarbonate (HCO_3^-) ion, either free-bicarbonate is very soluble in water (~100 g/L at 25° C) -or bound to hemoglobin.

Table 2. Solubilities of some gases in water

Gas	Structure*	Polarity	Solubility in water (g/L)†
Nitrogen	N≡N	Nonpolar	0.018 (40 °C)
Oxygen	O—O	Nonpolar	0.035 (50 °C)
Carbon dioxide	O—C—O	Nonpolar	0.97 (45 °C)
Ammonia		Polar	900 (10 °C)
Hydrogen sulfide		Polar	1,860 (40 °C)

*The arrow represents electric dipole; there is a partial negative charge (δ⁻) on the head of the arrow, a partial positive charge (δ⁺) on the tail of the arrow.

j. Water has a higher melting point, boiling point, and heat of vaporization than most other common solvents.

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Table 2. Solubilities of some gases in water

Gas	Structure*	Polarity	Solubility in water (g/L)†
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Oxygen	O—O	Nonpolar	0.035 (50 °C)
Carbon dioxide	O—C—O	Nonpolar	0.97 (45 °C)
Ammonia	H, N, H, H	Polar	900 (10 °C)
Hydrogen sulfide	H, S, H	Polar	1.860 (40 °C)

*The arrows represent polar covalent bonds; δ+ points toward the more electropositive atom and δ− toward the more electronegative atom.
†Grams of gas dissolved in 1 L of water.

1.5.2 Hydrogen bonding between two water molecules

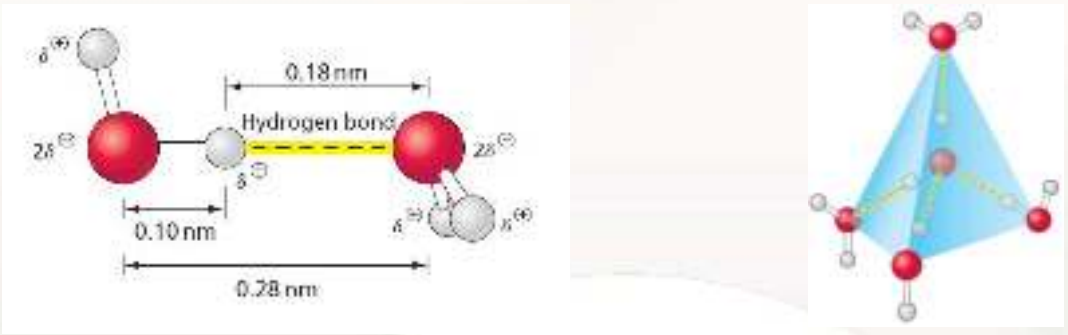

Fig. 14 Hydrogen bond

k. About 20 kJ mol^{-1} of heat is given off when hydrogen-bonded water molecules form in water under standard conditions (1 atm pressure and 25°C temperature). This value is the standard enthalpy of formation (ΔHf). It means that the change in enthalpy when hydrogen bonds form is about –20 kJ per mole of water.
l. This is equivalent to saying that +20 kJ mol^{-1} of heat energy is required to disrupt hydrogen bonds between water molecules.
m. In contrast, the energy required to break a covalent O—H bond in water is about 460 kJ mol^{-1}, and energy required to break a covalent C—H bond is about 410 kJ mol^{-1}. Thus, the strength of hydrogen bonds is less than 5% of the strength of typical covalent bonds.
n. Water molecules are unusual because they can form four O—H—O aligned hydrogen bonds with up to four other water molecules.

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o. They can donate each of their two hydrogen atoms to two other water molecules and accept two hydrogen atoms from two other water molecules.
p. Each hydrogen atom can participate in only one hydrogen bond.
q. In the common form of ice, every molecule of water participates in four hydrogen bonds, as expected.
r. Each of the hydrogen bonds points to the oxygen atom of an adjacent water molecule and these four adjacent hydrogen-bonded oxygen atoms occupy the vertices of a tetrahedron.
s. The bond angles are all equal (109.5°) in ice because the polarity of individual water molecules, which distorts the bond angles, is cancelled by the presence of hydrogen bonds.
t. Average energy required to break hydrogen bond in ice has been estimated to be 23 kJ mol^{-1}, making those bonds a bit stronger than those formed in water.
u. The ability of water molecules in ice to form four hydrogen bonds and the strength of these hydrogen bonds give ice an unusually high melting point because a large amount of energy, in the form of heat, is required to disrupt the hydrogen-bonded lattice of ice.
v. When ice melts most of the hydrogen bonds are retained by liquid water.
w. Each molecule of liquid water can form up to four hydrogen bonds with its neighbors but most participate in only two or three at any given moment. (Average value is 3.4)

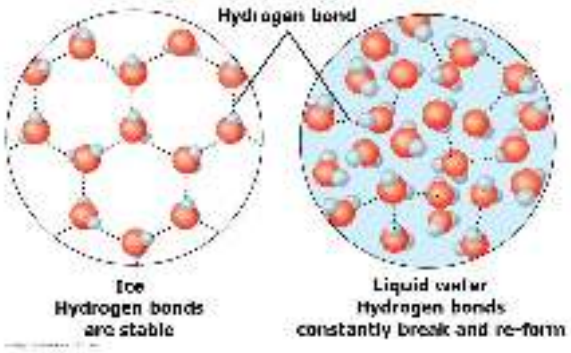

Fig. 15: Hydrogen bond formation in water molecules

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x. This means that the structure of liquid water is less ordered than that of ice.
y. The fluidity of liquid water is primarily a consequence of the constantly fluctuating pattern of hydrogen bonding as hydrogen bonds break and re-forms.
z. At any given time, there will be many water molecules participating in two, three, or four hydrogen bonds with other water molecules. There will also be many that participate in only one hydrogen bond or none at all. This is a dynamic structure—the average hydrogen bond life-time in water is only 1 to 20 picoseconds (1 ps =10^{-12} s).

The apt phrase "flickering clusters" has been applied to the short-lived groups of water molecules interlinked by hydrogen bonds in liquid water.

Directionality of the hydrogen bond

a. Hydrogen bonds are strongest when the bonded molecules are oriented to maximize electrostatic interaction, which occurs when the hydrogen atom and the two atoms that share it are in a straight line i.e. when the acceptor atom is in line with the covalent bond between the donor atom and H.
b. Hydrogen bonds are thus highly directional and capable of holding two hydrogen-bonded molecules or groups in a specific geometric arrangement.

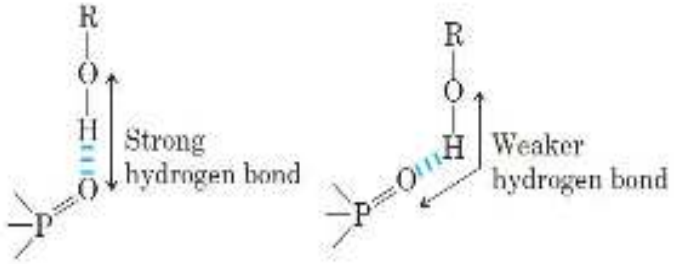

Fig. 16: Directionality of the Hydrogen bonds

c. The density of most substances increases upon freezing as molecular motion slows and tightly packed crystals form.
d. The density of water also increases as it cools—until it reaches a maximum of 1.0 g ml^{-1} at 4°C (277 K). (Grams are defined as the weight of 1 milliliter of water at 4°C.)

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e. Water expands as the temperature drops below 4°C. This expansion is caused by the formation of the more open hydrogen-bonded ice crystal in which each water molecule is hydrogen-bonded rigidly to four others. As a result, ice is slightly less dense (0.924 g ml^{-1}) than liquid water whose molecules can move enough to pack more closely.
f. Because ice is less dense than liquid water it floats and water freezes from the top down. This has important biological implications since a layer of ice on a pond insulates the creatures below from extreme cold.
g. Two additional properties of water are related to its hydrogen-bonding characteristics—its specific heat and its heat of vaporization.
h. The specific heat of a substance is the amount of heat needed to raise the temperature of 1 gram of the substance by 1°C. This property is also called the heat capacity.
i. In case of water, a relatively large amount of heat is required to raise the temperature because each water molecule participates in multiple hydrogen bonds that must be broken in order for the kinetic energy of the water molecules to increase (4.17 $J \cdot g^{-1} \cdot K^{-1}$) (1 Cal/g).
j. Abundance of water in cells and tissues of all large multicellular organisms means that temperature fluctuations within cells are minimized. This feature is of critical biological importance since the rates of most biochemical reactions are sensitive to temperature.
k. The heat of vaporization of water (~2260 J g^{-1}) is also much higher than that of many other liquids. (40.8 KJ/mol, 55.5)
l. A large amount of heat is required to convert water from a liquid to a gas because hydrogen bonds must be broken to permit water molecules to dissociate from one another and enter the gas phase.
m. Because the evaporation of water absorbs so much heat, perspiration is an effective mechanism for decreasing body temperature.

REFERENCES

Course Name	Fundamentals of Biochemistry
Lesson 2	Carbohydrate chemistry: Structure, classification, functions (mono, di and polysaccharides), isomerism and mutarotation
Content Creator Name	Dr. Ajay Pal
University/College Name	Chaudhary Charan Singh Haryana Agricultural University, Hisar
Course Reviewer Name	Sucheta Sharma
University/College Name	Punjab Agricultural University, Ludhiana

Lesson-2

Carbohydrate chemistry: Structure, classification, functions (mono, di and polysaccharides), isomerism and mutarotation

Objectives:

a. To study the classification and structures of carbohydrates
b. To study the physiological importance of carbohydrates
c. To study various reactions of monosaccharides

Glossary:

a. **Carbohydrates:** Polyhydroxy aldehydes or ketones, or compounds that can be derived from them.
b. **Homopolysaccharides:** polysaccharides composed of a single type of sugar monomer.
c. **Glycosaminoglycans (GAGs) or mucopolysaccharides:** Long linear polysaccharides consisting of repeating disaccharide units (i.e. two-sugar units).
d. **Enantiomer:** Stereoisomers that are non-superimposable mirror images of each other.
e. **Epimers:** Two sugars that differ in configuration only around one carbon atom.
f. **Racemic mixture:** A mixture that has equal amounts of left- (laevorotatory) and right-handed (dextrorotatory) enantiomers of a chiral molecule.
g. **Anomer:** An epimer at the hemiacetal/hemiketal carbon in a cyclic saccharide.
h. **Pyranose:** A chemical structure that includes a six-membered ring consisting of five carbon atoms and one oxygen atom.
i. **Furanose:** A chemical structure that includes a five-membered ring system consisting of four carbon atoms and one oxygen atom.
j. **Mutarotation:** Change in specific rotation of an optically active compound in solution with time, to an equilibrium value.

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2.1 Introduction and definition of carbohydrates

a. Carbohydrates are the most abundant biomolecules on Earth.
b. Carbohydrates played a key role in the establishment and evolution of life on earth by creating a direct link between the sun and chemical energy.
c. Carbohydrates are produced during the process of photosynthesis:

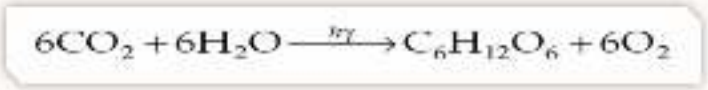

Fig. 1: Formation of carbohydrates

d. Each year, photosynthesis converts more than 100 billion metric tons of CO_2 and H_2O into glucose and other plant products.
e. Compounds with empirical formula, $(CH_2O)_n$, were initially called as carbohydrates (hydrates of carbons, old definition).
f. But, there are some carbohydrates (derivatives) that do not possess $(CH_2O)n$ formula e.g. 2-deoxyribose ($C_5H_{10}O_4$).
g. On the other hand, there are a few non-carbohydrate compounds like lactic acid ($C_3H_6O_3$) with empirical formula $(CH_2O)n$.
h. Hence, carbohydrates are polyhydroxy aldehydes or ketones, or compounds that can be derived from them.
i. Some carbohydrates contain nitrogen, phosphorous or sulphur also.

2.2 Classification and functions of carbohydrates

There are three major classes of carbohydrates:

1. Monosaccharides
2. Oligosaccharides
3. Polysaccharides

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2.2.1 Monosaccharides or simple sugars consist of a single polyhydroxy aldehyde or ketone unit.

a. The most abundant monosaccharide in nature is the six-carbon sugar D-glucose, sometimes referred to as dextrose.
b. Monosaccharides of more than four carbons tend to have cyclic structures.
c. Monosaccharides are colorless, crystalline solids that are freely soluble in water but insoluble in non-polar solvents.
d. Most have a sweet taste. Backbones of common monosaccharide molecules are unbranched carbon chains in which all the carbon atoms are linked by single bonds.
e. In open-chain form, one of the carbon atoms is double-bonded to an oxygen atom to form a carbonyl group; each of the other carbon atoms has a hydroxyl group.
f. If carbonyl group is at an end of the carbon chain (that is, in an aldehyde group) the monosaccharide is an aldose; if the carbonyl group is at any other position (in a ketone group) the monosaccharide is a ketose.
g. Simplest monosaccharides are the two three-carbon trioses: glyceraldehyde, an aldotriose, and dihydroxyacetone, a ketotriose.

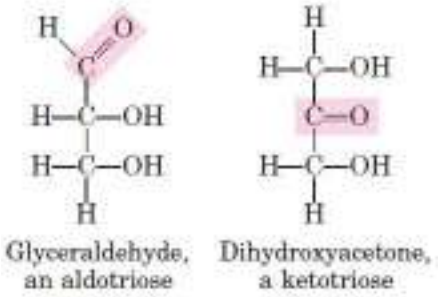

Fig. 2: Trioses

h. Monosaccharides with four, five, six, and seven carbon atoms in their backbones are called, respectively, tetroses, pentoses, hexoses, and heptoses.

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Classification of Monosaccharides

Number of Carbon Atoms	Kind of Carbonyl Group	
	Aldehyde	Ketone
3	Aldotriose	Triulose
4	Aldotetrose	Tetrulose
5	Aldopentose	Pentulose
6	Aldohexose	Hexulose
7	Aldoheptose	Heptulose
8	Aldooctose	Octulose
9	Aldononose	Nonulose

Table 1: Classification of monosaccharides

i. There are aldoses and ketoses of each of these chain lengths: aldotetroses and ketotetroses, aldopentoses and ketopentoses, and so on.
j. Aldopentoses D-ribose and 2-deoxy-D-ribose are components of nucleotides and nucleic acids.

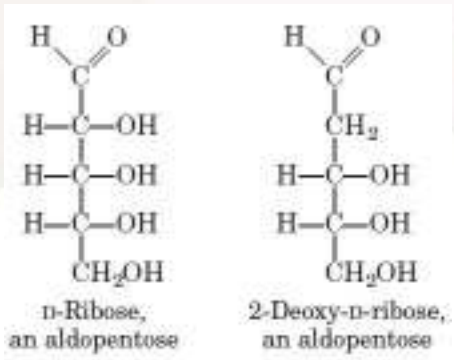

Fig. 3: Pentoses

k. Hexoses, which include the aldohexose D-glucose and the ketohexose D-fructose, are the most common monosaccharides in nature.

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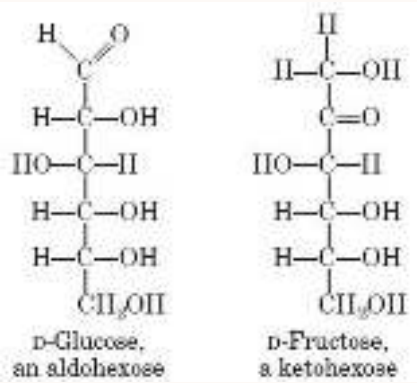

Fig. 4: Hexoses

2.2.2 Oligosaccharides consist of short chains of monosaccharide units, or residues, joined by characteristic linkages called glycosidic bonds.

a. Most abundant are the disaccharides, with two monosaccharide units.
b. Typical is sucrose (cane sugar), which consists of the six-carbon sugars D-glucose and D-fructose.
c. All common monosaccharides and disaccharides have names ending with the suffix "-ose."
d. In cells, most oligosaccharides consisting of three or more units do not occur as free entities but are joined to non-sugar molecules (lipids or proteins) in glycoconjugates.
e. Stachyose is typical of the oligosaccharide components found in substantial quantities in beans, peas, bran, white jasmine, yellow lupine and whole grains. This is metabolized readily by bacteria in the intestines.
f. Another notable glycoside is amygdalin, which occurs in bitter almonds (*Amygdalus communis* L.) and in the kernels or pits of cherries, peaches, and apricots.

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2.2.3 Polysaccharides are sugar polymers containing more than 20 or so monosaccharide units, and some have hundreds or thousands of units.

a. Some polysaccharides, such as cellulose, are linear chains; others, such as glycogen, are branched.
b. Most carbohydrates found in nature occur as polysaccharides, polymers of medium to high molecular weight.
c. Polysaccharides, also called glycans, differ from each other (1) in identity of their recurring monosaccharide units, (2) in length of their chains, (3) in the types of bonds linking the units, and (4) in degree of branching.
d. Homopolysaccharides contain only a single type of monomer; heteropolysaccharides contain two or more different kinds.
e. Some homopolysaccharides serve as storage forms of monosaccharides that are used as fuels; starch and glycogen are homopolysaccharides of this type.
f. Other homopolysaccharides (e.g. cellulose and chitin) serve as structural elements in plant cell walls and animal exoskeletons.
g. Heteropolysaccharides provide extracellular support for organisms of all kingdoms e.g. rigid layer of the bacterial cell envelope (the peptidoglycan) is composed in part of a heteropolysaccharide built from two alternating monosaccharide units.
h. In animal tissues, the extracellular space is occupied by several types of heteropolysaccharides, which form a matrix that holds individual cells together and provides protection, shape, and support to cells, tissues, and organs.
i. Unlike proteins, polysaccharides generally do not have definite molecular weights. This difference is a consequence of the mechanisms of assembly of the two types of polymers.

2.2.3.1 Some homopolysaccharides are stored forms of fuel

a. Most important storage polysaccharides are starch in plant cells and glycogen (animal starch) in animal cells.
b. Both polysaccharides occur intracellularly as large clusters or granules.
c. Most plant cells have the ability to form starch, but it is especially abundant in tubers, such as potatoes, and in seeds.
d. Starch is stored in plant cells in the form of granules in the stroma of plastids (plant cell organelles) of two types: chloroplasts, in which photosynthesis takes place, and amyloplasts, plastids that are specialized starch accumulation bodies.
e. When starch is to be mobilized and used by the plant that stored it, it must be broken down into its component monosaccharides.
f. Glycogen is main storage polysaccharide of animal cells.
g. Glycogen is especially abundant in the liver, where it may constitute as much as 7-10% of the wet weight; it is also present in skeletal muscle (0.5-2%).
h. In hepatocytes glycogen is found in large granules, which are themselves clusters of smaller granules composed of single, highly branched glycogen molecules with an average molecular weight of several million.

2.2.3.2 Some homopolysaccharides serve structural roles

a. Cellulose, a fibrous, tough, water-insoluble substance, is found in cell walls of plants, particularly in stalks, stems, trunks, and all the woody portions of the plant body.
b. It constitutes much of the mass of wood, and cotton is almost pure cellulose.
c. Chitin is a linear homopolysaccharide composed of N-acetylglucosamine (NAG).
d. The only chemical difference from cellulose is the replacement of the hydroxyl group at C-2 with an acetylated amino group.
e. Chitin is the principal component of the hard exoskeletons of nearly a million species of arthropods—insects, lobsters, and crabs, for e.g.—and is probably the second most abundant polysaccharide, next to cellulose, in nature.

f. Bacterial and algal cell walls contain structural heteropolysaccharides. Rigid component of bacterial cell walls is a heteropolymer of alternating N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAMA) residues.
g. Enzyme lysozyme kills bacteria by hydrolyzing the glycosidic bond between NAG and NAMA.

2.2.3.3 Glycosaminoglycans/GAGs are heteropolysaccharides of the extracellular matrix

a. Extracellular space in the tissues of multicellular animals is filled with a gel-like material, the extracellular matrix, also called ground substance, which holds the cells together and provides a porous pathway for the diffusion of nutrients and oxygen to individual cells.
b. Extracellular matrix is composed of an interlocking meshwork of heteropolysaccharides and fibrous proteins such as collagen, elastin, fibronectin, and laminin.
c. These heteropolysaccharides (GAGs) are a family of linear polymers composed of repeating disaccharide units.
d. One of the two monosaccharides is always either N-acetylglucosamine or N-acetylgalactosamine; the other is in most cases a uronic acid, usually D-glucuronic or L-iduronic acid.

Glycosaminoglycans include:

2.2.3.3.1 Hyaluronic acid (hyaluronate at physiological pH)

a. Contains alternating residues of D-glucuronic acid and N-acetylglucosamine.
b. Hyaluronate is also an essential component of the extracellular matrix of cartilage and tendons, to which it contributes tensile strength and elasticity as a result of its strong interactions with other components of the matrix.
c. Other glycosaminoglycans differ from hyaluronate in two respects: they are generally much shorter polymers and they are covalently linked to specific proteins (proteoglycans).

2.2.3.3.2 Chondroitin sulfate (Greek chondros, “cartilage”)

a. Contributes to the tensile strength of cartilage, tendons, ligaments, and the walls of the aorta.

2.2.3.3.3 Dermatan sulfate (Greek derma, “skin”)

a. Contributes to the pliability of skin and is also present in blood vessels and heart valves.
b. In this polymer, many of the glucuronate (GlcA) residues present in chondroitin sulfate are replaced by their epimer, iduronate (IdoA).

2.2.3.3.4 Keratan sulfates (Greek keras, “horn”)

a. Don’t have uronic acid and their sulfate content is variable.
b. They are present in cornea, cartilage, bone, and a variety of horny structures formed of dead cells: horn, hair, hoofs, nails, and claws.

2.2.3.3.5 Heparin (Greek hepar, “liver”)

a. Heparin is a natural anticoagulant made in mast cells and released into the blood, where it inhibits blood coagulation by binding to the protein antithrombin.
b. It has the highest negative charge density of any known biological macromolecule.

2.3 Structures of various carbohydrates

2.3.1 Monosaccharides

a. All the monosaccharides except dihydroxyacetone contain one or more asymmetric (chiral) carbon atoms and thus occur in optically active isomeric forms.
b. Simplest aldose, glyceraldehyde, contains one chiral center (the middle carbon atom) and therefore has two different optical isomers, or enantiomers.
c. By convention, one of these two forms is designated the D-isomer, the other the L-isomer.
d. To represent 3-D sugar structures on paper, we often use Fischer projection formulas.

e. In general, a molecule with n chiral centers can have 2^n stereoisomers (stereoisomers have the same chemical formula but differ in the position of the hydroxyl group on one or more of their asymmetric carbons).
f. Glyceraldehyde exists as two enantiomers, first identified by their opposite rotation of plane polarized light.
g. Naturally occurring glyceraldehyde rotates plane polarized light in a clockwise direction, and is designated (+) or d-glyceraldehyde. The enantiomer gives the opposite rotation and has a (-) or l- (levorotatory) prefix.

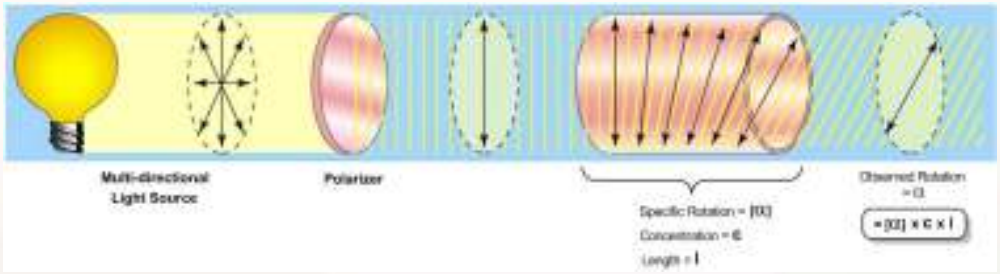

Fig. 5: Polarization by a chiral compound

D and L Monosaccharides

a. In 1891, Emil Fischer made the arbitrary assignments of D- and L- to the enantiomers of glyceraldehyde.

Fig. 6: D- and L- Glyceraldehyde

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b. A mixture of equal parts of an optically active isomer and its enantiomer is termed racemic and has zero net rotation of plane-polarized light because the positive rotation of each (+) form is exactly counteracted by the negative rotation of a (−) one.
c. The two enantiomers exhibit equal and opposite light rotation; thus, enantiomers are also called optical isomers.
d. Diastereomers have different physical and chemical reactivity.
e. Glyceraldehyde has $2^1 = 2$; the aldohexoses, with four chiral centers, have $2^4 = 16$ stereoisomers. (Vant Hoff's Rule of 'n')
f. Stereoisomers of monosaccharides of each carbon-chain length can be divided into two groups that differ in the configuration about the chiral center most distant from the carbonyl carbon. Those in which the configuration at this reference carbon is the same as that of D-glyceraldehyde are designated D-isomers, and those with the same configuration as L-glyceraldehyde are L-isomers.
g. When the hydroxyl group on the reference carbon is on the right in the projection formula, the sugar is the D-isomer; when on the left, it is the L-isomer.
h. Of 16 possible aldohexoses, eight are D forms and eight are L. Most of the hexoses of living organisms are D isomers.

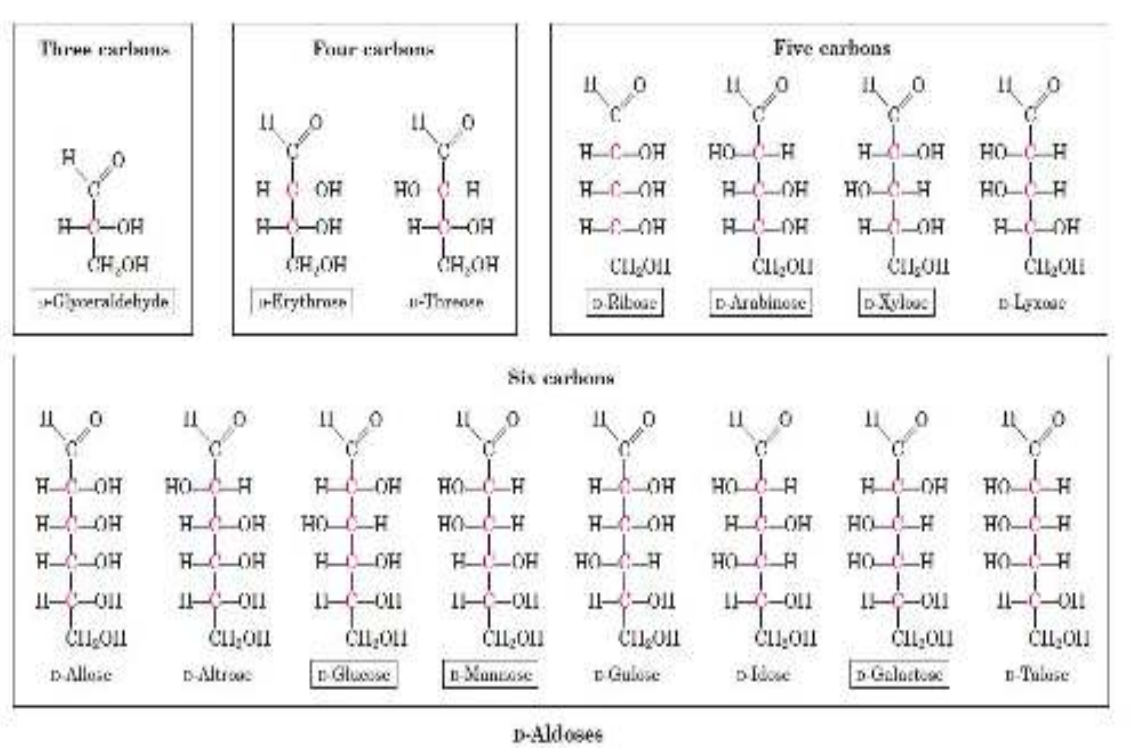

Fig 7: Series of D-aldoses from three to six carbon atoms. The carbon atoms in red are chiral centers. The sugars named in boxes are the most common in nature.

i. Four- and five-carbon ketoses are designated by inserting “ul” into the name of a corresponding aldose; for example, D-ribulose is the ketopentose corresponding to the aldopentose D-ribose.
j. Ketohexoses are named otherwise. E.g. fructose (from the Latin fructus, “fruit”; fruits are rich in this sugar) and sorbose (from the genus Sorbus which has berries rich in the related sugar alcohol sorbitol).

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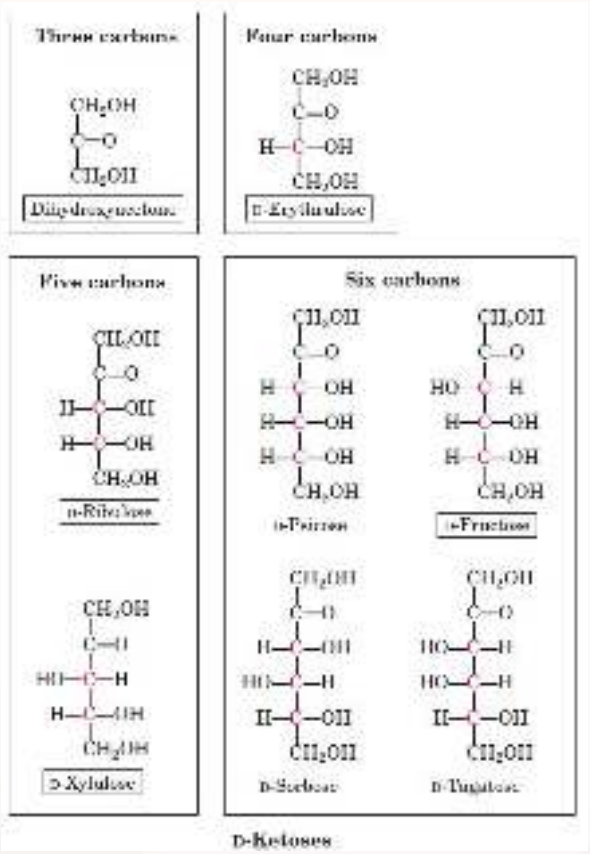

Fig. 8: Series of D-ketoses from three to six carbon atoms. The carbon atoms in red are chiral centers.

Epimers: Two sugars that differ only in the configuration around one carbon atom are called epimers; D-glucose and D-mannose, which differ only in the stereochemistry at C-2, are epimers, as are D-glucose and D-galactose (which differ at C-4).

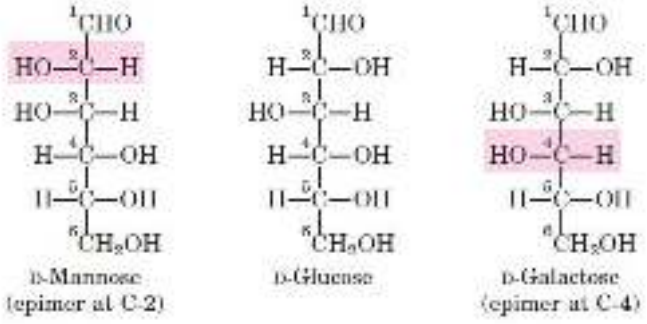

Fig. 9: D-Glucose and two of its epimers. Each epimer differs from D-glucose in the configuration at one chiral center (shaded red).

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2.3.1.1 Cyclic structures of glucose

a. In aqueous solution, aldotetroses and all monosaccharides with five or more carbon atoms in the backbone occur predominantly as cyclic (ring) structures in which the carbonyl group has formed a covalent bond with the oxygen of a hydroxyl group along the chain.
b. Formation of these ring structures is the result of a general reaction between alcohols and aldehydes or ketones to form derivatives called hemiacetals or hemiketals, which contain an additional asymmetric carbon atom and thus can exist in two stereoisomeric forms.
c. D-glucose exists in solution as an intramolecular hemiacetal in which the free hydroxyl group at C-5 has reacted with the aldehydic C-1, rendering the latter carbon asymmetric and producing two stereoisomers, designated α and β.
d. These six-membered ring compounds are called pyranoses because they resemble the six membered ring compound pyran.
e. Aldohexoses also exist in cyclic forms having five membered rings, which, because they resemble the five membered ring compound furan, are called furanoses.
f. However, the six-membered aldopyranose ring is much more stable than the aldofuranose ring and predominates in aldohexose solutions.
g. **Anomers:** Isomeric forms of monosaccharides that differ only in their configuration about the hemiacetal or hemiketal carbon atom are called anomers.
h. **Anomeric carbon:** Hemiacetal (or carbonyl) carbon atom is called the anomeric carbon.

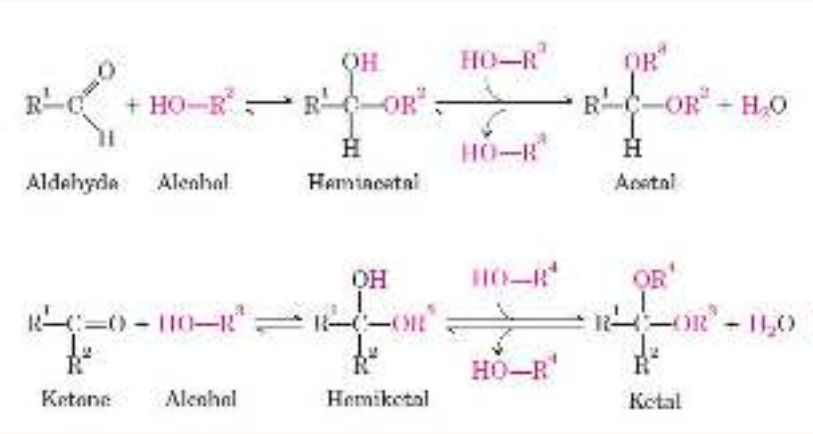

Fig 10: Formation of hemiacetals and hemiketals

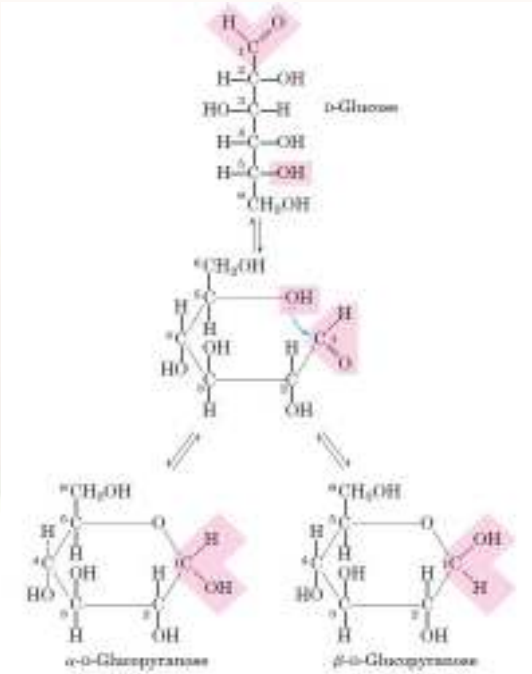

Fig. 11: Cyclic structure of glucose

2.3.1.2 Cyclic structure of fructose

a. Ketohexoses also occur in α and β anomeric forms.
b. In these compounds the hydroxyl group at C-5 (or C-6) reacts with the keto group at C-2, forming a furanose (or pyranose) ring containing a hemiketal linkage.

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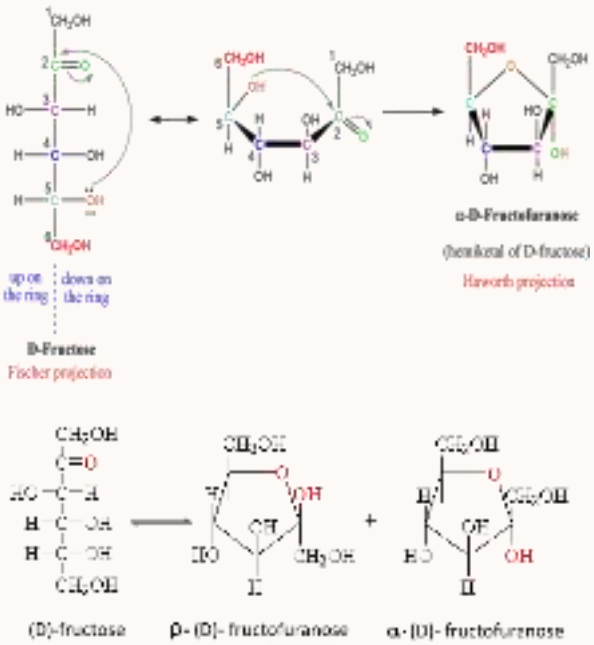

Fig. 12: Cyclic structure of fructose

2.3.2 Disaccharides

a. Disaccharides (such as maltose, lactose, and sucrose) consist of two monosaccharides joined covalently by an O-glycosidic bond, which is formed when a hydroxyl group of one sugar reacts with the anomeric carbon of the other.
b. This reaction represents the formation of an acetal from a hemiacetal (such as glucopyranose) and an alcohol (a hydroxyl group of the second sugar molecule).
c. Glycosidic bonds are readily hydrolyzed by acid but resist cleavage by base. Thus, disaccharides can be hydrolyzed to yield their free monosaccharide components by boiling with dilute acid.
d. When the anomeric carbon is involved in a glycosidic bond, that sugar residue cannot take the linear form and therefore becomes a non-reducing sugar.
e. In describing disaccharides or polysaccharides, the end of a chain with a free anomeric carbon (one not involved in a glycosidic bond) is commonly called the reducing end.

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Maltose

a. Disaccharide maltose contains two D-glucose residues joined by a glycosidic linkage between C-1 (the anomeric carbon) of one glucose residue and C-4 of the other.
b. Because the disaccharide retains a free anomeric carbon (C-1 of the glucose residue on the right, maltose is a reducing sugar.

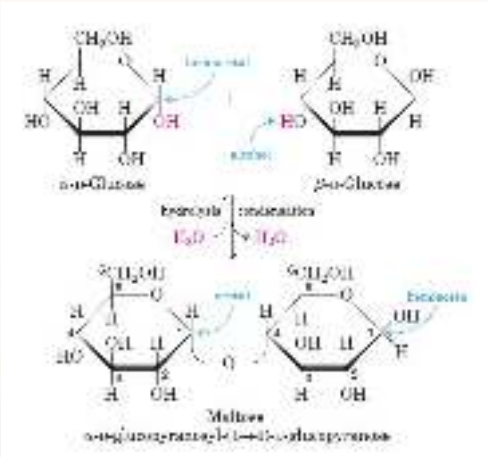

Fig. 13: Structure of maltose

Lactose

a. Lactose is a reducing disaccharide found only in **milk**.
b. It is made up of galactose at the non-reducing end and glucose at the reducing end.
c. They are connected by a beta **(1→ 4) linkage.**

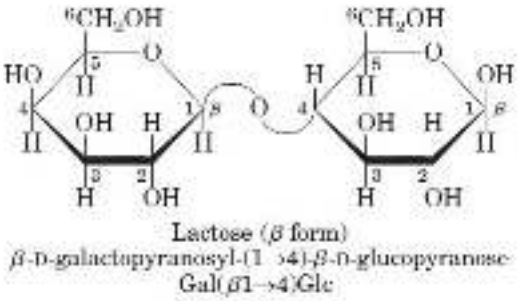

Fig. 14: Structure of lactose

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Sucrose

a. Sucrose, a sugar of commercial importance, is widely distributed in higher plants.
b. Sugarcane and sugar beet are the sole commercial sources.
c. It is made up of glucose and fructose.
d. The anomeric carbon atom of glucose (C-1) and fructose (C-2) are involved in linkage and is therefore a non-reducing disaccharide.
e. Sucrose is a major intermediate product of photosynthesis and it is the principal form in which sugar is transported from the leaves to other portions of plants via their vascular systems.

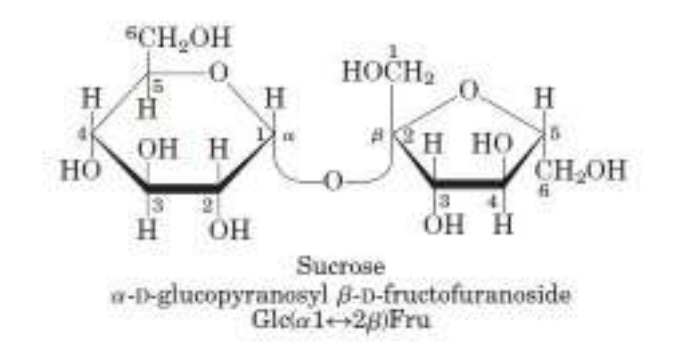

Fig. 15: Structure of sucrose

2.3.3 Polysaccharides

2.3.3.1 Starch

a. Starch contains two types of glucose polymer, amylose and amylopectin.

b. Amylose consists of long, unbranched chains of D-glucose residues connected by (α 1→4) linkages.

c. Such chains vary in molecular weight from a few thousand to more than a million.

d. Amylopectin also has a high molecular weight (up to 100 million) and is highly branched.

e. Glycosidic linkages joining successive glucose residues in amylopectin chains are (α1 → 4); the branch points (occurring every 24 to 30 residues) are (α1 → 6) linkages.

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f. Most forms of starch in nature are 10 to 30% α-amylose and 70 to 90% amylopectin.

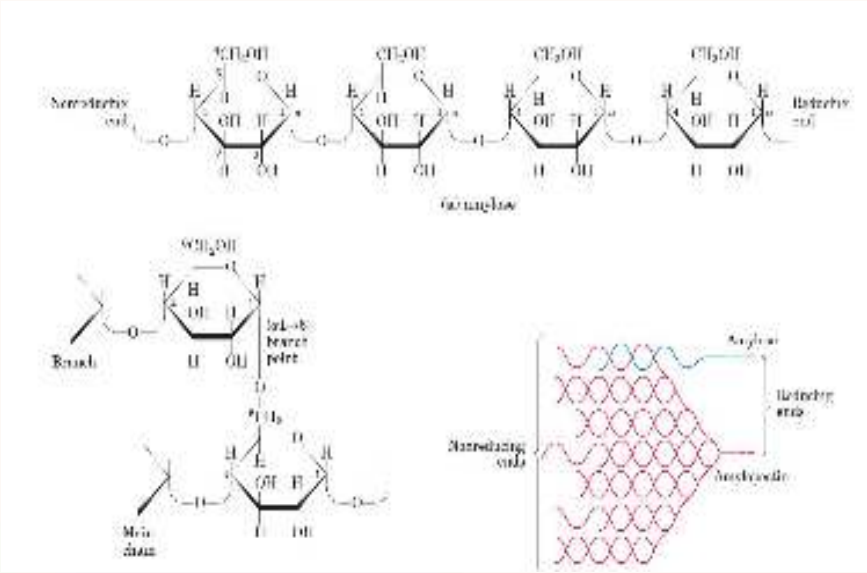

Fig. 16: A cluster of amylose and amylopectin like that believed to occur in starch granules. Strands of amylopectin (red) form double helical structures with each other or with amylose strands (blue)

2.3.3.2 Glycogen

a. Like amylopectin, glycogen is a polymer of (α1→4)-linked subunits of glucose, with (α1→6)-linked branches, but glycogen is more extensively branched (every 8 to 12 residues) and more compact than starch.

b. Glycogen is especially abundant in the liver, where it may constitute as much as 7-10% of the wet weight; it is also present in skeletal muscle (0.5-2%).

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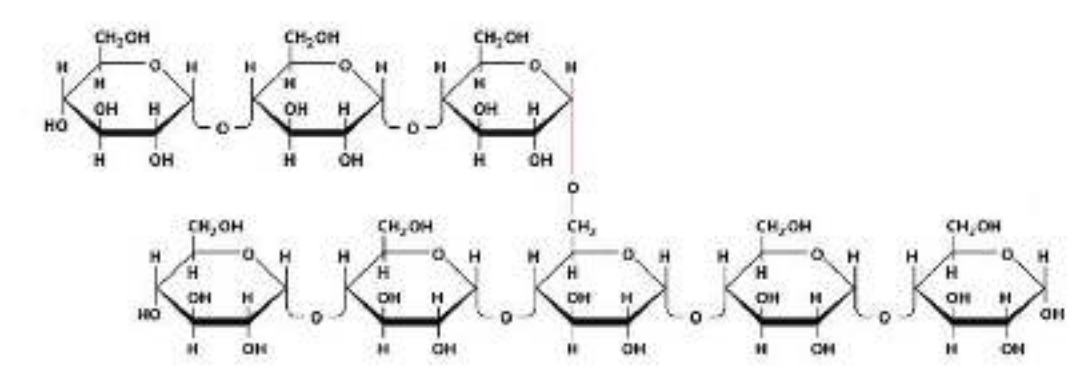

Fig. 17: Structure of glycogen

2.3.3.3 Cellulose

a. Cellulose, a fibrous, tough, water-insoluble substance, is found in cell walls of plants, particularly in stalks, stems, trunks, and all the woody portions of the plant body.

b. It constitutes much of the mass of wood, and cotton is almost pure cellulose.

c. Cellulose molecule is a linear, unbranched homopolysaccharide, consisting of 10,000 to 15,000 D-glucose units in β-configuration.

d. Glucose residues in cellulose are linked by (β1→4) glycosidic bonds, in contrast to the (α1→4) bonds of amylose, starch, and glycogen.

