UNIT 9

BIOMOLECULES

Structure

9.1 INTRODUCTION

In the present unit of biochemistry, we will study the chemical nature of molecules that form the part of any living system, whether it is plant or animal. The chemistry of living organisms is organized around carbon, hydrogen, oxygen and nitrogen which together make up to more than 90% of the mass of most cells. Biomolecules contain covalently bonded carbon backbone to which other carbon, hydrogen, oxygen or nitrogen atoms may be attached. As you know, carbon can form single bonds with hydrogen atoms and both single and double bonds with oxygen and nitrogen atoms. Covalently linked carbon atoms in biomolecules can form linear, branched chains and cyclic structures as well. Most of the biomolecules can be regarded as derivatives of hydrocarbons. Hydrogen atoms in the hydrocarbon chain may be substituted by a variety of functional groups to yield different families of organic compounds, which include alcohols, amines, aldehydes, ketones and carboxylic acids.

Biomolecules comprise large macromolecules such as carbohydrates, lipids and proteins as well as small molecules such as primary metabolites, secondary metabolites and natural products. Large macromolecules are assembled from small simple precursors having high molecular weights. Synthesis of macromolecules is energy consuming activity of cells. The covalent bonds and functional groups of biomolecules such as carbohydrates, lipids and proteins are basically central to their function. We will discuss three main classes of biomolecules-carbohydrates, lipids and proteins in this unit.

Objectives

After studying this unit, you should be able to:

- explain the monomeric units of carbohydrates and proteins,
- illustrate the structure of several biomolecules,
- describe the classification of carbohydrates, lipids and proteins, and
- discuss the general properties of biomolecules.

9.2 CARBOHYDRATES

Carbohydrates form one of the major classes of biomolecules along with proteins and lipids in the living system and as such they are the most abundant class of organic molecules found on the earth. Carbohydrates are generally distributed both in plants and animals. Energy from the sun utilized by green plants, algae, and some bacteria during photosynthesis is stored in the form of carbohydrates. You come across them in your daily life in numerous forms of food items such as bread, rice, potatoes, cookies, candy, table sugar, soft drinks, jams and fruit juices, salad etc.

Carbohydrates are also known as saccharides, a name derived from Greek word “Sakchron” that means sweet due to their sweet taste. The word carbohydrate literally means ‘carbon hydrates’ and is expressed by a general formula (CH_2O), where n is any positive number. Many but not all of the molecules belonging to carbohydrates follow empirical formula $(CH_2O)n$, that implies that the ratio of carbon to hydrogen and to oxygen atom is 1:2:1. Some of them contain nitrogen, phosphorus or sulfur also.

Carbohydrates are chemically polyhydroxy aldehydes or ketones. A carbohydrate molecule which contains an aldehyde group is called an aldose and the molecule having ketone group is called ketose. They have been divided into four major classes depending on the number of monomer units they contain. Monosaccharides are the simplest carbohydrates in this classification as they cannot be broken to smaller carbohydrates. These molecules (mono- single) have only one unit. Disaccharides (di- two) contain two monosaccharide units linked together. Oligosaccharides (oligo- few) consists of 3-20 monosaccharide units. Polysaccharides (poly- many) are the most abundant carbohydrate found in food, it has more than 20 to hundreds or thousands of monosaccharide units bound together.

9.2.1 Monosaccharides-"The simple Sugars"

Monosaccharides are crystalline, colorless compounds. These molecules are either polyhydroxy aldehydes or polyhydroxy ketones which cannot be hydrolyzed to simpler compounds. These molecules are readily soluble in water and insoluble in nonpolar solvents such as diethyl ether and benzene. These molecules have sweet taste. Monosaccharides are further subdivided based on

a) the number of carbon atoms present in their structure,

b) the type of carbonyl group, and

c) chirality.

Most of the natural monosaccharides exhibit a chain length of 3-7 carbons, for that reason the names have been given for these classes (Figs 9.1 and 9.2).

1. Trioses ($C_3H_6O_3$) - These carbohydrates are the first and smallest monosaccharide having three carbon chains. Triose with aldehyde group is called as aldotriose (glyceraldehyde) and if it contains ketone group, it is called as ketotriose (dihydroxyacetone).
2. Tetroses ($C_4H_8O_4$) – Four carbon chain monosaccharide molecules are called as aldotetroses (Erythrose, Threose) or ketotetroses. (Erythrulose).
3. Pentoses ($C_5H_{10}O_5$) - Monosaccharide molecules with five carbon chains are known as aldopentoses (Ribose, Xylose, Arabinose, D-Lyxose) or ketopentoses (Ribulose, Xylulose).
4. Hexoses ($C_6H_{12}O_6$) – Six carbon chain monosaccharide molecules are present as aldohexoses (Glucose, Allose, Altrose, Mannose, Gulose, Idose, Galactose, Talose) or ketohexoses (Fructose, Sorbose, Tagatose, Psicose).
5. Heptoses ($C_7H_{14}O_7$) – Seven chain monosaccharide molecules are present as aldoheptoses and ketoheptoses.

A) Monosaccharides have asymmetric carbon

You may recall from your school chemistry text books, carbon atom has a valency of four. So carbon atom whose four valencies are fulfilled by four different groups is called asymmetric or *chiral*. Monosaccharides due to presence of one or more chiral carbon atom in their structure are optically active; they can rotate the plane of polarized light. When a beam of plane polarized light is passed through their solution, these compounds will rotate the light either to right or to left. The phenomenon (degree of rotation of plane polarized light) is measured using an instrument known as polarimeter. If solution rotates the plane of polarized light towards right, it is said to be dextrorotatory and is denoted by + or d sign. Similarly solution of compounds rotating the plane of the light towards left side are called levorotatory and denoted by – or l sign. If a solution has equimolar amounts of dextro and laevo forms, then the resultant solution exhibit no optical activity and such a mixture is called a **racemic mixture**.