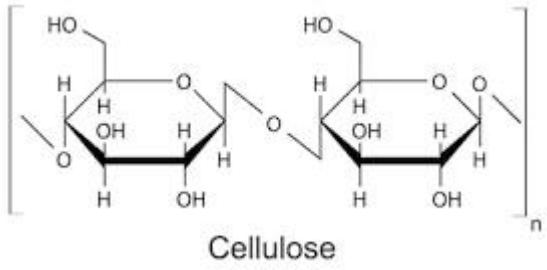

Fig. 18: Structure of cellulose

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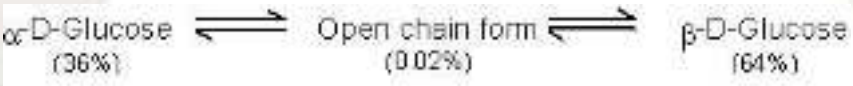
2.4 Polarimetry and Mutarotation

a. Sugar solutions can be analyzed by polarimetry (a method based on the interaction between chiral centers and linearly polarized light).
b. Linearly polarized light can be produced by passing normal light through a special filter (a polarizer).
c. Solutions of chiral substances rotate the plane of polarized light by an angle, α (either to the left or to the right).
d. A solution's optical rotation depends on the type of chiral compound, its concentration, and the thickness of the layer of the solution.
e. Two stereoisomeric forms of glucose i.e. α-D-glucose and β-D-glucose exist in separate crystalline forms and thus have different melting points and specific rotations.
f. For example α-D-glucose has a melting point of 419°K with a specific rotation of +112° while β-D-glucose has a melting point of 424°K and has a specific rotation of +19°.
g. However, when either of these two forms is dissolved in water and allowed to stand, it gets converted into an equilibrium mixture of α-and β-forms through a small amount of the open chain form.



h. As a result of this equilibrium, the specific rotation of a freshly prepared solution of α-D-glucose gradually decreases from of +112° to +52.7° and that of β-D-glucose gradually increases from +19° to +52.7°.
i. This change in specific rotation of an optically active compound in solution with time, to an equilibrium value (due to change in molecular form), is called mutarotation.
j. During mutarotation, the ring opens and then recloses either in the inverted position or in the original position giving a mixture of α- and β- forms. All

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reducing carbohydrates, i.e., monosaccharides and disaccharides (maltose, lactose etc.) undergo mutarotation in aqueous solution.

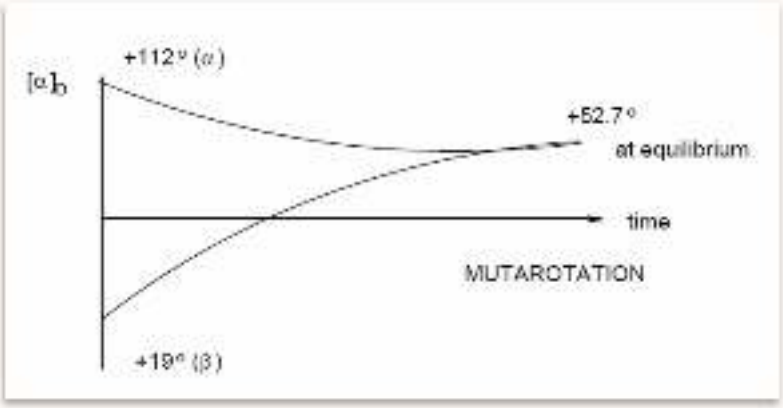

Fig. 19: Process of mutarotation

2.5 Important reactions of carbohydrates/Monosaccharides

2.5.1 Oxidation

a. An important reaction of monosaccharides is the oxidation of aldehyde group, one of the most easily oxidized organic functional groups.
b. Aldehyde oxidation can be accomplished with any mild oxidizing agent, such as Tollens' reagent or Benedict's reagent.
c. With the latter, complexed copper (II) ions are reduced to copper (I) ions that form a brick-red precipitate of copper (I) oxide.
d. Any carbohydrate capable of reducing either Tollens' or Benedict's reagents without first undergoing hydrolysis is said to be a reducing sugar.

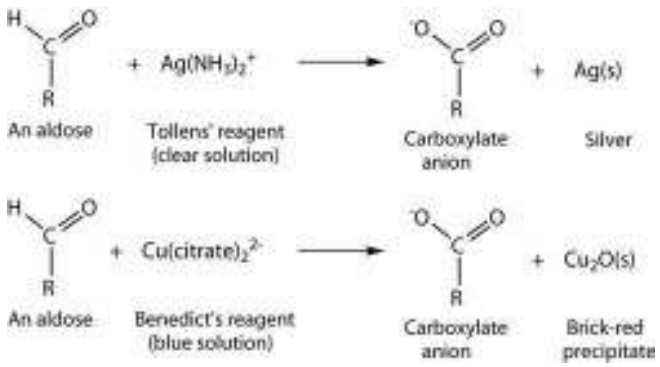

Fig. 20: Tollen's and Benedict's test

2.5.2 Glycoside formation

a. Acetal derivatives formed when a monosaccharide reacts with an alcohol in the presence of an acid catalyst are called glycosides.
b. This reaction is illustrated for glucose and methanol in the diagram below:

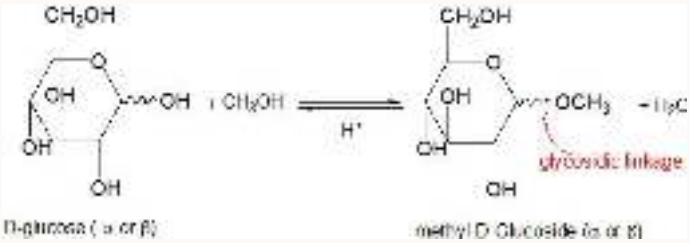

Fig. 21 Formation of glycoside

c. In naming of glycosides, the "ose" suffix of the sugar name is replaced by "oside", and the alcohol group name is placed first.
d. As is generally true for most acetals, glycoside formation involves the loss of an equivalent of water.
e. The product is stable to base and alkaline oxidants such as Tollen's reagent.
f. Since acid-catalyzed formation of acetals is reversible, glycosides may be hydrolyzed back to their alcohol and sugar components by aqueous acid.

2.5.3 Biological ester formation: Phosphorylation

a. Almost all biomolecules are charged species, which (1) keeps them water soluble, and (2) prevents them from diffusing across lipid bilayer membranes.
b. Although many biomolecules are ionized by virtue of negatively charged carboxylate and positively charged amino groups, the most common ionic group in biologically important organic compounds is phosphate. Thus, the phosphorylation of alcohol groups is a critical metabolic step.
c. In alcohol phosphorylations, ATP is almost always the phosphate donor, and the mechanism is very consistent: the alcohol oxygen acts as a nucleophile, attacking the gamma-phosphorus of ATP and expelling ADP.

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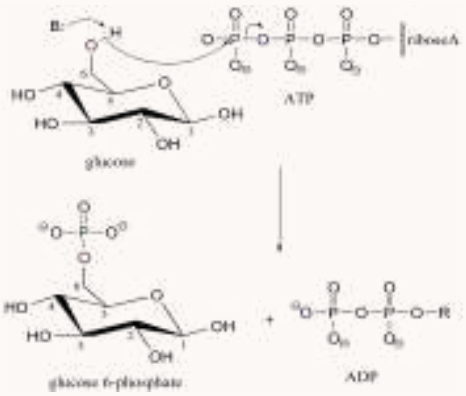

Fig. 22: Formation of biological ester

2.5.4 Derived monosaccharides

a. In sugars, the hydroxyl group is replaced by a hydrogen to produce deoxy sugars.
b. The important deoxy sugar is **2-deoxy ribose** that occurs in deoxy ribonucleic acid.
c. Other important deoxy sugars are L-fucose and L. rhamnose. The substitution of the hydroxyl group at C-6 of L-galactose or L-mannose with hydrogen produces fucose or rhamnose respectively.
d. L-fucose occurs in the cell wall polysaccharides namely hemicelluloses and L-rhamnose occurs in pectic polysaccharides namely rhamnogalacturonan. These deoxy sugars are also found in the complex oligosaccharide components of glycoproteins and glycolipids.

Amino sugars

a. The hydroxyl group, usually at **C-2**, is replaced by an amino group to produce aminosugars such as glucosamine, galactosamine and mannosamine.
b. The amino group may be condensed with acetic acid to produce N-acetyl amino sugars, for example, N-acetyl glucosamine. This glucosamine derivative is important constituent of many structural polymers (chitin, bacterial cell wall polysaccharides).

Polyols (alditols)

a. Both aldoses and ketoses are reduced to **polyhydric alcohols (polyols)** when treated with enzymes, sodium amalgam, and hydrogen under high pressure with catalyst or sodium borohydride.
b. Each aldose yields the corresponding alcohol upon reduction. A ketose forms two alcohols because of the appearance of a new asymmetric carbon atom in the process.
c. By this reduction process, the following sugars give rise to their respective alcohols under specified conditions.

Glucose	Sorbitol
Fructose	Sorbitol and mannitol
Mannose	Mannitol
Glyceraldehyde	Glycerol
Erythrose	Erythritol
Ribose	Ribitol
Galactose	Dulcitol

d. Polyols occur in many plant products. Sorbitol was first isolated from the berries of mountain ash (Sorbus aucuparia). Commercially sorbitol is manufactured by the hydrogenation of glucose.
e. Mannitol occurs in many terrestrial and marine plants.
f. Potential food applications of polyols include confectionery products, bakery products, deserts, jams and marmalade.

g. Sorbitol is an excellent moisture conditioner and is used in pharmaceutical preparations such as elixirs and syrups.
h. Sorbitol, as a humectant in creams and lotions helps to stabilize the water content, providing better moisture control.
i. The use of sorbitol or xylitol in toothpaste and mouthwashes is highly desirable.

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Course Name	Fundamentals of Biochemistry
Lesson 3	Metabolism of carbohydrates: glycolysis, gluconeogenesis, glycogenolysis, glycogenesis, TCA cycle, central role of TCA cycle in metabolism
Content Creator Name	Dr. Ajay Pal
University/College Name	Chaudhary Charan Singh Haryana Agricultural University, Hisar
Course Reviewer Name	Sucheta Sharma
University/College Name	Punjab Agricultural University, Ludhiana

Lesson 3:

Metabolism of carbohydrates: glycolysis, gluconeogenesis, glycogenolysis, glycogenesis, TCA cycle, central role of TCA cycle in metabolism

Objectives:

a. To study the major pathways of glucose metabolism
b. To study glycolysis and gluconeogenesis
c. To study catabolism and anabolism of glycogen
d. To study TCA cycle and its importance in metabolism

Glossary:

a. **Metabolism:** Sum of all chemical transformations taking place in a cell or organism.
b. **Catabolism:** Set of metabolic pathways that break down molecules into smaller units that are either oxidized to release energy or used in other anabolic reactions.
c. **Anabolism:** Synthesis of complex molecules in living organisms from simpler ones together with the storage of energy.
d. **Glycolysis:** Process by which one molecule of glucose is converted into two molecules of pyruvate.
e. **Glycogenolysis:** Breakdown of glycogen into glucose, a simple sugar that body uses to produce energy.
f. **Glycogenesis:** Process of glycogen synthesis, in which glucose molecules are added to chains of glycogen for storage.
g. **Cellular respiration:** Molecular processes by which cells consume O2 and produce CO2.

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3.1 Metabolism

a. Metabolism is the sum of all chemical transformations taking place in a cell or organism. It occurs through a series of enzyme-catalyzed reactions that constitute metabolic pathways.
b. Each consecutive step in a metabolic pathway brings about a specific (since enzymatic), small chemical change (usually the removal, transfer, or addition of a particular atom/functional group).
c. The precursor is converted into a product through a series of metabolic intermediates called metabolites.

3.1.1 Catabolism

a. Degradative phase of metabolism.
b. Organic nutrient molecules (carbohydrates, fats, and proteins) are converted into smaller, simpler end products (such as lactic acid, CO2, NH3).
c. Catabolic pathways release energy.
d. Some energy is conserved in formation of ATP and reduced electron carriers (NADH, NADPH, and FADH2) and the rest is lost as heat.

3.1.2 Anabolism

a. Synthetic (biosynthetic) phase of metabolism.
b. Small, simple precursors are built up into larger and more complex molecules like lipids, polysaccharides, proteins, and nucleic acids.
c. Anabolic reactions require an input of energy, generally in the form of the phosphoryl group transfer potential of ATP and the reducing power of NADH, NADPH, and FADH2.

3.2 Metabolism of carbohydrates

a. Carbohydrate is utilized by cells mainly in the form of glucose.
b. The three principal monosaccharides resulting from the digestive processes are glucose, fructose and galactose.
c. Both fructose and galactose are readily converted to glucose by the liver.
d. Since glucose is the compound formed from starch and glycogen, the carbohydrate metabolism commences with this monosaccharide.

3.3 Glycolysis

a. A molecule of glucose (glc) is degraded in a series of enzyme-catalyzed reactions to yield two molecules of 3-carbon compound pyruvate.
b. During the sequential reactions of glycolysis, some of the free energy released from glucose is conserved in the form of ATP and NADH.
c. Glycolysis was the first metabolic pathway to be elucidated and is probably the best understood.
d. It is the primary pathway occurring in the cytoplasm of all the tissues of biological systems.
e. Glycolysis is an almost universal central pathway of glc catabolism.
f. It is the pathway with the largest flux of carbon in most cells.
g. Glycolytic breakdown of glc is the sole source of metabolic energy in some mammalian tissues and cell types (like erythrocytes).
h. Many anaerobic microorganisms are entirely dependent on glycolysis.
i. The term fermentation is generally used for anaerobic degradation of glucose or other organic nutrients to obtain energy, conserved as ATP.
j. Glycolysis differs among species only in the details of its regulation and in the subsequent metabolic fate of the pyruvate formed.

In plants, glucose and fructose are the main monosaccharides catabolised by glycolysis although others are also converted into these sugars.

a. Glucose entering the glycolysis is derived from starch or sucrose, and fructose is derived from sucrose.
b. The starch is either from seeds or chloroplasts of matured plants.
c. Glycolysis normally takes place in the presence of O2 in higher plant cells.

Glycolysis has two phases: The breakdown of the 6-carbon glucose into two molecules of the 3-carbon pyruvate occurs in ten steps.

a. The first five of which constitute the preparatory phase.
b. "lysis/lyase" step gives the pathway its name.
c. Two molecules of ATP are invested before the cleavage of glucose into two 3-carbon pieces.
d. The second phase is the payoff phase. It gives energy.
e. The net yield is two molecules of ATP per molecule of glucose used, because two molecules of ATP were invested in the preparatory phase. Energy is also conserved in the payoff phase in the formation of two molecules of NADH per molecule of glucose.

Three types of chemical transformations during glycolysis:

a. Degradation of the carbon skeleton of glucose to yield pyruvate,
b. Phosphorylation of ADP to ATP by high-energy phosphate compounds formed during glycolysis, and
c. Transfer of a hydride ion to NAD+ to form NADH.

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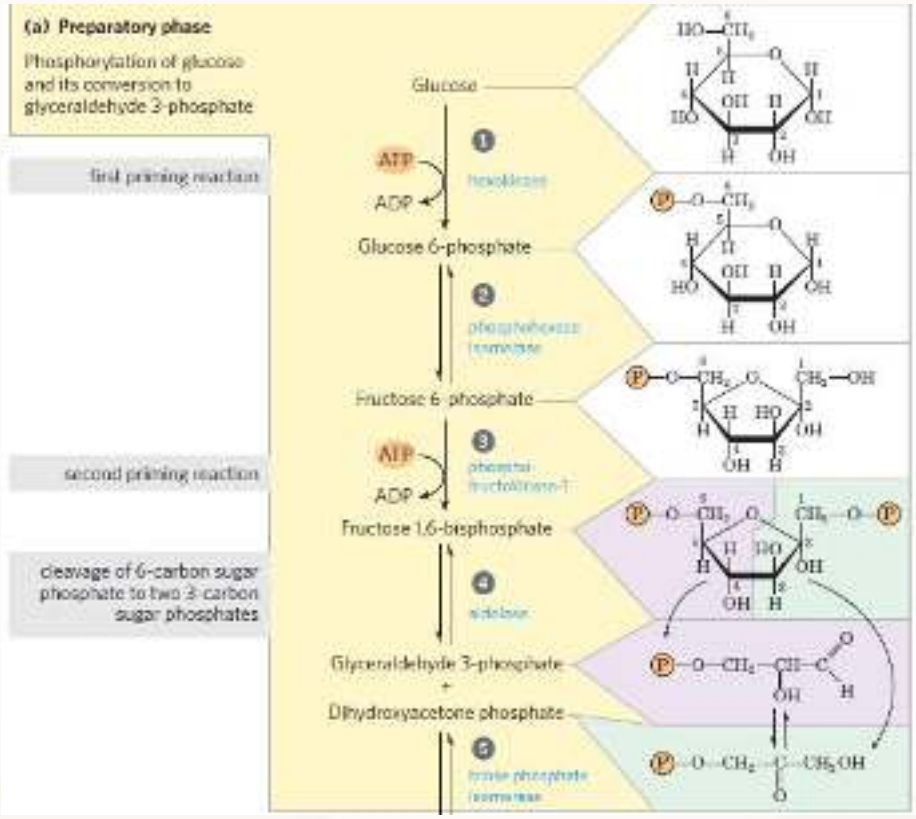

Fig. 1 Preparatory phase of Glycolysis

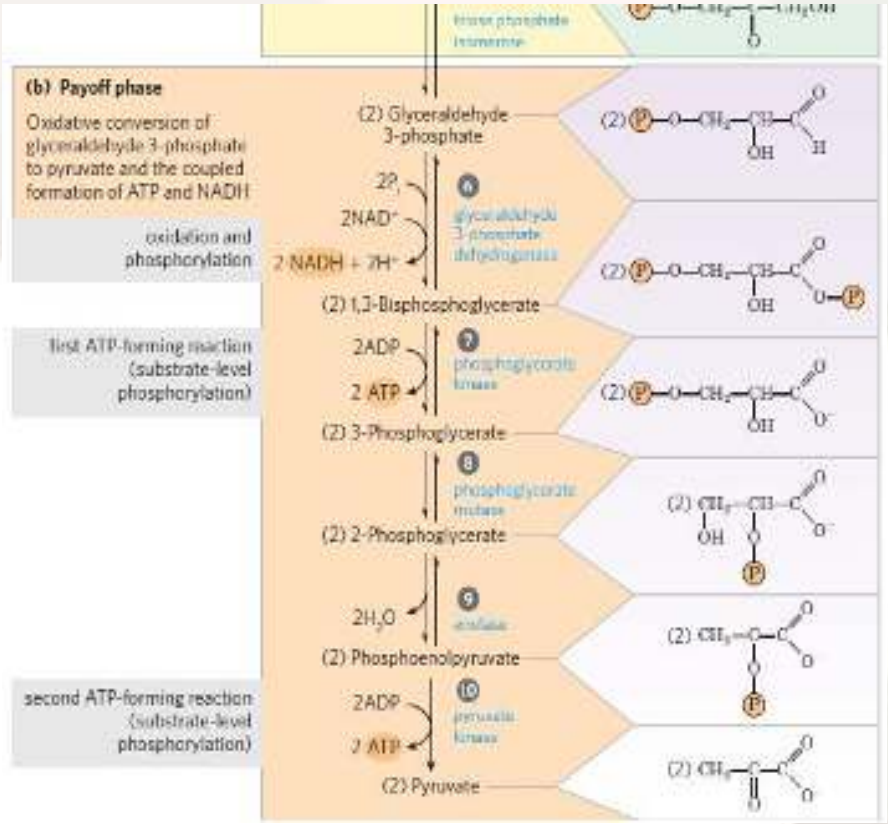

Fig. 2 Pay-off phase of Glycolysis

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Net reaction of Glycolysis:

Glucose + 2 NAD+ + 2 Pi + 2 ADP → 2 pyruvate + 2 ATP + 2 NADH + 2 H++ 2 H2O

3.4 Three catabolic fates of pyruvate:

a. Pyruvate is oxidized, with loss of its carboxyl group as CO2, to yield the acetyl group of acetyl-coenzyme A; The acetyl group is then oxidized completely to CO2 by the TCA/citric acid cycle. The energy from the electron-transfer reactions drives the synthesis of ATP in the mitochondrion.
b. Pyruvate is reduced to lactate via lactic acid fermentation. In vigorously contracting skeletal muscle, under low oxygen conditions (hypoxia), NADH can't be reoxidized to NAD+, but NAD+ is required to continue glycolysis (G3PDH). Under these conditions, pyruvate is reduced to lactate, accepting electrons from NADH and thereby regenerating the NAD+ necessary for glycolysis to continue.
c. The third route of pyruvate catabolism leads to ethanol. In some plant tissues and in certain invertebrates, protists, and microorganisms such as brewer's yeast, pyruvate is converted under hypoxic or anaerobic conditions into ethanol and CO2, a process called ethanol (alcohol) fermentation.
d. The oxidation of pyruvate is an important catabolic process, but pyruvate has anabolic fates as well. It can, for example, provide the carbon skeleton for the synthesis of the amino acid alanine.

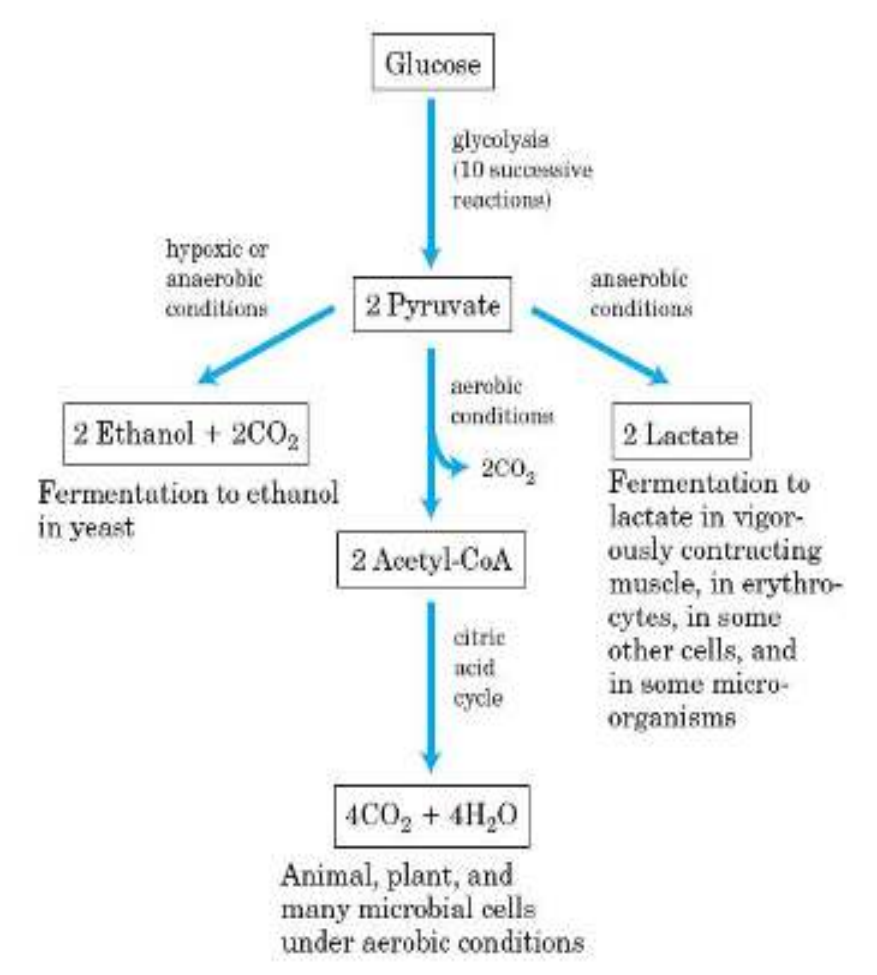

Fig. 3 Three possible catabolic fates of the pyruvate formed in glycolysis.

3.5 Gluconeogenesis

a. In mammals, some tissues depend almost completely on glucose for their metabolic energy. For human brain and nervous system, as well as erythrocytes, testes, renal medulla, and embryonic tissues, glc from blood is the sole or major source of fuel.
b. However, supply of glc from these stores is not always sufficient; between meals and during longer fasts, or after vigorous exercise glycogen is depleted.
c. For these times, organisms need a method for synthesizing glc from non-carbohydrate precursors. This is accomplished by a pathway called gluconeogenesis (GNG) (“formation of new sugar”), which converts pyruvate and related three- and four-carbon compounds to glc.
d. GNG occurs in all animals, plants, fungi, and microorganisms.

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e. Reactions are essentially same in all tissues and all species.
f. Important precursors of glc in animals are three-carbon compounds such as lactate, pyruvate, and glycerol, as well as certain amino acids.
g. In mammals, GNG takes place mainly in liver, and to a lesser extent in renal cortex.
h. GNG and glycolysis are not identical pathways running in opposite directions, although they do share several steps; seven of the ten enzymatic reactions of GNG are reverse of glycolytic reactions.
i. However, three reactions of glycolysis are essentially irreversible in vivo and can't be used in GNG.
    1. Conversion of glc to glc-6-phosphate by hexokinase (HK).
    2. Phosphorylation of fru-6-phosphate to fru-1,6-bisphosphate by phosphofructo kinase-1 (PFK-1), and
    3. Conversion of phosphoenol pyruvate (PEP) to pyruvate by pyruvate kinase (PK).

In GNG, three irreversible steps are by-passed by a separate set of enzymes, catalyzing reactions that are sufficiently exergonic to be effectively irreversible in the direction of glc synthesis.

Net reaction of Gluconeogenesis:

2 Pyruvate + 4ATP + 2GTP + 2NADH + 6H2O → Glucose + 4ADP + 2GDP + 6Pi + 2NAD+ + 2H+

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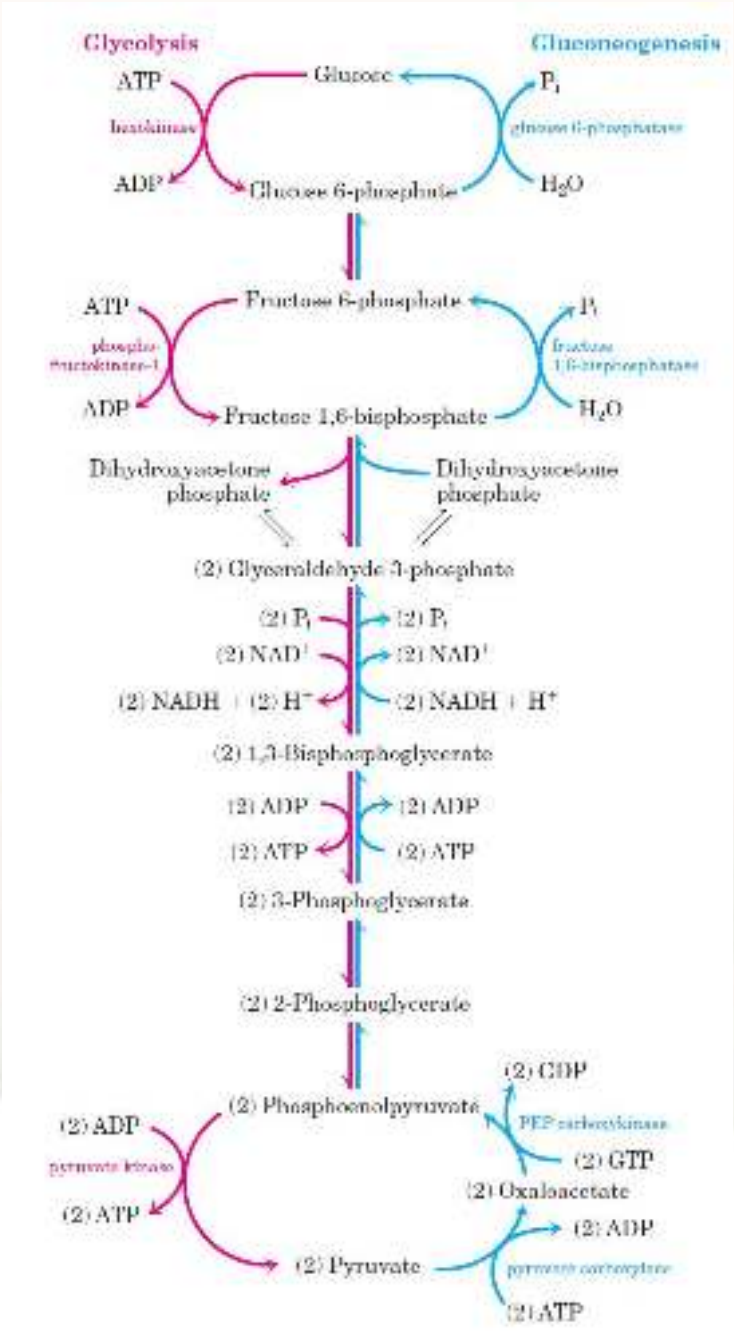

Fig. 4 Glycolysis and gluconeogenesis

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3.6 Glycogen metabolism in animals

a. In a wide range of organisms, excess glc is converted to polymeric forms for storage—glycogen in vertebrates and many microorganisms, starch in plants.
b. In vertebrates, glycogen is found primarily in liver and skeletal muscle; it may represent up to 10% of the fresh weight of liver and 1-2% of the fresh weight of muscle.
c. Glycogen is stored in large cytosolic granules.
d. Glycogen in muscle is there to provide a quick source of energy for either aerobic or anaerobic metabolism. Muscle glycogen can be exhausted in less than an hour during vigorous activity. Liver glycogen serves as a reservoir of glucose for other tissues when dietary glucose is not available (between meals or during a fast); this is especially important for neurons of brain, which can't use fatty acids as fuel. Liver glycogen can be depleted in 12-24 hours.
e. In humans, total amount of energy stored as glycogen is far less than the amount stored as fat (TAG) but fats can't be converted to glc in mammals and can't be catabolized anaerobically.

3.6.1 Glycogenolysis

a. It is the pathway for breakdown of glycogen into glucose, a simple sugar that body uses to produce energy.
b. Glycogen breakdown is catalyzed by the enzyme, glycogen phosphorylase.
c. In skeletal muscle and liver, glucose units of outer branches of glycogen enter glycolytic pathway through action of three enzymes:
    1. Glycogen phosphorylase: catalyzes reaction in which an α1 → 4 glycosidic linkage between two glc residues at nonreducing (NR) end of glycogen undergoes attack by inorganic phosphate (Pi), removing terminal glc residue as D-glc 1-Ⓟ. Glycogen phosphorylase acts repetitively on NR ends of glycogen branches until it reaches a point four glc residues away from an α1→6 branch point, where its action stops. Glc 1-Ⓟ can enter glycolysis or, in liver, replenish blood glc.

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2. Glycogen debranching enzyme: Further degradation by glycogen phosphorylase can occur only after the debranching enzyme catalyzes two successive reactions that transfer branches. Once these branches are transferred and the glucosyl residue at C-6 is hydrolyzed, glycogen phosphorylase activity can continue.
3. Phosphoglucomutase: Glc 1-Ⓟ, the end product of the glycogen phosphorylase reaction, is converted to glc 6-Ⓟ by phosphoglucomutase, which catalyzes the reversible reaction:

d. Glc 6-Ⓟ formed from glycogen in skeletal muscle can enter glycolysis and serve as an energy source to support muscle contraction.
e. In liver, glycogen breakdown serves a different purpose: to release glc into blood when blood glc level drops, as it does between meals. This requires an enzyme, glc-6-phosphatase, that is present in liver and kidney but not in other tissues.
f. Because muscle and adipose tissue lack glc 6-phosphatase, they can't convert glc-6-Ⓟ formed by glycogen breakdown to glc, and these tissues therefore do not contribute glc to blood.

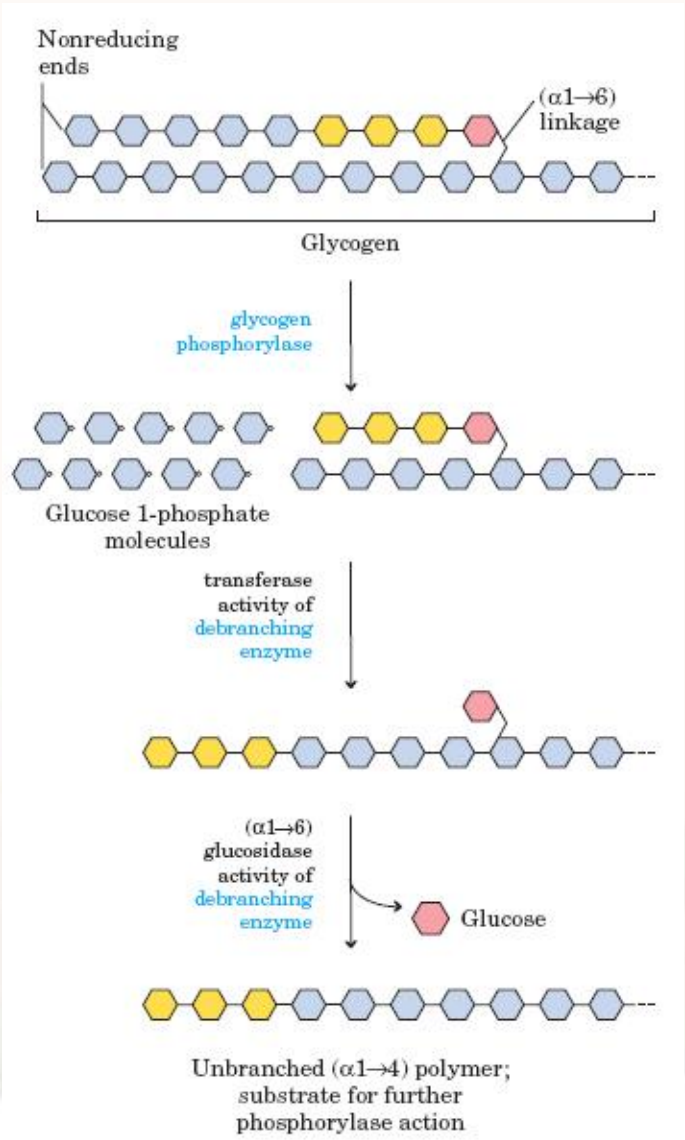

Fig. 5 Glycogenolysis

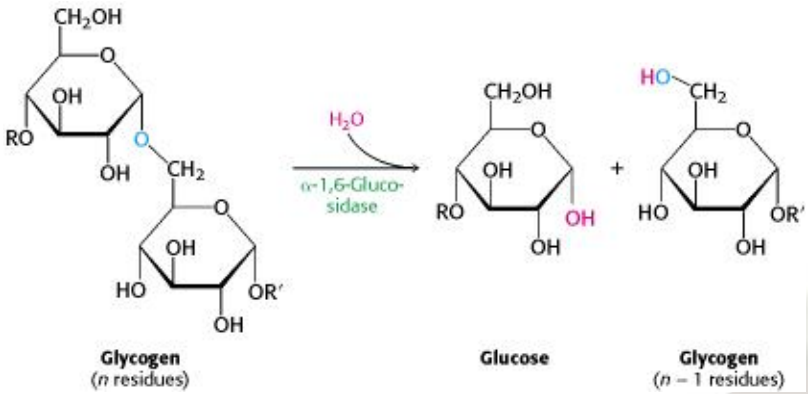

Fig. 6 Breakdown of glycogen

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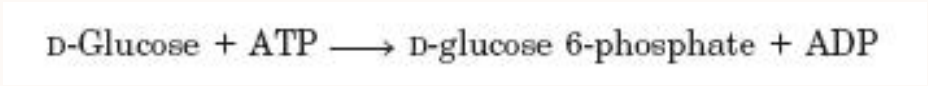
3.6.2 Glycogenesis:

a. Glycogen synthesis takes place in virtually all animal tissues but is especially prominent in liver and skeletal muscles.
b. Starting point for synthesis of glycogen is glc-6-Ⓟ which can be derived from free glc in a reaction catalyzed by isozymes HK-I and HK-II in muscle and HK-IV (glucokinase) in liver:



c. To initiate glycogen synthesis, glc-6-Ⓟ is converted to glc-1-Ⓟ in phosphoglucomutase reaction:

d. Product of this reaction is converted to UDP-glucose by action of UDP-glc pyrophosphorylase, in a key step of glycogen biosynthesis. This enzyme is named for the reverse reaction; in the cell, reaction proceeds in direction of UDP-glc formation, because pyrophosphate is rapidly hydrolyzed by inorganic pyrophosphatase

e. UDP-glc is immediate donor of glc residues in reaction catalyzed by glycogen synthase, which promotes the transfer of glc residue from UDP-glucose to a NR end of a branched glycogen molecule.
f. Glycogen synthase can't make α1-→6 bonds found at the branch points of glycogen; these are formed by glycogen-branching enzyme.
g. Glycogen branching enzyme catalyzes transfer of a terminal fragment of 6 or 7 glc residues from NR end of a glycogen branch having at least 11 residues to the C-6 hydroxyl group of a glc residue at a more interior position of the same or another glycogen chain, thus creating a new branch.
h. Further glc residues may be added to new branch by glycogen synthase.
i. Biological effect of branching is to make the glycogen molecule more soluble and to increase the number of NR ends.

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j. This increases the number of sites accessible to glycogen phosphorylase and glycogen synthase, both of which act only at NR ends.

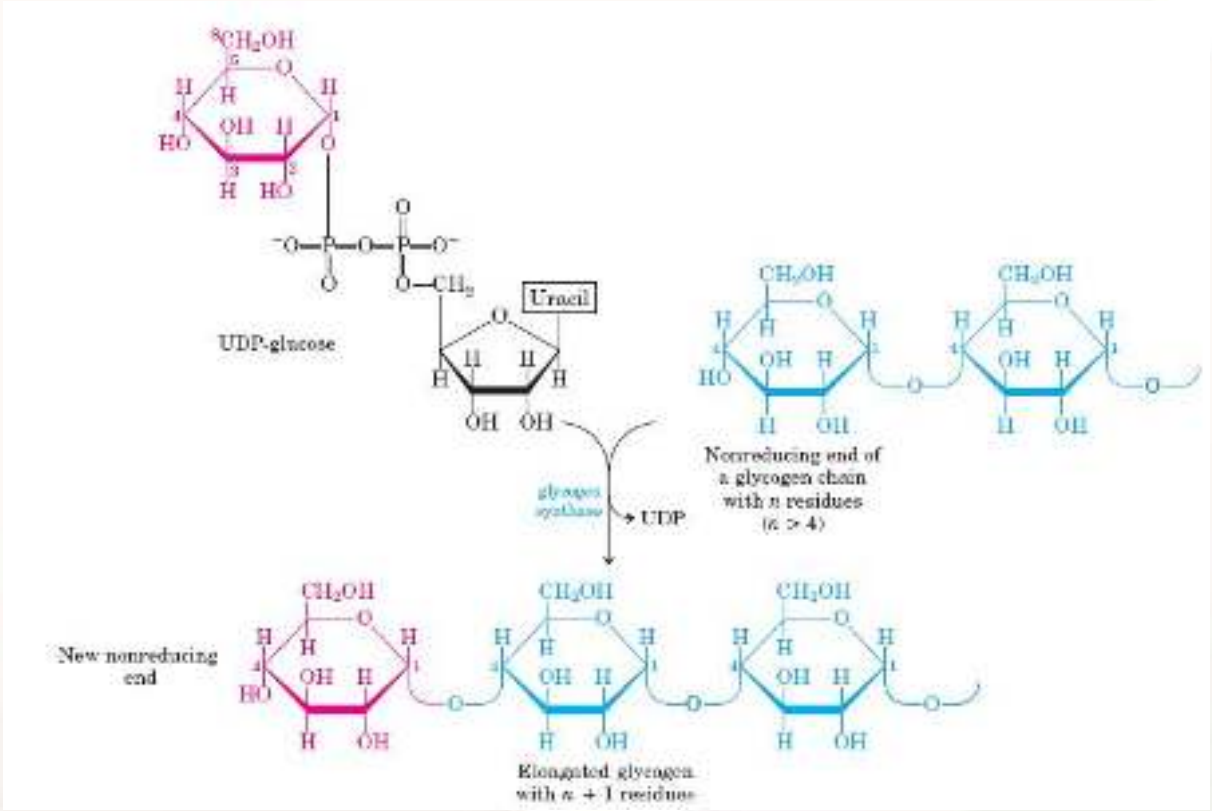

Fig. 7 Glycogenesis

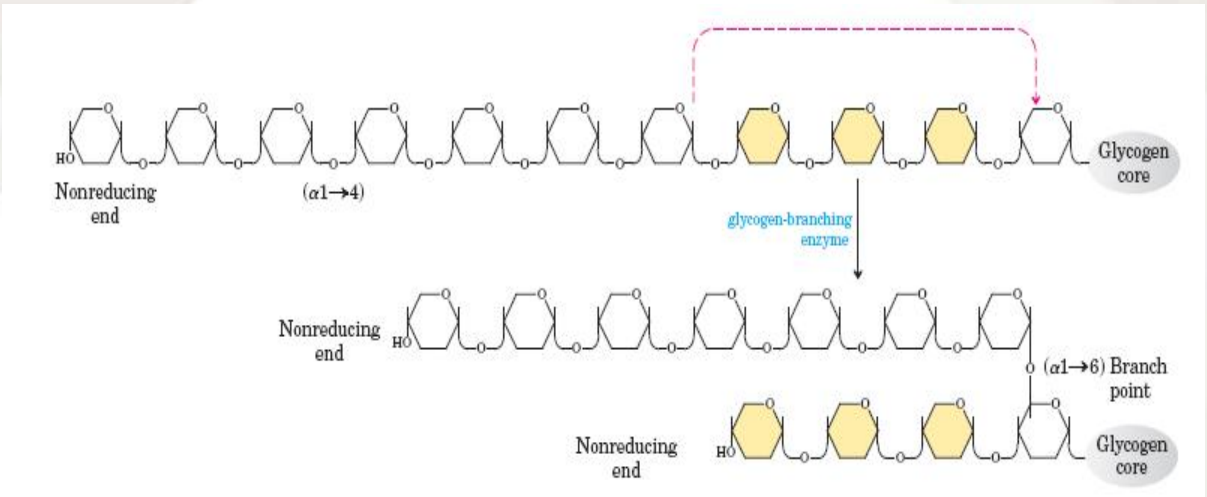

Fig. 8 Branching in glycogen

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3.7 Cellular respiration

a. Cellular respiration occurs in three major stages.

In the first, organic fuel molecules—glc, FAs, and some AAs—are oxidized to yield two-carbon fragments in the form of acetyl group of acetyl-CoA.

In second stage, acetyl groups are fed into TCA cycle, which enzymatically oxidizes them to CO2; the energy released is conserved in the reduced electron carriers NADH and FADH2.

In third stage of respiration, these reduced coenzymes are themselves oxidized, giving up protons and electrons.

1. The electrons are transferred to O2—the final electron acceptor—via a chain of electron-carrying molecules known as the respiratory chain.
2. In the course of electron transfer, large amount of energy released is conserved in the form of ATP, by a process called oxidative phosphorylation.

3.7.1 Tricarboxylic acid (TCA) cycle / Citric acid cycle / Krebs cycle (after its discoverer, Hans Krebs)

a. TCA cycle is a hub in metabolism, with degradative pathways leading in and anabolic pathways leading out, and it is closely regulated in coordination with other pathways.
b. Pyruvate is oxidized to acetyl-CoA and CO2
c. Overall reaction catalyzed by Pyruvate dehydrogenase (PDH) complex is an oxidative decarboxylation, an irreversible oxidation process in which the carboxyl group is removed from pyruvate as a molecule of CO2 and two remaining carbons become the acetyl group of acetyl-CoA. The reaction involves six cofactors: coenzyme A, NAD+, lipoic acid, FAD, thiamine pyrophosphate (TPP) and Mg2+.

$$CH_3\text{-}CO\text{-}COOH + CoASH + NAD^+ \xrightarrow[\text{Lipoate, } Mg^{2+}]{\text{TPP, FAD}} CH_3\text{-}COSCoA + NADH + H^+ + CO_2$$

d. NADH formed in this reaction gives up a hydride ion (:H) to respiratory chain, which carries two electrons to oxygen or, in anaerobic microorganisms, to an alternative electron acceptor such as nitrate or sulfate.
e. Eugene Kennedy and Albert Lehninger showed in 1948 that, in eukaryotes, the entire set of reactions of TCA cycle takes place in mitochondria.
f. In most prokaryotes, enzymes of TCA cycle are in cytosol, and plasma membrane plays a role analogous to that of the inner mitochondrial membrane in ATP synthesis.

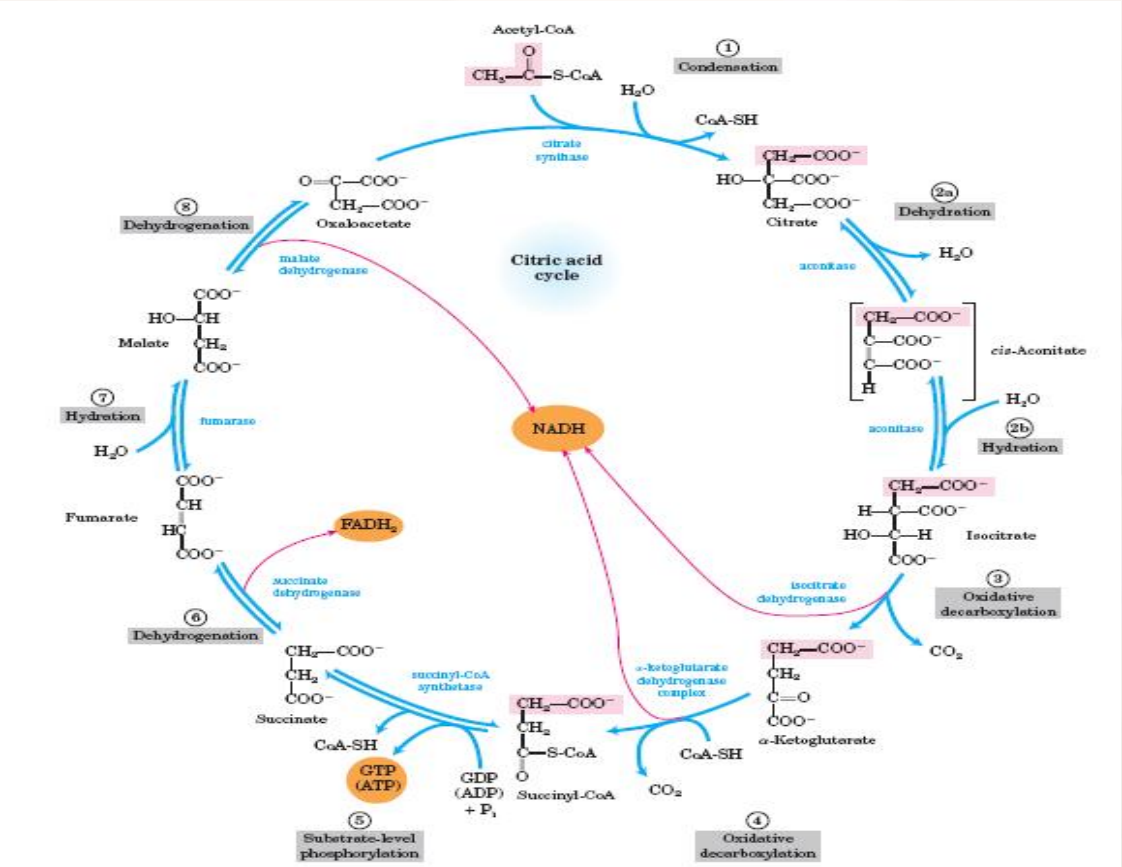

Fig. 9 TCA cycle

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Net reaction of TCA cycle:

Acetyl CoA + 3 NAD+ + FAD + ADP + Pi → 2 CO_2 + 3 NADH + 3 H+ + $FADH_2$ + ATP

3.7.2 Role of TCA cycle in metabolism:

a. No net removal of oxalo acetic acid (OAA) occurs; one molecule of OAA can theoretically bring about oxidation of an infinite number of acetyl groups, and, in fact, OAA is present in cells in very low concentrations.
b. Four of the eight steps in this process are oxidations, in which the energy of oxidation is very efficiently conserved in the form of the reduced coenzymes NADH and $FADH_2$.
c. Although TCA cycle is central to energy-yielding metabolism its role is not limited to energy conservation.
d. 4- and 5-carbon intermediates of the cycle serve as precursors for a wide variety of products. To replace intermediates removed for this purpose, cells employ anaplerotic (replenishing) reactions.
e. In aerobic organisms, TCA cycle is an amphibolic pathway, one that serves in both catabolic and anabolic processes.
f. Besides its role in oxidative catabolism of carbohydrates, FAs, and AAs, TCA cycle provides precursors for many biosynthetic pathways.

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Course Name	Fundamentals of Biochemistry
Lesson 3	Metabolism of carbohydrates: glycolysis, gluconeogenesis, glycogenolysis, glycogenesis, TCA cycle, central role of TCA cycle in metabolism
Content Creator Name	Dr. Ajay Pal
University/College Name	Chaudhary Charan Singh Haryana Agricultural University, Hisar
Course Reviewer Name	Sucheta Sharma
University/College Name	Punjab Agricultural University, Ludhiana

Lesson 3:

Metabolism of carbohydrates: glycolysis, gluconeogenesis, glycogenolysis, glycogenesis, TCA cycle, central role of TCA cycle in metabolism

Objectives:

a. To study the major pathways of glucose metabolism
b. To study glycolysis and gluconeogenesis
c. To study catabolism and anabolism of glycogen
d. To study TCA cycle and its importance in metabolism

Glossary:

a. **Metabolism:** Sum of all chemical transformations taking place in a cell or organism.
b. **Catabolism:** Set of metabolic pathways that break down molecules into smaller units that are either oxidized to release energy or used in other anabolic reactions.
c. **Anabolism:** Synthesis of complex molecules in living organisms from simpler ones together with the storage of energy.
d. **Glycolysis:** Process by which one molecule of glucose is converted into two molecules of pyruvate.
e. **Glycogenolysis:** Breakdown of glycogen into glucose, a simple sugar that body uses to produce energy.
f. **Glycogenesis:** Process of glycogen synthesis, in which glucose molecules are added to chains of glycogen for storage.
g. **Cellular respiration:** Molecular processes by which cells consume O2 and produce CO2.

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3.1 Metabolism

a. Metabolism is the sum of all chemical transformations taking place in a cell or organism. It occurs through a series of enzyme-catalyzed reactions that constitute metabolic pathways.
b. Each consecutive step in a metabolic pathway brings about a specific (since enzymatic), small chemical change (usually the removal, transfer, or addition of a particular atom/functional group).
c. The precursor is converted into a product through a series of metabolic intermediates called metabolites.

3.1.1 Catabolism

a. Degradative phase of metabolism.
b. Organic nutrient molecules (carbohydrates, fats, and proteins) are converted into smaller, simpler end products (such as lactic acid, CO2, NH3).
c. Catabolic pathways release energy.
d. Some energy is conserved in formation of ATP and reduced electron carriers (NADH, NADPH, and FADH2) and the rest is lost as heat.

3.1.2 Anabolism

a. Synthetic (biosynthetic) phase of metabolism.
b. Small, simple precursors are built up into larger and more complex molecules like lipids, polysaccharides, proteins, and nucleic acids.
c. Anabolic reactions require an input of energy, generally in the form of the phosphoryl group transfer potential of ATP and the reducing power of NADH, NADPH, and FADH2.

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3.2 Metabolism of carbohydrates

a. Carbohydrate is utilized by cells mainly in the form of glucose.
b. The three principal monosaccharides resulting from the digestive processes are glucose, fructose and galactose.
c. Both fructose and galactose are readily converted to glucose by the liver.
d. Since glucose is the compound formed from starch and glycogen, the carbohydrate metabolism commences with this monosaccharide.

3.3 Glycolysis

a. A molecule of glucose (glc) is degraded in a series of enzyme-catalyzed reactions to yield two molecules of 3-carbon compound pyruvate.
b. During the sequential reactions of glycolysis, some of the free energy released from glucose is conserved in the form of ATP and NADH.
c. Glycolysis was the first metabolic pathway to be elucidated and is probably the best understood.
d. It is the primary pathway occurring in the cytoplasm of all the tissues of biological systems.
e. Glycolysis is an almost universal central pathway of glc catabolism.
f. It is the pathway with the largest flux of carbon in most cells.
g. Glycolytic breakdown of glc is the sole source of metabolic energy in some mammalian tissues and cell types (like erythrocytes).
h. Many anaerobic microorganisms are entirely dependent on glycolysis.
i. The term fermentation is generally used for anaerobic degradation of glucose or other organic nutrients to obtain energy, conserved as ATP.
j. Glycolysis differs among species only in the details of its regulation and in the subsequent metabolic fate of the pyruvate formed.

In plants, glucose and fructose are the main monosaccharides catabolised by glycolysis although others are also converted into these sugars.

a. Glucose entering the glycolysis is derived from starch or sucrose, and fructose is derived from sucrose.
b. The starch is either from seeds or chloroplasts of matured plants.
c. Glycolysis normally takes place in the presence of O2 in higher plant cells.

Glycolysis has two phases: The breakdown of the 6-carbon glucose into two molecules of the 3-carbon pyruvate occurs in ten steps.

a. The first five of which constitute the preparatory phase.
b. "lysis/lyase" step gives the pathway its name.
c. Two molecules of ATP are invested before the cleavage of glucose into two 3-carbon pieces.
d. The second phase is the payoff phase. It gives energy.
e. The net yield is two molecules of ATP per molecule of glucose used, because two molecules of ATP were invested in the preparatory phase. Energy is also conserved in the payoff phase in the formation of two molecules of NADH per molecule of glucose.

Three types of chemical transformations during glycolysis:

a. Degradation of the carbon skeleton of glucose to yield pyruvate,
b. Phosphorylation of ADP to ATP by high-energy phosphate compounds formed during glycolysis, and
c. Transfer of a hydride ion to NAD+ to form NADH.

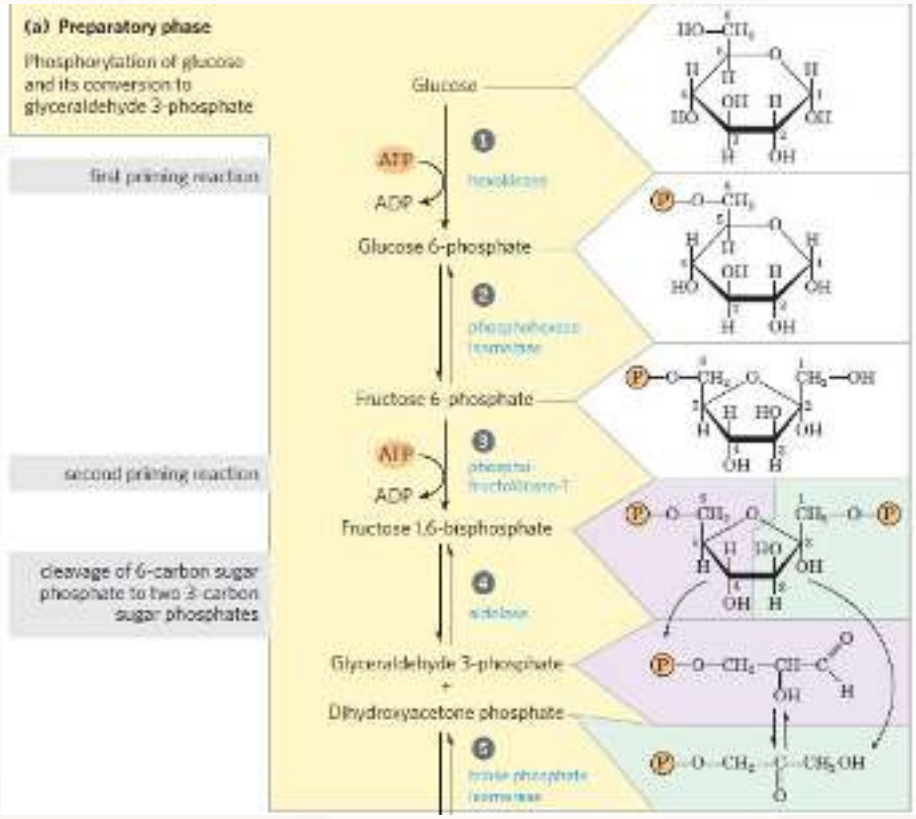

Fig. 1 Preparatory phase of Glycolysis

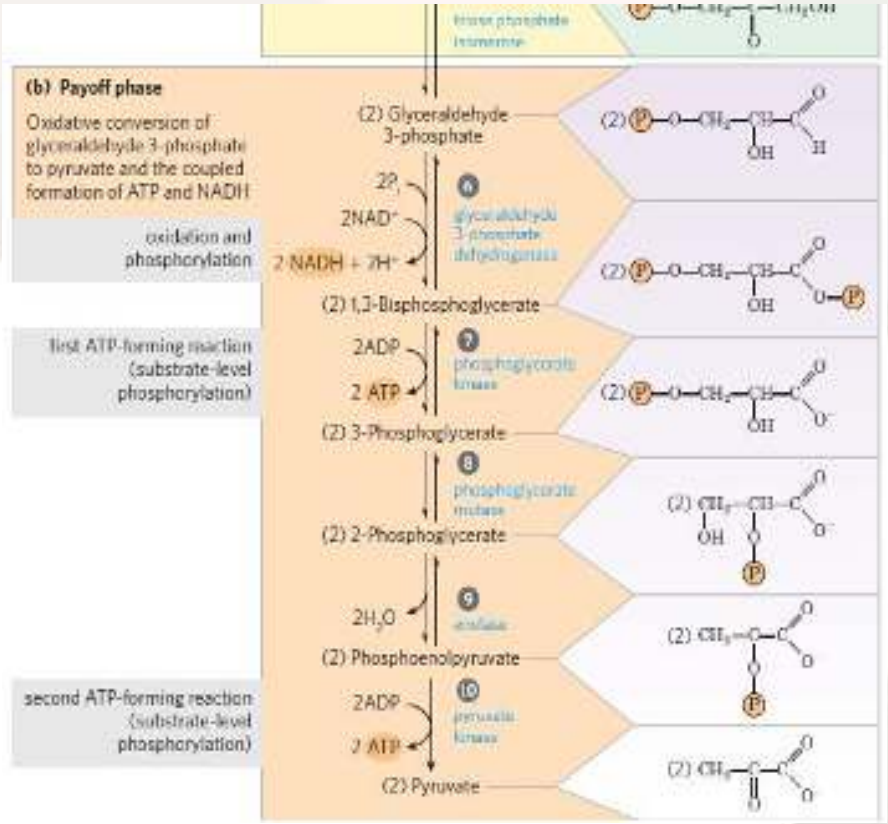

Fig. 2 Pay-off phase of Glycolysis

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Net reaction of Glycolysis:

Glucose + 2 NAD+ + 2 Pi + 2 ADP → 2 pyruvate + 2 ATP + 2 NADH + 2 H++ 2 H2O

3.4 Three catabolic fates of pyruvate:

a. Pyruvate is oxidized, with loss of its carboxyl group as CO2, to yield the acetyl group of acetyl-coenzyme A; The acetyl group is then oxidized completely to CO2 by the TCA/citric acid cycle. The energy from the electron-transfer reactions drives the synthesis of ATP in the mitochondrion.
b. Pyruvate is reduced to lactate via lactic acid fermentation. In vigorously contracting skeletal muscle, under low oxygen conditions (hypoxia), NADH can't be reoxidized to NAD+, but NAD+ is required to continue glycolysis (G3PDH). Under these conditions, pyruvate is reduced to lactate, accepting electrons from NADH and thereby regenerating the NAD+ necessary for glycolysis to continue.
c. The third route of pyruvate catabolism leads to ethanol. In some plant tissues and in certain invertebrates, protists, and microorganisms such as brewer's yeast, pyruvate is converted under hypoxic or anaerobic conditions into ethanol and CO2, a process called ethanol (alcohol) fermentation.
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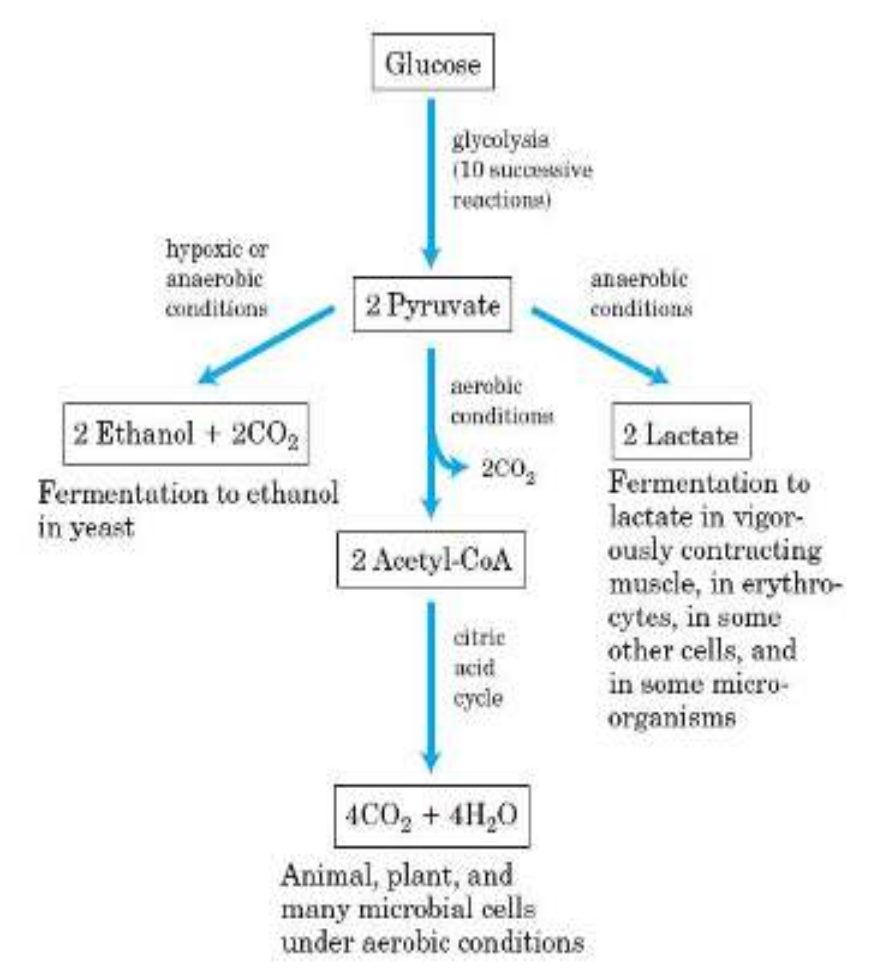

Fig. 3 Three possible catabolic fates of the pyruvate formed in glycolysis.

3.5 Gluconeogenesis

a. In mammals, some tissues depend almost completely on glucose for their metabolic energy. For human brain and nervous system, as well as erythrocytes, testes, renal medulla, and embryonic tissues, glc from blood is the sole or major source of fuel.
b. However, supply of glc from these stores is not always sufficient; between meals and during longer fasts, or after vigorous exercise glycogen is depleted.
c. For these times, organisms need a method for synthesizing glc from non-carbohydrate precursors. This is accomplished by a pathway called gluconeogenesis (GNG) (“formation of new sugar”), which converts pyruvate and related three- and four-carbon compounds to glc.
d. GNG occurs in all animals, plants, fungi, and microorganisms.

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e. Reactions are essentially same in all tissues and all species.
f. Important precursors of glc in animals are three-carbon compounds such as lactate, pyruvate, and glycerol, as well as certain amino acids.
g. In mammals, GNG takes place mainly in liver, and to a lesser extent in renal cortex.
h. GNG and glycolysis are not identical pathways running in opposite directions, although they do share several steps; seven of the ten enzymatic reactions of GNG are reverse of glycolytic reactions.
i. However, three reactions of glycolysis are essentially irreversible in vivo and can't be used in GNG.
    1. Conversion of glc to glc-6-phosphate by hexokinase (HK).
    2. Phosphorylation of fru-6-phosphate to fru-1,6-bisphosphate by phosphofructo kinase-1 (PFK-1), and
    3. Conversion of phosphoenol pyruvate (PEP) to pyruvate by pyruvate kinase (PK).

In GNG, three irreversible steps are by-passed by a separate set of enzymes, catalyzing reactions that are sufficiently exergonic to be effectively irreversible in the direction of glc synthesis.

Net reaction of Gluconeogenesis:

2 Pyruvate + 4ATP + 2GTP + 2NADH + 6H2O → Glucose + 4ADP + 2GDP + 6Pi + 2NAD+ + 2H+

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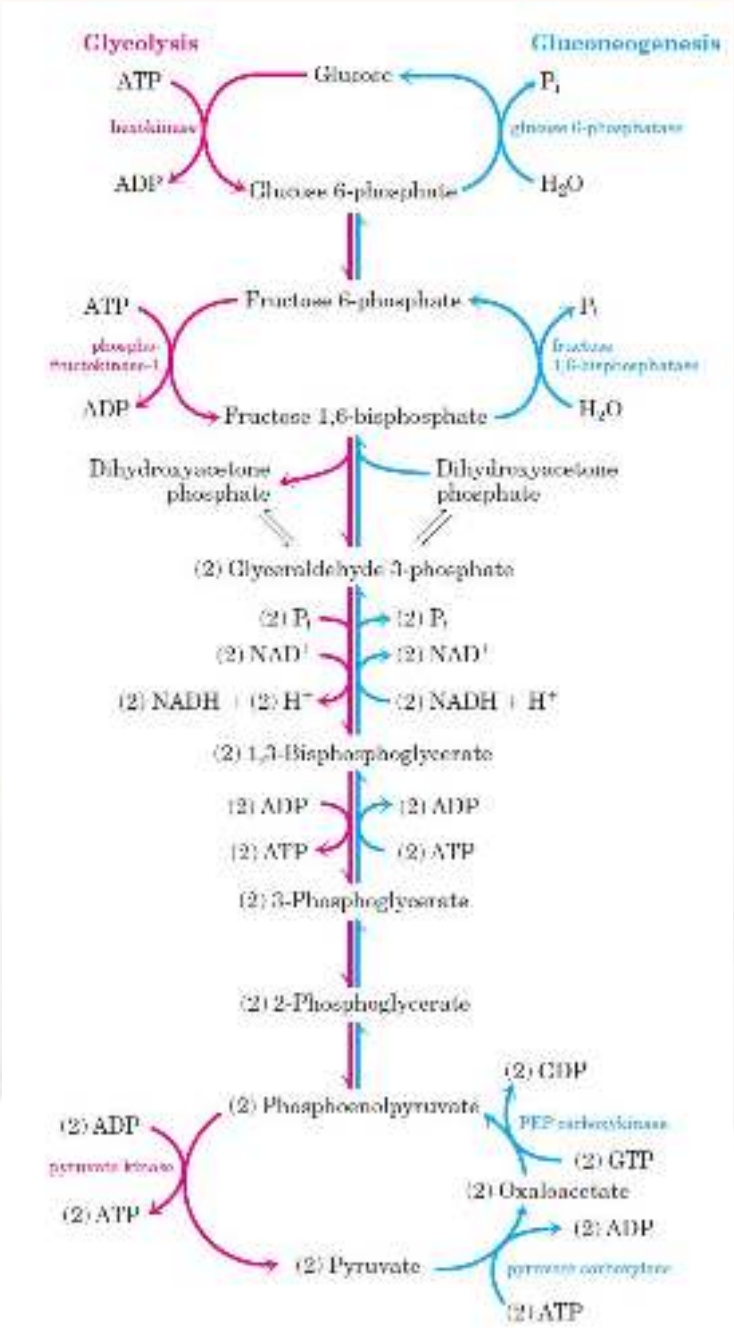

Fig. 4 Glycolysis and gluconeogenesis

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3.6 Glycogen metabolism in animals

a. In a wide range of organisms, excess glc is converted to polymeric forms for storage—glycogen in vertebrates and many microorganisms, starch in plants.
b. In vertebrates, glycogen is found primarily in liver and skeletal muscle; it may represent up to 10% of the fresh weight of liver and 1-2% of the fresh weight of muscle.
c. Glycogen is stored in large cytosolic granules.
d. Glycogen in muscle is there to provide a quick source of energy for either aerobic or anaerobic metabolism. Muscle glycogen can be exhausted in less than an hour during vigorous activity. Liver glycogen serves as a reservoir of glucose for other tissues when dietary glucose is not available (between meals or during a fast); this is especially important for neurons of brain, which can't use fatty acids as fuel. Liver glycogen can be depleted in 12-24 hours.
e. In humans, total amount of energy stored as glycogen is far less than the amount stored as fat (TAG) but fats can't be converted to glc in mammals and can't be catabolized anaerobically.

3.6.1 Glycogenolysis

a. It is the pathway for breakdown of glycogen into glucose, a simple sugar that body uses to produce energy.
b. Glycogen breakdown is catalyzed by the enzyme, glycogen phosphorylase.
c. In skeletal muscle and liver, glucose units of outer branches of glycogen enter glycolytic pathway through action of three enzymes:
    1. Glycogen phosphorylase: catalyzes reaction in which an α1 → 4 glycosidic linkage between two glc residues at nonreducing (NR) end of glycogen undergoes attack by inorganic phosphate (Pi), removing terminal glc residue as D-glc 1-Ⓟ. Glycogen phosphorylase acts repetitively on NR ends of glycogen branches until it reaches a point four glc residues away from an α1→6 branch point, where its action stops. Glc 1-Ⓟ can enter glycolysis or, in liver, replenish blood glc.

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2. Glycogen debranching enzyme: Further degradation by glycogen phosphorylase can occur only after the debranching enzyme catalyzes two successive reactions that transfer branches. Once these branches are transferred and the glucosyl residue at C-6 is hydrolyzed, glycogen phosphorylase activity can continue.
3. Phosphoglucomutase: Glc 1-Ⓟ, the end product of the glycogen phosphorylase reaction, is converted to glc 6-Ⓟ by phosphoglucomutase, which catalyzes the reversible reaction:

d. Glc 6-Ⓟ formed from glycogen in skeletal muscle can enter glycolysis and serve as an energy source to support muscle contraction.
e. In liver, glycogen breakdown serves a different purpose: to release glc into blood when blood glc level drops, as it does between meals. This requires an enzyme, glc-6-phosphatase, that is present in liver and kidney but not in other tissues.
f. Because muscle and adipose tissue lack glc 6-phosphatase, they can't convert glc-6-Ⓟ formed by glycogen breakdown to glc, and these tissues therefore do not contribute glc to blood.

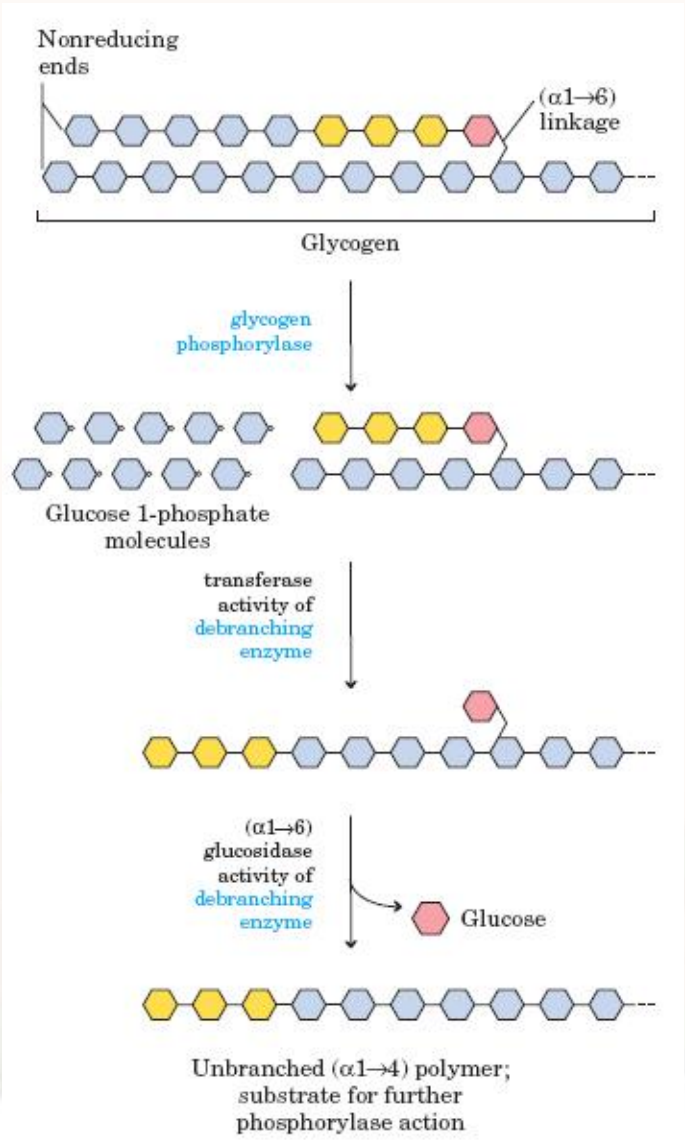

Fig. 5 Glycogenolysis

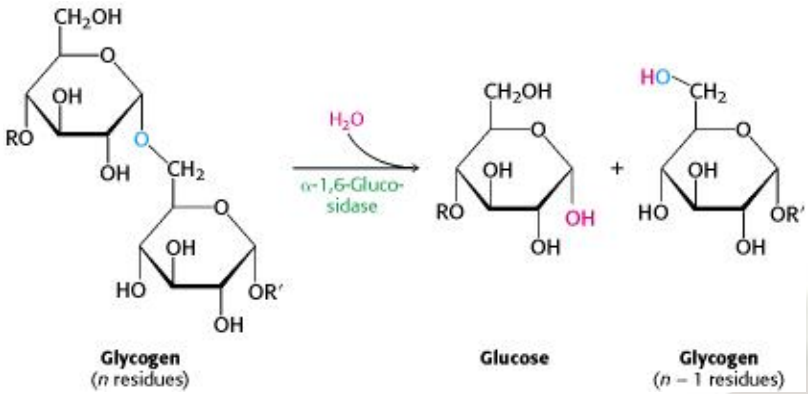

Fig. 6 Breakdown of glycogen

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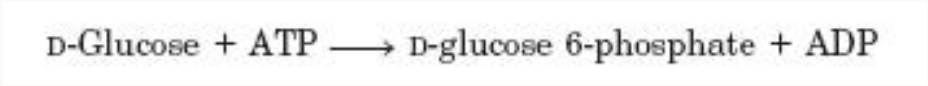
3.6.2 Glycogenesis:

a. Glycogen synthesis takes place in virtually all animal tissues but is especially prominent in liver and skeletal muscles.
b. Starting point for synthesis of glycogen is glc-6-Ⓟ which can be derived from free glc in a reaction catalyzed by isozymes HK-I and HK-II in muscle and HK-IV (glucokinase) in liver:



c. To initiate glycogen synthesis, glc-6-Ⓟ is converted to glc-1-Ⓟ in phosphoglucomutase reaction:

d. Product of this reaction is converted to UDP-glucose by action of UDP-glc pyrophosphorylase, in a key step of glycogen biosynthesis. This enzyme is named for the reverse reaction; in the cell, reaction proceeds in direction of UDP-glc formation, because pyrophosphate is rapidly hydrolyzed by inorganic pyrophosphatase

e. UDP-glc is immediate donor of glc residues in reaction catalyzed by glycogen synthase, which promotes the transfer of glc residue from UDP-glucose to a NR end of a branched glycogen molecule.
f. Glycogen synthase can't make α1-→6 bonds found at the branch points of glycogen; these are formed by glycogen-branching enzyme.
g. Glycogen branching enzyme catalyzes transfer of a terminal fragment of 6 or 7 glc residues from NR end of a glycogen branch having at least 11 residues to the C-6 hydroxyl group of a glc residue at a more interior position of the same or another glycogen chain, thus creating a new branch.
h. Further glc residues may be added to new branch by glycogen synthase.
i. Biological effect of branching is to make the glycogen molecule more soluble and to increase the number of NR ends.

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j. This increases the number of sites accessible to glycogen phosphorylase and glycogen synthase, both of which act only at NR ends.

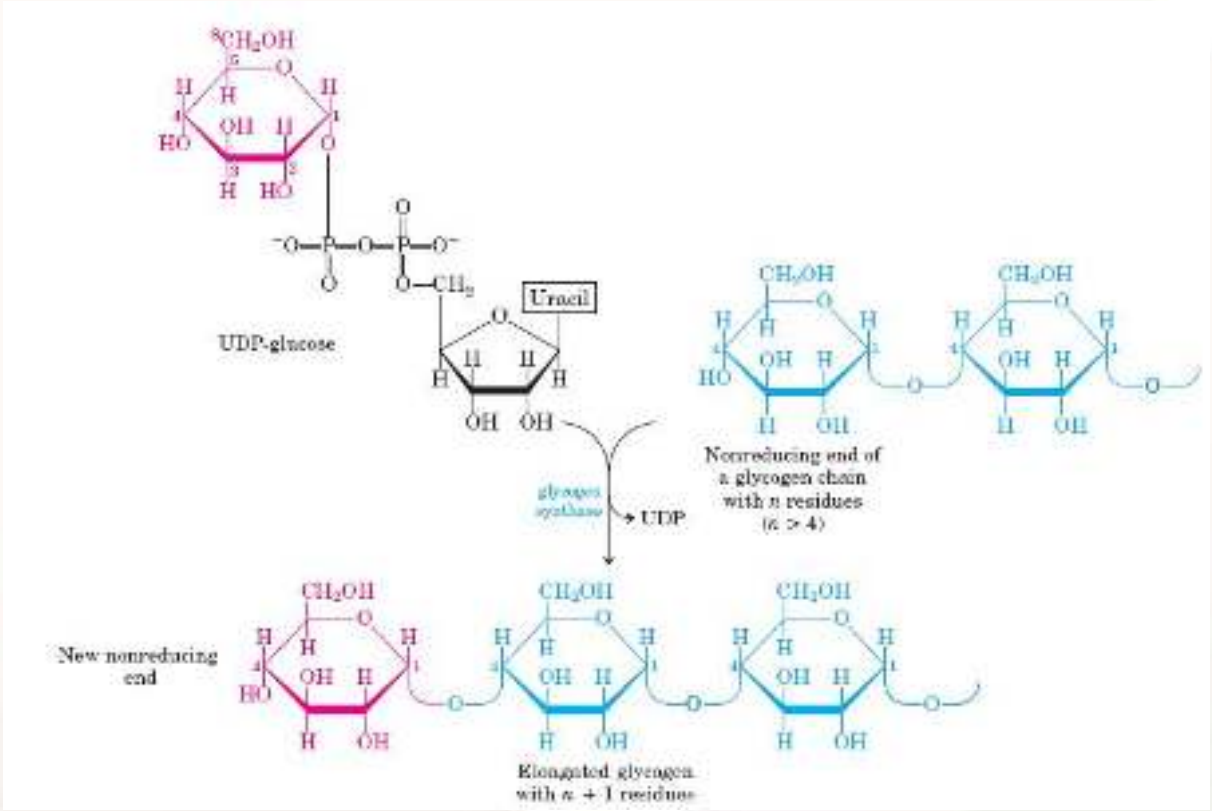

Fig. 7 Glycogenesis

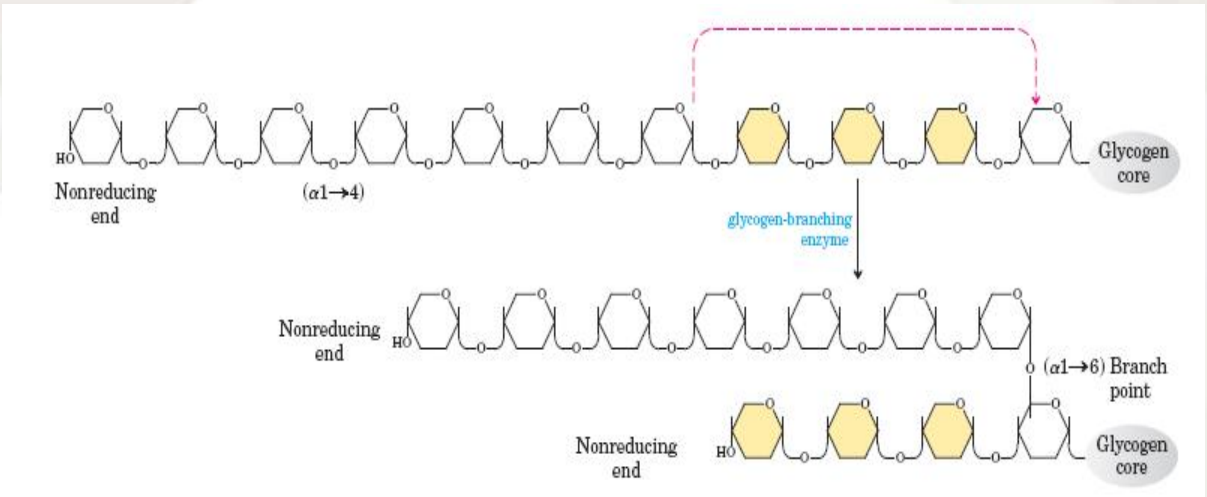

Fig. 8 Branching in glycogen

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3.7 Cellular respiration

a. Cellular respiration occurs in three major stages.

In the first, organic fuel molecules—glc, FAs, and some AAs—are oxidized to yield two-carbon fragments in the form of acetyl group of acetyl-CoA.

In second stage, acetyl groups are fed into TCA cycle, which enzymatically oxidizes them to CO2; the energy released is conserved in the reduced electron carriers NADH and FADH2.

In third stage of respiration, these reduced coenzymes are themselves oxidized, giving up protons and electrons.

1. The electrons are transferred to O2—the final electron acceptor—via a chain of electron-carrying molecules known as the respiratory chain.
2. In the course of electron transfer, large amount of energy released is conserved in the form of ATP, by a process called oxidative phosphorylation.

3.7.1 Tricarboxylic acid (TCA) cycle / Citric acid cycle / Krebs cycle (after its discoverer, Hans Krebs)

a. TCA cycle is a hub in metabolism, with degradative pathways leading in and anabolic pathways leading out, and it is closely regulated in coordination with other pathways.
b. Pyruvate is oxidized to acetyl-CoA and CO2
c. Overall reaction catalyzed by Pyruvate dehydrogenase (PDH) complex is an oxidative decarboxylation, an irreversible oxidation process in which the carboxyl group is removed from pyruvate as a molecule of CO2 and two remaining carbons become the acetyl group of acetyl-CoA. The reaction involves six cofactors: coenzyme A, NAD+, lipoic acid, FAD, thiamine pyrophosphate (TPP) and Mg2+.

$$CH_3\text{-}CO\text{-}COOH + CoASH + NAD^+ \xrightarrow[\text{Lipoate, } Mg^{2+}]{\text{TPP, FAD}} CH_3\text{-}COSCoA + NADH + H^+ + CO_2$$

d. NADH formed in this reaction gives up a hydride ion (:H) to respiratory chain, which carries two electrons to oxygen or, in anaerobic microorganisms, to an alternative electron acceptor such as nitrate or sulfate.
e. Eugene Kennedy and Albert Lehninger showed in 1948 that, in eukaryotes, the entire set of reactions of TCA cycle takes place in mitochondria.
f. In most prokaryotes, enzymes of TCA cycle are in cytosol, and plasma membrane plays a role analogous to that of the inner mitochondrial membrane in ATP synthesis.

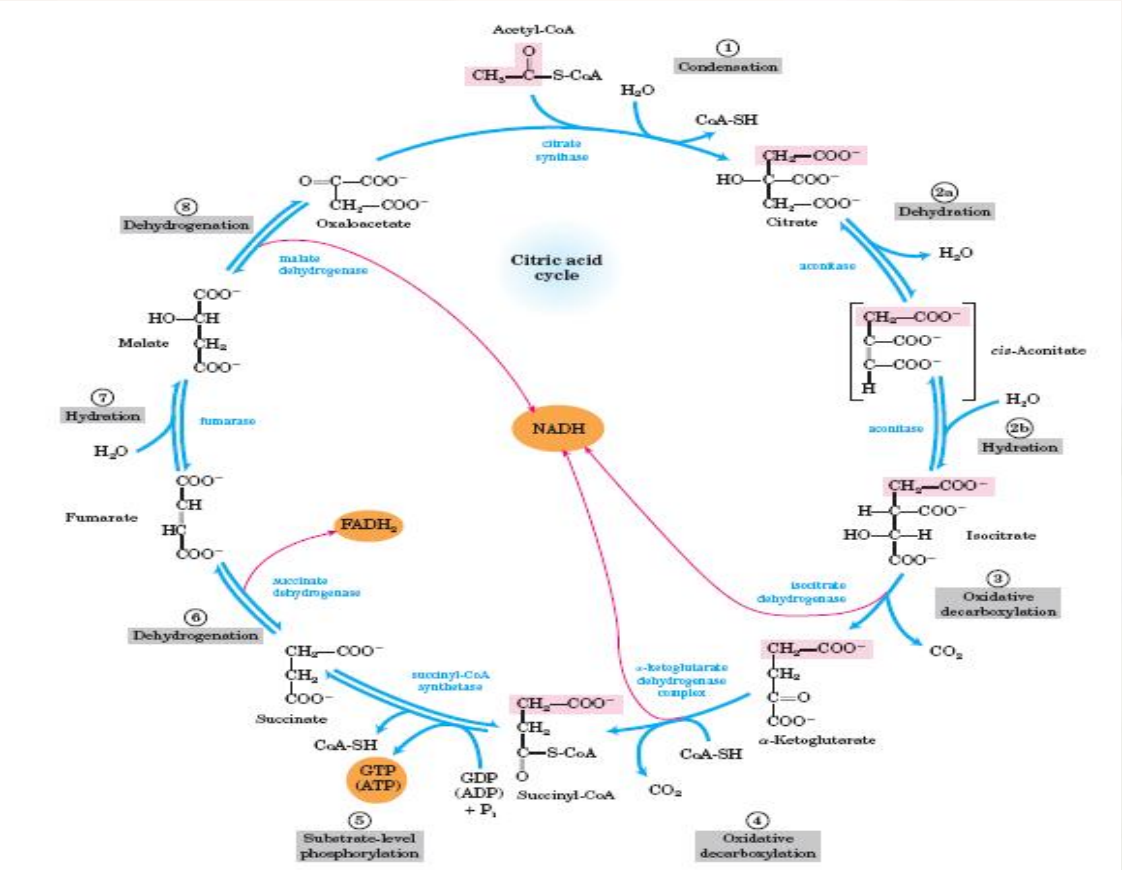

Fig. 9 TCA cycle

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Net reaction of TCA cycle:

Acetyl CoA + 3 NAD+ + FAD + ADP + Pi → 2 CO_2 + 3 NADH + 3 H+ + $FADH_2$ + ATP

3.7.2 Role of TCA cycle in metabolism:

a. No net removal of oxalo acetic acid (OAA) occurs; one molecule of OAA can theoretically bring about oxidation of an infinite number of acetyl groups, and, in fact, OAA is present in cells in very low concentrations.
b. Four of the eight steps in this process are oxidations, in which the energy of oxidation is very efficiently conserved in the form of the reduced coenzymes NADH and $FADH_2$.
c. Although TCA cycle is central to energy-yielding metabolism its role is not limited to energy conservation.
d. 4- and 5-carbon intermediates of the cycle serve as precursors for a wide variety of products. To replace intermediates removed for this purpose, cells employ anaplerotic (replenishing) reactions.
e. In aerobic organisms, TCA cycle is an amphibolic pathway, one that serves in both catabolic and anabolic processes.
f. Besides its role in oxidative catabolism of carbohydrates, FAs, and AAs, TCA cycle provides precursors for many biosynthetic pathways.

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Course Name	Fundamentals of Biochemistry
Lesson 5	Primary, secondary, tertiary and quaternary structure of proteins, amphoteric property, biuret reaction and xanthoproteic reaction, digestion and absorption of protein.
Content Creator Name	Dr. Ajay Pal
University/College Name	Chaudhary Charan Singh Haryana Agricultural University, Hisar
Course Reviewer Name	Sucheta Sharma
University/College Name	Punjab Agricultural University, Ludhiana

Lesson5:

Primary, secondary, tertiary and quaternary structure of proteins, amphoteric property, biuret reaction and xanthoproteic reaction, digestion and absorption of protein.

Objectives:

a. To study the different levels of structural organization of proteins
b. To study amphoteric properties of amino acids
c. To study the chemical reactions for detection of proteins
d. To study digestion and absorption of proteins

Glossary:

a. **Peptide bonds:** A peptide bond is a chemical bond formed between two molecules when the carboxyl group of one molecule reacts with the amino group of other molecules, releasing a molecule of water.
b. **Native proteins:** Proteins in any of their functional, folded conformations.
c. Primary structure of a protein: Sequence and composition of amino acids in a protein.
d. **Denaturation:** Denaturation is a process in which proteins lose the quaternary, tertiary and secondary structure which is present in their native state, by application of some external stress or compound such as a strong acid or base, a concentrated inorganic salt or an organic solvent.
e. **Zymogen:** A zymogen, also called a proenzyme, is an inactive precursor of an enzyme. It requires a biochemical change for it to become an active enzyme.
f. **Endopeptidase:** Endopeptidase or endoproteinase are proteolytic peptidases that break peptide bonds of non-terminal amino acids.

E-Lecture

5.1 Protein structure:

a. Spatial arrangement of atoms in a protein is called its conformation.
b. Possible conformations of a protein include any structural state that can be achieved without breaking covalent bonds.
c. A change in conformation can occur, for e.g., by rotation about single bonds.
d. Of the numerous conformations that are theoretically possible in a protein containing hundreds of single bonds, one or (more commonly) a few generally predominate under biological conditions.
e. Need for multiple stable conformations reflects the changes that must occur in most proteins as they bind to other molecules or catalyze reactions.
f. Conformations existing under a given set of conditions are usually the ones that are thermodynamically the most stable, having the lowest Gibbs free energy (G).
g. Proteins in any of their functional, folded conformations are called native proteins.
h. About 200 to 460 kJ/mol are required to break a single covalent bond, whereas weak interactions can be disrupted by a mere 4 to 30 kJ/mol.
i. Individual covalent bonds that contribute to the native conformations of proteins, such as disulfide bonds linking separate parts of a single polypeptide chain, are much stronger than individual weak interactions. Yet, because they are so numerous, it is the weak interactions that predominate as a stabilizing force in protein structure.
j. In general, protein conformation with lowest free energy (that is, the most stable conformation) is the one with the maximum number of weak interactions.
k. Most of the structural patterns in proteins under physiological condition reflect two simple rules:

    1. Hydrophobic residues are largely buried in the protein interior, away from water; and
    2. Number of hydrogen bonds within the protein is maximized.

Four levels of structural organization can be distinguished in proteins:

1. Primary
2. Secondary
3. Tertiary
4. Quaternary

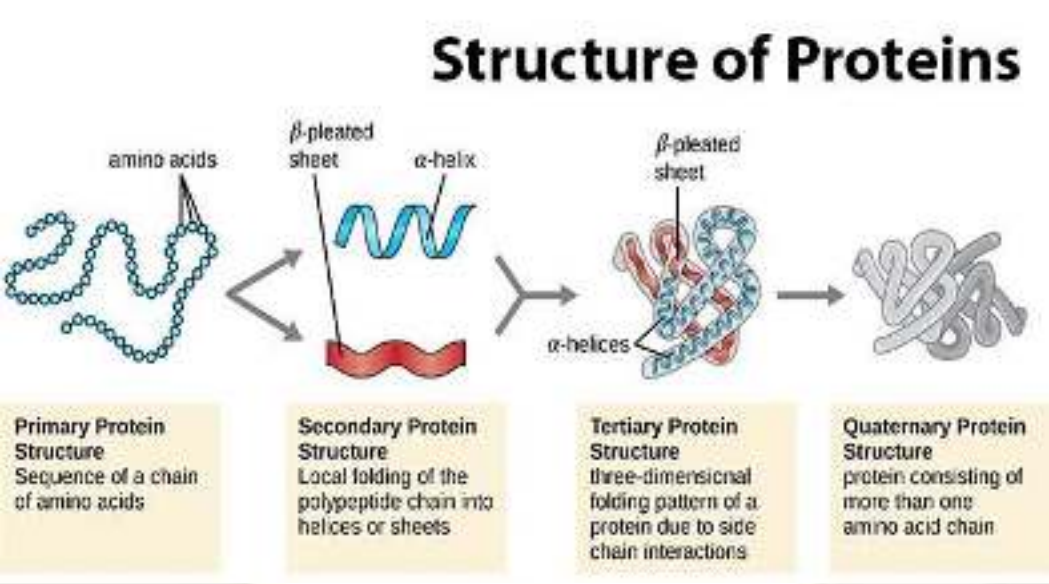


Fig. 1: Different levels of protein structure

5.1.1 Primary structure:

a. Primary structure of a protein refers to the sequence of amino acids in polypeptide chain and the order in which they are covalently linked together.
b. Primary structure is held together by peptide bonds that are made during the process of protein biosynthesis. Two ends of the polypeptide chain are referred as carboxyl terminus (C-terminus) and amino terminus (N-terminus) based on the nature of the free group on each extremity.
c. Counting of residues always starts at the N-terminal end (NH2-group), which is the end where the amino group is not involved in a peptide bond.
d. It also refers to the location of disulfide bridges, if there are any, in a polypeptide chain.

e. Primary structure of a protein is determined by the gene corresponding to the protein.
f. A specific sequence of nucleotides in DNA is transcribed into mRNA, which is read by ribosome in a process called translation.
g. Sequence of amino acids in insulin was discovered by Frederick Sanger, establishing that proteins have defining amino acid sequences. It is composed of 51 amino acids in 2 chains. One chain has 31 amino acids, and the other has 20 amino acids.
h. Sequence of a protein is unique to a protein, and defines its structure and function.
i. Sequence of a protein can be determined by methods such as Edman degradation or tandem mass spectrometry.
j. Often, however, it is read directly from the sequence of gene using the genetic code.
k. The words "amino acid residues" are used while discussing proteins because when a peptide bond is formed, a water molecule is lost, and therefore proteins are made up of amino acid residues.
l. Post-translational modifications suchas phosphorylation and glycosylation are usually also considered as a part of the primary structure, and cannot be read from the gene.
m. Peptide bond has a partial double bond nature. So, conformation of peptide group is restricted to one of two possible conformations, either Trans or Cis.

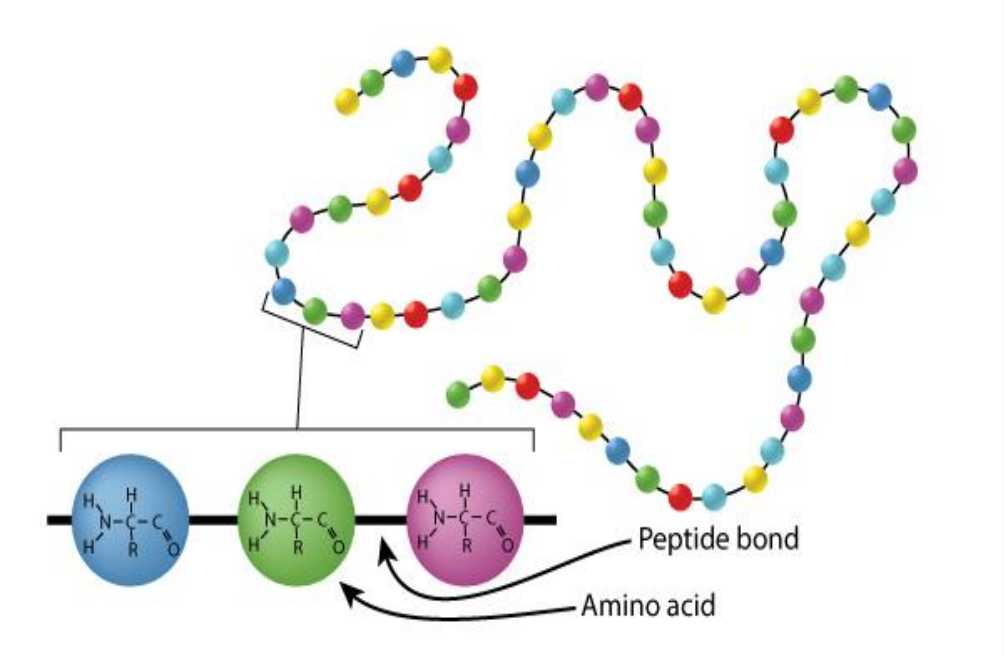


Fig. 2: Primary structure of protein

5.1.2. Secondary structure

Secondary structure refers to the steric relationship of amino acids that are close to one another in the linear sequence.

a. The folding of a linear polypeptide chain occurs to form a specific coiled structure.
b. Such coiling or folding is maintained by hydrogen bonds and hydrogen bond is the only bond responsible for secondary structure.
c. A few types of secondary structure are particularly stable and occur widely in proteins.
d. The most prominent are the α-helix and β-conformations.
e. Pauling and Corey predicted the existence of these secondary structures in 1951 by considering the dimensions of peptide groups, possible steric constraints, and opportunities for stabilization by formation of hydrogen bonds. They revealed that the peptide group has a rigid, planar structure which is a consequence of resonance interactions that give the peptide bond a 40% double bond character.
f. Peptide groups mostly assume the trans conformation in which successive C2 atoms are on opposite sides of peptide bond joining them. The cis configuration creates steric interference.

5.1.2.1. α-Helix:

a. The α-helical conformation was proposed in 1951 by Linus Pauling and Robert Corey.
b. The simplest arrangement the polypeptide (PP) chain could assume with its rigid peptide bonds (but other single bonds free to rotate) is a helical structure, which Pauling and Corey called the α-helix.
c. The helix structure of proteins is stabilized by intramolecular hydrogen bonding.
d. In this structure, hydrogen bonds are formed between the C=O group of one peptide bond and the N-H group of another after 3 amino acid units.
e. In this structure the PP backbone is tightly wound around an imaginary axis drawn longitudinally through the middle of the helix, and the R groups of the AA residues protrude outward from the helical backbone.
f. The repeating unit is a single turn of the helix, which extends about 5.4 Å (0.50 to 0.55 nm) along the long axis. (5.4Å is known as pitch (the axial distance per turn) of the α helix.
g. The AA residues in an α helix have conformations with Ψ = -45° to -50° and φ = -60°, and each helical turn includes 3.6 AA residues.
h. AA residues are plotted every (360/3.6) 100° around the spiral.
i. The helical twist of the α helix found in all proteins is right-handed.
j. The α helix is the predominant structure in α-keratins.
k. More generally, about one-fourth of all AA residues in PPs are found in α-helices, the exact fraction varying greatly from one protein to the next.
l. Certain amino acids tend to disrupt the alpha-helix. Among these are proline (the N atoms is part of the rigid ring and no rotation of the N-C bond can occur) and amino acid with charged or bulk R groups that either electrostatically or physically interferes with helix formation.

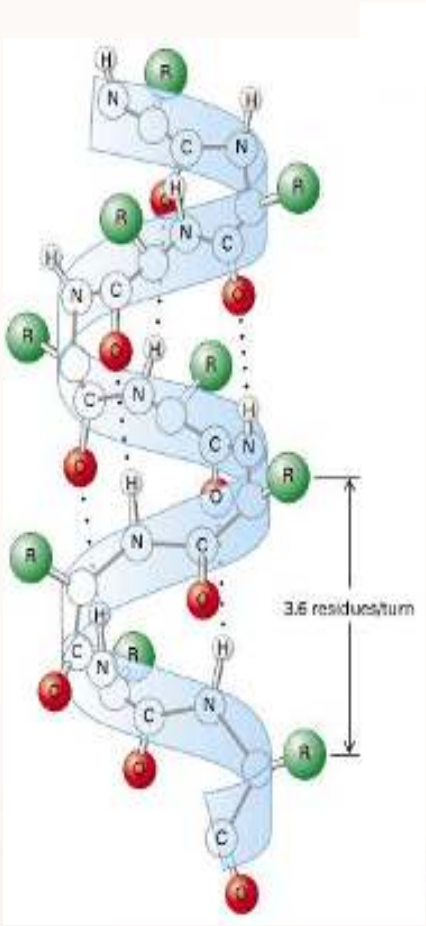


Fig. 3: Alpha helix structure

5.1.2.2. β pleated sheets

a. The β-conformation organizes polypeptide chains into sheets:
b. Pauling and Corey predicted a second type of repetitive structure, the β-conformation.
c. Besides alpha helix, they are another major structural element in globular proteins containing 20-28% of all residues.
d. This is a more extended conformation of PP chains, and its structure has been confirmed by x-ray analysis.
e. In the β-conformation, the backbone of the PP chain is extended into a zigzag rather than helical structure.
f. Zigzag PP chains can be arranged side by side to form a structure resembling a series of pleats.
g. Average length of beta sheets in a protein is 6 AA residues. The actual length ranges from 2 to 22 residues and most beta sheets contain less than 6 strands.

h. Side chains from adjacent residues of a strand in a beta sheet are found on opposite sides of the sheet and do not interact with one another.
i. In a β-sheet, hydrogen bonds are formed between adjacent segments of PP chain.
j. The individual segments that form a β-sheet are usually nearby on PP chain, but can also be quite distant from each other in the linear sequence of the PP; they may even be segments in different polypeptide chains.
k. β-sheets are formed from strands that are very often from distant portions of the PP sequence.
l. Hydrogen bonds in β-sheets are on average 0.1 Angstrom shorter than those found in alpha helices.
m. R groups of adjacent AAs protrude from zigzag structure in opposite directions, creating the alternating pattern. The adjacent polypeptide chains in a β-sheet can be either parallel or antiparallel (having the same or opposite amino-to-carboxyl orientations, respectively).
n. The N-H and C=O groups on the outer edge of the β-sheet structure is not hydrogen bonded to other strands of the primary sequence.
o. Hydrogen bonds in a parallel beta sheet are not perpendicular (but oblique) to the individual strands.
p. Antiparallel beta sheets are intrinsically more stable than parallel sheets due to more optimal orientation of the inter-strand hydrogen bonds.
q. The secondary structure of a PP segment can be completely defined if the φ and Ψ angles are known for all AA residues in that segment.

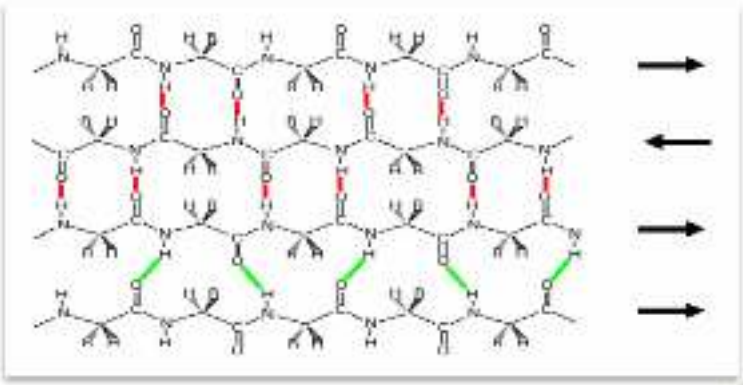


Fig. 4: β-sheet structure

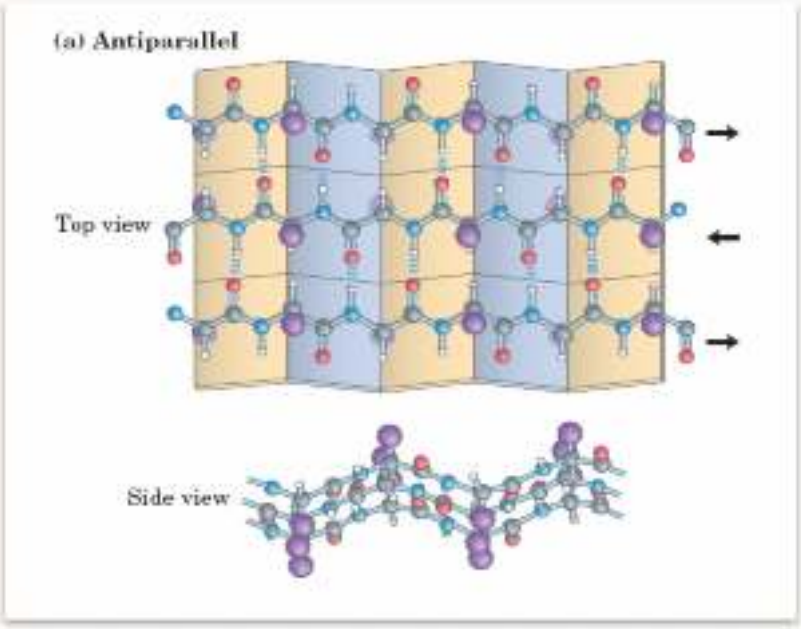


Fig. 5: Antiparallel β-sheet

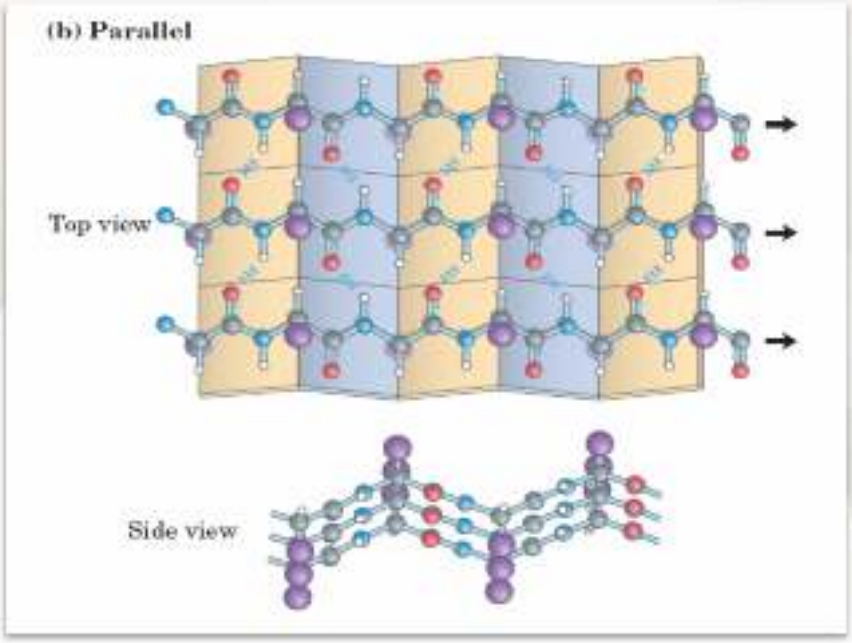


Fig. 6: Parallel β-sheet structure

5.1.2.3. β-Turns

a. β-Turns are common in proteins.
b. In globular proteins, which have a compact folded structure, nearly one-third of the AA residues are in turns or loops where the PP chain reverses direction.
c. These are the connecting elements that link successive runs of α-helix or β-conformation.
d. Particularly common are β-turns that connect the ends of two adjacent segments of an antiparallel β-sheet.
e. Structure is a 180° turn involving four AA residues, with the carbonyl oxygen of the first residue forming a hydrogen bond with the amino-group hydrogen of the fourth.
f. Peptide groups of the central two residues do not participate in any inter-residue hydrogen bonding.
g. Gly and Pro residues often occur in β-turns, the former because it is small and flexible, the latter because peptide bonds involving the imino nitrogen of proline readily assume the cis configuration, a form that is particularly amenable to a tight turn.

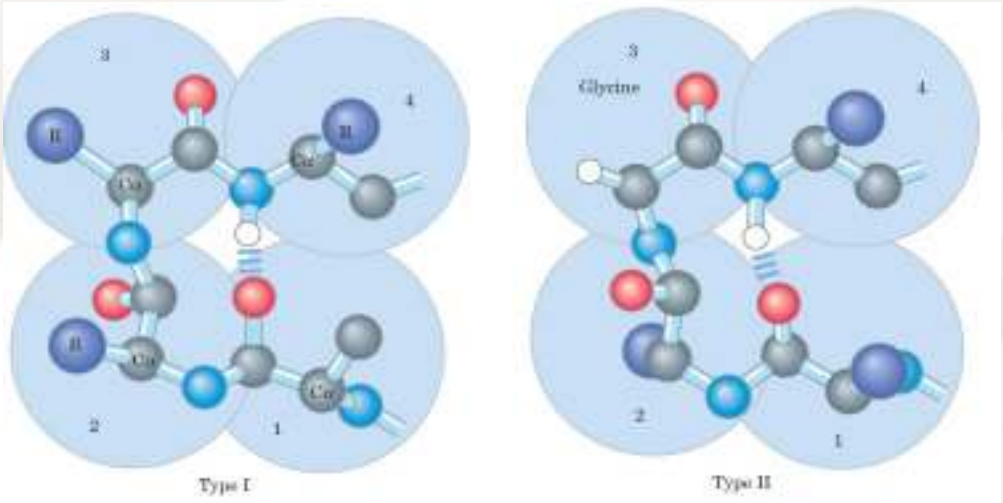


Fig. 7: ***β-Turns***

5.1.3 Protein tertiary structure

a. Overall, 3-D arrangement of all atoms in a protein is referred to as the protein's tertiary structure.
b. AAs that are far apart in PP sequence and that reside in different types of secondary structure may interact within the completely folded structure of a protein.
c. Location of bends (including β-turns) in PP chain and the direction and angle of these bends are determined by the number and location of specific bend-producing residues, such as Pro, Thr, Ser, and Gly.
d. Interacting segments of PP chains are held in their characteristic tertiary positions by different kinds of weak bonding interactions (and sometimes by covalent bonds such as disulfide cross-links) between the segments.

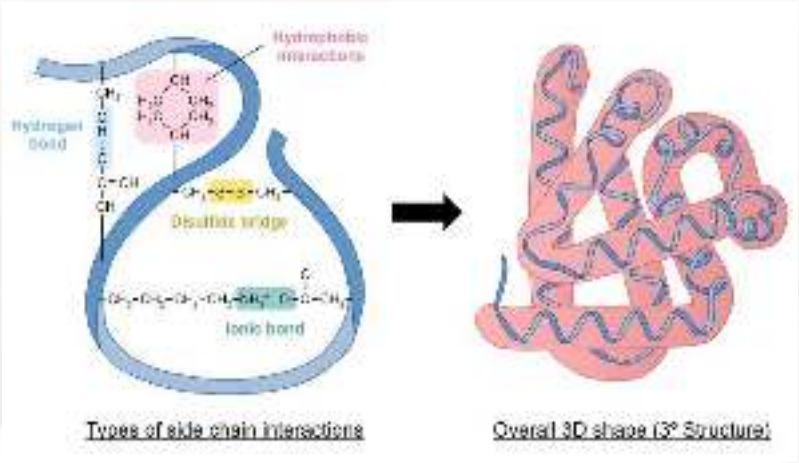


Fig. 8: Tertiary structure of protein

e. Hydrogen bonds: These are formed between the side chain (R group) of amino acids having a hydrogen donor group and an acceptor group.
f. Salt-linkages (electrostatic forces; ionic bonds): These are due to the interaction between amino groups of basic amino acids and the carboxyl group of acidic amino acids present in the R group.
g. Disulfide bonds (S-S linkages): The S-S linkages are formed by the oxidation of sulfhydryl (-SH) group of two cysteine side chains.

h. Hydrophobic bonds: These are formed as a result of interaction between non-polar side chains.
i. Dipole-dipole interaction: This interaction occurs between polar unionized side chains.

5.1.4 Protein quaternary structure

a. Some proteins contain two or more PP chains, or subunits, which may be identical or different. Arrangement of these protein subunits in three-dimensional complexes constitutes quaternary structure.
b. These subunits are held together by noncovalent surface interaction between the polar side chains. Proteins formed like above are termed oligomers and the individual polypeptide chains are variously termed protomers, monomers or subunits.
c. In considering the higher levels of structure, it is useful to classify proteins into two major groups: (1) fibrous proteins, having polypeptide chains arranged in long strands or sheets, and (2) globular proteins, having polypeptide chains folded into a spherical or globular shape.
d. Two groups are structurally distinct: fibrous proteins usually consist largely of a single type of secondary structure; globular proteins often contain several types of secondary structures.
e. Two groups differ functionally in that the structures that provide support, shape, and external protection to vertebrates are made of fibrous proteins, whereas most enzymes and regulatory proteins are globular proteins.
f. Fibrous proteins are adapted for a structural function and keratin, collagen, and silk fibroin illustrate the relationship between protein structure and biological function.
g. Fibrous proteins share properties that give strength and/or flexibility to the structures in which they occur. In each case, the fundamental structural unit is a simple repeating element of secondary structure.
h. All fibrous proteins are insoluble in water, a property conferred by a high concentration of hydrophobic AA residues both in the interior of the protein and on its surface.
i. Protein quaternary structures range from simple dimers to large complexes.

j. Many proteins have multiple PP subunits and association of PP chains can serve a variety of functions.
k. Many multi-subunit proteins have regulatory roles; the binding of small molecules may affect the interaction between subunits, causing large changes in the protein's activity in response to small changes in the concentration of substrate or regulatory molecules.
l. In other cases, separate subunits can take on separate but related functions, such as catalysis and regulation.
m. Some associations, such as the fibrous proteins and the coat proteins of viruses, serve primarily structural roles.
n. Some very large protein assemblies are the site of complex, multistep reactions. e.g. ribosome, the site of protein synthesis, which incorporates dozens of protein subunits along with a number of RNA molecules.
o. Multimeric proteins can have from two to hundreds of subunits. A multimer with just a few subunits is often called an oligomer.
p. If a multimer is composed of a number of non-identical subunits, the overall structure of the protein can be asymmetric and quite complicated.
q. However, most multimers have identical subunits or repeating groups of non-identical subunits, usually in symmetric arrangements.
r. The repeating structural unit in such a multimeric protein, whether it is a single subunit or a group of subunits, is called a protomer.
s. The first oligomeric protein for which the 3-D structure was determined was hemoglobin (Mr 64,500), which contains four polypeptide chains and four heme prosthetic groups, in which the iron atoms are in the ferrous (Fe2+) state.
t. The protein portion, called globin, consists of two α chains (141 residues each) and two β chains (146 residues each).
u. The subunits of hemoglobin are arranged in symmetric pairs, each pair having one α and β subunit.
v. Hemoglobin can therefore be described either as a tetramer or as a dimer of α-β protomers.

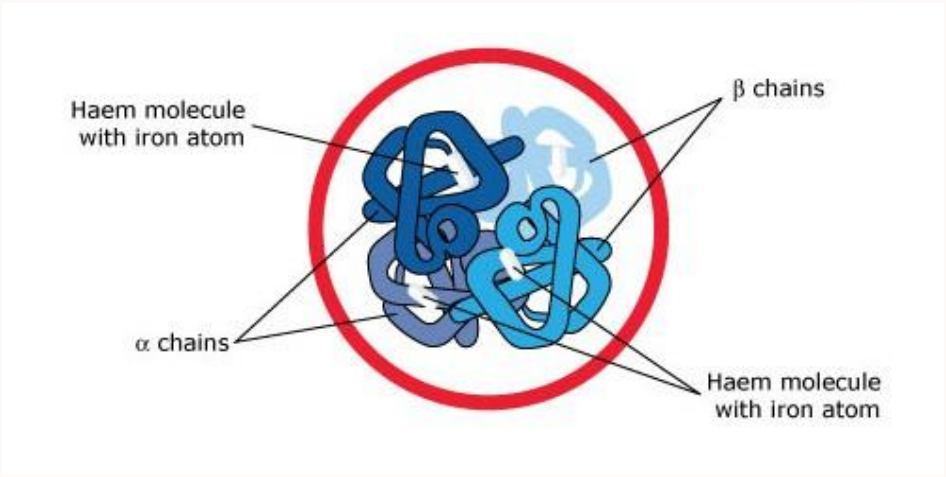


Fig. 9: Haemoglobin molecule

5.2 Biuret and xanthoproteic reaction

5.2.1 Biuret test for proteins:

a. The biuret test, also known as Piotrowski's test, is a chemical test used for detecting the presence of the peptide bonds. In the presence of peptides, a copper (II) ion forms mauve-colored coordination complex in an alkaline solution. Several variants on the test have been developed, such as the BCA test and the Modified Lowry test.

b. Biuret reaction can be used to assess the concentration of proteins because peptide bonds occur with the same frequency per amino acid in the peptide. The intensity of the color at 540 nm is directly proportional to the protein concentration, according to the Beer–Lambert law.

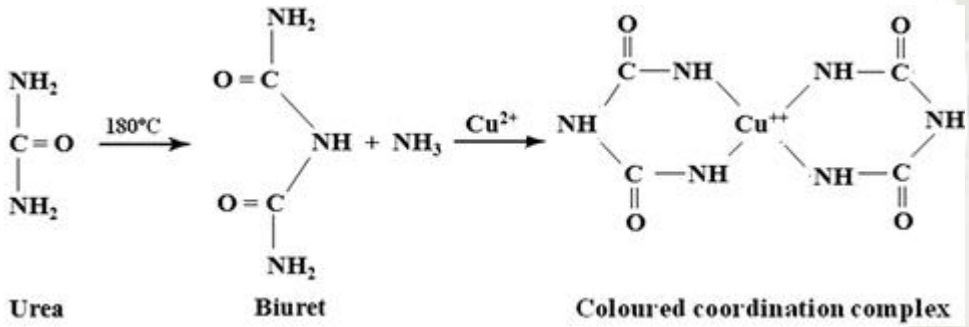


Fig. 10 Biuret reaction

5.2.2 Xanthoproteic reaction:

a. Xanthoproteic reaction is a method that can be used to detect the presence of protein soluble in a solution, using concentrated nitric acid. The test gives a positive result in amino acids carrying aromatic groups, especially in the presence of tyrosine.
b. If the test is positive the proof is neutralized with an alkali, turning dark yellow. The yellow colour is due to xanthoproteic acid which is formed due to nitration of phenyl rings of aromatic amino acids, examples being tyrosine and tryptophan. This chemical reaction is a qualitative test, determining the presence or absence of proteins.

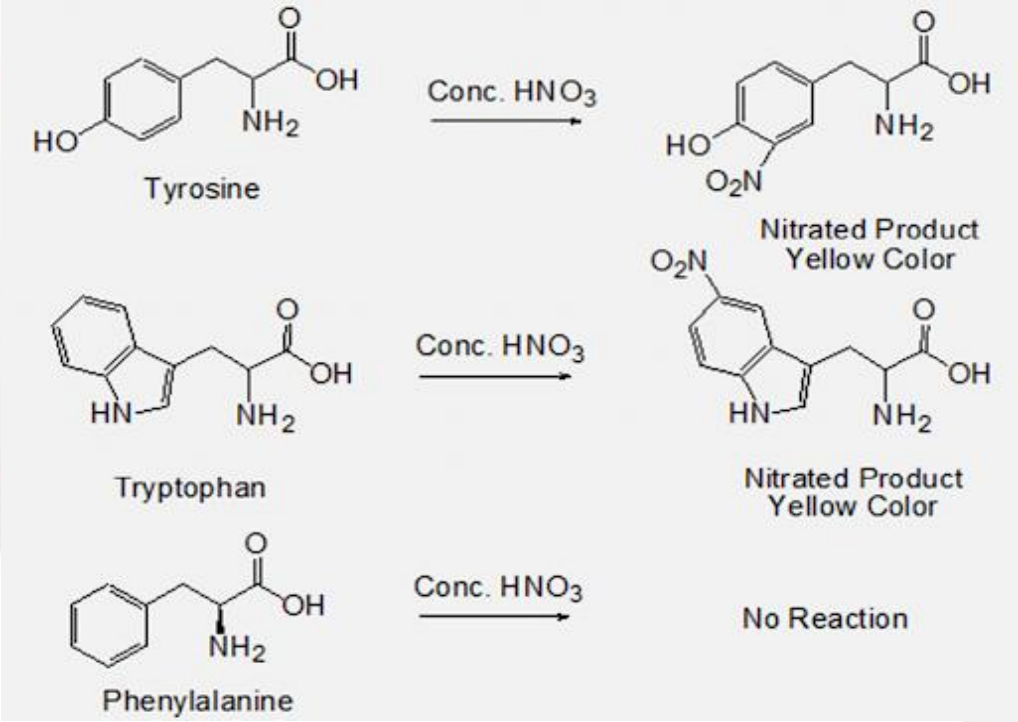


Fig. 11 Xanthoproteic reaction

5.3 Amphoteric nature of amino acids:

a. Amino and carboxyl groups of amino acids, along with the ionizable R groups of some amino acids, function as weak acids and bases.
b. When an amino acid lacking an ionizable R group is dissolved in water at neutral pH, it exists in solution as dipolar ion, or zwitterion (German for “hybrid ion”), which can act as either an acid or a base.
c. Substances having this dual (acid-base) nature are amphoteric and are often called ampholytes (from “amphoteric electrolytes”). A simple monoamino monocarboxylic -amino acid, such as alanine, is a diprotic acid when fully protonated; it has two groups, the —COOH group and the —NH3 group, that can yield protons:

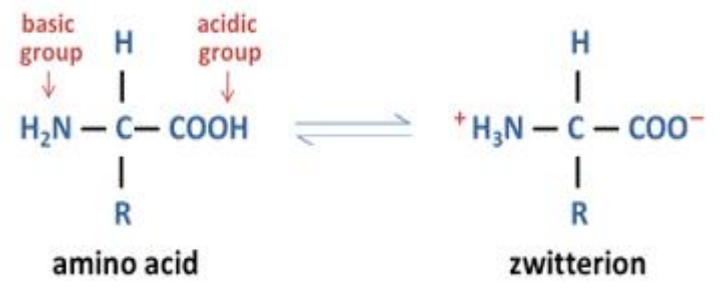


Fig. 12: Zwitterion

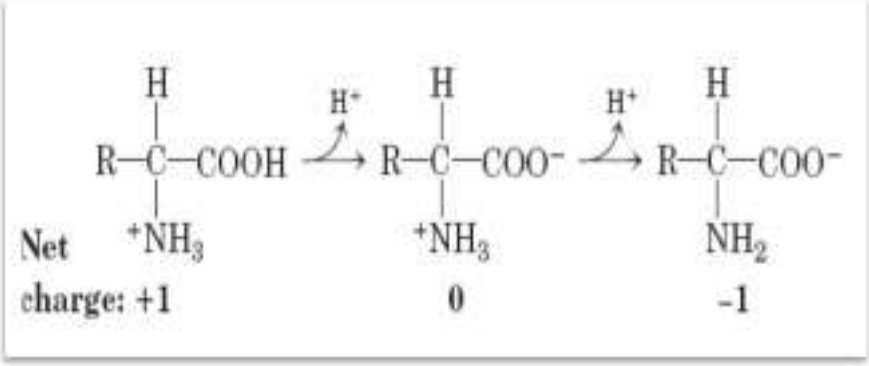


Fig. 13: Amphoteric nature of Amino Acid

d. Proteins, like amino acids, are amphoteric and contain both acidic and basic groups.
e. They possess electrically charged groups and hence migrate in an electric field.

5.4 Digestion and absorption of proteins:

5.4.l. Digestion of proteins by gastric secretion-

a. Protein digestion begins in the stomach. Gastric juice produced by stomach contains hydrochloric acid (HCl) and a protease proenzyme named pepsinogen.
b. Hydrochloric acid: The pH of stomach is < 2 due to the presence of HCl, secreted by parietal (oxyntic) cells of gastric gland. This acid performs two important functions-denaturation of proteins and killing of certain microorganisms.
c. The denatured proteins are more susceptible to proteases for digestion.
d. Pepsin: Pepsin (Greek: pepsis-digestion) is produced by serous cells of stomach as pepsinogen, the inactive zymogen or proenzyme. Pepsinogen is converted to active pepsin either by autocatalysis, brought about by other pepsin molecules or by gastric HCI.
e. Removal of a fragment of polypeptide chain (44 amino acids in case of pig enzyme) makes the inactive enzyme active after attaining a proper conformation.
f. Pepsin is an acid-stable endopeptidase optimally active at a very low pH (2.0). Active site of the enzyme contains two carboxyl groups which are maintained at low pH.
g. Pepsin digestion of proteins results in peptides and a few amino acids which act as stimulants for the release of the hormone cholecystokinin from the duodenum.
h. Rennin: This enzyme, also called chymosin, is found in stomach of infants and children. Rennin is involved in the curdling of milk. It converts milk protein casein to calcium-paracaseinate which can be effectively digested by pepsin. Rennin is absent in adults.

5.4.2. Digestion of proteins by pancreatic proteases:

a. The proteases of pancreatic juice are secreted as zymogens (proenzymes) and then converted to active forms. These processes are initiated by the release of two polypeptide hormones, namely cholecystokinin and secretin from the intestine.

1. Release and activation of zymogens:

I. The key enzyme for activation of zymogen is enteropeptidase produced by intestinal (mostly duodenal) mucosal epithelial cells.

II. Enteropeptidase leaves off a hexapeptide from the N-terminal end of trypsinogen to produce trypsin, the active enzyme. Trypsin, in turn, activates other trypsinogen molecules (autocatalysis).

III. Further, trypsin is the common activator of all other pancreatic zymogens to produce the active proteases, namely chymotrypsin, elastase and carboxypeptidase (A and B).

2. Action of carboxypeptidases:

I. The pancreatic carboxypeptidase (A and B) are metalloenzymes that are dependent on Zn2+ for their catalytic activity, hence they are sometimes called Zn-proteases. They also possess certain degree of substrate specificity in their action.

II. Carboxypeptidase B acts on peptide bonds of COOH- terminal amino acid, the amino group of which is contributed by arginine or lysine. Whereas Carboxypeptidase A acts on peptide bonds of COOH- terminal amino acid, the amino group of which should not be contributed by arginine, lysine or proline.

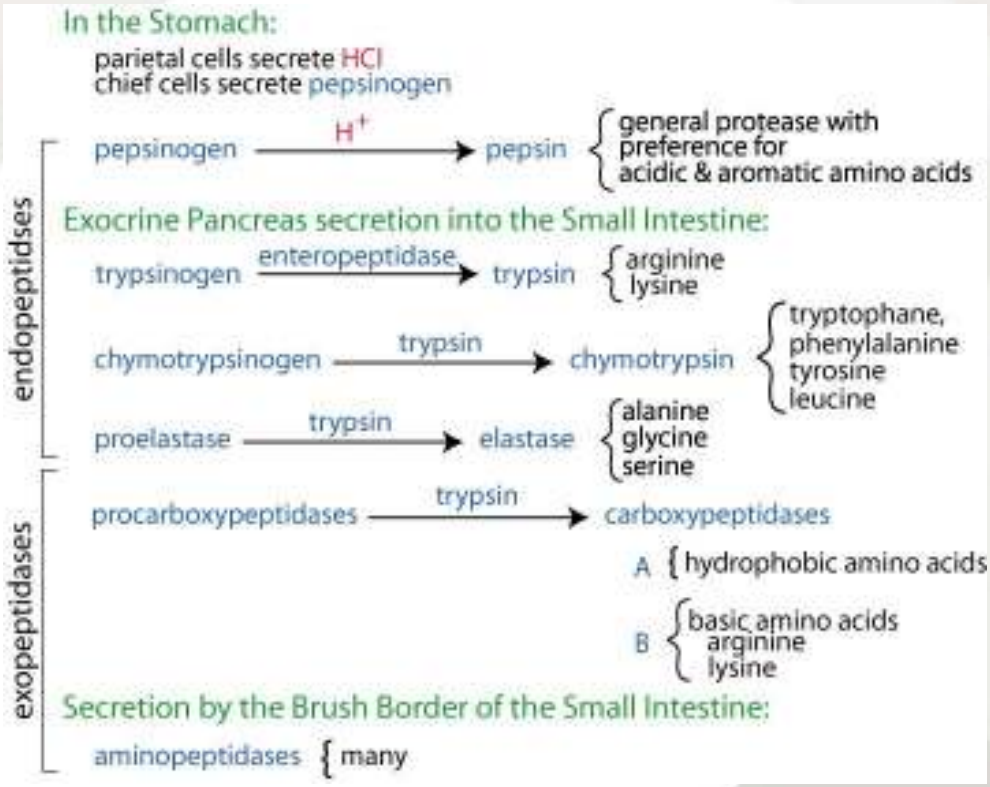
III. The combined action of pancreatic proteases results in formation of free amino acids and small peptides (2-8 amino acids).

5.4.3. Digestion of proteins by small intestinal enzymes:

a. The luminal surface of intestinal epithelial cells contains aminopeptidases and dipeptidases.
b. Aminopeptidase is a non-specific exopeptidase which repeatedly cleaves N-terminal amino acids one by one to produce free amino acids and smaller peptides. The dipeptidases act on different dipeptides to liberate amino acids.

5.4.4 Absorption of amino acids and dipeptides:

a. Free amino acids, dipeptides and to some extent tripeptides are absorbed by intestinal epithelial cells.
b. The di- and tripeptides, after being absorbed are hydrolysed into free amino acids in the cytosol of epithelial cells. The activities of dipeptidases are high in these cells. Therefore, after a protein meal, only the free amino acids are found in the portal vein.
c. The small intestine possesses an efficient system to absorb free amino acids. L-Amino acids are more rapidly absorbed than D-amino acids.
d. The transport of L-amino acids occurs by an active process (against a concentration gradient), in contrast to D-amino acids which takes place by a simple diffusion.



1. Mechanism of amino acids absorption:

I. Amino acids are primarily absorbed by a mechanism similar for the transport of D-glucose.
II. It is basically a Na+-dependent active process linked with the transport of Na+.
III. As the Na+ diffuses along the concentration gradient, the amino acid also enters the intestinal cell.
IV. Both Na+ and amino acids share a common carrier and are transported together. The energy is supplied indirectly by ATP.
V. A Na+-independent system of amino acid transport across intestinal cells has also been identified.
VI. The compound cytochalasin I inhibit Na+-independent transport system.

The direct absorption of intact proteins is very important for the transfer of maternal immunoglobulins (y-globulins) to the offspring. The intact proteins and polypeptides are not absorbed by the adult intestine. However, the macromolecular absorption in certain individuals appears to be responsible for antibody formation that often causes food allergy.

Course Name	Fundamentals of Biochemistry
Lesson 6	Classification, structure, functions and properties of lipids, essential fatty acids and phospholipids
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Lesson 6:

Classification, structure, functions and properties of lipids, essential fatty acids and phospholipids

Objectives:

a. To study the classification of lipids.
b. To study the structure and functions of various categories of lipids.
c. To study the importance of essential fatty acids.

Glossary:

a. **Hydrocarbons:** A hydrocarbon is an organic compound consisting entirely of hydrogen and carbon.
b. **Saponification:** Saponification is a process that involves the conversion of fat, oil, or lipid, into soap and alcohol by the action of heat in the presence of aqueous alkali. Soaps are salts of fatty acids and fatty acids are monocarboxylic acid having long carbon chains e.g. sodium palmitate.
c. **Hydrogenation:** Hydrogenation is a chemical reaction between molecular hydrogen and another compound or element, usually in the presence of a catalyst such as nickel, palladium or platinum. The process is commonly employed to reduce or saturate organic compounds.
d. **Glycolipids:** Glycolipids are lipids with a carbohydrate attached by a glycosidic bond.
e. **Triacylglycerols/triglyceride:** A triglyceride is an ester derived from glycerol and three fatty acids.
f. **Waxes:** Waxes are 'monoesters' of long-alcohols and long-chain carboxylic acids.
g. **Essential fatty acids:** The fatty acids that can't be synthesized by the body and, therefore, should be supplied in the diet.
h. **Saponifiable lipids:** Lipids comprising of one or more ester groups, enabling it to undergo hydrolysis in the presence of a base, acid, or enzymes, e.g. waxes, triglycerides etc.

6.1 Lipids:

a. Lipids are a heterogeneous group of organic compounds that are insoluble in water and soluble in non-polar organic solvents.
b. They naturally occur in most plants, animals, microorganisms and are used as cell membrane components, energy storage molecules, insulation, and hormones.
c. In human body, these molecules can be synthesized in liver and are found in oil, butter, whole milk, cheese, fried foods, and also in some red meats.

6.2 Properties of lipids:

Lipids are a family of organic compounds, composed of fats and oils. These molecules yield high energy and are responsible for different functions within human body. Listed below are some important characteristics of lipids.

a. Lipids are oily or greasy nonpolar molecules, stored in adipose tissue of body.
b. Unlike polysaccharides and proteins, lipids are not polymers—they lack a repeating monomeric unit.
c. Lipids are energy-rich organic molecules, which provide energy for different life processes.
d. Lipids are a class of compounds characterised by their solubility in nonpolar solvents like alcohol, chloroform, acetone, benzene, etc and insolubility in water.
e. They have lesser specific gravity (density) than water and therefore float in water.
f. Though fats are insoluble in water, they can be broken down into minute droplets and dispersed in water. This is called emulsification.
g. Examples of naturally occurring emulsions are milk and yolk of egg.
h. Emulsification greatly increases the surface area of the fat and this is an essential requisite for digestion of fat in the intestine.
i. Lipids are significant in biological systems as they form for a mechanical barrier dividing a cell from the external environment known as the cell membrane.
j. Lipids may be either liquids or non-crystalline solids at room temperature.
k. Pure fats and oils are colorless, odorless, and tasteless.
l. Solid triacylglycerols/TAGs (fats) have high proportions of saturated fatty acids.
m. Liquid triacylglycerols/TAGs (oils) have high proportions of unsaturated fatty acids.

n. Hydrolysis of TAGs like any other esters form their carboxylic acid and alcohol– a process known as hydrolysis.
o. Saponification-TAGs may be hydrolyzed by several procedures, the most common of which utilizes alkali or enzymes called lipases. Alkaline hydrolysis is termed saponification because one of the products of the hydrolysis is a soap, generally sodium or potassium salts of fatty acids.
p. Hydrogenation-The carbon-carbon double bonds in unsaturated fatty acids can be hydrogenated by reacting with hydrogen to produce saturated fatty acids.
q. Rancidity- The term rancid is applied to any fat or oil that develops a disagreeable odor. Hydrolysis and oxidation reactions are responsible for causing rancidity. Oxidative rancidity occurs in TAGs containing unsaturated fatty acids.
r. Hydrolytic rancidity involves partial hydrolysis of the triacylglycerol to mono and Diacylglycerol.
s. Hydrolysis is hastened by the presence of moisture, warmth and lipases present in fats or air.

6.3 Fatty acids:

a. Fatty acids are carboxylic acids (or organic acid), usually with long aliphatic tails (long chains) which are either unsaturated or saturated.
b. The non-polar hydrocarbon chain accounts for the poor solubility of fatty acids in water.
c. Lack of carbon-carbon double bond indicates that the fatty acid is saturated. The saturated fatty acids have higher melting points as compared to unsaturated acids of corresponding size due to their ability to pack their molecules together thus leading to a straight rod-like shape.
d. Unsaturated fatty acids, on the other hand, do contain C=C bonds. Monounsaturated fatty acids have one C=C bond, and polyunsaturated have more than one C=C bond.

e. Often, naturally occurring fatty acids possesses an even number of carbon atoms and are unbranched. On the other hand, unsaturated fatty acids contain a cis-

double bond(s) which creates a structural kink that disables them to group their molecules in straight rod-like shape.

f. The structure of fatty acids is written as a symbol of two numbers separated by a colon: the first number denotes the carbon atoms in the chain and the second number denotes the number of unsaturation centres. The positions of double bonds are specified by superscript numbers following (delta). Thus 18:2 (Δ9, 12) indicates an eighteen carbon fatty acid with two double bonds between C-9 and C-10, and between C-12 and C-13.

Table 1: Structure, properties and nomenclature of different fatty acids

Carbon skeleton	Structure*	Systematic name†	Common name (derivation)	Melting point (°C)	Solubility at 30 °C (mg/g solvent)	
					Water	Benzene
12:0	$CH_3(CH_2)_{10}COOH$	*n*-Dodecanoic acid	Lauric acid (Latin *laurus*, "laurel plant")	44.2	0.063	2,600
14:0	$CH_3(CH_2)_{12}COOH$	*n*-Tetradecanoic acid	Myristic acid (Latin *Myristica*, nutmeg genus)	53.9	0.024	874
16:0	$CH_3(CH_2)_{14}COOH$	*n*-Hexadecanoic acid	Palmitic acid (Latin *palma*, "palm tree")	63.1	0.0083	348
18:0	$CH_3(CH_2)_{16}COOH$	*n*-Octadecanoic acid	Stearic acid (Greek *stear*, "hard fat")	69.6	0.0034	124
20:0	$CH_3(CH_2)_{18}COOH$	*n*-Eicosanoic acid	Arachidic acid (Latin *Arachis*, legume genus)	76.5		
24:0	$CH_3(CH_2)_{22}COOH$	*n*-Tetracosanoic acid	Lignoceric acid (Latin *lignum*, "wood" + *cera*, "wax")	86.0		
16:1(Δ^9)	$CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH$	*cis*-9-Hexadecenoic acid	Palmitoleic acid	1 to −0.5		
18:1(Δ^9)	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$	*cis*-9-Octadecenoic acid	Oleic acid (Latin *oleum*, "oil")	13.4		
18:2($\Delta^{9,12}$)	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$	*cis*-,*cis*-9,12-Octadecadienoic acid	Linoleic acid (Greek *linon*, "flax")	1–5		
18:3($\Delta^{9,12,15}$)	$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$	*cis*-,*cis*-,*cis*-9,12,15-Octadecatrienoic acid	α-Linolenic acid	−11		
20:4($\Delta^{5,8,11,14}$)	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_3COOH$	*cis*-,*cis*-,*cis*-,*cis*-5,8,11,14-Icosatetraenoic acid	Arachidonic acid	−49.5		

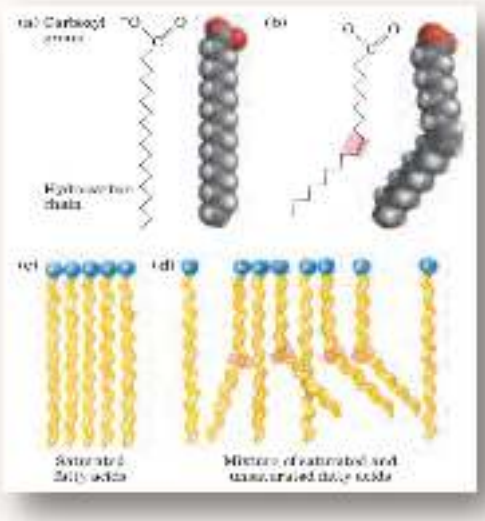

Fig. 1: Packing of saturated and unsaturated fatty acids

6.4 Some unusual fatty acids present in plants

a. The unusual fatty acids are found only in few individual species or genus or a whole family.
b. Castor bean (Ricinus communis) seed oil is rich in ricinoleic acid (90%) which is 12-hydroxy oleic acid CH3(CH2)5-CH(OH)-CH2-CH=CH-(CH2)7-COOH.
c. Rape seed (Brassica napus) is rich in erucic acid (cis-13-docosenoic acid CH3(CH2)7-CH=CH-(CH2)11-COOH).
d. Hydnocarpic and chaulmoogric acids are found in chaulmoogra oil which is used in the treatment of leprosy.

6.5 Classification of lipids:

Lipids can be classified into two main classes:

a. **Non-saponifiable lipids-** A nonsaponifiable lipid can't be disintegrated into smaller molecules through hydrolysis. Nonsaponifiable lipids include cholesterol, prostaglandins etc.
b. **Saponifiable lipids-** A saponifiable lipid comprises one or more ester groups, enabling it to undergo hydrolysis in the presence of a base, acid, or enzymes, e.g. waxes, triglycerides, sphingolipids, and phospholipids.

Further, these categories can be divided into non-polar and polar lipids.

a. Nonpolar lipids, namely triglycerides, are utilized as fuel and to store energy.
b. Polar lipids, that could form a barrier with an external water environment, are utilized in membranes. Polar lipids comprise sphingolipids and glycerophospholipids.
c. Fatty acids are pivotal components of all these lipids.
d. Within these two major classes of lipids, there are numerous specific types of lipids important to live, including fatty acids, triglycerides, glycerophospholipids, sphingolipids, and steroids. These are broadly classified as simple lipids and complex lipids.

1. **Simple lipids: Esters of fatty acids with various alcohols. These include:**
   a. Fats/oils: Esters of fatty acids (saturated and unsaturated) with glycerol.
   b. Waxes: Esters of long chain fatty acid of higher molecular weight with long chain monohydric alcohols.

Fats and oils

a. Triacylglycerols are the simplest lipids constructed from fatty acids and glycerol.
b. They are also referred as triglycerides, fats or neutral fats.
c. Triacylglycerols are composed of three fatty acids esterified to the three hydroxyl groups of glycerol
d. When all the 3 fatty acid molecules are of the same kind the triacylglycerol is said to be simple triacylglycerol.
e. Mixed triacylglycerol possesses two or more different fatty acids.
f. Triacylglycerol that are solid at room temperature are called as fats
g. Liquid triacylglycerols are called as oils.
h. Neutral fats or oils are mostly composed of mixed triacyl glycerol.
i. Fats are usually rich in saturated fatty acids and the unsaturated fatty acids predominate in oils.

j. Most oil-producing plants store their lipids in the form of triacylglycerols. Triacylglycerols are normally stored in the endosperm of the seed although some plants store appreciable quantities of fat in the fleshy fruit mesocarp, for example, avocado.
k. Some plants like the oil palm, store oils in both the mesocarp (Palm oil) and the endosperm (Palm kernel oil).
l. Most of the common edible oils (groundnut, sunflower, gingelly, soybean, safflower, rice bran) contain limited number of the common fatty acids such as palmitic, stearic, oleic, linoleic and linolenic acids.
m. Palm kernel and coconut oils contain higher amount of medium chain saturated fatty acids.

Waxes

a. Waxes are “esters” (an organic compound made by replacing the hydrogen with acid by an alkyl or another organic group) formed from long-alcohols and long-chain carboxylic acids.
b. Waxes are found almost everywhere. Fruits and leaves of many plants possess waxy coatings that can safeguard them from small predators and dehydration.
c. Fur of a few animals and the feathers of birds possess same coatings serving as water repellents.
d. Carnauba wax is known for its water resistance and toughness (significant for car wax).

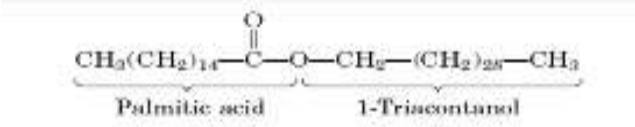

Fig. 2: Structure of beeswax (triacontanoylpalmitate)

e. The outermost surface of the cell walls of epidermal cells are covered with a hydrophobic cuticle which contains wax called cuticular wax.

f. The main components of cuticular waxes are hydrocarbon (odd chain alkanes) and its derivatives, wax esters, free aldehydes, free acids, free alcohols and other components like mono esters of phenolic acids and aliphatic alcohols.
g. The main function of the cuticular wax is to reduce the excessive losses and gains of water by the underlying tissue.
h. It also helps in protecting the tissues from chemical, physical and biological attack.

2. **Complex lipids:** Esters of fatty acids containing groups in addition to alcohol and a fatty acid.

a. Phospholipids: These are lipids containing, in addition to fatty acids and alcohol, a phosphoric acid residue. They frequently have nitrogen-containing bases and other substituents, e.g. in glycerophospholipids the alcohol is glycerol and in sphingophospholipids the alcohol is sphingosine. Glycolipids (glycosphingolipids) are lipids containing a fatty acid, sphingosine, and carbohydrate.

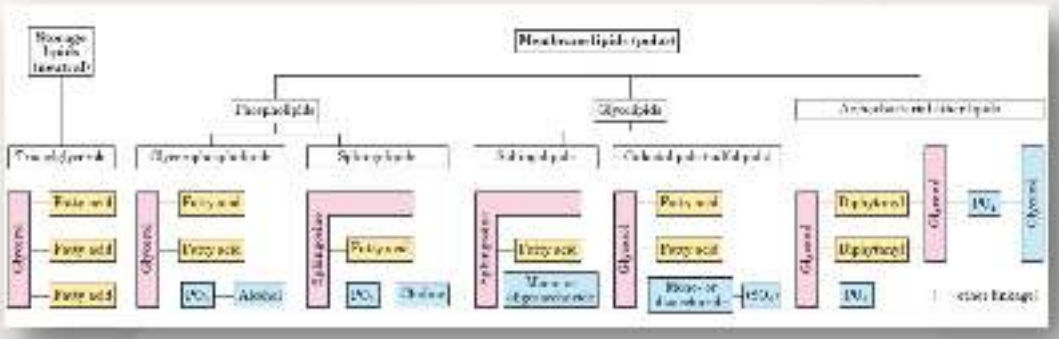

Fig. 3: Classification/types of lipids

Phospholipids

a. Phospholipids (PL) are a class of lipids whose molecule has a hydrophilic "head" containing a phosphate group, and two hydrophobic "tails" derived from fatty acids, joined by an alcohol residue.
b. The phosphate group can be modified with simple organic molecules such as choline, ethanolamine or serine.
c. Phospholipids are a key component of all cell membranes. They can form lipid bilayers because of their amphiphilic characteristic.

Glycerophospholipids are derivatives of phosphatidic acid

a. Glycerophospholipids, also called phosphoglycerides, are membrane lipids in which two fatty acids are attached in ester linkage to the first and second carbons of glycerol, and a highly polar or charged group is attached through a phosphodiester linkage to the third carbon.
b. Glycerophospholipids are named as derivatives of the parent compound, phosphatidic acid, according to the polar alcohol in the head group.
c. Phosphatidylcholine and phosphatidylethanolamine have choline and ethanolamine in their polar head groups.
d. In all these compounds, the head group is joined to glycerol through a phosphodiester bond, in which the phosphate group bears a negative charge at neutral pH.
e. The polar alcohol may be negatively charged (as in phosphatidylinositol 4,5-bisphosphate), neutral (phosphatidylserine), or positively charged (phosphatidylcholine, phosphatidylethanolamine).
f. These charges contribute greatly to the surface properties of membranes.
g. The FAs in glycerophospholipids can be any of a wide variety, so a given phospholipid (phosphatidylcholine, for example) may consist of a number of molecular species, each with its unique complement of FAs.
h. The distribution of molecular species is specific for different organisms, different tissues of the same organism, and different glycerophospholipids in the same cell or tissue.
i. In general, glycerophospholipids contain a C16 or C18 saturated FA at C-1 and a C18 to C20 unsaturated FA at C-2.
j. The biological significance of the variation in FAs and head groups is not yet understood.

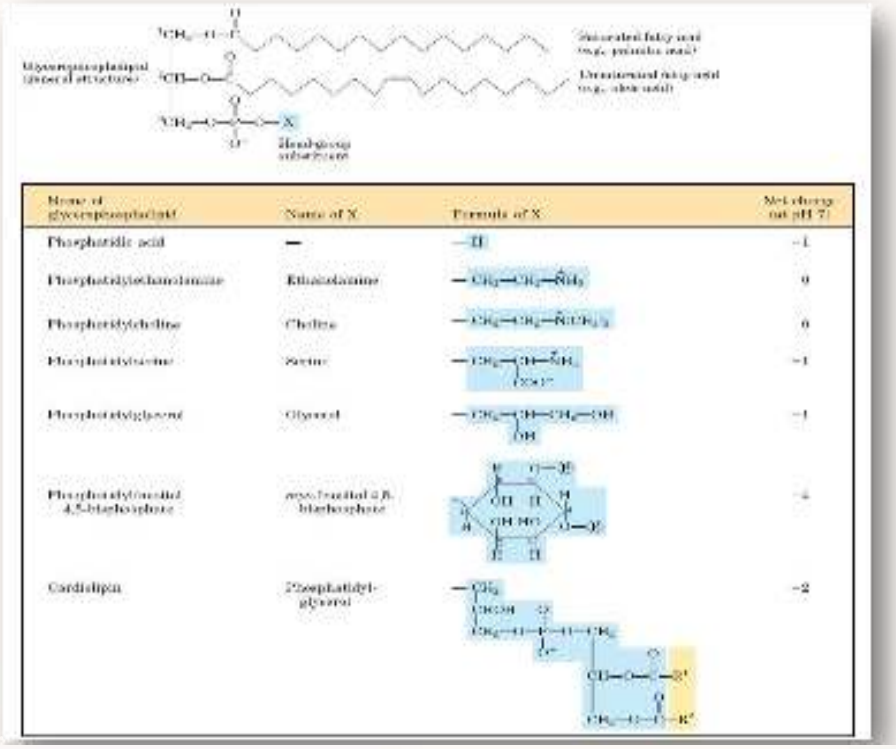

Fig. 4. Types of glycerophospholipids

Sphingophospholipids

a. The phosphate and fatty acids are attached to the alcohol sphingosine instead of glycerol in sphingophospholipids.
b. The fatty acids are attached through an amide linkage rather than the ester linkage.
c. The base present is normally choline.

d. When a fatty acid is attached by an amide linkage to the -NH2, group the resulting compound is a ceramide.
e. Ceramide is the fundamental structural unit common to all sphingophospholipids.
f. Sphingophospholipids are found in the seeds of several plant species.
g. The sphingomyelins, the main sphingophospholipids of animals, are not present in plants.

3. **Other complex lipids:** Lipids such as sulfolipids and amino lipids. Lipoproteins may also be placed in this category.

Glycolipids and sulpholipids

a. Glycolipids are structurally characterised by the presence of one or more monosaccharide residues and the absence of a phosphate.
b. They are O-glycoside of either sphingosine or glycerol derivative. The monosaccharides commonly attached are D-glucose, D-galactose or N-acetyl D-galactosamine.
c. Monogalactosyl diglycerides and digalactosyl diglycerides have been shown to be present in a wide variety of higher plant tissues
d. The 3 position of 1, 2-diacylglycerol is linked to 6- sulpho-6-deoxy D-glucose by glycosidic bond in plant sulpholipid.
e. The sulpholipid is mostly present in chloroplasts, predominantly in the membranes of thylakoid.

Lipoprotein

a. Protein molecules associated with triacylglycerol, cholesterol or phospholipids are called lipoproteins.
b. Triacylglycerols derived from intestinal absorption or from the liver are not transported in the free form in circulating blood plasma, but move as chylomicrons, as very low density lipoproteins (VLDL) or as free fatty acids (FFA) - albumin complexes.

c. Besides, two more physiologically important groups of lipoproteins are low density lipoprotein (LDL) and high density lipoprotein (HDL).
d. The major lipid components of chylomicrons and VLDL are triacylglycerol, whereas the predominant lipids in LDL and HDL are cholesterol and phospholipid respectively.
e. The protein part of lipoprotein is known as apoprotein.
f. Lipoproteins occur in milk, egg-yolk and also as components of cell membranes.

Steroids

a. Our bodies possess chemical messengers known as hormones that are basically organic compounds synthesized in glands and transported by bloodstream to various tissues in order to trigger or hinder the desired process.
b. Sterols are structural lipids present in the membranes of most eukaryotic cells.
c. Characteristic structure of sterol is the steroid nucleus, consisting of four fused rings, three with six carbons and one with five.
d. Steroid nucleus is almost planar and is relatively rigid; the fused rings do not allow rotation about C-C bonds.
e. Bacteria can't synthesize sterols; a few bacterial species, however, can incorporate exogenous sterols into their membranes.
f. Sterols of all eukaryotes are synthesized from simple five carbon isoprene subunits, as are the fat-soluble vitamins, quinones, and dolichols.
g. In addition to their roles as membrane constituents, the sterols serve as precursors for a variety of products with specific biological activities.
h. Steroid hormones, for e.g., are potent biological signals that regulate gene expression.
i. Bile acids are polar derivatives of cholesterol that act as detergents in the intestine, emulsifying dietary fats to make them more readily accessible to digestive lipases.
j. Cholesterol is a wax-like substance, found only in animal source foods. Triglycerides, LDL, HDL, VLDL etc. are different types of cholesterol found in blood.

k. Cholesterol is an important lipid found in the cell membrane. It is a sterol, which means that cholesterol is a combination of steroid and alcohol. In the human body, cholesterol is synthesized in the liver.
l. These compounds are biosynthesized by all living cells and are essential for the structural component of the cell membrane.
m. In the cell membrane, the steroid ring structure of cholesterol provides a rigid hydrophobic structure that helps to boost the rigidity of the cell membrane. Without cholesterol, the cell membrane would be too fluid.
n. It is an important component of cell membranes and is also the basis for the synthesis of other steroids, including the sex hormones estradiol and testosterone, as well as other steroids such as cortisone and vitamin D.

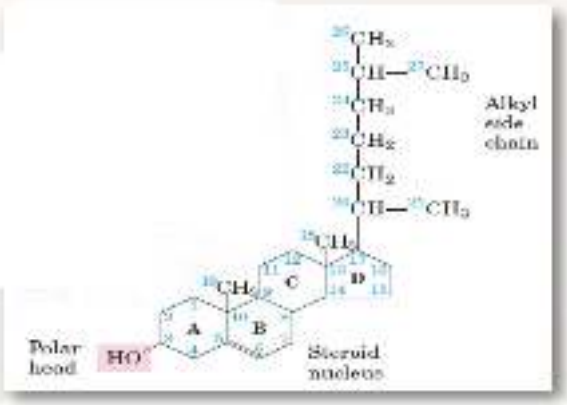

Fig. 5: Structure of cholesterol

6.6 Functions of lipids:

It is established that lipids play extremely important roles in the normal functions of a cell. Not only do lipids serve as highly reduced storage forms of energy, but they also play an intimate role in the structure of cell membrane and organellar membranes. Lipids perform many functions, such as:

1. Energy storage
2. Making biological membranes
3. Insulation
4. Protection – e.g. protecting plant leaves from drying up
5. Buoyancy

6. Acting as hormones
7. Act as the structural component of the body and provide the hydrophobic barrier that permits partitioning of the aqueous contents of the cell and subcellular structures.
8. Lipids are major sources of energy in animals and high lipid-containing seeds.
9. Activators of enzymes eg. glucose-6-phosphatase, stearyl CoA desaturase and ω-monooxygenase, and β-hydroxybutyric dehydrogenase (a mitochondrial enzyme) require phosphatidylcholine micelles for activation.

6.7 Essential fatty acids:

a. The fatty acids that can't be synthesized by the body and, therefore, should be supplied in the diet are known as essential fatty acids (EFA).
b. Chemically, they are polyunsaturated fattyacids, namely linoleic acid (18:2Δ9,12) and linolenic acid (18:3Δ9,12,15).
c. Arachidonic acid (20:4Δ5,8,11,14) becomes essential if its precursor linoleic acid is not provided in the diet in sufficient amounts.
d. Biochemical basis for essentiality: Linoleic acid and linolenic acid are essential since humans lack the enzymes that can introduce double bonds beyond carbon

Functions of EFA:

a. Essential fatty acids are required for the membrane structure and function, transport of cholesterol, formation of lipoproteins, prevention of fatty liver etc.
b. They are also needed for the synthesis of another important group of compounds namely eicosanoids.
c. Deficiency of EFA: The deficiency of EFA results in phrynoderma or toad skin, characterized by the presence of horny eruptions on the posterior and lateral parts of limbs, on the back and buttocks, loss of hair and poor wound healing.

Omega-3 and Omega-6 fatty acids:

a. Omega-3 and omega-6 both are fatty acids of polyunsaturated type. The difference is in where the first of the double bonds occurs.
b. In omega-3 fatty acids, the first double bond occurs on the third carbon atom, but in omega-6 fatty acids, the first double bond is on the sixth carbon atom, counting from the methyl end (denoted as omega)
c. Both omega-3 (ω-3) and omega-6 (ω-6) fatty acids are important components of cell membranes and are precursors to many other substances in the body such as those involved in regulating blood pressure and inflammatory responses.
d. The human body is capable of producing all the fatty acids it needs, except for two: linoleic acid (LA) - an omega-6 fatty acid, and alpha-linolenic acid (ALA) - an omega-3 fatty acid.
e. These have to be consumed from the diet and are termed “essential fatty acids”. Both of these fatty acids are needed for growth and repair but can also be used to make other fatty acids.

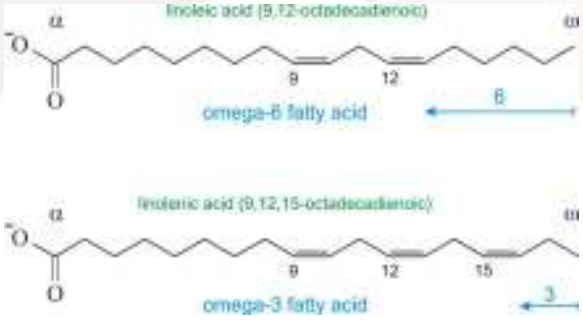

Fig. 6: Omega-3 and 6 fatty acids

Course Name	Fundamentals of Biochemistry
Lesson 7	Digestion and absorption of lipids, lipid auto-oxidation, significance of Omega-3 and Omega-6 fatty acids
Content Creator Name	Dr. Ajay Pal
University/College Name	Chaudhary Charan Singh Haryana Agricultural University, Hisar
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Division of Computer Applications, ICAR-Indian Agricultural Statistics Research Institute

Lesson 7:

Digestion and absorption of lipids, lipid auto-oxidation, significance of Omega-3 and Omega-6 fatty acids

Objectives:

a. To study digestion and absorption of lipids
b. To study lipid auto-oxidation and significance of Omega fatty acids

Glossary

a. **Digestion:** Process in alimentary canal by which food is broken down physically (by action of teeth) and chemically (by action of enzymes) and converted into a substance suitable for absorption and assimilation into the body.
b. **Amphiphilic:** An amphiphile is a chemical compound possessing both hydrophilic and lipophilic properties. Such a compound is called amphiphilic or amphipathic.
c. **Emulsification:** It is the phenomenon of dispersion of lipids into smaller droplets due to reduction in the surface tension.
d. **Detergents:** Detergents are surface-active molecules that self-associate and bind to hydrophobic surfaces in a concentration-dependent manner. The amphipathic character of detergents is evident in their structures, which consist of a polar (or charged) head group and a hydrophobic tail.
e. **Peristalsis:** Peristalsis, involuntary movements of the longitudinal and circular muscles, primarily in the digestive tract but occasionally in other hollow tubes of the body that occur in progressive wavelike contractions. Peristaltic waves occur in the oesophagus, stomach, and intestines.
f. **Lipid peroxidation:** It is the oxidative degradation of lipids where free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage.

7.1 Digestion of lipids

a. There is considerable variation in the daily consumption of lipids which mostly depends on the economic status and dietary habits. The intake of lipids is much less (often < 60 /day) in poorer sections of the society, particularly in the less developed countries.
b. In the developed countries, an adult ingests about 60-150 g of lipids per day. Of this, more than 90% is fat (triacylglycerol/TAG). The rest of the dietary lipid is made up of phospholipids, cholesterol, cholesteryl esters and free fatty acids.
c. Lipids are insoluble or sparingly soluble in aqueous solution. The digestive enzymes, however, are present in aqueous medium. This poses certain problems for the digestion and absorption of lipids.
d. Fortunately, the digestive tract possesses specialized machinery to increase the surface area of lipids for digestion; solubilize the digested products for absorption.

7.1.1 Minor digestion of lipids in the stomach

a. The digestion of lipids is initiated in the stomach, catalysed by acid-stable lipase.
b. This enzyme (also called lingual lipase) is believed to originate from the glands at the back of tongue.
c. Stomach contains a separate gastric lipase which can degrade fat containing short chain fatty acids at neutral pH.
d. The digestion of lipids in the stomach of an adult is almost negligible, since lipids are not emulsified and made ready for lipase action.
e. Further, the low pH in the stomach is unfavourable for the action of gastric lipase.
f. In case of infants, the milk fat (with short chain fatty acids) can be hydrolysed by gastric lipase to some extent. This is because the stomach pH of infants is close to neutrality, ideal for gastric lipase action.

7.1.2 Emulsification of lipids in the small intestine

a. Emulsification is the phenomenon of dispersion of lipids into smaller droplets due to reduction in the surface tension. This is accompanied by increase in the surface area of lipid droplets.
b. Emulsification is essential for effective digestion of lipids, since the enzymes can act only on the surface of lipid droplets.
c. More correctly, lipases act at the interfacial area between the aqueous and lipid phase.
d. The process of emulsification occurs by three complementary mechanisms-

7.1.2.1 Detergent action of bile salts

a. The terms bile salts and bile acids are often used inter-changeably.
b. At physiological pH, the bile acids are mostly present as anions.
c. Bile salts are the biological detergents synthesized from cholesterol in the liver. They are secreted with bile into the duodenum.
d. Bile salts possess steroid nucleus, the side chain of which is attached to either glycine (glycocholic acid) or taurine (taurocholic acid).
e. Bile salts are the most effective biological emulsifying agents.
f. They interact with lipid particles and the aqueous duodenal contents and convert them into smaller particles (emulsified droplets).
g. Further, bile salts stabilize the smaller particles by preventing them from coalescing.

7.1.2.2 Surfactant action of degraded lipids

a. The initial digestive products of lipids (catalysed by lipase) namely free fatty acids, monoacyl glycerols promote emulsification.
b. These compounds along with phospholipids are known as surfactants. They are characterized by possessing polar and non-polar groups.
c. Surfactants get absorbed to the water-lipid interfaces and increase the interfacial area of lipid droplets. Thus, the initial action of lipase helps in further digestion of lipids.

Mechanical mixing due to peristalsis

a. Besides the action of bile salts and surfactants, the mechanical mixing due to peristalsis also helps in emulsification of lipids.
b. The smaller lipid emulsion droplets are good substrates for digestion.

7.1.3 Digestion of lipids by pancreatic enzymes:

a. The pancreatic enzymes are primarily responsible for degradation of dietary triacylglycerols, cholesteryl esters and phospholipids.

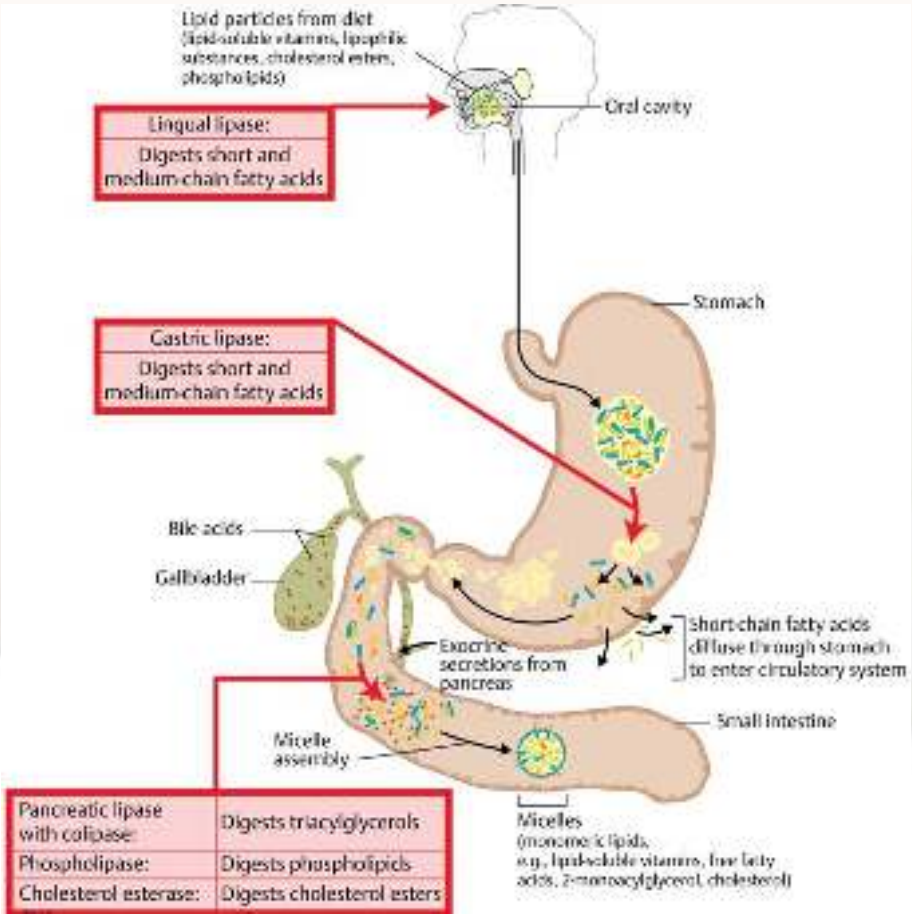
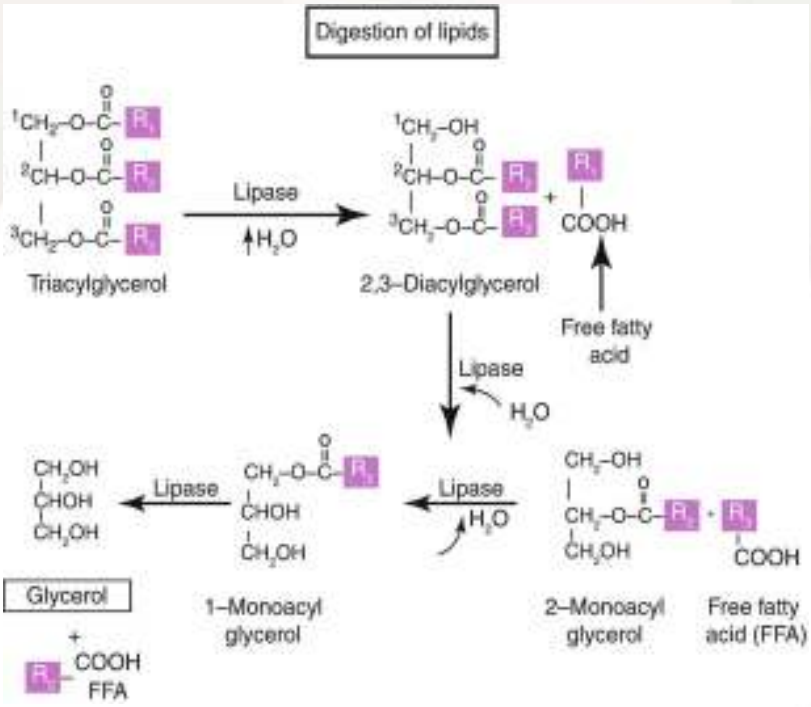


Fig. 1 Digestion of lipids

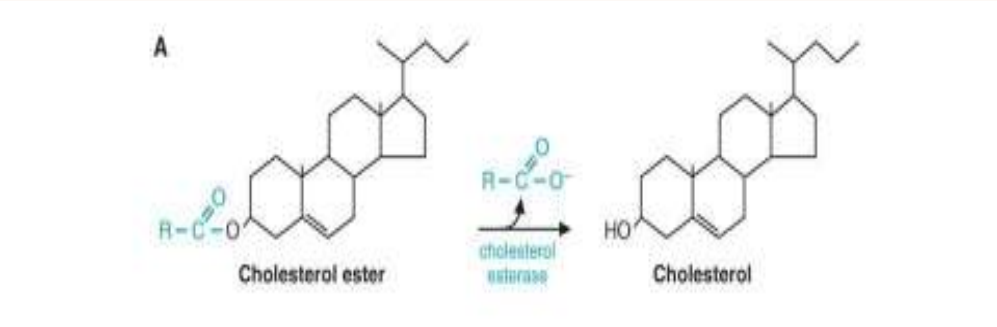
Degradation of triacylglycerols:

a. Pancreatic lipase is the major enzyme that digests dietary fats.
b. This enzyme preferentially cleaves fatty acids (particularly long chain, above 10 carbons) at position 1 and 3 of triacylglycerols.
c. The products are 2-monoacylglycerol and free fatty acids.
d. The activity of pancreatic lipase is inhibited by bile acids which are present alongwith the enzyme in the small intestine.
e. This problem is overcome by a small protein, colipase (mol. wt. 12,000).
f. It is also secreted by pancreas as procolipase and converted to active form by trypsin.
g. Colipase binds at the lipid-aqueous interface and helps to anchor and stabilize lipase.
h. Lipid esterase is a less specific enzyme present in pancreatic juice. It acts on monoacylglycerols, cholesteryl esters, vitamin esters etc. to liberate free fatty acids.
i. The presence of bile acids is essential for the activity of lipid esterase.



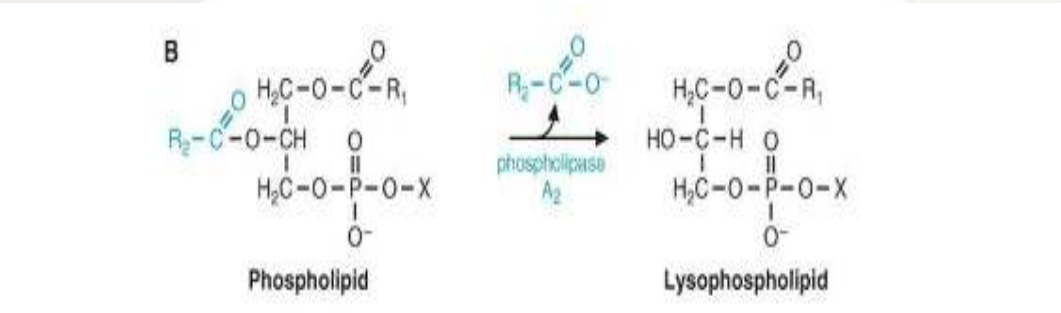
7.1.3.2 Degradation of cholesteryl esters

a. A specific enzyme namely pancreatic cholesterol esterase (cholesteryl ester hydrolase) cleaves cholesteryl esters to produce cholesterol and free fatty acids.



1.3.3 Degradation of phospholipids

a. Phospholipase are enzymes responsible for the hydrolysis of phospholipids.
b. Pancreatic juice is rich in phospholipase A2 which cleaves the fatty acid at the 2nd position of phospholipids.
c. The products are a free fatty acid and a lysophospholipid.
d. Phospholipase A2 is secreted as a zymogen which is activated in the intestineby the action of trypsin.



7.2 Absorption of lipids:

The former and present theories to explain the absorption of lipids were given by Verzar and Frazer, respectively.

7.2.1 Lipolytic theory put forth by Verzar: According to this, fats are completely hydrolysed to glycerol and free fatty acids. The latter are absorbed either as soaps or in association with bile salts.

7.2.1 Partition theory proposed by Frazer: This theory states that the digestion of triacylglycerols is partial and not complete. The partially digested triacylglycerols, in association with bile salts form emulsions. The lipids are taken up by the intestinal mucosal cells. As per this theory, re-synthesis of lipids is not necessary for their entry into the circulation.

7.2.1 Bergstrom theory: This is a more recent and comprehensive theory to explain lipid absorption. It has almost replaced the earlier theories, and is briefly described here under. The primary products obtained from the lipid digestion are 2-monoacylglycerol, free fatty acids and free cholesterol.

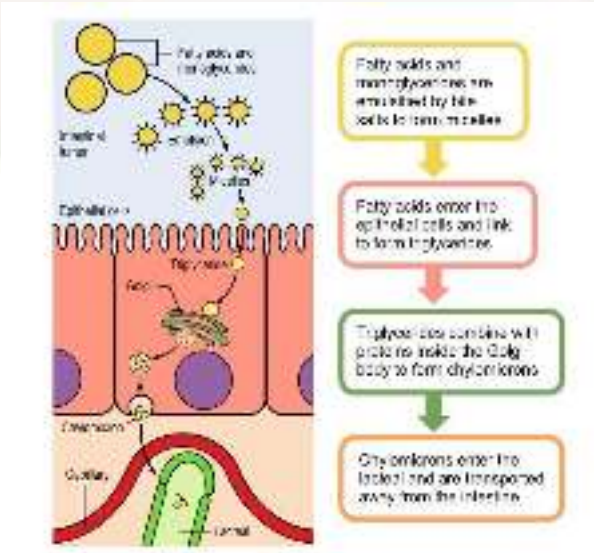


Fig. 2 Absorption of lipids

7.3 Lipid auto-oxidation

a. Lipid auto-oxidation or peroxidation is the oxidative degradation of lipids.
b. It is the process in which free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage.
c. This process proceeds by a free radical chain reaction mechanism.
d. It most often affects polyunsaturated fatty acids, because they contain multiple double bonds in between which lie methylene bridges (-CH2-) that possess especially reactive hydrogen atoms.
e. As with any radical reaction, the reaction consists of three major steps: initiation, propagation, and termination.
f. The chemical products of this oxidation are known as lipid peroxides or lipid oxidation products (LOPs).
g. Initiation is the step in which a fatty acid radical is produced. The most notable initiators in living cells are reactive oxygen species (ROS), such as OH· and HOO·, which combines with a hydrogen atom to make water and a fatty acid radical.
h. The fatty acid radical is not a very stable molecule, so it reacts readily with molecular oxygen, thereby creating a peroxyl-fatty acid radical. This radical is also an unstable species that reacts with another free fatty acid, producing a different fatty acid radical and a lipid peroxide, or a cyclic peroxide if it had reacted with itself. This cycle continues, as the new fatty acid radical reacts in the same way.
i. When a radical reacts with a non-radical, it always produces another radical, which is why the process is called a "chain reaction mechanism". The radical reaction stops when two radicals react and produce a non-radical species. This happens only when the concentration of radical species is high enough for there to be a high probability of collision of two radicals. Living organisms have different molecules that speed up termination by neutralizing free radicals and, therefore, protecting the cell membrane. One important antioxidant is vitamin E. Another important antioxidant is vitamin C. Other anti-oxidants made within the body include the enzymes superoxide dismutase, catalase, and peroxidase.

7.3.1 Final products of lipid auto-oxidation

a. The end products of lipid peroxidation are reactive aldehydes, such as malondialdehyde and 4-hydroxynonenal, the second one being known also as "second messenger of free radicals" and major bioactive marker of lipid peroxidation, due to its numerous biological activities resembling activities of reactive oxygen h species.

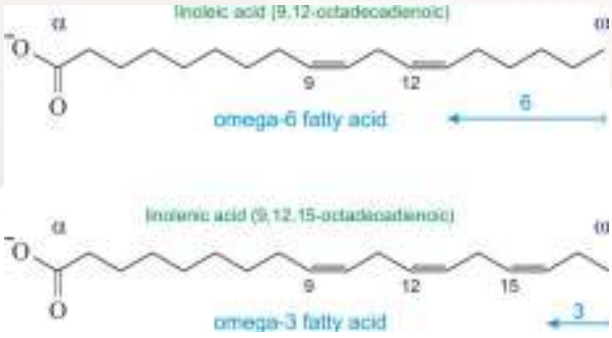
7.3.2 Inhibition of lipid peroxidation

a. Antioxidants such as vitamin C and vitamin E may inhibit lipid peroxidation. An alternative method employs deuteration of polyunsaturated fatty acids (PUFA) at the methylene bridges (bis-allylic sites) between double bonds, which leads to the inhibition of the chain reaction courtesy of a kinetic isotope effect. Such D-PUFAs, for example, 11,11-D2-ethyl linoleate, suppress lipid peroxidation even at relatively low levels of incorporation into membranes.

7.4 Omega-3 and Omega-6 fatty acids

a. The fat is termed “monounsaturated” if there is one double bond, and “polyunsaturated” if there are two or more double bonds.
b. The omega-3 and omega-6 both are fatty acids of polyunsaturated type. The difference is in where the first of the double bonds occurs.
c. In omega-3 fatty acids, the first double bond occurs on the third carbon atom, but in omega-6 fatty acids, the first double bond is on the sixth carbon atom, counting from the methyl end (denoted as omega)
d. Both omega-3 (ω-3) and omega-6 (ω-6) fatty acids are important components of cell membranes and are precursors to many other substances in the body such as those involved in regulating blood pressure and inflammatory responses.
e. There is increasing support for omega-3 fatty acids in protecting against fatal heart disease and it is known that they have anti-inflammatory effects, which may be important in this and other diseases.
f. There is also growing interest in the role of omega-3 fatty acids in the prevention of diabetes and certain types of cancer.

g. The human body is capable of producing all the fatty acids it needs, except for two: linoleic acid (LA) - an omega-6 fatty acid, and alpha-linolenic acid (ALA) - an omega-3 fatty acid.
h. These have to be consumed from the diet and are termed “essential fatty acids”. Both of these fatty acids are needed for growth and repair but can also be used to make other fatty acids.
i. For example, the omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), can be synthesised from ALA.
j. However, as conversion is limited, it is recommended that sources of these are also included in the diet.
k. ALA and LA are found in plant and seed oils.
l. Although the levels of LA are usually much higher than those of ALA, rapeseed oil and walnut oil are very good sources of the latter.
m. EPA and DHA are found in oily fish (e.g., salmon, mackerel, herring).
n. AA can be obtained from animal sources, such as meat and egg yolk.



Enzymes: nomenclature; classification; specificity; mechanism of enzyme action; kinetics and regulation of enzyme activity

Course Name	Fundamentals of Biochemistry
Lesson 8	Enzymes: nomenclature; classification; specificity; mechanism of enzyme action; kinetics and regulation of enzyme activity
Content Creator Name	Dr. Ajay Pal
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Lesson 8:

Enzymes: nomenclature; classification; specificity; mechanism of enzyme action; kinetics and regulation of enzyme activity

Objectives:

a. To study history, nomenclature and classification of enzymes
b. To study enzyme specificity and mechanism of enzyme action
c. To study enzyme kinetics and regulation of enzyme activity

Glossary:

a. **Thermolabile:** Thermolabile refers to a substance which is subject to destruction, decomposition, or change in response to heat.
b. **Stereoisomers:** They are the compounds which have the same molecular formula, but differ in their structural configuration.
c. **Stereospecificity:** Stereospecificity is the property of a reaction mechanism that leads to different stereoisomeric reaction products from different stereoisomeric reactants, or which operates on only one of the stereoisomers.
d. **Activation energy:** It is the minimum amount of energy needed to activate or energize molecules or atoms so that they can undergo a chemical reaction or transformation.
e. **Iso(en)zymes:** Isozymes are enzymes that differ in amino acid sequence but catalyze the same chemical reaction. These enzymes usually display different kinetic parameters or different regulatory properties.
f. **Allosteric site:** Site other than active site which serves a regulatory function when bound by ligands (a general term used to describe small molecules like substrate, product, inhibitor, or activator).

Designed and developed under the aegis of NAHEP Component-2 Project "Investments in ICAR Leadership in Agricultural Higher Education"
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8.1 Introduction to enzymes

a. Enzymes may be defined as biocatalysts synthesized by living cells that accelerate the rates of biological reactions.
b. They are protein in nature (exception - RNA acting as ribozyme), colloidal and thermolabile in character, and specific in their action.
c. Enzymes are the largest class of proteins and more than 3000 different enzymes are listed in Enzyme Nomenclature.
d. Each enzyme is very specific in its function and acts only in a particular metabolic reaction. Virtually every step in metabolism is catalyzed by an enzyme.
e. The catalytic power of enzymes far exceeds that of synthetic catalysts.
f. Enzymes can enhance reaction rates in cells as much as 1016 times the uncatalyzed rate.

8.2 History of enzymes

a. Wilhelm Kühne (1877–78), a German Physiologist coined/used the term enzyme.
b. Emil Fischer (1894) proposed ‘Lock and Key’ hypothesis.
c. Eduard Buchner (1897) found that sucrose was fermented by yeast extracts even when there were no living yeast cells in the mixture. He named the enzyme that brought about the fermentation of sucrose "zymase".
d. Michaelis and Menten (1913) proposed the ‘kinetic theory of enzyme action’.
e. James B. Sumner (1926) showed that enzyme urease was a pure protein and crystallized it.
f. John Howard Northrop and Wendell Meredith Stanley (1930) worked on digestive enzymes pepsin, trypsin and chymotrypsin and concluded that pure proteins can be enzymes.
g. Koshland (1958) proposed the ‘induced fit’ model.
h. Monod (1965) proposed ‘allosteric model’ of enzyme.
i. W. Arber, D. Nathans, and H.O. Smith (1978) discovered ‘Restriction endonucleases’.
j. R.B. Merrifield (1984) chemically synthesized Ribonuclease A.
k. S. Altman and T.R. Cech (1989) discovered catalysis by RNA molecules (Ribozymes).

l. P.D. Boyer, J.E. Walker, and J.C. Skou (1997) studied ATP synthase and Na/K-ATPase.
    A. Warshel and others (2013) studied 'Computational Enzymology', branch of science describing the functions of enzymes and other biological molecules.
m. F.H. Arnold (2018) a winner of the 2018 Nobel Prize in Chemistry, pioneered the use of evolution to create enzymes with entirely new functions.

8.3 Nomenclature and classification of enzyme:

a. In the early days, the enzymes were given names by their discoverers in an arbitrary manner.
b. For example, the names pepsin, trypsin and chymotrypsin convey no information about the function of the enzyme or the nature of the substrate on which they act.
c. Sometimes, the suffix-ase was added to the substrate for naming the enzymes e.g. lipase acts on lipids; nuclease on nucleic acids; lactase on lactose.
d. These are known as trivial names of the enzymes which, however, fail to give complete information of enzyme reaction (type of reaction, cofactor requirement etc.)
e. The International Union of Biochemistry (IUB) appointed an Enzyme Commission in 1961.
f. This committee made a thorough study of the existing enzymes and devised some basic principles for the classification and nomenclature of enzymes.
g. Since 1964, the IUB system of enzyme classification has been in force.
h. Enzymes are divided into six major classes (in that order).
i. Each class on its own represents the general type of reaction brought about by the enzymes of that class.

Six classes of enzymes:

a. Oxidoreductases: Enzymes involved in oxidation-reduction reactions.
b. Transferases: Enzymes that catalyse the transfer of functional groups.
c. Hydrolases: Enzymes that bring about hydrolysis of various compounds.
d. Lyases: Enzymes specialised in the addition or removal of water, ammonia, CO2 etc.
e. Isomerases: Enzymes involved in isomerisation reactions.
f. Ligases: Enzymes catalysing the synthetic reactions (Greek : ligate-to bind) where two molecules are joined together and ATP/GTP is used.

Table 1. Different classes of enzymes

*Enzyme class with examples**	*Reaction catalysed*
1. **Oxidoreductases** Alcohol dehydrogenase (alcohol : NAD^+ oxidoreductase E.C. 1.1.1.1.), cytochrome oxidase, L- and D-amino acid oxidases	Oxidation ⟶ Reduction $AH_2 + B \longrightarrow A + BH_2$
2. **Transferases** Hexokinase (ATP : D-hexose 6-phosphotransferase, E.C. 2.7.1.1.), transaminases, transmethylases, phosphorylase	Group transfer $A - X + B \longrightarrow A + B - X$
3. **Hydrolases** Lipase (triacylglycerol acyl hydrolase E.C. 3.1.1.3), choline esterase, acid and alkaline phosphatases, papain, urease	Hydrolysis $A - B + H_2O \longrightarrow AH + BOH$
4. **Lyases** Aldolase (ketose 1-phosphate aldehyde lyase, E.C. 4.1.2.7), fumarase, histidase	Addition ⟶ Elimination $A - B + \overset{\vee}{X} - \hat{Y} \longrightarrow AX - BY$
5. **Isomerases** Triose phosphate isomerase (D-glyceraldehyde 3-phosphate ketoisomerase, E.C. 5.3.1.1), retinol isomerase, phosphohexose isomerase	Interconversion of isomers $A \longrightarrow A'$
6. **Ligases** Glutamine synthetase (L-glutamate ammonia ligase, E.C. 6.3.1.2), acetyl CoA carboxylase, succinate thiokinase	Condensation (usually dependent on ATP) $A + B \xrightarrow[ATP \quad ADP + Pi]{} A - B$

*For one enzyme in each class, systematic name along with E.C. number is given in the brackets

g. In this classification each enzyme is given a code number consisting of a four-number system.
h. On this system the first number indicates the main class and the second and third show the subclass and sub-subclass, respectively, thus defining the type of reaction.

i. The fourth number is the actual number of that enzyme within its sub-subclass.
j. For example, alcohol dehydrogenase is given the code “EC 1.1.1.1.”
k. The first number indicates that it belongs to oxidoreductase class (EC 1.x.x.x).
l. Within this class, enzymes acting on CH-OH group of donors bear the same subclass number (EC 1.1.x.x).
m. Within this subclass, enzymes that use NAD+ or NADP+ as electron acceptor are given the number EC 1.1.1.x.
n. Since alcohol dehydrogenase is the first enzyme in this category, it gets its fourth number (EC 1.1.1.1).

8.4 Active Site:

a. Each enzyme is a structural microcosm capable of catalysis, specificity, and regulation.
b. Chemical reactions performed by an enzyme occur at specific location on the enzyme protein called the active site (or catalytic center).
c. Enzyme active sites are small relative to its total molecular volume.
d. Architecture of active site is responsible for imparting specificity and catalytic potency to each enzyme.
e. Active sites are usually clefts and crevices in the protein; they create a unique three-dimensional microenvironment by (a) aligning an array of amino acid side chains and cofactors and (b) excluding bulk solvent, i.e. water.
f. Substrates are assembled at the enzyme active site.
g. Once the reaction is complete, the products leave the active site.
h. For a reversible reaction however, products become substrates and vice versa.
i. Enzyme active sites may also accommodate inhibitors – molecules structurally resembling substrates or products.

8.5 Allosteric Site

a. Apart from the active site, some enzymes may display an additional site – the allosteric site (allos in Greek means another).
b. Such sites serve a regulatory function when bound by ligands (a general term used to describe small molecules like substrate, product, inhibitor, or activator).
c. Allosteric site and active site could exist on the same subunit (e.g., phosphofructokinase) or on distinct subunits (e.g., aspartate transcarbamylase) of an oligomeric protein.
d. Ligand binding to an allosteric site may influence the active site geometry and function. Communication between sites can be achieved by conformational coupling across the protein matrix.

8.6 Cofactors

a. An additional non-protein molecule that is needed by some enzymes to help the reaction.
b. Tightly bound cofactors are called prosthetic groups
c. Cofactors that are bound and released easily are called coenzymes.
d. Many vitamins are coenzymes.

Table 2: Some coenzymes that serve as transient carriers of specific atoms or functional groups

Coenzyme	Example of chemical group transferred	Dietary precursor
Biocytin	CO2	Biotin
Coenzyme A	Acyl groups	Pantothenic acid
FAD	electrons	Riboflavin
TPP	Aldehydes	Thiamine

8.7 Enzyme specificity:

a. Enzymes are highly specific in their action when compared with the chemical catalysts.
b. The occurrence of thousands of enzymes in the biological system might be due to the specific nature of enzymes.
c. Specificity is a characteristic property of the active site.
d. Three types of enzyme specificity are well-recognised: stereospecificity, reaction specificity and substrate specificity.

8.6.1 Stereospecificity or optical specificity:

a. The enzymes act only on one isomer and, therefore, exhibit stereospecificity. e.g. L-amino acid oxidase and D-amino acid oxidase act on L- and D-amino acids, respectively. Hexokinase acts on D-hexoses; Glucokinase on D-glucose; Amylase acts on α-glycosidic linkages; Cellulase cleaves p-glycosidic bonds.
b. Stereospecificity explained by considering three distinct regions of substrate molecule specifically binding with three complementary regions on the surface of the enzyme.
c. The class of enzymes belonging to isomerases do not exhibit stereospecificity, since they are specialized in the interconversion of isomers.

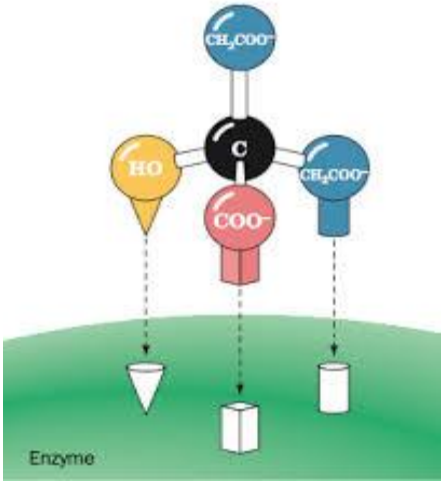


Fig. 1: Stereospecificity of an enzyme

8.7.2 Reaction specificity:

a. The same substrate can undergo different types of reactions, each catalysed by a separate enzyme and this is referred to as reaction specificity.
b. An amino acid can undergo transamination, oxidative deamination, decarboxylation, racemization etc.
c. The enzymes however, are different for each of these reactions.

8.7.3Substrate specificity:

1. The substrate specificity varies from enzyme to enzyme.
2. It maybe absolute, relative or broad.
   a. Absolute substrate specificity: Certain enzymes act only on one substrate e.g. glucokinase acts on glucose to give glucose-6-phosphate, urease cleaves urea to ammonia and carbon dioxide.
   b. Relative substrate specificity: Some enzymes act on structurally related substances. This, in turn, may be dependent on the specific group or a bond present. The action of trypsin is a good example for group specificity. Trypsin hydrolyses peptide linkage involving arginine or lysine. Chymotrypsin cleaves peptide bonds attached to aromatic amino acids (phenylalanine, tyrosine andtryptophan).
   c. Broad specificity: Some enzymes act on closely related substrates which is commonly known as broad substrate specificity, e.g. hexokinase acts on glucose, fructose/mannose and glucosamine and not on galactose.

8.8 Mechanism of enzyme action:

a. Catalysis is the prime function of enzymes. The nature of catalysis taking place in the biological system is similar to that of non-biological catalysis.
b. For any chemical reaction to occur, the reactants have to be in an activated state or transition state.

c. Enzymes lower activation energy: The energy required by the reactants to undergo the reaction is known as activation energy.
d. The reactants when heated attain the activation energy. The catalyst (or the enzyme in the biological system) reduces the activation energy and this causes the reaction to proceed at a lower temperature.
e. Enzymes do not alter the equilibrium constant; they only enhance the velocity of the reaction. The role of catalyst or enzyme is comparable with a tunnel made in a mountain to reduce the barrier.
f. The enzyme lowers energy barrier of reactants, thereby making the reaction go faster.
g. The enzymes reduce the activation energy of the reactants in such a way that all the biological systems occur at body temperature (below 40ºC).

Enzyme-substrate complex formation:

a. The prime requisite for enzyme catalysis is that the substrate (S) must combine with the enzyme (E) at the active site to form enzyme-substrate complex (ES) which ultimately results in the product formation (P).

$$E + S \rightarrow E\text{-}S \rightarrow E + P$$

b. A few theories have been put forth to explain mechanism of enzyme-substrate complex formation.

Lock and key model or Fischer's template theory:

a. This theory was proposed by a German Biochemist Emil Fischer. This is in fact the very first model proposed to explain an enzyme catalysed reaction.
b. According to this model, the structure or conformation of the enzyme is rigid. The substrate fits to the binding site (now active site) just as a key fits into the proper lock or a hand into the proper glove. Thus, the active site of an enzyme is a rigid and pre-shaped template where only a specific substrate can bind.

c. This model does not give any scope for the flexible nature of enzymes hence the model totally fails to explain many facts of enzymatic reactions, the most important being the effect of allosteric modulators.

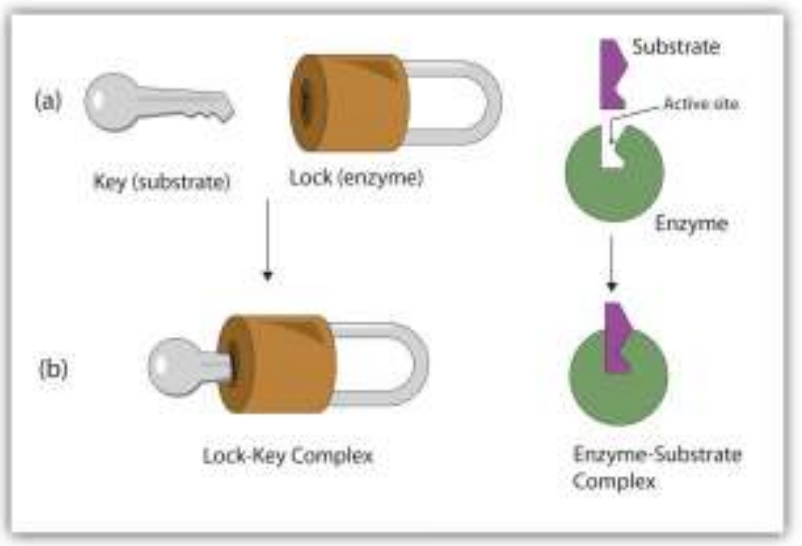


Fig. 2: Lock and Key model

Induced fit theory or Koshland's model:

a. Koshland, in 1958, proposed a more acceptable and realistic model for enzyme-substrate complex formation. As per this model, the active site is not rigid and pre-shaped. The essential features of the substrate binding site are present at the nascent active site.
b. The interaction of the substrate with the enzyme induces a fit or a conformation change in the enzyme, resulting in the formation of a strong substrate binding site.
c. Further, due to induced fit, the appropriate amino acids of the enzyme are repositioned to form the active site and bring about the catalysis.
d. Induced fit model has sufficient experimental evidence from the X-ray diffraction studies.
e. Koshland's model also explains the action of allosteric modulators and competitive inhibition on enzymes.

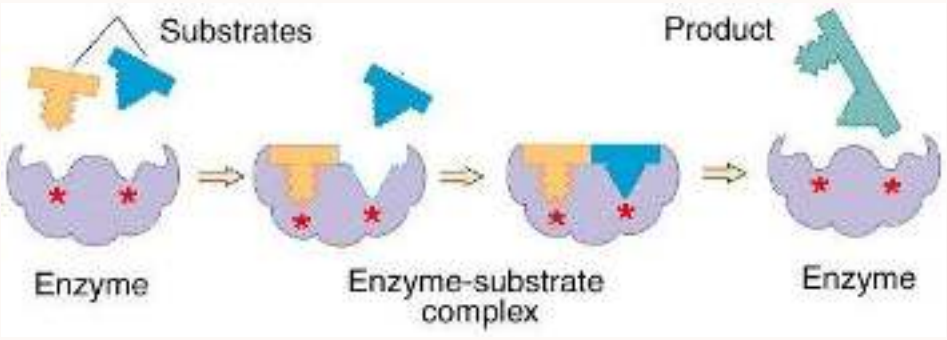


Fig. 3: Induced fit theory

Substrate strain theory:

a. In this model, the substrate is strained due to the induced conformation change in the enzyme.
b. It is also possible that when a substrate binds to the preformed active site, the enzyme induces a strain to the substrate. The strained substrate leads to the formation of product.
c. In fact, a combination of the induced fit model with the substrate strain is considered to be operative in the enzymatic action.

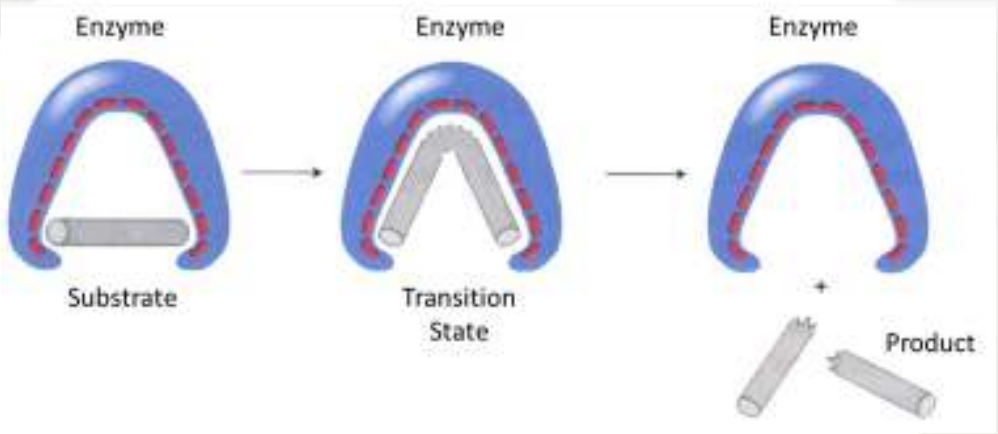


Fig. 4: Substrate strain theory

8.9 Enzyme Unit

a. To facilitate comparison of enzyme activities from various samples (and from values reported in the literature), an international unit is recommended.
b. The standard enzyme unit (U) is the amount that catalyzes the formation of one micromole of product per minute, under defined assay conditions.
c. This unit has the dimensions of µmol x min-1.
d. The more the number of units in a sample means the more enzyme catalyst present in that sample.
e. The enzyme activity unit may also be expressed in terms of µmol substrate consumed per min.
f. The International Union of Biochemistry has recommended the use of katal – according to SI units.
g. A katal corresponds to the amount of enzyme that produces one mole of product per second. It is obvious that katal is a very large unit and hence is not in common use.

$$
\begin{aligned}
1\ \text{katal} &= 1\ \text{mol} \times \text{sec}^{-1} \\
&= 10^6\ \mu\text{mol} \times 60\ \text{min}^{-1} \\
&= 6 \times 10^7\ \mu\text{mol} \times \text{min}^{-1} \\
&= 6 \times 10^7\ \text{U}
\end{aligned}
$$

Similarly, $1\ \text{U} = 16.67\ \text{nkatal}$

8.10 Specific Activity

a. A way to express the amount and concentration of enzyme is through U and U x ml-1, respectively. These units reflect on the enzyme content of the given sample but do not tell us anything about the purity of the enzyme.
b. The units of enzyme in a sample can be same regardless of the quantity and diversity of other proteins present. We could however present the quantity (U) of enzyme present in a known amount of protein.

c. Specific activity is thus defined as the number of units per mg of protein.
d. It is an index of the purity of the enzyme sample – the higher the proportion of enzyme protein in a given protein sample, the greater will be its specific activity.
e. The purer the enzyme sample, the higher is its specific activity.
f. If this is extended logically to the stage of highest enzyme purity, then that sample must have every protein molecule representing only that enzyme. Beyond this point (of limit of highest U x mg-1 protein), it is not possible to enhance the specific activity by any method of purification.
g. Conversely, achieving highest constant specific activity is considered a necessary criterion of enzyme purity.

8.11 Turnover Number

a. With a pure enzyme (possessing highest limiting specific activity), the amount of enzyme protein (say in mg) can also be expressed as number of moles of that enzyme (say in µmol).
b. However, to do this we need to know one additional bit of information – the molecular mass of the enzyme. When this is available, we can present the specific activity of the pure enzyme.
c. This quantity – called the turnover number – has the units of dimension "time-1" (more commonly, sec-1). It indicates the number of times a single enzyme molecule converts substrate into product in 1 min. In this definition it is assumed that substrate is saturating and that the enzyme has one active site per molecule.

Table 3. Range of enzyme turnover numbers

Enzyme (substrate)	Turnover number (s^{-1})
Catalase (for H_2O_2)	1.0×10^7
Carbonic anhydrase (for CO_2)	0.6×10^6
Ketosteroid isomerase	0.7×10^5
Urease	1.0×10^4
Triosephosphate isomerase	4.3×10^3
DNA polymerase I (*E. coli*)	6.0×10^2
Adenosine deaminase	3.7×10^2
Chorismate mutase	5.0×10^1

8.12 Regulation of enzyme activity: In biological system, regulation of enzyme activities occurs at different stages in one or more of the following ways to achieve cellular economy.

8.12.1 Allosteric regulation:

a. Some of the enzymes possess additional sites known as allosteric sites (Greek: allo-other), besides the active site. Such enzymes are known as allosteric enzymes. The allosteric sites are unique places on the enzyme molecule. Certain substances referred to as allosteric modulators (effectors or modifiers) bind at the allosteric site and regulate the enzyme activity. The enzyme activity is increased when a positive (+) allosteric effector binds at the allosteric site known as activator site. On the other hand, a negative (-) allosteric effector binds at the allosteric site called inhibitor site and inhibits the enzyme activity.

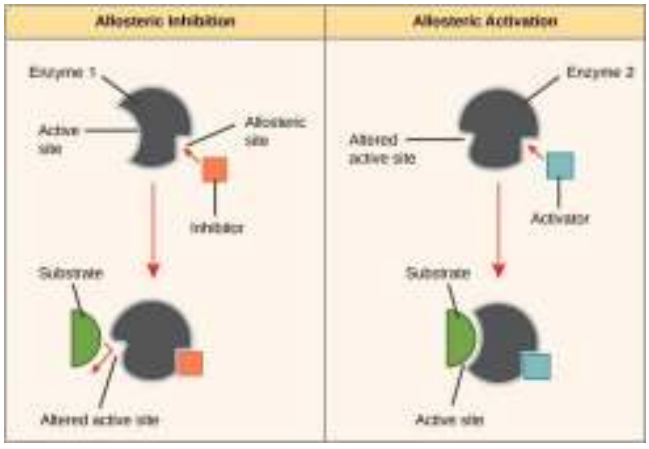


Fig. 5: Allosteric regulation

8.12.2 Activation of latent enzymes:

a. Latent enzymes, as such, are inactive. Some enzymes are synthesized as Proenzymes or zymogens which undergo irreversible covalent activation by the breakdown of one or more peptide bonds. For instance, proenzymes namely chymotrypsinogen, pepsinogen and plasminogen are respectively, converted to the active enzymes chymotrypsin, pepsin and plasmin. Certain enzymes exist in the active and inactive forms which are inter-convertible depending on the needs of the body. The inter-conversion is brought about by the reversible covalent modifications, namely phosphorylation and dephosphorylation, and oxidation and reduction of disulfide bonds.

8.12.3 Compartmentation

a. There are certain substances in the body (e.g., fatty acids, glycogen) which are synthesized and also degraded. There is no point for simultaneous occurrence of both the pathways. Generally, the synthetic (anabolic) and breakdown (catabolic) pathways are operative in different cellular organelles to achieve maximum economy. For instance, enzymes for fatty acid synthesis are found in the cytosol whereas enzymes for fatty acid oxidation are present in the mitochondria. Depending on the needs of the body – through the mediation of hormonal and other controls - fatty acids are either synthesized or oxidized.

Table 4. Compartmentation

Organelle	*Enzyme/metabolic pathway*
Cytoplasm	Aminotransferases; peptidases; glycolysis; hexose monophosphate shunt; fatty acid synthesis; purine and pyrimidine catabolism.
Mitochondria	Fatty acid oxidation; amino acid oxidation; Krebs cycle; urea synthesis; electron transport chain and oxidative phosphorylation.
Nucleus	Biosynthesis of DNA and RNA.
Endoplasmic reticulum (microsomes)	Protein biosynthesis; triacylglycerol and phospholipid synthesis; steroid synthesis and reduction; cytochrome P_{450}; esterase.
Lysosomes	Lysozyme; phosphatases; phospholipases; hydrolases; proteases; lipases; nucleases.
Golgi apparatus	Glucose 6-phosphatase; 5′-nucleotidase; glucosyl- and galactosyl-transferases.
Peroxisomes	Catalase; urate oxidase; D-amino acid oxidase; long chain fatty acid oxidation.

8.12.4 Control of enzyme synthesis:

a. Most of the enzymes, particularly the rate limiting ones, are present in very low concentration. Nevertheless, the amount of the enzyme directly controls the velocity of the reaction, catalysed by that enzyme. Many rate limiting enzymes have short half-lives. This helps in the efficient regulation of the enzyme levels.

8.12.5 Enzyme degradation:

a. Enzymes are not immortal, since it will create a series of problems. There is a lot of variability in the half-lives of individual enzymes. For some, it is in days while for others in hours or in minutes, e.g. LDH4- 5 to 6 days; LDH1 - 8 to12 hours; amylase -3 to 5 hours. In general, the key and regulatory enzymes are most rapidly degraded. If not needed, they immediately disappear and, as and when required, they are quickly synthesized. Though not always true, an enzyme with long half-life is usually sluggish in its catalytic activity.

8.12.6 Isoenzymes:

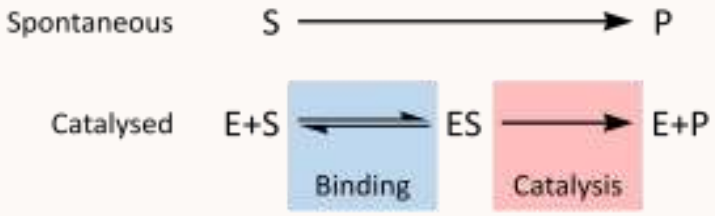
a. Multiple forms of the same enzyme will also help in the regulation of enzyme activity, many of the isoenzymes are tissue-specific. Although isoenzymes of a given enzyme catalyse the same reaction, they differ in Km, Vmax or both. e.g. isoenzymes of LDH and CPK.

8.13 Enzyme Kinetics

a. Enzyme kinetics is the investigation of how enzymes bind substrates and turn them into products. The rate data used in kinetic analyses are commonly obtained from enzyme assays. Enzyme kinetics is arguably the most time and cost-effective way to study enzymes.
b. It is the primary way to study enzyme catalysis, because no other approach allows one to test whether a chemically or spectrophotometrically detected intermediate is formed and turned over on the catalytic time scale.

Designed and developed under the aegis of NAHEP Component-2 Project "Investments in ICAR Leadership in Agricultural Higher Education"
Division of Computer Applications, ICAR-Indian Agricultural Statistics Research Institute

I. Kinetic studies provide the information on the mechanism, mode of regulation, kinetic parameters that are essential for an understanding of enzyme specificity and physiological function.
II. After the initiation of an enzyme-catalyzed reaction, the concentrations of the various components change with time.



8.13.1 Michaelis–Menten equation

a. A rate equation (or the rate law) gives the experimentally observed dependence of rate on the concentration of reactants.
b. Michaelis and Menten provided the conceptual breakthrough and derived the now famous rate equation for an enzyme-catalyzed reaction.
c. They assumed that the formation of ES complex from E and S is at equilibrium.
d. Initially, the reaction is first order reaction i.e. rate of reaction is directly proportional to substrate concentration.
e. At the end, the reaction follows zero order i.e. rate of reaction is independent of substrate concentration.

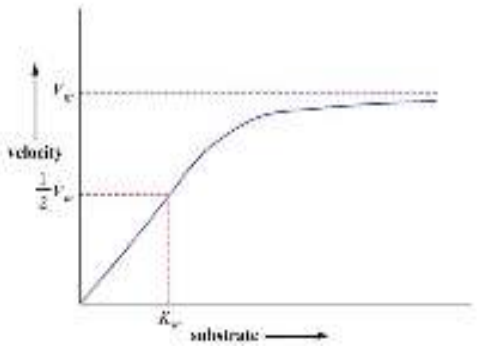


Fig. 6: Velocity – Substrate concentration graph

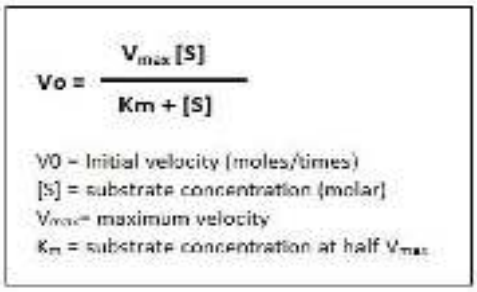


Fig.7: Michaelis–Menten equation

f. Both Vmax and Km (and therefore Vmax/Km) are constants for a given enzyme.
g. Units for Km and Vmax will be those in which [S] and v, respectively, are measured.
h. Km describes the likelihood that substrate will dissociate from enzyme. The Michaelis constant Km describes the substrate concentration that is needed to achieve a reaction rate that is exactly half of the Vmax.
i. Km value is used as a measure of an enzyme's affinity for its substrate. Lower the Km value the higher the enzyme's affinity for the substrate and vice versa.
j. Km value also provides an idea of the strength of binding of the substrate to the enzyme molecule.

8.13.2 Factors affecting enzyme activity

Enzyme activity can be affected by a variety of factors, such as temperature, pH and concentration of enzyme and substrate.

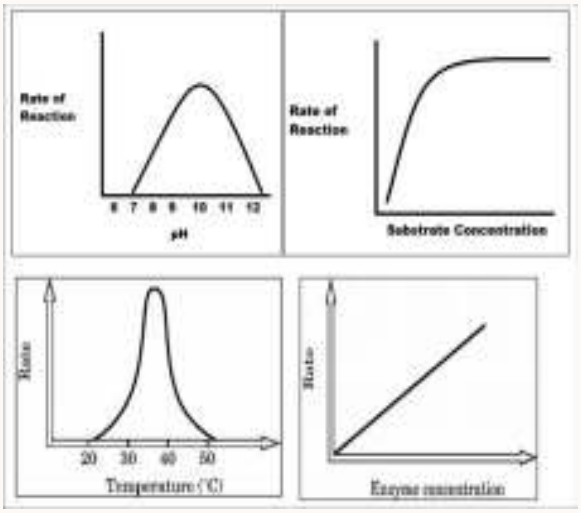


Fig.8: Factors affecting enzyme activity

8.13.2.1 Concentration of Enzyme

a. As the concentration of the enzyme is increased, the velocity of the reaction proportionately increases.
b. This property is used for determining the activities of serum enzymes during the diagnosis of diseases.

8.13.2.2 Concentration of Substrate

a. In the presence of a given amount of enzyme, the rate of enzymatic reaction increases as the substrate concentration increases until a limiting rate is reached, after which further increase in the substrate concentration produces no significant change in the reaction rate.
b. At this point, so much substrate is present that essentially all of the enzyme active sites have substrate bound to them.
c. In other words, the enzyme molecules are saturated with substrate. The excess substrate molecules cannot react until the substrate already bound to the enzymes has reacted and been released (or been released without reacting).

8.13.2.3 Effect of Temperature

a. The protein nature of the enzymes makes them extremely sensitive to thermal changes.
b. Enzyme activity occurs within a narrow range of temperatures compared to ordinary chemical reactions.
c. Each enzyme has a certain temperature at which it is more active. This point is called the optimal temperature, which ranges between 37 to 40C°.
d. The enzyme activity gradually lowers as the temperature rises more than the optimal temperature until it reaches a certain temperature at which the enzyme activity stops completely due to the change of its natural composition.
e. On the other hand, if the temperature lowers below the optimal temperature, the enzyme activity lowers until the enzyme reaches a minimum temperature at which the enzyme activity is the least.
f. The enzyme activity stops completely at 0°C, but if the temperature rises again, then the enzyme gets reactivated once more.

8.13.2.4 Effect of pH

a. Enzymes are protein substances that contain acidic carboxylic groups (COOH) and basic amino groups (NH2). So, the enzymes are affected by changing the pH value.
b. Each enzyme has a pH value that it works at with maximum efficiency called the optimal pH.
c. If the pH is lower or higher than the optimal pH, the enzyme activity decreases until it stops working.
d. For example, pepsin works at a low pH, while trypsin works at a high pH. Most enzymes work at neutral pH 7.4.

8.13.2.5 Effect of Activators

a. Some of the enzymes require certain inorganic metallic cations like Mg2+, Mn2+, Zn2+, Ca2+, Co2+, Cu2+, Na+, K+ etc., for their optimum activity.
b. Rarely, anions are also needed for enzyme activity, e.g. a chloride ion (Cl–) for amylase.

8.13.2.6 Inhibitors

a. Any molecule that acts directly on an enzyme to lower its catalytic rate. They are usually specific and they work at low concentrations. They block the enzyme but they do not usually destroy it.
b. These can be cellular metabolites, or foreign substances such as drugs or toxins that have either a therapeutic or toxic (can be lethal) effect.

There are two major types of inhibition:

(1) Irreversible inhibition

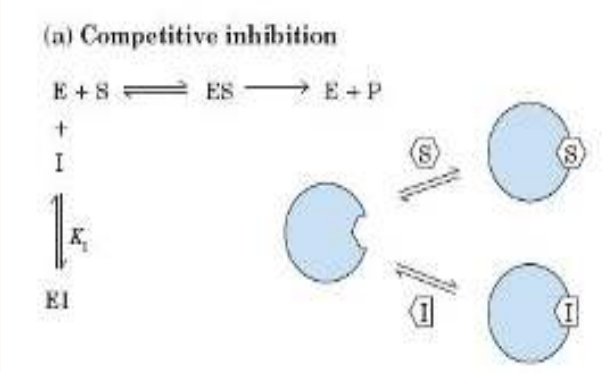
(2) Reversible inhibition

a) Competitive

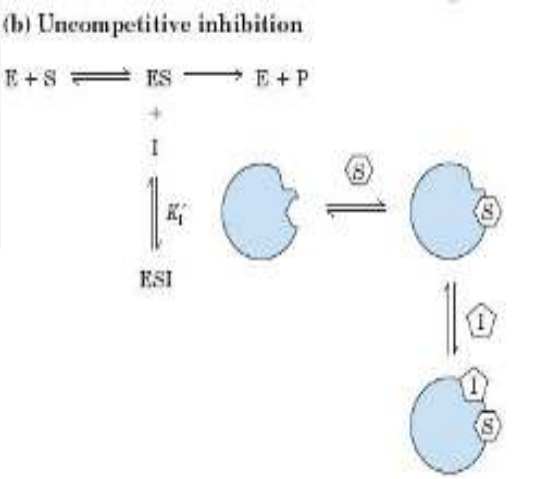
b) Un-competitive

c) Mixed

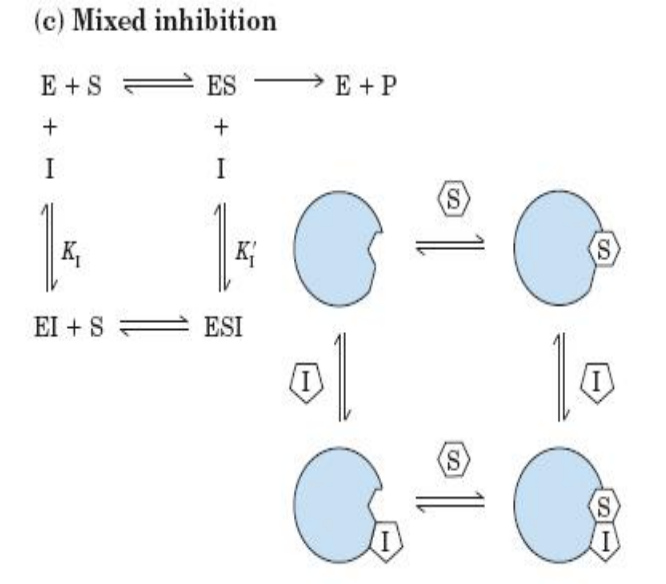
c. Irreversible inhibitors combine with the functional groups of the amino acids in the active site, irreversibly. Examples include nerve gases and pesticides containing organophosphorus, combine with serine residues in the enzyme acetylcholine esterase.
d. A competitive inhibitor competes with the substrate for the active site of an enzyme. While the inhibitor (I) occupies the active site, it prevents binding of the substrate to the enzyme. Many competitive inhibitors are compounds that resemble the substrate and combine with the enzyme to form an EI complex, but without leading to catalysis.



e. In competitive inhibition, inhibition can be overcome by high [S]. Vmax does not change, but Km increases (Km, app = aKm).
f. An uncompetitive inhibitor binds at a site distinct from the substrate active site and, unlike a competitive inhibitor, binds only to the ES complex.



g. Since I does not share the binding site with S, uncompetitive inhibition cannot be overcome by high [S]. So, both Vmax, app and Km, app – decrease.
h. A mixed inhibitor also binds at a site distinct from the substrate active site, but it binds to either E or ES.



i. Inhibitor binds at a site other than the active site (E or ES) and causes changes in the overall 3-D shape of the enzyme that leads to a decrease in activity.
j. Mixed inhibition cannot be overcome by high [S]. Here Vmax,app – decrease and Km,app remains unchanged.

Course Name	Fundamentals of Biochemistry
Lesson 9	Steroid and peptide hormones- chemistry and function. Structure and functions of fat- and water-soluble vitamins. Vitamins – classification- functions. Minerals – classification – functions
Content Creator Name	Dr. Ajay Pal
University/College Name	Chaudhary Charan Singh Haryana Agricultural University, Hisar
Course Reviewer Name	Sucheta Sharma
University/College Name	Punjab Agricultural University, Ludhiana

Lesson 9:

Steroid and peptide hormones- chemistry and function. Structure and functions of fat- and water-soluble vitamins. Vitamins – classification- functions. Minerals – classification – functions.

Objectives

a. To study the chemistry and functions of steroid and peptide hormones
b. To study structure and functions of vitamins
c. To study classification and functions of minerals

Glossary

a. **Endocrine system:** The endocrine system is a chemical messenger system comprising feedback loops of hormones released by internal glands of an organism directly into the circulatory system, regulating distant target organs.
b. **Steroids:** A steroid is a biologically active organic compound with four rings arranged in a specific molecular configuration.
c. Osteoporosis: Osteoporosis means “porous bone.” It is a bone disease that occurs when the body loses too much bone, makes too little bone, or both.
d. **Tonicity:** Tonicity is a measure of the effective osmotic pressure gradient; the water potential of two solutions separated by a semipermeable cell membrane.
e. Coenzyme: A coenzyme is an organic non-protein compound that binds with an enzyme to catalyze a reaction.
f. **Megaloblasticanemia:** It's known as vitamin B-12 or folate deficiency anemia, or macrocytic anemia, as well. It is caused when red blood cells are not produced properly. It is characterized by red blood cells that are larger than normal.
g. **Antioxidant:** Antioxidants are substances that can prevent or slow down the cell damage caused by free radicals, unstable molecules that the body produces as a reaction to environmental and other pressures. They are sometimes called “free-radical scavengers.

Hormones

9.1 Introduction

a. Hormones are the organic substances, produced in small amounts by specific tissues (endocrine glands), secreted into the blood stream to control the metabolic and biological activities in the target cells.
b. Hormones may be regarded as the chemical messengers involved in the transmission of information from one tissue to another and from cell to cell.
c. Hormones can be classified as either amino acid–based hormones (amine, peptide, or protein) or steroid hormones.
d. The former are water-soluble and act on the surface of target cells via second messengers; the latter, being lipid-soluble, move through the plasma membranes of target cells (both cytoplasmic and nuclear) to act within their nuclei.

9.1.1 Steroid hormones

a. Steroid hormone, any of a group of hormones that belong to the class of chemical compounds known as steroids; they are secreted by three “steroid glands”—the adrenal cortex, testes, and ovaries—and during pregnancy by the placenta.
b. All steroid hormones are derived from cholesterol.
c. They are transported through the blood-stream to the cells of various target organs where they carry out the regulation of a wide range of physiological functions.
d. Steroid hormones can be grouped into two classes: corticosteroids (typically made in the adrenal cortex) and sex steroids (typically made in the gonads or placenta).
e. Within those two classes are five types of hormones, according to the receptors to which they bind: glucocorticoids and mineralocorticoids (both corticosteroids) and androgens, estrogens, and progestogens (sex steroids).
f. Adreno-corticosteroids are classified into three groups according to their dominant biological action. However, there is some overlap in their functions.

9.1.1.1 Glucocorticoids: These are 21-carbon steroids, produced mostly by zona fasciculata.

a. Cortisol (also known as hydrocortisone) is the most important glucocorticoid in humans.
b. Corticosterone is predominantly found in rats.

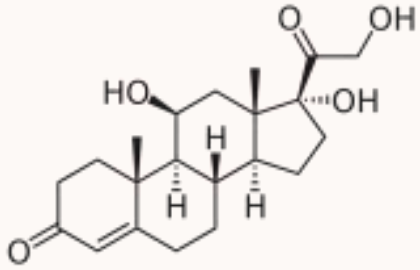


Fig. 1. Structure of cortisol

Biochemical functions of glucocorticoid hormones

a. They affect glucose, amino acid and fat metabolism in a manner that is opposite to the action of insulin.
b. Glucocorticoids promote the synthesis of glucose (gluconeogenesis).
c. Glucocorticoids increase the circulating free fatty acids. This is caused by increased breakdown of storage triacylglycerol (lipolysis) in adipose tissue and reduced utilization of plasma free fatty acids for the synthesis of triacylglycerols.
d. Glucocorticoids exhibit both catabolic and anabolic effects on protein and nucleic acid metabolism. They promote both transcription and translation processes in the liver.
e. Glucocorticoids (particularly at high concentration) cause catabolic effects in extra hepatic tissues suchas muscle, adipose tissue, bone etc. This results in enhanced degradation of proteins.
f. The influence of glucocorticoids on water metabolism is mediated through antidiuretic hormone (ADH). Deficiency of glucocorticoids causes increased production of ADH.
g. They stimulate the fight and flight response (to face sudden emergencies) of catecholamines.
h. They increase the production of gastric HCl and pepsinogen.
i. They inhibit the bone formation hence the subjects are at a risk for osteoporosis.

9.1.1.2 Mineralocorticoids: These are also 21-carbon containing steroids produced by zona glomerulosa.

a. They regulate water and electrolyte balance. Aldosterone is the most prominent mineralocorticoid.

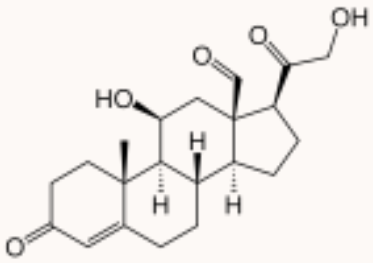


Fig. 2. Structure of aldosterone

Biochemical functions of mineralocorticoid hormones

a. Aldosterone promotes Na+ reabsorption at the distal convoluted tubules of kidney. Na+ retention is accompanied by corresponding excretion of K+, H+ and NH4+ ions.
b. The production of aldosterone is regulated by different mechanisms. These include renin-angiotensin, potassium, sodium and adrenocorticotropic hormone.
c. The steroid hormones are metabolized in the liver and excreted in urine as conjugates of glucuronides or sulfates.

9.1.1.3 Androgens and estrogens: The innermost adrenal cortex zona reticularis produces small quantities of androgens (19-carbon) and estrogens (18-carbon).

a. These hormones affecting sexual development and functions are mostly produced by gonads.
b. Dehydroepiandrosterone, a precursor for androgens is synthesized in adrenal cortex.
c. The male sex hormones or androgens are produced by the Leydig cells of the testes and to a minor extent by the adrenal glands in both the sexes.
d. Ovaries also produce small amounts of androgens.

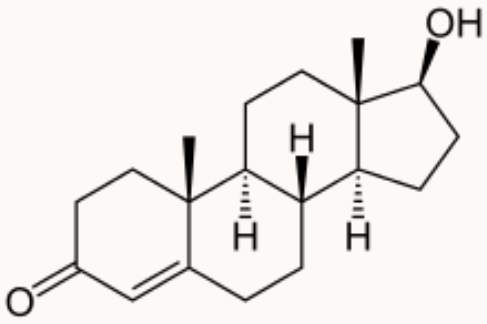


Fig. 3. Structure of testosterone (the major androgen)

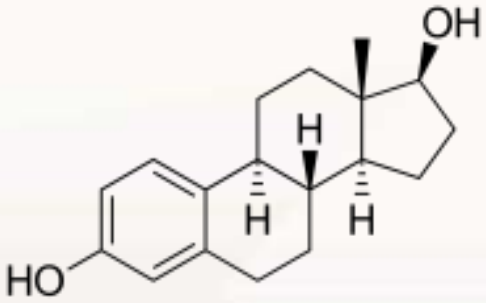


Fig. 4. Structure of estradiol (the major estrogen)

Biochemical functions of androgens

a. The androgens, primarily dihydrotestosterone and testosterone influence growth, development and maintenance of male reproductive organs.
b. Androgens are anabolic in nature.
c. Androgens promote transcription and translation processes.
d. Androgens cause positive nitrogen balance and increase the muscle mass.
e. Androgens increase glycolysis, fatty acid synthesis and citric acid cycle.
f. Androgens promote mineral deposition and bone growth before the closure of epiphyseal cartilage.

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Division of Computer Applications, ICAR-Indian Agricultural Statistics Research Institute

g. The estrogens are primarily concerned with growth, development and maintenance of female reproductive organs, maintenance of menstrual cycles and development of female sexual characteristics.
h. Estrogens increase lipogenesis in adipose tissue and, for this reason, women have relatively more fat (about 5%) than men.
i. Transhydrogenase is an enzyme activated by estrogen. It is capable of transferring reducing equivalents from NADPH to NAD+.
j. Progesterone is essentially required for the implantation of fertilized ovum and maintenance of pregnancy.
k. It promotes the growth of glandular tissue in uterus and mammary gland.
l. Progesterone increases the body temperature by 0.5-1.5°C. The exact mechanism of this thermogenic effect is not clearly known. The measurement of temperature was used as anindicator for ovulation.

9.1.2 Peptide hormones

a. Peptide or protein hormones are hormones whose molecules peptide or proteins, respectively. The latter have longer amino acid chain lengths than the former.
b. These hormones have an effect on the endocrine system of animals, including humans.
c. Peptide and protein hormones are synthesized in cells from amino acids according to mRNA transcripts, which are synthesized from DNA templates inside the cell nucleus.

9.1.2.1 Adrenocorticotropic hormone (ACTH; also adrenocorticotropin, corticotrophin)

a. It is a polypeptide tropic hormone produced by the anterior pituitary gland.
b. ACTH stimulates secretion of glucocorticoid steroid hormones from adrenal cortex cells, especially in the zona fasciculata of the adrenal glands.
c. ACTH is also related to the circadian rhythm in many organisms.

9.1.2.2 Amylin, or islet amyloid polypeptide (IAPP)

a. It is a 37-residue peptide hormone, co-secreted with insulin from the pancreatic β-cells.
b. It thus functions as a synergistic partner to insulin, with which it is co-secreted from pancreatic beta cells in response to meals.
c. The overall effect is to slow the rate of appearance of glucose in the blood after eating; this is accomplished via coordinate slowing down of gastric emptying, inhibition of digestive secretion [gastric acid, pancreatic enzymes, and bile ejection], and a resulting reduction in food intake.

9.1.2.3 Angiotensin

a. It is a peptide hormone that causes vasoconstriction and an increase in blood pressure.
b. It is part of the renin–angiotensin system, which regulates blood pressure.
c. Angiotensin also stimulates the release of aldosterone from the adrenal cortex to promote sodium retention by the kidneys.

9.1.2.4 Atrial natriuretic peptide (ANP) or atrial natriuretic factor (ANF)

a. It is a natriuretic peptide hormone.
b. ANP is synthesized and secreted by cardiac muscle cells in the walls of the atria in the heart.
c. These cells contain volume receptors which respond to increased stretching of the atrial wall due to increased atrial blood volume.
d. Reduction of blood volume by ANP can result in secondary effects such as reduction of extracellular fluid volume, improved cardiac ejection fraction with resultant improved organ perfusion, decreased blood pressure, and increased serum potassium.

9.1.2.5 Calcitonin

a. It is a 32 amino acid peptide hormone secreted by parafollicular cells (also known as C cells) of the thyroid gland in humans, and in many other animals in the ultimopharyngeal body.
b. It acts to reduce blood calcium, opposing the effects of parathyroid hormone.

9.1.2.6 Cholecystokinin

a. It is a peptide hormone of the gastrointestinal system responsible for stimulating the digestion of fat and protein.
b. Cholecystokinin, officially called pancreozymin, is synthesized and secreted by enteroendocrine cells in the duodenum, the first segment of the small intestine.
c. Its presence causes the release of digestive enzymes and bile the pancreas and gall bladder, respectively, and also acts as a hunger suppressant.

9.1.2.7 Gastrin

a. It is a peptide hormone that stimulates secretion of gastric acid (HCl) by the parietal cells of the stomach and aids in gastric motility.
b. It is released by G cells in the pyloric antrum of the stomach, duodenum, and the pancreas.

9.1.2.8 Ghrelin (or lenomorelin, INN)

a. It is a hormone produced by enteroendocrine cells of the gastrointestinal tract, especially the stomach, and is often called a "hunger hormone" because it increases food intake.
b. Blood levels of ghrelin are highest before meals when hungry, returning to lower levels after mealtimes.
c. Ghrelin may help to prepare for food intake by increasing gastric motility and gastric acid secretion.

9.1.2.9 Glucagon

a. It is a peptide hormone, produced by alpha cells of the pancreas.
b. It works to raise the concentration of glucose and fatty acids in the bloodstream, and is considered to be the main catabolic hormone of the body.
c. It is also used as a medication to treat a number of health conditions.
d. Its effect is opposite to that of insulin, which lowers extracellular glucose.

9.1.2.10 Growth hormone or somatotropin

a. It, also known as human growth hormones (hGH or HGH) in its human form, is a peptide hormone that stimulates growth, cell reproduction, and cell regeneration in humans and other animals.
b. It is thus important in human development.

9.1.2.11 Follicle-stimulating hormone (FSH)

a. It is a gonadotropin, glycoprotein polypeptide hormone. FSH is synthesized and secreted by the gonadotropic cells of the anterior pituitary gland, and regulates the development, growth, pubertal maturation, and reproductive processes of the body.
b. FSH and luteinizing hormone work together in the reproductive system.

9.1.2.12 Insulin (from Latin insula, 'island')

a. It is a peptide hormone produced by beta cells of the pancreatic islets; it is considered to be the main anabolic hormone of the body.
b. It regulates the metabolism of carbohydrates, fats and protein by promoting the absorption of glucose from the blood into liver, fat and skeletal muscle cells.

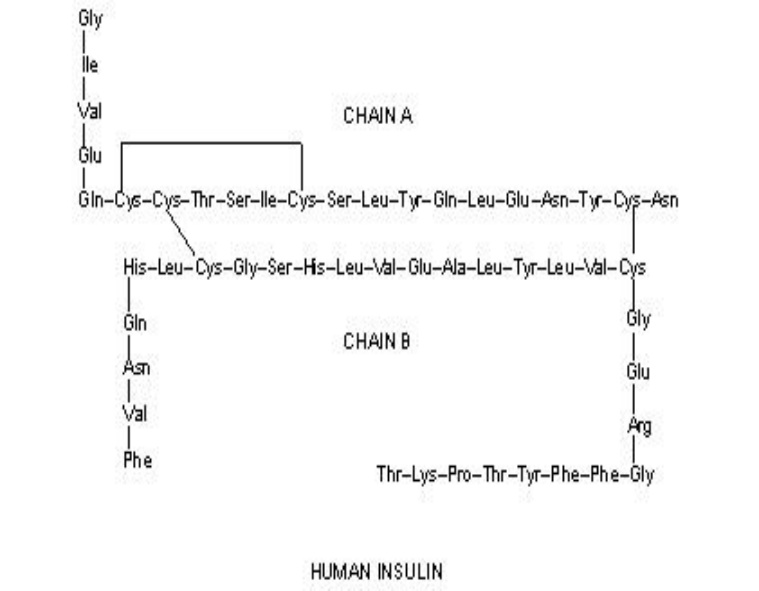

Fig. 5. Structure of insulin

9.1.2.13 Leptin (from Greek leptos, "thin")

a. It is a hormone predominantly made by adipose cells and enterocytes in the small intestine that helps to regulate energy balance by inhibiting hunger, which in turn diminishes fat storage in adipocytes.

9.1.2.14 The melanocyte-stimulating hormones

a. They, collectively known as MSH, melanotropins or intermedins, are a family of peptide hormones and neuropeptides consisting of α-melanocyte-stimulating hormone (α-MSH), β-melanocyte-stimulating hormone (β-MSH), and γ-melanocyte-stimulating stimulating hormone (γ-MSH), produced by cells in the pars intermedia of the anterior lobe of the pituitary gland.
b. An increase in MSH causes darker skin in humans.
c. MSH increases in humans during pregnancy.
d. This, along with increased estrogens, causes increased pigmentation in pregnant women.

9.1.2.15 Oxytocin

a. It is a peptide hormone and neuropeptide.
b. It is normally produced in the hypothalamus and released by the posterior pituitary.
c. It plays a role in social bonding, reproduction, childbirth, and the period after childbirth.
d. Oxytocin is released into the bloodstream as a hormone in response to love and in labor. This helps with birth, bonding with the baby, and milk production.

9.1.2.16 Parathyroid hormone (PTH)

a. It, also called parahormone or parathyrin, is a hormone secreted by the parathyroid glands that regulates the serum calcium concentration through its effects on bone, kidney, and intestine.

9.1.2.17 Prolactin (PRL)

a. It, also known as lactotropin, is a protein best known for its role in enabling mammals (and birds), usually females, to produce milk.

9.1.2.18 Renin

a. It, also known as an angiotensinogenase, is an aspartic protease protein and enzyme secreted by kidneys that participates in the body's renin–angiotensin–aldosterone system, also known as the renin–angiotensin–aldosterone axis—that mediates the volume of extracellular fluid (blood plasma, lymph and interstitial fluid) and arterial vasoconstriction. Thus, it regulates the body's mean arterial blood pressure.

9.1.2.19 Somatostatin

a. It, also known as growth hormone-inhibiting hormone (GHIH), is a peptide hormone that regulates endocrine system and affects neurotransmission and cell proliferation via interaction with G-protein-coupled somatostatin receptors and inhibition of the release of numerous secondary hormones.
b. Somatostatin inhibits insulin and glucagon secretion.

9.1.2.20 Thyroid-stimulating hormone (TSH, or thyrotropin, thyrotropic hormone)

a. It is a pituitary hormone that stimulates the thyroid gland to produce thyroxine (T4), and then triiodothyronine (T3) which stimulates the metabolism of almost every tissue in the body.

9.1.2.21 Thyrotropin-releasing hormone (TRH)

a. It is a hypophysiotropic hormone, produced by neurons in the hypothalamus, that stimulates the release of thyroid-stimulating hormone and prolactin from the anterior pituitary.

9.1.2.22 Vasopressin (antidiuretic hormone (ADH), arginine vasopressin or argipressin)

a. It is a hormone synthesized as a peptide prohormone in the hypothalamus, and is converted to AVP.
b. Vasopressin regulates the tonicity of body fluids.
c. It is released from the posterior pituitary in response to hypertonicity and causes the kidneys to reabsorb solute-free water and return it to the circulation from the tubules of the nephron, thus returning the tonicity of the body fluids toward normal.

9.1.2.23 Vasoactive intestinal peptide (VIP)

a. It is a peptide hormone that is vasoactive in the intestine.
b. VIP stimulates contractility in the heart, causes vasodilation, increases glycogenolysis lowers arterial blood pressure and relaxes the smooth muscle of trachea, stomach and gallbladder.

Vitamins

9.2 Introduction

a. Vitamins may be regarded as organic compounds required in the diet in small amounts to perform specific biological functions for normal maintenance of optimum growth and health of the organism.

9.2.1 Classification of vitamins

a. There are about 15 vitamins, essential for humans. They are classified as fat soluble (A, D, E and K) and water soluble (C and B-group) vitamins.
b. The B-complex vitamins may be sub-divided into energy-releasing (B1, B6, biotin etc.) and hematopoietic (folic acid and B12).
c. Most of the water-soluble vitamins exert the functions through their respective coenzymes while only one fat soluble vitamin (K) has been identified to function as a coenzyme.

9.2.2 Water-soluble vitamins must come from our food each day; they

a. are soluble in aqueous solutions and can’t be stored in the body.
b. are cofactors for many enzymes.
c. are excreted in urine each day.
d. are easily destroyed by heat, oxygen, and ultraviolet light, so care must be taken in food preparation.

9.2.2.1 Thiamin (Vitamin B1)

It consists of a substituted pyrimidine joined by a methylene bridge to a substituted thiazole.

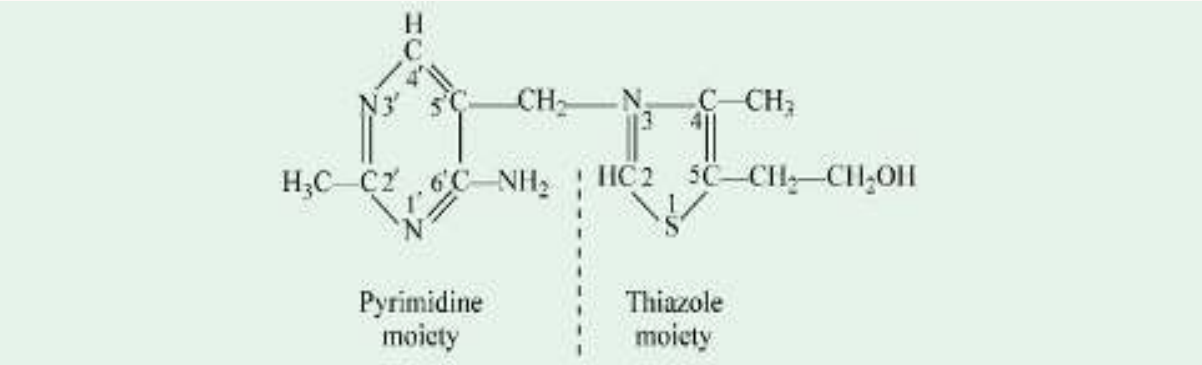

Fig. 6. Structure of Vitamin B1

The active form is thiamine pyrophosphate.

Thiamine performs the following functions in the human body:

a. It is directly involved in carbohydrate, protein, and fat metabolism.
b. It participates in synthesis of ATP- energy necessary for the implementation of intracellular processes.
c. It promotes the transition of carbohydrates to glucose, which is required by the body for active activity.
d. It promotes the breakdown of carbohydrates and fats that come with food; helps form functional blood cells.
e. It promotes the full growth and development of systems and organs.
f. It is responsible for the normal functioning of the digestive system.
g. It normalizes heart function.
h. It protects the nervous system from stress factors, as it participates in the formation of nerve endings of the myelin sheath, which protects cells from destruction.

i. It improves nutrient absorption by maintaining smooth muscle tone in the digestive system; it has a positive effect on the central nervous system, and the lack of this compound leads to negative consequences for cognitive abilities.
j. It is responsible for the normal state of the visual organs.
k. Thiamine is often also called an anti-stress vitamin, which fully reflects its important role for the human body. Apathy against the background of a lack of this substance develops due to a general decline in strength and weakness, which leads to a depressive state.
l. Deficiency disease: Beriberi.

9.2.2.2 Riboflavin (Vitamin B2)

It consists of a isoalloxazine ring attached to sugar alcohol, ribitol.

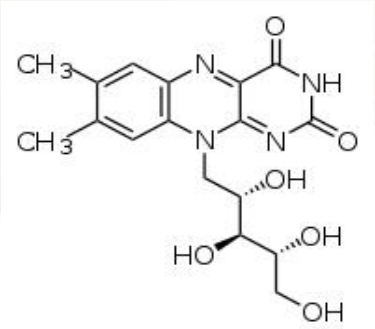

Fig. 7. Structure of Vitamin B2

Coenzyme form is FMN and FAD.

Function of Vitamin B2:

a. It assists in energy production.
b. It helps to synthesize normal fatty acids and amino acids.
c. It helps the nervous system to function efficiently.
d. It aids in cellular growth.
e. It assists in the metabolism of certain other vitamins.

9.2.2.3 Niacin (Vitamin B3)

Nicotinic acid is a monocarboxylic acid derivative of pyrimidine.

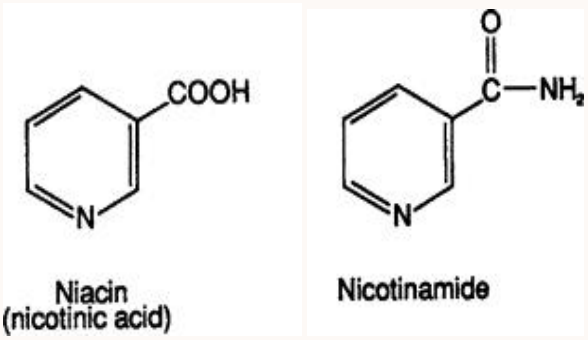

Fig. 8. Structure of Vitamin B3

a. Both nicotinic acid and nicotinamide act as source of vitamin in diet.
b. Coenzyme form is NADH and NADPH.

The major functions of vitamin B3 include:

a. This nutrient helps the body to convert food into glucose, used to produce energy. Niacin contributes to the normal function of the nervous system and normal psychological function.
b. It also contributes to the reduction of tiredness and fatigue.
c. Niacin is used to fortify grain, including corn and bran breakfast cereals and wheat flour.
d. It lowers LDL cholesterol.
e. It may help prevent heart disease.
f. Brain needs niacin — as a part of the coenzymes NAD+ and NADP+ — to get energy and function properly.
g. Niacin helps protect skin cells from sun damage, whether it's used orally or applied as a lotion.
h. Severe niacin deficiency causes a condition called pellagra.

9.2.2.4 Pantothenic acid (Vitamin B5)

It is formed by combination of pantoic acid and beta alanine.

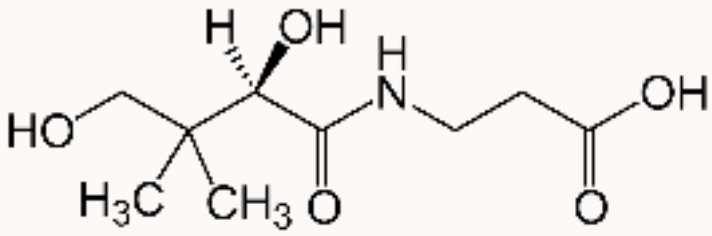

Fig. 9. Structure of Vitamin B5

- Active form is coenzyme A.

Some functions of Vitamin B5 include:

- It is a component of CoA (transfer of acyl groups, most commonly acetyl).
- Component of fatty acid synthase complex.

9.2.2.5 Pyridoxin (Vitamin B6)

It consists of three closely related pyridine derivatives: pyridoxine, pyridoxal and pyridoxamine.

Fig. 10. Structure of Vitamin B6

- Active form is pyridoxal phosphate.

The functions of Vitamin B6 are:

a. It is necessary for creating neurotransmitters that regulate emotions, including serotonin, dopamine and gamma-aminobutyric acid.
b. It acts as a coenzyme in transamination and decarboxylation reactions of amino acids.
c. Vitamin B6 play a role in improving brain function and preventing Alzheimer's disease.
d. Due to its role in hemoglobin production, vitamin B6 is helpful in preventing and treating anemia caused by deficiency.
e. It is used to treat symptoms of premenstrual syndrome including anxiety, depression and irritability.
f. It is used for decades to treat nausea and vomiting during pregnancy.
g. It prevents clogged arteries and minimizes heart disease risk.
h. Vitamin B6 play a role in preventing eye diseases, especially a type of vision loss that affects older adults called age-related macular degeneration.

9.2.2.6 Biotin (Vitamin B7)

It is an imidazole derivative.

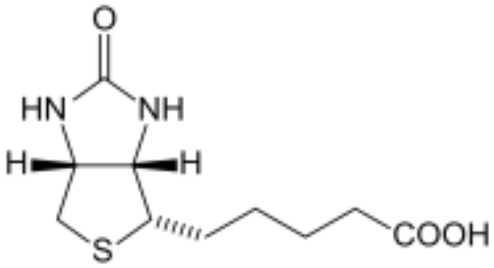

Fig. 11. Structure of Vitamin B7

Vitamin B7 plays important roles as follows:

a. It acts as a coenzyme in a number of metabolic pathways involving fatty acids and essential amino acids, as well as in gluconeogenesis.
b. It promotes appropriate function of the nervous system and is essential for liver metabolism as well.

c. It is advised as a dietary supplement for strengthening hair and nails, as well as in skin care.
d. It aids cell growth and the maintenance of mucous membranes.
e. Since biotin is an important factor in the synthesis of glucose, it helps to maintain an appropriate blood sugar level in patients suffering from type 2 diabetes.

9.2.2.7 Folic acid (Vitamin B9)

It consists of the base pteridine attached to one molecule each of para-amino-benzoic acid and glutamic acid.

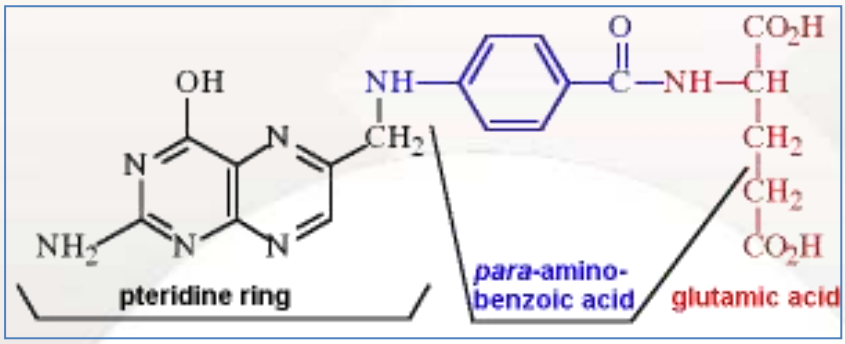

Fig. 12. Structure of Vitamin B9

- Active form is tetrahydro folic acid.

Vitamin B9 is crucial as:

a. It is necessary for the production of red blood cells.
b. It is required for the synthesis of DNA.
c. It helps in tissue growth and cell function.
d. It helps to increase appetite when needed and stimulates the formation of digestive acids.

9.2.2.8 Cobalmin (Vitamin B12)

It has a corrin ring to which a cobalt atom is added in the center.

Fig. 13. Structure of Vitamin B12

- The active forms are methyl cobalamin and deoxyadenosylcobalamin.

The major functions of Vitamin B12 include:

a. It helps to keep the body's nerve and blood cells healthy.
b. It prevents a type of anemia called megaloblasticanemia that makes people tired and weak.
c. It plays a part in the synthesis of fatty acids and energy production.
d. It enables the release of energy by helping the human body absorb folic acid.
e. It helps to prevent the risk of age-related macular degeneration.
f. The vitamin plays a role in preventing brain atrophy, which is the loss of neurons in the brain and often associated with memory loss or dementia.

9.2.2.9 Vitamin C (Ascorbic acid)

Derived from glucose in number of mammals. In humans required in diet due to absence of the enzyme L-gulonolactone oxidase.

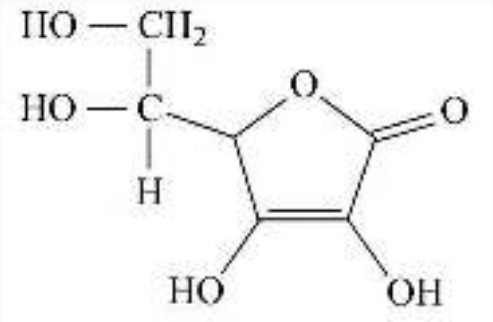

Fig. 18. Structure of Vitamin C

Functions of Vitamin C:

a. Vitamin C is required for bone formation.
b. Ascorbic acid enhances iron absorption by keeping it in the ferrous form. This is due to the reducing property of vitamin C.
c. The degradation of hemoglobin to bile pigments requires ascorbic acid.
d. Vitamin C is essential for the hydroxylation of tryptophan (enzyme-hydroxylase) to hydroxyl tryptophan in the synthesis of serotonin.
e. Ascorbic acid is required for the oxidation of p-hydroxy phenylpyruvate (enzyme hydroxylase) to homogentisic acid in tyrosine metabolism.
f. Ascorbic acid is involved in the maturation of erythrocytes.
g. Vitamin C is necessary for the hydroxylation reactions in the synthesis of corticosteroid hormones.
h. Ascorbic acid is a strong antioxidant. It spares vitamin A, vitamin E, and some B-complex vitamins from oxidation.
i. Vitamin C enhances the synthesis of immunoglobulins (antibodies) and increases the phagocytic action of leucocytes.
j. Vitamin C reduces the risk of cataract formation.
k. Deficiency of vitamin C causes scurvy.

9.2.3 Fat Soluble Vitamins

a. include A, D, E, and K and are not involved as coenzymes in catalytic reactions.
b. are soluble in lipids but not in aqueous solutions.
c. are stored in the body and not eliminated in urine.
d. are important in vision, bone formation, antioxidants, and blood clotting.

9.2.3.1 Retinol (Vitamin A)

It is a polyisoprenoid compound containing a cyclohexenyl ring. The main form is retinol. Others are retinal and retinoic acid. Main source is beta carotene.

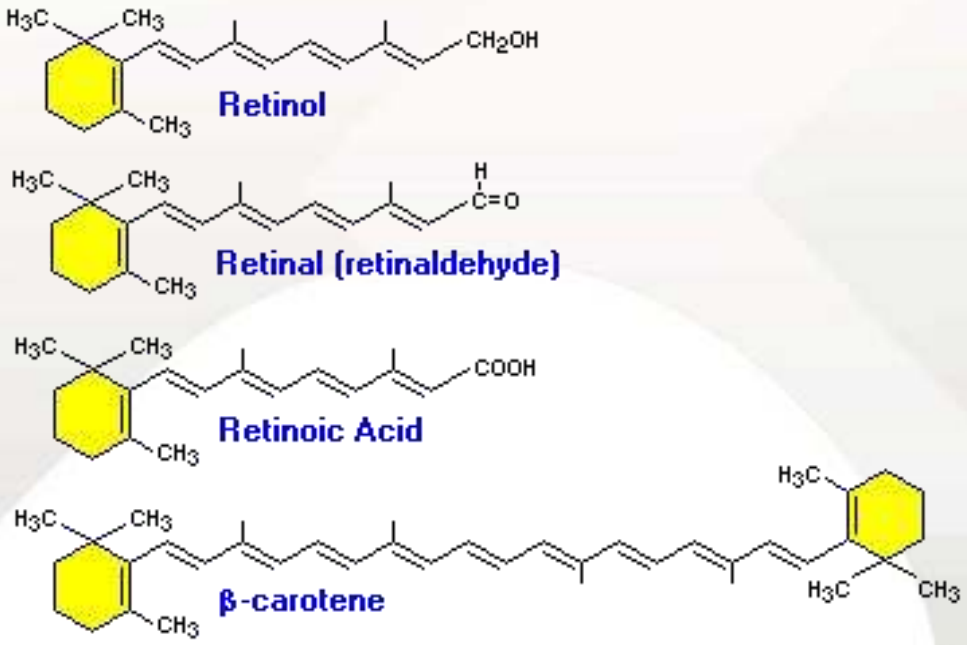

Fig. 14. Structure of Vitamin A

Functions of Vitamin A:

a. Retinol and retinoic acid function almost like steroid hormones. They regulate the protein synthesis and thus are involved in the cell growth and differentiation.
b. Retinol and retinoic acid are required to prevent keratin synthesis (responsible for horny surface).

c. Retinyl phosphate synthesized from retinol is necessary for the synthesis of certain glycoproteins which are required for growth and mucus secretion.
d. Retinol and retinoic acid are involved in the synthesis of transferrin, the iron transport protein.
e. Vitamin A is essential for the maintenance of proper immune system to fight against various infections.
f. Cholesterol synthesis requires vitamin A.
g. Carotenoids (most important β-carotene) function as antioxidants and reduce the risk of cancers initiated by free radicals and strong oxidants.
h. **Role in visual cycle.**

Vitamin A is a component of the visual pigments of rod and cone cells. Rhodopsin, the visual pigment of the rod cells in the retina, consists of 11-cis retinal specifically bound to the protein opsin. When rhodopsin is exposed to light, a series of photochemical isomerizations occur, which results in the bleaching of the visual pigment and release of all-trans retinal and opsin.

This process triggers a nerve impulse that is transmitted by the optic nerve to the brain. Regeneration of rhodopsin requires isomerization of all-trans retinal back to 11-cis retinal. All-trans retinal, after being released from rhodopsin, is reduced to all-trans retinol, esterfied, and isomerized to 11-cis retinol that is oxidized to 11-cis retinal. The latter combines with opsin to form rhodopsin, thus completing the cycle. Similar reactions are responsible for color vision in the cone cells.

9.2.3.2 Cholecalciferol (Vitamin D)

a. It is a steroid prohormone that is converted to calcitriol hormone that plays a central role in calcium and phosphate metabolism.
b. Synthesized from ergosterol in plants and 7-dehydrocholesterol in animals.
c. Transport of vit D occurs by Vitamin D binding proteins and it is converted to 1, 25 dihydroxy D3 after hydroxylation in liver and kidney.

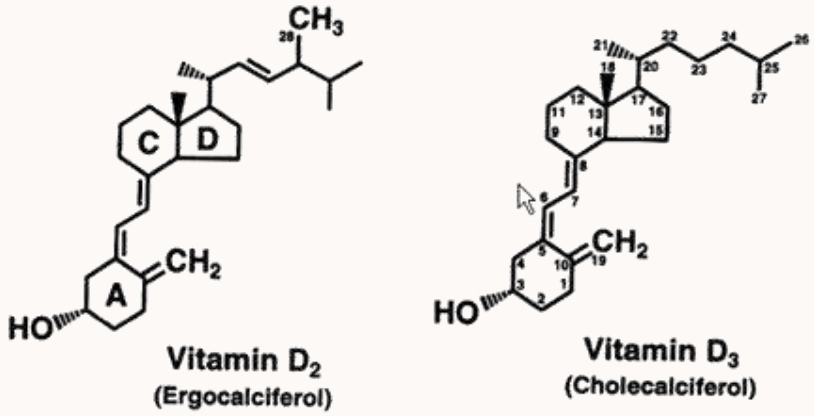

Fig. 15. Structure of Vitamin D

Functions of Vitamin D:

a. Vitamin D plays a significant role in the regulation of calcium and maintenance of phosphorus levels in the blood.
b. It has a protective effect against the influenza virus.
c. It is needed to keep bones, teeth and muscles healthy.
d. It enhances muscle contraction.
e. It possesses a range of anti-inflammatory properties.
f. It helps to regulate kidney function.
g. Deficiency causes rickets in young children and osteomalacia in adults.

9.2.3.3 Tocopherol (Vitamin E)

There are several naturally occurring tocopherols. All are isoprenoid substituted 6-hydroxychromanes.

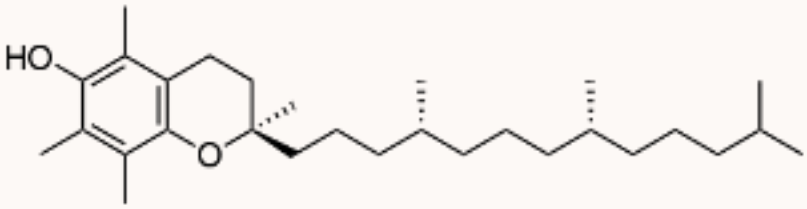

Fig. 16. Structure of Vitamin E

a. Vitamin E is essential for the membrane structure and integrity of the cell. It is regarded as a membrane antioxidant.
b. It prevents the peroxidation of polyunsaturated fatty acids in various tissues and membranes.
c. It protects RBC from hemolysis by oxidizing agents (e.g. H2O2).
d. It is closely associated with reproductive functions and prevents sterility.
e. Vitamin E preserves and maintains germinal epithelium of gonads for proper reproductive function.
f. It increases the synthesis of heme by enhancing the activity of enzymes 6-aminolevulinic acid (ALA) synthase and ALA dehydratase.
g. It is required for cellular respiration through electron transport chain (believed to stabilize coenzyme Q).
h. Vitamin E prevents the oxidation of vitamin A and carotenes.
i. It is required for proper storage of creatine in skeletal muscle.
j. It is needed for optimal absorption of amino acids from the intestine.
k. It is involved in proper synthesis of nucleic acids.
l. It protects liver from being damaged by toxic compounds such as carbon tetrachloride.

9.2.3.4 Phylloquinone (Vitamin K)

Vitamins belonging to K group are polyisoprenoid-substituted napthoquinones.

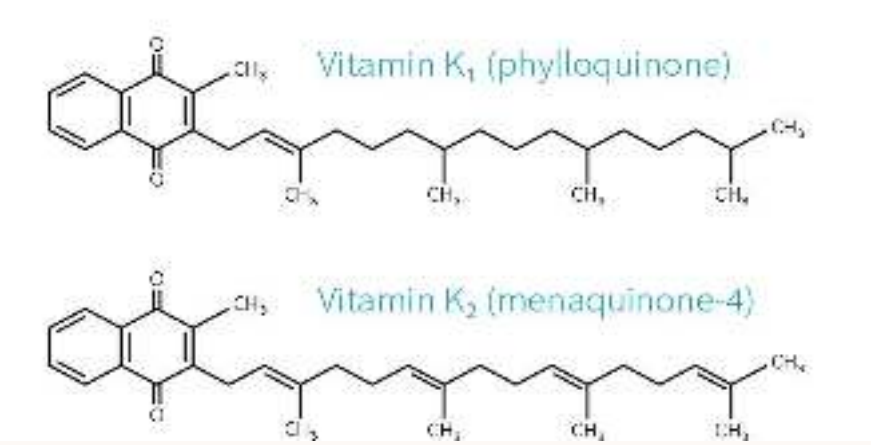

Fig. 17. Structure of Vitamin K

Functions of Vitamin K:

a. It is needed for the synthesis of several clotting factors II, VII, IX and X.
b. It is important for blood clotting and healthy bones.
c. It supports the maintenance of strong bones, improves bone density and decreases the risk of fractures.
d. Increased blood levels of vitamin K have been linked with improved episodic memory in older adults.
e. Vitamin K help to keep blood pressure lower by preventing mineralization, where minerals build up in the arteries.

Minerals

9.3 Introduction

a. A mineral is an element required as an essential nutrient by organisms to perform functions necessary for life.
b. The human body needs about twenty different minerals in order to function properly.
c. These elements can be classified into macro and microminerals.
d. Macro minerals are needed in amounts higher than 100 mg/day and include calcium (Ca), phosphorus (P), magnesium (Mg), sulfur (S), sodium (Na), chloride (Cl) and potassium (K).
e. Micro minerals (needed in amounts less than 100 mg/day) include elements such as iron (Fe), zinc (Zn), iodine (I), selenium (Se), manganese (Mn), chromium (Cr), copper (Cu), molybdenum (Mo), fluorine (F), boron (B), cobalt (Co), silicon (Si), aluminum (Al), arsenic (Ar), tin (Sn), lithium (Li) and nickel (Ni).

Table 1: Macro-minerals

Mineral	Function	Sources	Recommended Dietary Allowance (RDA)
Sodium	Needed for proper fluid balance, nerve transmission, and muscle contraction. Act as osmoticum in plants.	Table salt, soy sauce; large amounts in processed foods; small amounts in milk, breads, vegetables, and unprocessed meats	500 mg/day
Chloride	Needed for proper fluid balance, stomach acid. Important for plant photosynthesis as it is involved in opening and closing of stomata.	Table salt, soy sauce; large amounts in processed foods; small amounts in milk, meats, breads, and vegetables	750 mg/day
Potassium	Needed for proper fluid balance, nerve transmission, and muscle contraction. Associated with movement of water, nutrients and carbohydrates in plant tissue.	Meats, milk, fresh fruits and vegetables, whole grains, legumes	2000 mg/day
Calcium	Important for healthy bones and teeth; helps muscles relax and contract; important in	Milk and milk products; canned fish with bones	1000-1200 mg/day

	nerve functioning, blood clotting, blood pressure regulation, immune system health. Needed in large amounts by all plants for formation of cell walls and cell membranes.	(salmon, sardines); fortified tofu and fortified soy milk; greens (broccoli, mustard greens); legumes	
Phosphorus	Important for healthy bones and teeth; found in every cell; part of the system that maintains acid-base balance. Involved in several key plant functions, including energy transfer, photosynthesis, transformation of sugars and starches, nutrient movement within the plant and transfer of genetic characteristics from one generation to the next.	Meat, fish, poultry, eggs, milk, processed foods (including soda pop)	700 mg/day
Magnesium	Found in bones; needed for making protein, muscle contraction, nerve transmission, immune system health. It is central core of the chlorophyll molecule in plant tissue.	Nuts and seeds; legumes; leafy, green vegetables; seafood; chocolate; artichokes; "hard" drinking water	310-400 mg/day
Sulfur	Found in protein molecules. Plays an important role in	Occurs in foods as part of protein: meats, poultry, fish,	No universal RDA

	plant defense against stresses and pests.	eggs, milk, legumes, nuts	

Trace minerals (micro-minerals): The body needs trace minerals in very small amounts. Note that iron is considered to be a trace mineral, although the amount needed is somewhat more than for other micro-minerals.

Table 2: Micro-minerals

Mineral	Function	Sources	Recommended Dietary Allowance (RDA)
Iron	Part of a molecule (hemoglobin) found in red blood cells that carries oxygen in the body; needed for energy metabolism. Involved in chlorophyll synthesis, and is essential for the maintenance of chloroplast structure and function.	Organ meats; red meats; fish; poultry; shellfish (especially clams); egg yolks; legumes; dried fruits; dark, leafy greens; iron-enriched breads and cereals; and fortified cereals	10-15 mg/day
Zinc	Required for the activity of >300 enzymes, covering all six classes of enzymes (e.g. carbonic anhydrase, Alcohol	Meats, fish, poultry, leavened whole grains,	12-15 mg/day

	dehydrogenase, phospholipase C etc.). Needed for making protein and genetic material; has a function in taste perception, wound healing, normal fetal development, production of sperm, normal growth and sexual maturation, immune system health. Plays a pivotal function in plant response to pests and diseases.	vegetables	
Iodine	Found in thyroid hormone, which helps regulate growth, development, and metabolism. Little is known about the role of iodine in plant physiology	Seafood, foods grown in iodine-rich soil, iodized salt, bread, dairy products	150 micrograms/day
Selenium	Antioxidant. Reported to mitigate stress in plants because of its capacity to induce the synthesis of S- and N- compounds, in addition to stimulating the activity of antioxidant enzymes and metabolites.	Meats, seafood, grains	55-70 micrograms/day
Copper	Part of many enzymes (superoxide dismutase, ceruloplasmin, amine oxidases, cytochrome-c oxidase etc.); needed for iron metabolism.	Legumes, nuts and seeds, whole grains, organ meats, drinking water	1.5 - 3.0 mg/day

	Activates some enzymes in plants which are involved in lignin synthesis.		
Manganese	Required for many enzymes (e.g. pyruvate carboxylase, lipase, superoxide dismutase etc.). Plays role in bone formation, blood clotting, and reducing inflammation. An essential cofactor for oxygen-evolving complex of photosynthetic machinery, catalyzing the water-splitting reaction in photosystem II.	Widespread in foods, especially plant foods	2-5 mg/day
Fluoride	Involved in formation of bones and teeth; helps prevent tooth decay.	Drinking water (either fluoridated or naturally containing fluoride), fish, and most teas	3.1 to 3.8 mg/day
Chromium	Works closely with insulin to regulate blood sugar (glucose) levels. Stimulator for plants, promotes growth and increases biomass at low concentration.	Unrefined foods, especially liver, brewer's yeast, whole grains, nuts, cheeses	50-200 microgram/day

Molybdenum	Required for some enzymes (e.g. xanthine oxidase, sulfite oxidase, and aldehyde oxidase etc.). It is an essential component in two enzymes that convert nitrate into nitrite and then into ammonia before it is used to synthesize amino acids within the plant. It also needed by symbiotic nitrogen fixing bacteria in legumes to fix atmospheric nitrogen.	Legumes; breads and grains; leafy greens; leafy, green vegetables; milk; liver	75-260 microgram/day

Other trace nutrients known to be essential in tiny amounts include nickel, silicon, vanadium, and cobalt.

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Nucleic acids: Structure function and importance. genetic code. Transcription and translation. Protein synthesis. Energy changes in chemical reactions, reversible and irreversible reactions in metabolism

Course Name	Fundamentals of Biochemistry
Lesson 10	Nucleic acids: Structure function and importance. genetic code. Transcription and translation. Protein synthesis. Energy changes in chemical reactions, reversible and irreversible reactions in metabolism
Content Creator Name	Dr. Ajay Pal
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Lesson 10:

Nucleic acids: Structure function and importance. genetic code. Transcription and translation. Protein synthesis. Energy changes in chemical reactions, reversible and irreversible reactions in metabolism

Objectives

a. To study structures and functions of nucleic acids.
b. To study the features of genetic code, transcription and translation processes.
c. To study energy changes in chemical reactions, reversible and irreversible reactions in metabolism.

Glossary

a. **Phosphodiester bond:** A phosphodiester bond occurs when exactly two of the hydroxyl groups in phosphoric acid react with hydroxyl groups on other molecules to form two ester bonds.
b. **Transcription:** Transcription is the first of several steps of DNA based gene expression in which a particular segment of DNA is copied into RNA by the enzyme RNA polymerase.
c. **Translation:** Translation is the process in which ribosomes in the cytoplasm or endoplasmic reticulum synthesize proteins after transcription of DNA to RNA in the cell's nucleus.
d. **Codon:** A codon is a trinucleotide sequence of DNA that corresponds to a specific amino acid. The genetic code describes the relationship between the sequence of DNA bases (A, C, G, and T) in a gene and the corresponding protein sequence that it encodes.
e. **Mutation:** Change in the sequence of a gene or DNA which alters the protein formation is known as mutation.
f. **Ribozymes:** Ribozymes are RNA molecules that have the ability to catalyze specific biochemical reactions, including RNA splicing in gene expression, similar to the action of protein enzymes.
g. **Promoter:** A promoter is a sequence of DNA needed to turn a gene on or off. The process of transcription is initiated at the promoter.

10.1 Introduction to nucleic acids

a. Nucleic acid is a naturally occurring chemical compound that is capable of being broken down to yield phosphoric acids, sugars, and a mixture of organic bases (purines and pyrimidines).
b. Nucleic acids are the main information-carrying molecules of the cell, and, by directing the process of protein synthesis, they determine the inherited characteristics of every living thing.
c. The two main classes of nucleic acids are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).
d. DNA is the master blueprint for life and constitutes the genetic material in all free-living organisms and most viruses.
e. RNA is the genetic material of certain viruses, but it is also found in all living cells, where it plays an important role in certain processes such as the making of proteins.

10.2 Basic structure

a. Nucleic acids are polynucleotides—that is, long chain like molecules composed of a series of nearly identical building blocks called nucleotides.
b. Each nucleotide consists of a nitrogen-containing aromatic base attached to a pentose (five-carbon) sugar, which is in turn is attached to a phosphate group.
c. Each nucleic acid contains four of five possible nitrogen-containing bases: adenine (A), guanine (G), cytosine (C), thymine (T), and uracil (U).
d. A and G are categorized as purines, and C, T, and U are collectively called pyrimidines.
e. All nucleic acids contain the bases A, C, and G; T, however, is found only in DNA, while U is found in RNA.
f. The pentose sugar in DNA (2'-deoxyribose) differs from the sugar in RNA (ribose) by the absence of a hydroxyl group (—OH) on the 2' carbon of the sugar ring.
g. Without an attached phosphate group, the sugar attached to one of the bases is known as a nucleoside.

h. The phosphate group connects successive sugar residues by bridging the 5'-hydroxyl group on one sugar to the 3'-hydroxyl group of the next sugar in the chain.
i. These linkages are called phosphodiester bonds and are same in RNA and DNA.

10.3 Biosynthesis and degradation

a. Nucleotides are synthesized from readily available precursors in the cell.
b. Ribose phosphate portion of both purine and pyrimidine nucleotides is synthesized from glucose via pentose phosphate pathway (hexose monophosphate pathway).
c. Six-atom pyrimidine ring is synthesized first and subsequently attached to ribose phosphate.
d. Two rings in purines are synthesized while attached to the ribose phosphate during the assembly of adenine or guanine nucleosides.
e. In both cases the end product is a nucleotide carrying a phosphate attached to the 5' carbon on the sugar.
f. Finally, a specialized enzyme called a kinase adds two phosphate groups using adenosine triphosphate (ATP) as the phosphate donor to form ribonucleoside triphosphate, the immediate precursor of RNA.
g. For DNA, the 2'-hydroxyl group is removed from the ribonucleoside diphosphate to give deoxyribonucleoside diphosphate.
h. An additional phosphate group from ATP is then added by another kinase to form a deoxyribonucleoside triphosphate, the immediate precursor of DNA.

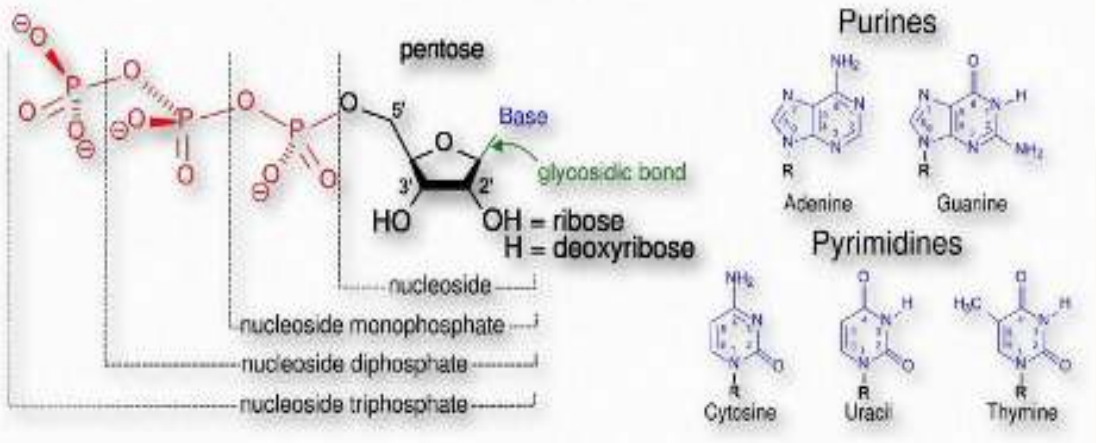


Fig.1 Precursors of DNA

i. During normal cell metabolism, RNA is constantly being made and broken down.
j. The purine and pyrimidine residues are reused by several salvage pathways to make more genetic material.
k. Purine is salvaged in the form of the corresponding nucleotide, whereas pyrimidine is salvaged as the nucleoside.

10.4 Deoxyribonucleic acid (DNA)

a. DNA is a polymer of four nucleotides A, C, G, and T, which are joined through a backbone of alternating phosphate and deoxyribose sugar residues.
b. These nitrogen-containing bases occur in complementary pairs as determined by their ability to form hydrogen bonds between them.
c. A always pairs with T through two hydrogen bonds, and G always pairs with C through three hydrogen bonds.
d. Spans of A:T and G:C hydrogen-bonded pairs are nearly identical, allowing them to bridge the sugar-phosphate chains uniformly.
e. This structure, along with the molecule's chemical stability, makes DNA the ideal genetic material.
f. The bonding between complementary bases also provides a mechanism for the replication of DNA and the transmission of genetic information.

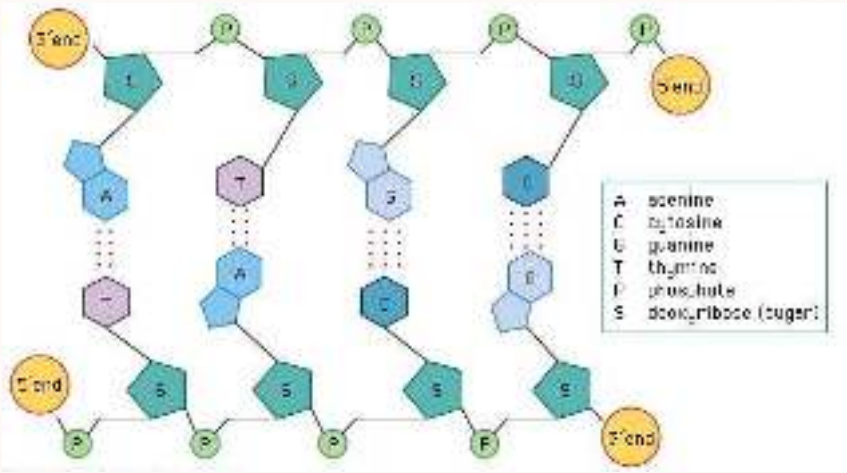


Fig. 2 Structure of DNA

g. In 1953 James D. Watson and Francis H.C. Crick proposed a 3-dimensional structure for DNA based on low-resolution X-ray crystallographic data and on Erwin Chargaff's observation that, in naturally occurring DNA, the amount of T equals the amount of A and the amount of G equals the amount of C.
h. Watson and Crick, who shared a Nobel Prize in 1962 for their efforts, postulated that two strands of polynucleotides coil around each other, forming a double helix.
i. The two strands, though identical, run in opposite directions as determined by the orientation of the 5' to 3' phosphodiester bond.
j. Sugar-phosphate chains run along the outside of helix, and the bases lie on the inside, where they are linked to complementary bases on the other strand through hydrogen bonds.

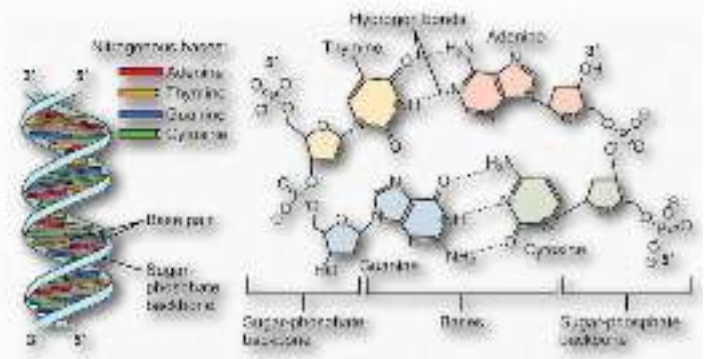


Fig. 3 Hydrogen bonding between nitrogenous bases in DNA

k. The double helical structure of normal DNA takes a right-handed form called the B-helix.
l. The helix makes one complete turn approximately every 10 base pairs. B-DNA has two principal grooves, a wide major groove and a narrow minor groove.
m. Many proteins interact in the space of the major groove, where they make sequence-specific contacts with the bases.
n. In addition, a few proteins are known to make contacts via the minor groove.
o. B form is most stable structure for a random-sequence DNA molecule under physiological conditions and is therefore the standard point of reference in any study of the properties of DNA.
p. Two structural variants that have been well characterized in crystal structures are the A and Z forms.

n. Comparison of A, B, and Z forms of DNA is shown in Table 1.

Table 1. Comparison of A, B, and Z forms of DNA

	A form	*B form*	*Z form*
Helical sense	Right handed	Right handed	Left handed
Diameter	~26 Å	~20 Å	~18 Å
Base pairs per helical turn	11	10.5	12
Helix rise per base pair	2.6 Å	3.4 Å	3.7 Å
Base tilt normal to the helix axis	20°	6°	7°
Sugar pucker conformation	C-3′ endo	C-2′ endo	C-2′ endo for pyrimidines; C-3′ endo for purines
Glycosyl bond conformation	Anti	Anti	Anti for pyrimidines; syn for purines

10.5 Ribonucleic Acid (RNA)

a. RNA is a single-stranded nucleic acid polymer of the four nucleotides A, C, G, and U joined through a backbone of alternating phosphate and ribose sugar residues.
b. It is the first intermediate in converting the information from DNA into proteins essential for the working of a cell.
c. Some RNAs also serve direct roles in cellular metabolism.
d. RNA is made by copying the base sequence of a section of double-stranded DNA, called a gene, into a piece of single-stranded nucleic acid. This process, called transcription is catalyzed by an enzyme called RNA polymerase.

10.5.1Chemical structure

a. Whereas DNA provides the genetic information for the cell and is inherently quite stable, RNA has many roles and is much more reactive chemically.
b. RNA is sensitive to oxidizing agents such as periodate that leads to opening of the 3’-terminal ribose ring structure.
c. The 2’-hydroxyl group on the ribose ring is a major cause of instability in RNA, because the presence of alkali leads to rapid cleavage of phosphodiester bond linking ribose and phosphate groups.
d. In general, this instability is not a significant problem for the cell, because RNA is constantly being synthesized and degraded.
e. Interactions between the nitrogen-containing bases differ in DNA and RNA.
f. In DNA, which is usually double-stranded, the bases in one strand pair with complementary bases in a second DNA strand. But, in RNA, which is usually single-stranded, the bases pair with other bases within the same molecule, leading to complex 3-dimensional structures.
g. Occasionally, intermolecular RNA/RNA duplexes do form, but they form a right-handed A-type helix rather than the B-type DNA helix.
h. Depending on the amount of salt present, either 11 or 12 base pairs are found in each turn of the helix.
i. Single-stranded RNAs are flexible molecules that form a variety of structures through internal base pairing and additional non-base pair interactions.

j. They can form hairpin loops such as those found in transfer RNA (tRNA), as well as longer-range interactions involving both the bases and the phosphate residues of two or more nucleotides.
k. This leads to compact 3-dimensional structures.
l. In some types of RNA, a large number of bases are modified after the RNA is transcribed.
m. More than 90 different modifications have been documented, including extensive methylations and a wide variety of substitutions around the ring.
n. In some cases, these modifications are known to affect structure and are essential for function.

10.6 Types of RNA

10.6.1 Messenger RNA (mRNA)

a. An mRNA delivers the information encoded in one or more genes from DNA to ribosome, a specialized structure, or organelle, where that information is decoded into a protein.
b. In prokaryotes, mRNAs contain an exact transcribed copy of the original DNA sequence with a terminal 5’-triphosphate group and a 3’-hydroxyl residue.
c. In eukaryotes, the mRNA molecules are more elaborate. The 5’-triphosphate residue is further esterified, forming a structure called a cap. At 3’ ends, eukaryotic mRNAs typically contain long runs of adenosine residues (polyA) that are not encoded in the DNA but are added enzymatically after transcription.
d. Eukaryotic mRNA molecules are usually composed of small segments of original gene and are generated by a process of cleavage and rejoining from an original precursor RNA (pre-mRNA) molecule, which is an exact copy of the gene.
e. In general, prokaryotic mRNAs are degraded very rapidly, whereas the cap structure and the polyA tail of eukaryotic mRNAs greatly enhance their stability.

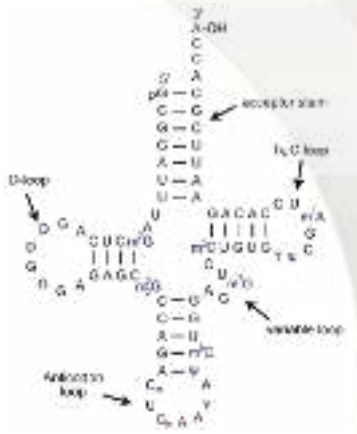
10.6.2 Ribosomal RNA (rRNA)

a. Ribosomal RNA (rRNA) molecules are structural components of the ribosome.
b. The rRNAs form extensive secondary structures and play an active role in recognizing conserved portions of mRNAs and tRNAs.
c. They also assist with the catalysis of protein synthesis.
d. In E. coli, seven copies of rRNA genes synthesize ~15,000 ribosomes per cell.
e. In eukaryotes, the numbers are much larger. From 50 to 5,000 sets of rRNA genes and as many as 10 million ribosomes may be present in a single cell.
f. In eukaryotes, these rRNA genes are looped out of the main chromosomal fibres and coalesce in the presence of proteins to form an organelle called the nucleolus.
g. The nucleolus is where the rRNA genes are transcribed and the early assembly of ribosomes takes place.

10.6.3 Transfer RNA (tRNA)

a. A tRNA carries individual amino acids into the ribosome for assembly into the growing polypeptide chain.
b. The tRNA molecules contain 70 to 80 nucleotides.
c. Comparisons of tRNAs from various species reveal many common denominators of structure. Eight or more of the nucleotide residues have modified bases and sugars, many of which are methylated derivatives of the principal bases.
d. Most tRNAs have a guanylate (pG) residue at the 5’ end, and all have the trinucleotide sequence CCA(3’) at the 3’ end.
e. When drawn in two dimensions, the hydrogen-bonding pattern of all tRNAs forms a cloverleaf structure with four arms; the longer tRNAs have a short fifth arm, or extra arm.
f. In three dimensions, a tRNA has the form of a twisted L.
g. Two of the arms of a tRNA are critical for its adaptor function.
h. The amino acid arm can carry a specific amino acid esterified by its carboxyl group to the 2’- or 3’-hydroxyl group of the A residue at the 3’ end of the tRNA.
i. The anticodon arm contains the anticodon.

j. The other major arms are the D arm, which contains the unusual nucleotide dihydrouridine (D), and the TΨC arm, which contains ribothymidine (T), not usually present in RNAs, and pseudouridine (Ψ), which has an unusual carbon–carbon bond between the base and ribose.
k. The D and TΨC arms contribute important interactions for the overall folding of tRNA molecules, and the TΨC arm interacts with the large-subunit rRNA.
l. Specialized tRNAs exist for each of the 20 amino acids needed for protein synthesis, and in many cases more than one tRNA for each amino acid is present.
m. The nucleotide sequence of mRNA is converted into a protein sequence by translating each three-base sequence (called a codon) with a specific protein.
n. The 61 codons used to code amino acids can be read by fewer than 61 distinct tRNAs (as will be described in 'translation' section).
o. In E. coli, a total of 40 different tRNAs are used to translate the 61 codons.
p. The amino acids are loaded onto the tRNAs by specialized enzymes called aminoacyltRNA synthetases, usually with one synthetase for each amino acid.
q. All tRNAs adopt similar structures because they all have to interact with the same sites on the ribosome.



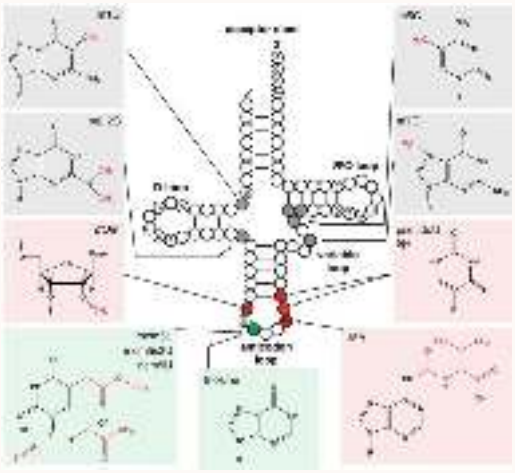


Fig. 4 Structure of t-RNA

10.6.4 Other RNAs

a. Many other small RNA molecules with specialized functions are present in cells.
b. For example, small nuclear RNAs (snRNAs) are involved in RNA splicing, and other small RNAs that form part of the enzymes telomerase or ribonuclease P are part of ribonucleoprotein particles.
c. The RNA component of telomerase contains a short sequence that serves as a template for the addition of small strings of oligonucleotides at the ends of eukaryotic chromosomes.
d. Other RNA molecules serve as guide RNAs for editing, or they are complementary to small sections of rRNA and either direct the positions at which methyl groups need to be added or mark U residues for conversion to the isomer pseudouridine.

10.7 Ribozymes

a. Not all catalysis within the cell is carried out exclusively by proteins.
b. Thomas Cech and Sidney Altman, jointly awarded the Nobel Prize in 1989, discovered that certain RNAs, now known as ribozymes, showed enzymatic activity.
c. Cech showed that a non-coding sequence in the small subunit rRNA of protozoans, which had to be removed before the rRNA was functional, can excise itself from a much longer precursor RNA molecule and rejoin the two ends in an autocatalytic reaction.

d. Altman showed that the RNA component of an RNA protein complex called ribonuclease P can cleave a precursor tRNA to generate a mature tRNA.
e. In addition to self-splicing RNAs similar to the one discovered by Cech, artificial RNAs have been made that show a variety of catalytic reactions.
f. It is now widely held that there was a stage during evolution when only RNA catalyzed and stored genetic information. This period, sometimes called “the RNA world,” is believed to have preceded the function of DNA as genetic material.
g. 10.8 Function of nucleic acids
h. The most important function of nucleic acids for living things is their role as carriers of information.
i. Because nucleic acids can be created with four “bases,” and because “base pairing rules” allow information to be “copied” by using one strand of nucleic acids as a template to create another, these molecules are able to both contain and copy information.
j. Protect the information: DNA source code is vital to a cell, so DNA must be protected from potential damages. To transport DNA’s instructions to other parts of the cell, copies of its information are made using another type of nucleic acid – RNA.
k. It is these RNA copies of genetic information which are sent out of the nucleus and around the cell to be used as instructions by cellular machinery.
l. Cells also use nucleic acids for other purposes. Ribosomes – the cellular machines that make protein – and some enzymes are made out of RNA.

m. DNA uses RNA as a sort of protective mechanism, separating the DNA from the chaotic environment of the cytoplasm. Within the nucleus, the DNA is protected. Outside of the nucleus, movements of organelles, vesicles, and other cellular components could easily damage the long, complex DNA strands.
n. RNA can act both as hereditary material and an enzyme. This strengthens the case for the idea that the very first life might have been a self-replicating, self-catalyzing RNA molecule.
o. 10.9 RNA metabolism

p. RNA provides the link between the genetic information encoded in DNA and the actual workings of the cell.
q. Some RNA molecules such as rRNAs and snRNAs become part of complicated ribonucleoprotein structures with specialized roles in the cell.
r. Others such as tRNAs play key roles in protein synthesis, while mRNAs direct the synthesis of proteins by the ribosome.
s. Three distinct phases of RNA metabolism occur.
t. First, selected segments of the genome are copied by transcription to produce the precursor RNAs.
u. Second, these precursors are processed to become functionally mature RNAs ready for use. When these RNAs are mRNAs, they are then used for translation.
v. Third, after use the RNAs are degraded, and the bases are recycled.
w. Transcription is the process where a specific segment of DNA, a gene, is copied into a specific RNA that encodes a single protein or plays a structural or catalytic role.
x. Translation is the decoding of information within mRNA molecules that takes place on a specialized structure called a ribosome. There are important differences in both transcription and translation between prokaryotic and eukaryotic organisms.

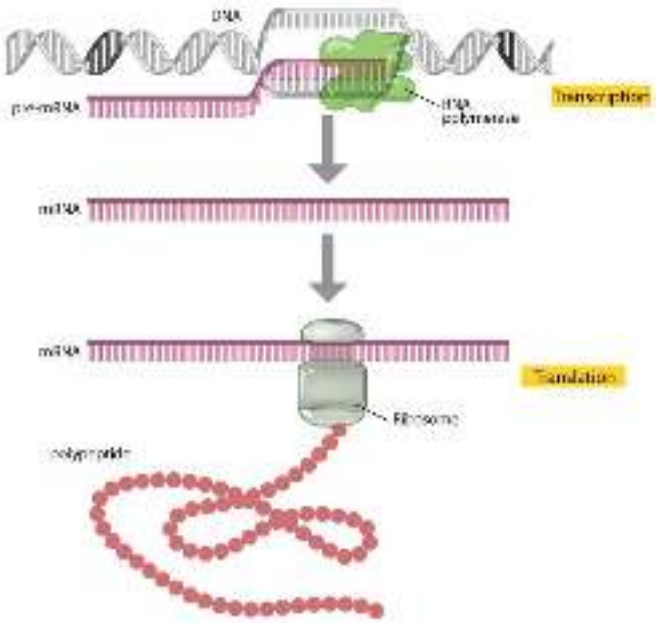


Fig. 5: RNA metabolism

10.9.1 Transcription

a. Process of synthesis of RNA from DNA is called transcription.
b. Small segments of DNA are transcribed into RNA by the enzyme RNA polymerase, which achieves this copying in a strictly controlled process.
c. During transcription, only one strand of the DNA is usually copied.
d. This is called the template strand, and the RNA molecules produced are single-stranded.
e. The DNA strand that would correspond to the mRNA is called the coding or sense strand.
f. Transcription involves three steps: Initiation, elongation and termination.
g. The first step is to recognize a specific sequence on DNA called a promoter that signifies the start of the gene.

Fig. 6 Promoter site in prokaryotes

h. The two strands of DNA become separated at this point, and RNA polymerase begins copying from a specific point on one strand of the DNA using a ribonucleoside 5’-triphosphate to begin the growing chain.
i. In elongation step, additional ribonucleoside triphosphates are used as the substrate, and, by cleavage of their high-energy phosphate bond, ribonucleoside mono-phosphates are incorporated into the growing RNA chain.
j. Each successive ribonucleotide is directed by the complementary base pairing rules of DNA.
k. Thus, a C in DNA directs the incorporation of a G into RNA, G is copied into C, T into A, and A into U.
l. Synthesis continues until a termination signal is reached, at that point the RNA polymerase drops off the DNA, and the RNA molecule is released.

m. In some cases, this RNA molecule is the final mRNA. In other cases, it is a pre-mRNA and requires further processing before it is ready for translation by the ribosome.
n. Ahead of many genes in prokaryotes, there are signals called “operators” where specialized proteins called repressors bind to the DNA just upstream of the start point of transcription and prevent access to the DNA by RNA polymerase.
o. These repressor proteins thus prevent transcription of the gene by physically blocking the action of the RNA polymerase.
p. Typically, repressors are released from their blocking action when they receive signals from other molecules in the cell indicating that the gene needs to be expressed.
q. Ahead of some prokaryotic genes are signals to which activator proteins bind that positively induce transcription.
r. Transcription in higher organisms requires the RNA polymerase, a more complicated enzyme than the relatively simple five-subunit enzyme of prokaryotes.
s. In addition, there are many more accessory factors that help to control the efficiency of the individual promoters.
t. These accessory proteins are called transcription factors and typically respond to signals from within the cell that indicate whether transcription is required.
u. In many human genes, several transcription factors may be needed before transcription can proceed efficiently.
v. A transcription factor can cause either repression or activation of gene expression in eukaryotes.
w. In eukaryotes the initial product of transcription is called a pre-mRNA, which is extensively spliced before the mature mRNA is produced, ready for translation by the ribosome.

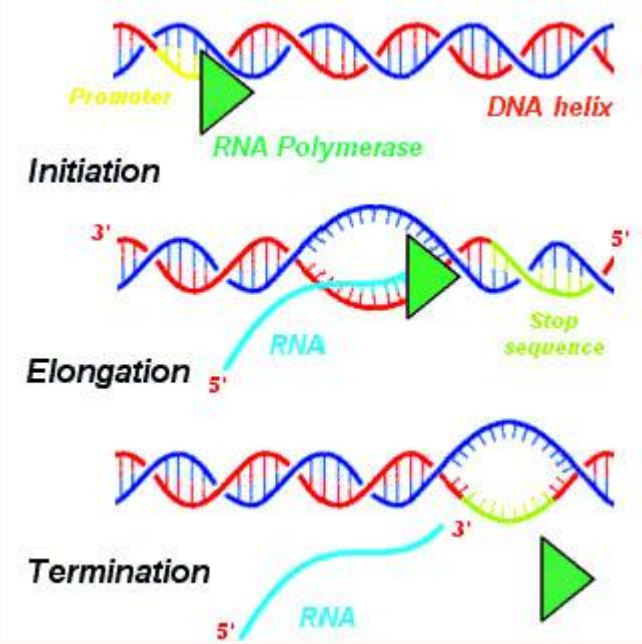


Fig. 7 Process of transcription

10.9.2 Genetic code

a. It is regarded as dictionary of nucleotide bases that determines the amino acid sequence in proteins.
b. Genetic code was elucidated during 1960s by Har Gobind Khorana and Marshall W. Nirenberg, who shared a Nobel Prize in 1968.
c. Khorana and Nirenberg used artificial templates and protein synthesizing systems in the test tube to determine the coding potential of all 64 possible triplet codons.
d. The key feature of the genetic code is that the 20 amino acids are encoded by 61 codons. Thus, there is degeneracy in the code such that one amino acid is often specified by more than one codon. Example: In the case of serine and leucine, six codons can be used for each.
e. The code appears to be almost universal, from bacteria through archaea to eukaryotes with some exceptions found in the mitochondria of humans and many other organisms as well as in some species of bacteria.

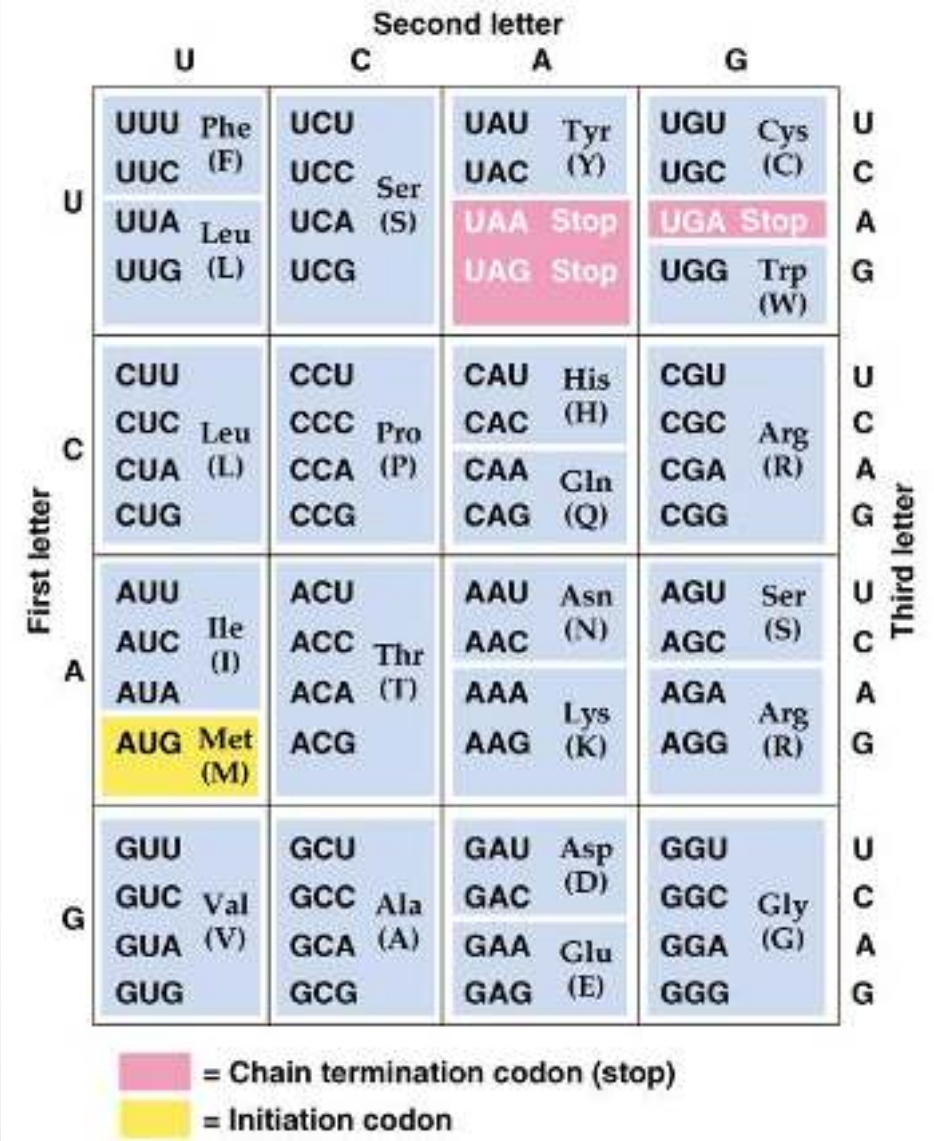


Fig. 8 Genetic code

Features of genetic code

a. **Genetic code is a triplet:** Genetic code is made up of three nucleotides and forms 64 different combinations. A single triplet is a codon.

b. **Universal:** The genetic code is nearly universal which means it is present in all organisms on earth. What amino acid a triplet forms in one organism, the same amino acid it forms in all organisms. Starting from single-cell prokaryotes to eukaryotes, all life has the same kind of genetic codes. All living organism forms all their 20 amino acids from these 64 permutations of a codon. Also, the start and stop codons are universally present in all.

c. **The genetic code is highly degenerate:** DNA has 4 letters and code has a triplet of it which means 64 different combinations can be possible. But only 20 different amino acids exist which literally means that more than one codon is for a single amino acid. For example, the proline is translated from 4 different codons viz. CCU, CCC, CCA, and CCG. Notably, AUG and UGG are the only two codons that encode only a single amino acid.
d. **The genetic code is non-overlapping:** A single code encodes only a single amino acid which means it can't be shared to form another amino acid.

Fig. 9 The non-overlapping property of genetic code

e. **The code is unambiguous:** This means that a single codon always forms an amino acid whenever it undergoes transcription. Also, it can't form another different amino acid.
f. **The code is continuous and comma less:** A chain of genetic codes or codons is written in a single continuous line without any comma. No lines or any indication can be allowed in an mRNA chain.

Fig. 10 The genetic code is comma less

g. **The reading frame:** The reading frame determines the order of mRNA from which a protein is formed. Every reading frame starts with the AUG start codon and then expends to the end codon. The thumb rule to read the reading frame is that it should be read from 5’ to 3’ direction from the start codon. However, in eukaryotes, the open reading frame sequences (exons) are interrupted by the introns which are removed during the mRNA synthesis. “The sequence starting from the AUG (start codon) and stop codon which translates into protein is known as an open reading frame (ORF)”.

Fig. 11 The example of a ORF with start and stop codon

h. **Start codon:** A genetic code is initiated by the defined three-nucleotide codon known as the start codon from where the translation initiates. AUG is one of the most common start codons present in all organisms and encodes for the amino acid methionine. Besides, GUG and UUG are also found in some organisms as the start codons which encode valine and leucine amino acids, respectively.

i. **Stop codon:** A stop codon gives a signal to stop the translation of protein. Three stop codons are known are named ‘amber (UAG)’, ‘opal (UGA)’ and ‘ochre (UAA)’. The stop codon is also known as a nonsense codon. If it is inserted into the DNA sequence prematurely, it creates mutations. Conclusively, the stop codon in the mRNA gives a signal to detach from the ribosome assembly and a truncated protein is formed.

j. **Codon redundancy:** The genetic code follows the mechanism of redundancy means; different codons form a single amino acid but a single codon can’t take part in the formation of different amino acids.

k. **Mutations and genetic code:** Mutations are the greatest things to induce new alterations in nature. Three of the common genetic mutations viz. point mutations, missense mutations and non-sense mutations can change the genetic code.

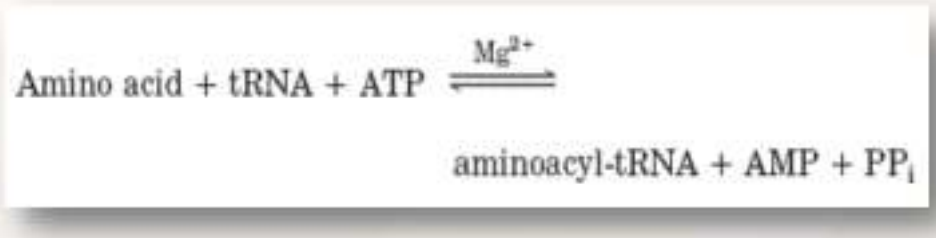
10.9.3 Translation

a. The process of translation uses the information present in the nucleotide sequence of mRNA to direct the synthesis of a specific protein for use by the cell.
b. Translation takes place on the ribosomes—complex particles in the cell that contain RNA and protein.
c. In prokaryotes the ribosomes are loaded onto the mRNA while transcription is still ongoing.
d. Near the 5′ end of the mRNA, a short sequence of nucleotides signals the starting point for translation. It contains a few nucleotides called a ribosome binding site, or Shine-Dalgarno sequence.
e. In E. coli the tetranucleotide GAGG is sufficient to serve as a binding site.
f. This typically lies five to eight bases upstream of an initiation codon.
g. The mRNA sequence is read three bases at a time from its 5′ end toward its 3′ end, and one amino acid is added to the growing chain from its respective aminoacyl tRNA, until the complete protein chain is assembled.
h. Translation stops when the ribosome encounters a termination codon, normally UAG, UAA, or UGA.
i. Special release factors associate with the ribosome in response to these codons, and the newly synthesized protein, tRNAs, and mRNA all dissociate.
j. The ribosome then becomes available to interact with another mRNA molecule.
k. In eukaryotes, the essence of protein synthesis is the same, although the ribosomes are more complicated. As with prokaryotic initiation, the signal sequence interacts with the 3′ end of the small subunit rRNA during formation of the initiation complex.
l. The issue of fidelity is important during protein synthesis, but it is not as crucial as fidelity during replication. Because, one mRNA molecule can be translated repeatedly to give many copies of the protein.

m. When an occasional protein is mistranslated, it usually does not fold properly and is then degraded by the cellular machinery. However, proofreading mechanisms exist within the ribosome to ensure accurate pairing between the codon in the mRNA and the anticodon in the tRNA.

Protein biosynthesis takes place in four stages

Stage 1: Activation of AAs: Aminoacyl-tRNA synthetases attach the correct amino acids to their tRNAs



Stage 2: A specific AA initiates protein synthesis

a. Protein synthesis begins at amino-terminal end and proceeds by stepwise addition of amino acids to carboxyl-terminal end of growing polypeptide.
b. AUG initiation codon specifies an amino-terminal formyl methionine (fMet) residue in prokaryotes.
c. Initiation of polypeptide synthesis in bacteria requires (1) 30S ribosomal subunit, (2) mRNA coding for polypeptide to be made, (3) initiating fMet-tRNAfMet, (4) a set of three proteins called initiation factors (IF-1, IF-2, and IF-3), (5) GTP, (6) 50S ribosomal subunit, and (7) Mg+2.
d. Formation of initiation complex takes place in three steps.
    I. In step 1 the 30S ribosomal subunit binds two initiation factors, IF-1 and IF-3.
    II. Factor IF-3 prevents the 30S and 50S subunits from combining prematurely.
    III. mRNA then binds to 30S subunit. Initiating (5') AUG is guided to its correct position by the Shine-Dalgarno sequence (named for Australian researchers John Shine and Lynn Dalgarno, who identified it) in mRNA.

IV. This mRNA-rRNA interaction positions the initiating (5') AUG sequence of mRNA in the precise position on the 30S subunit where it is required for initiation of translation.

V. Bacterial ribosomes have three sites that bind aminoacyl-tRNAs, the aminoacyl (A) site, the peptidyl (P) site, and the exit (E) site.

VI. Initiating (5') AUG is positioned at the P site, the only site to which fMet-tRNAfMet can bind.

VII. fMet-tRNAfMet is the only aminoacyl-tRNA that binds first to P site; during subsequent elongation stage, all other incoming aminoacyl-tRNAs (including the Met-tRNAMet that binds to interior AUG codons) bind first to the A site and only subsequently to P and E sites.

VIII. E site is the site from which the "uncharged" tRNAs leave during elongation.

IX. Factor IF-1 binds at A site and prevents tRNA binding at this site during initiation.

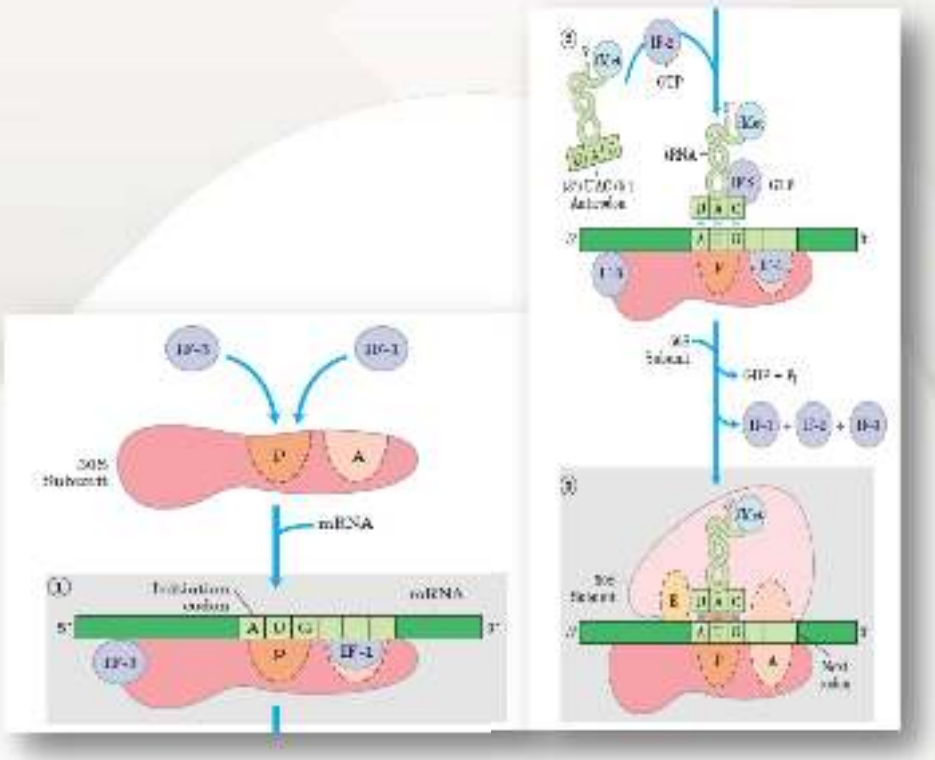


Fig. 12. Initiation phase of translation

X. In step 2, the complex consisting of 30S ribosomal subunit, IF-3, and mRNA is joined by both GTP-bound IF-2 and initiating fMet-tRNAfMet.

XI. Anticodon of this tRNA now pairs correctly with the mRNA's initiation codon.

XII. In step 3 this large complex combines with 50S ribosomal subunit; simultaneously, the GTP bound to IF-2 is hydrolyzed to GDP and Pi, which are released from the complex.

XIII. All three IFs depart from ribosome at this point. (IF3 is released causing GTP to hydrolyze to GDP, which in turn releases IF2-GDP). GTP hydrolysis promotes the release of IF1 and subsequent association of 30S subunit with 50S subunit.

XIV. Completion of these steps produces a functional 70S ribosome called the initiation complex, containing the mRNA and the initiating fMet-tRNAfMet.

Stage 3: Peptide bonds are formed in the elongation stage

a. Elongation requires (1) initiation complex, (2) aminoacyl-tRNAs, (3) three soluble cytosolic proteins called elongation factors (EF-Tu, EF-Ts, and EF-G), and (4) GTP.
b. Cells use three steps to add each amino acid residue, and steps are repeated as many times as there are residues to be added.

Elongation step 1: Binding of an incoming aminoacyl-tRNA

a. In first step of elongation cycle, the appropriate incoming aminoacyl-tRNA binds to a complex of GTP-bound EF-Tu.
b. The resulting aminoacyl-tRNA–EF-Tu–GTP complex binds to A site of 70S initiation complex.
c. GTP is hydrolyzed and an EF-Tu–GDP complex is released from 70S ribosome. EF-Tu–GTP complex is regenerated in a process involving EF-Ts and GTP.

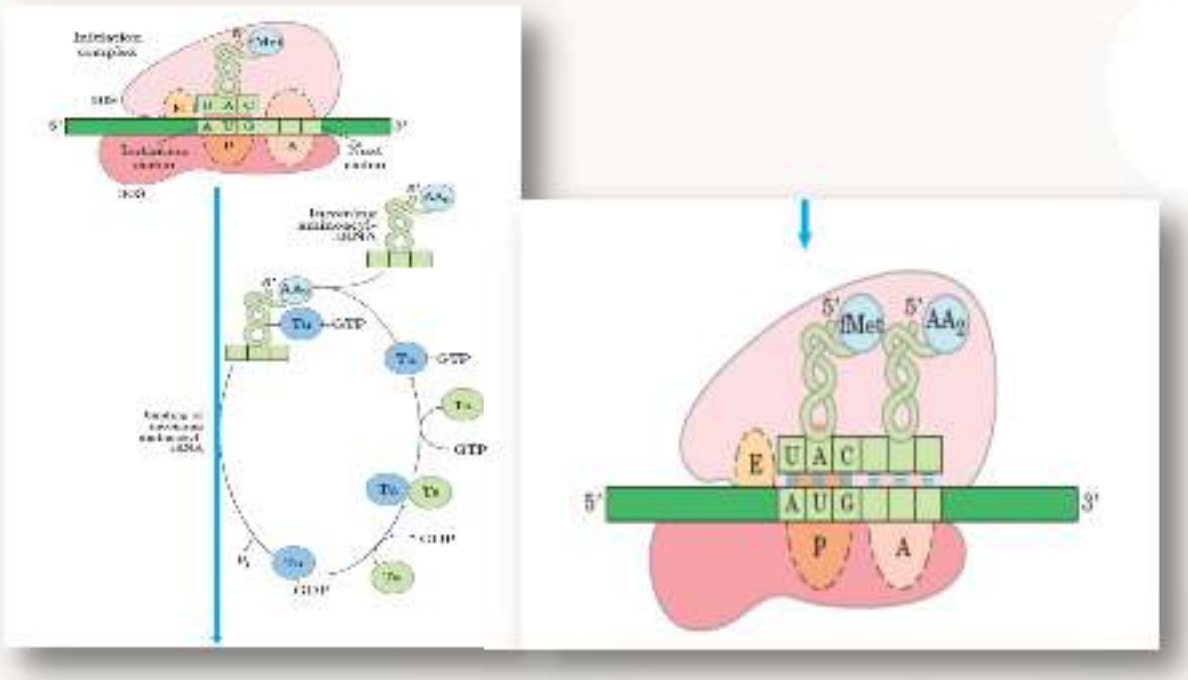
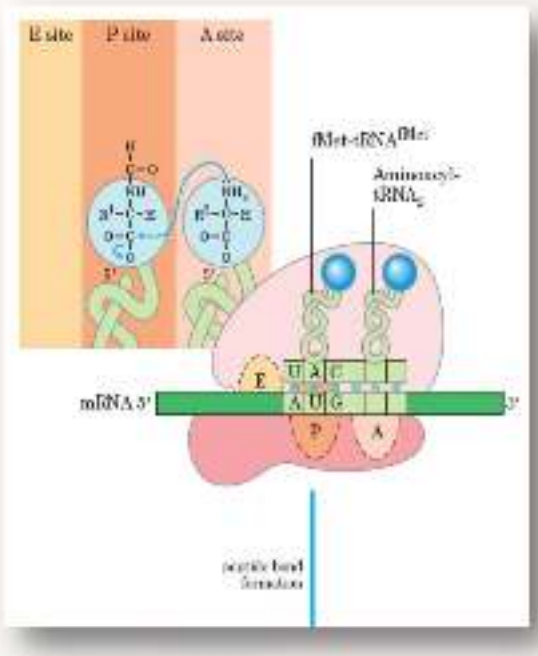
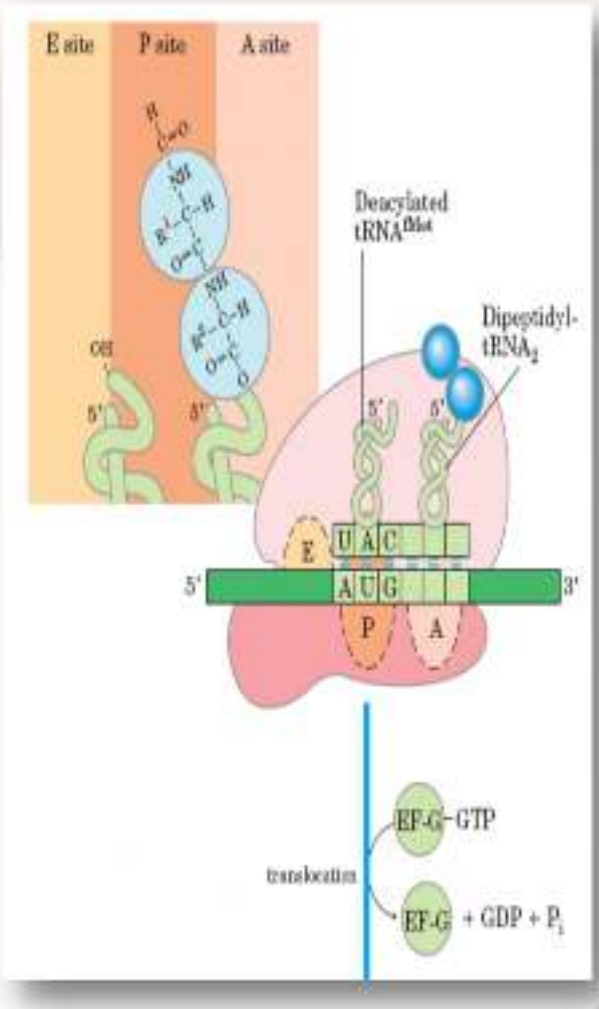


Fig 13: Step 1 -Elongation phase

Elongation step 2: Peptide bond formation

a. A peptide bond is now formed between two amino acids bound by their tRNAs to A and P sites on ribosome.
b. This occurs by transfer of initiating N-formyl-methionyl group from its tRNA to amino group of second amino acid, now in A site.
c. α-amino group of amino acid in A site acts as a nucleophile, displacing the tRNA in P site to form peptide bond. This reaction produces a dipeptidyl-tRNA in A site, and now “uncharged” (deacylated) tRNAfMet remains bound to P site.
d. Enzymatic activity that catalyses peptide bond formation has been referred to as peptidyl transferase and this reaction is catalysed by the 23S rRNA, a ribozyme.


E site
P site
A site
Aminoacyl-
mRNA 5'
3'
E
U A C
A U G
P
A


E site
P site
A site
Deacylated
Dipeptidyl-
OH
5'
3'
E
U A C
A U G
P
A
translocation
+ GDP + P_i

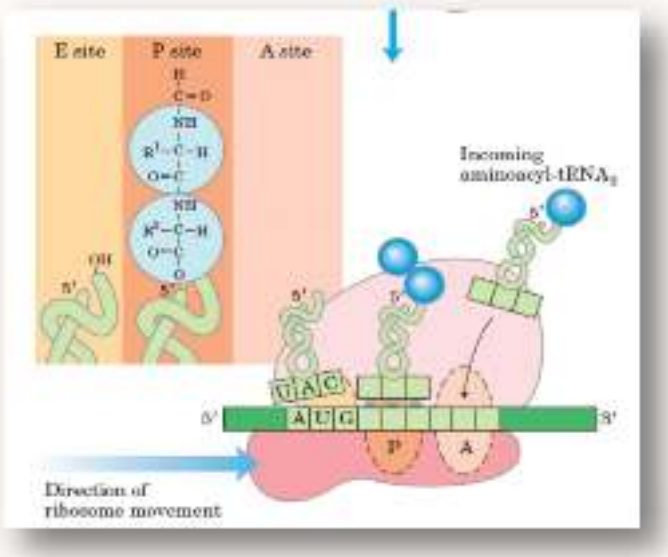


Fig. 14. Elongation phase of translation

Elongation step 3: Translocation

a. In translocation step of elongation cycle, ribosome moves one codon toward 3' end of mRNA.
b. This movement shifts the anticodon of dipeptidyl-tRNA (which is still attached to second codon of mRNA) from A site to P site, and shifts the deacylated tRNA from P site to E site, from where the tRNA is released into cytosol.
c. Third codon of mRNA now lies in A site and second codon in P site.
d. Movement of ribosome along mRNA requires EF-G (also known as translocase) and energy provided by hydrolysis of another molecule of GTP.
e. Ribosome, with its attached dipeptidyl-tRNA and mRNA, is now ready for the next elongation cycle and attachment of a third amino acid residue.
f. This process occurs in same way as addition of second residue.
g. For each amino acid residue correctly added to growing polypeptide, two GTPs are hydrolyzed to GDP and Pi as ribosome moves from codon to codon along mRNA toward 3' end.
h. Polypeptide remains attached to tRNA of most recent amino acid to be inserted.

Stage 4: Termination of polypeptide synthesis requires a special signal

a. Elongation continues until ribosome adds last amino acid coded by mRNA.
b. Termination is signalled by the presence of one of three termination codons in the mRNA (UAA, UAG, UGA), immediately following the final coded amino acid.
c. In bacteria, once a termination codon occupies ribosomal A site, three termination factors, or release factors—the proteins RF-1, RF-2, and RF-3—contribute to (1) hydrolysis of terminal peptidyl-tRNA bond; (2) release of free polypeptide and last tRNA, now uncharged, from P site; and (3) dissociation of the 70S ribosome into its 30S and 50S subunits, ready to start a new cycle of PP synthesis.

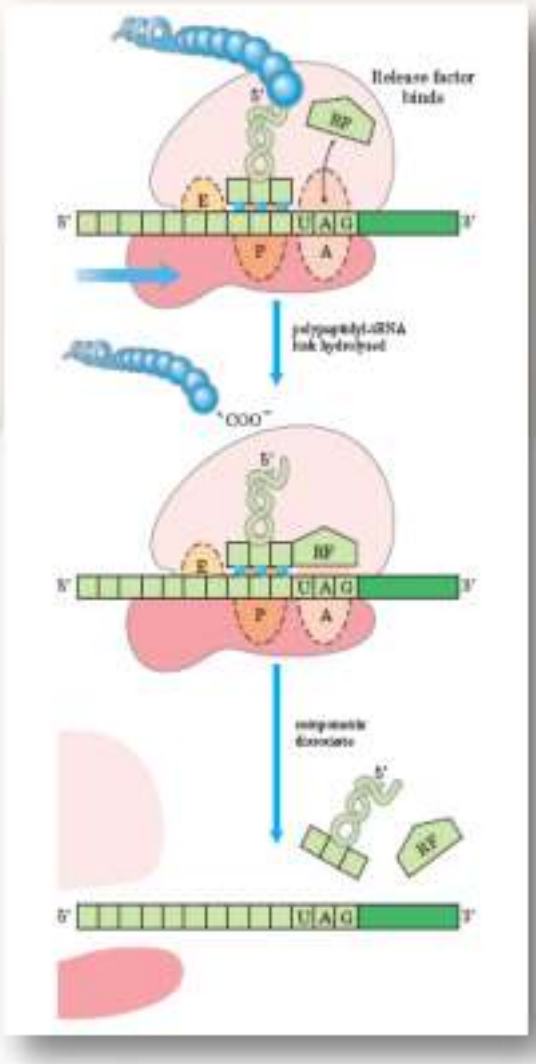


Fig. 15. Termination phase of translation

10.10 Bioenergetics

a. Living cells and organisms must perform work to stay alive, to grow, and to reproduce.
b. The ability to harness energy and to channel it into biological work is a fundamental property of all living organisms and it must have been acquired very early in cellular evolution.
c. Modern organisms carry out a remarkable variety of energy transductions, conversions of one form of energy to another.
d. They use the chemical energy of fuels to bring about the synthesis of complex, highly ordered macromolecules from simple precursors.
e. They also convert the chemical energy of fuels into concentration gradients (proton motive force) and electrical gradients, into motion and heat, and, in a few organisms such as fireflies and some deep-sea fish, into light.
f. Photosynthetic organisms transduce light energy into all these other forms of energy.
g. Bioenergetics is the quantitative study of the energy transductions that occur in living cells and of the nature and function of the chemical processes underlying these transductions.
h. Gibbs free energy (G): It expresses the amount of energy capable of doing work during a reaction at constant temperature and pressure.
i. When a reaction proceeds with release of free energy (i.e. when the system changes so as to possess less free energy), the free-energy change, ΔG, has a negative value and the reaction is said to be exergonic. In endergonic reactions, the system gains free energy and ΔG is positive.

- Endergonic - any reaction that requires an input of energy
- Exergonic - any reaction that releases free energy

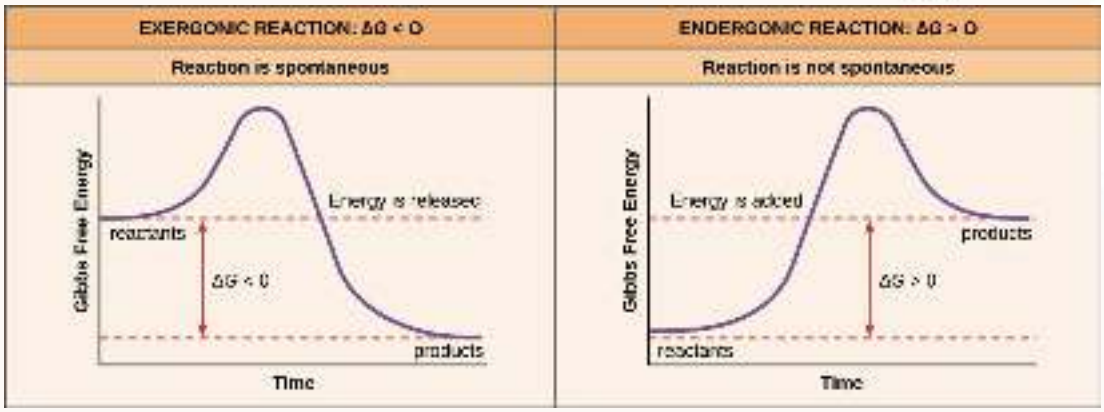


Fig. 16 Exergonic and Endergonic reaction

j. Enthalpy (ΔH):

- It is the heat content of the reacting system (P is constant).
- It reflects the number and kinds of chemical bonds in the reactants and products.
- When a chemical reaction releases heat, it is said to be exothermic and the heat content of the products is less than that of the reactants and ΔH has, by convention, a negative value.
- Reacting systems that take up heat from their surroundings are endothermic and have positive values of ΔH.

k. Entropy (ΔS)

- It is a quantitative expression for the randomness or disorder in a system.
- When the products of a reaction are less complex and more disordered than the reactants, the reaction is said to proceed with a gain in entropy.
- The units of ΔG and ΔH are joules/mole or calories/mole. (1 cal=4.184 J)
- The units of ΔS are joules/mole. Kelvin (J/mol. K) or Jmol-1K-1.
- Under the conditions existing in biological systems (including constant T and P), changes in free energy, enthalpy, and entropy are related to each other quantitatively by the equation-

$$\Delta G = \Delta H - T\Delta S$$

Where, T is the absolute temperature

- By convention, ΔS has a positive sign when entropy increases and ΔH has a negative sign when heat is released by the system to its surroundings.

- Either of these conditions, which are typical of favorable processes, tends to make ΔG negative. In fact, ΔG of a spontaneously reacting system is always negative.
- Cells require sources of free energy
- Cells are isothermal systems—they function at essentially constant temperature (and at constant pressure).
- Energy that cells can and must use is free energy, described by the Gibbs free-energy function G, which allows (1) prediction of the direction of chemical reactions, (2) their exact equilibrium position, and (3) the amount of work they can perform at constant temperature and pressure.

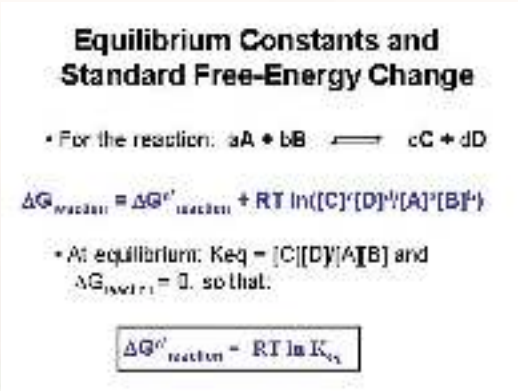


Table 2. Standard free energy changes of few chemical reactions at pH 7.0 and 25°C (298K)

10.10.1 Irreversible Reactions

a. A fundamental concept of chemistry is that chemical reactions occurred when reactants reacted with each other to form products.
b. These unidirectional reactions are known as irreversible reactions, reactions in which the reactants convert to products and where the products can't convert back to the reactants.
c. For example, glucose is converted to glucose-6-phosphate by the enzyme hexokinase which is an irreversible reaction. One molecule of ATP is used for this conversion.

10.10.2 Reversible Reactions

a. In reversible reactions, the reactants and products are never fully consumed; they are each constantly reacting and being produced.
b. For example, there are four binding sites on a hemoglobin protein.
c. Hemoglobin molecules can either bind to carbon dioxide or oxygen.
d. As blood travels through the alveoli of the lungs, hemoglobin molecules pick up oxygen-rich molecules and bind to the oxygen.
e. As the hemoglobin travels through the rest of the body, it drops off oxygen at the capillaries for the organ system to use oxygen.
f. After expelling the oxygen, it picks up carbon dioxide.
g. Because this process is constantly carried out through the body, there are always hemoglobin molecules picking or expelling oxygen and other hemoglobin molecules that are picking up or expelling carbon dioxide.
h. Therefore, the hemoglobin molecules, oxygen, and carbon dioxide are reactants while the hemoglobin molecules with oxygen or carbon dioxide bound to them are the products.
i. In this closed system, some reactants convert into products as some products are changing into reactants, making it similar to a reversible reaction.

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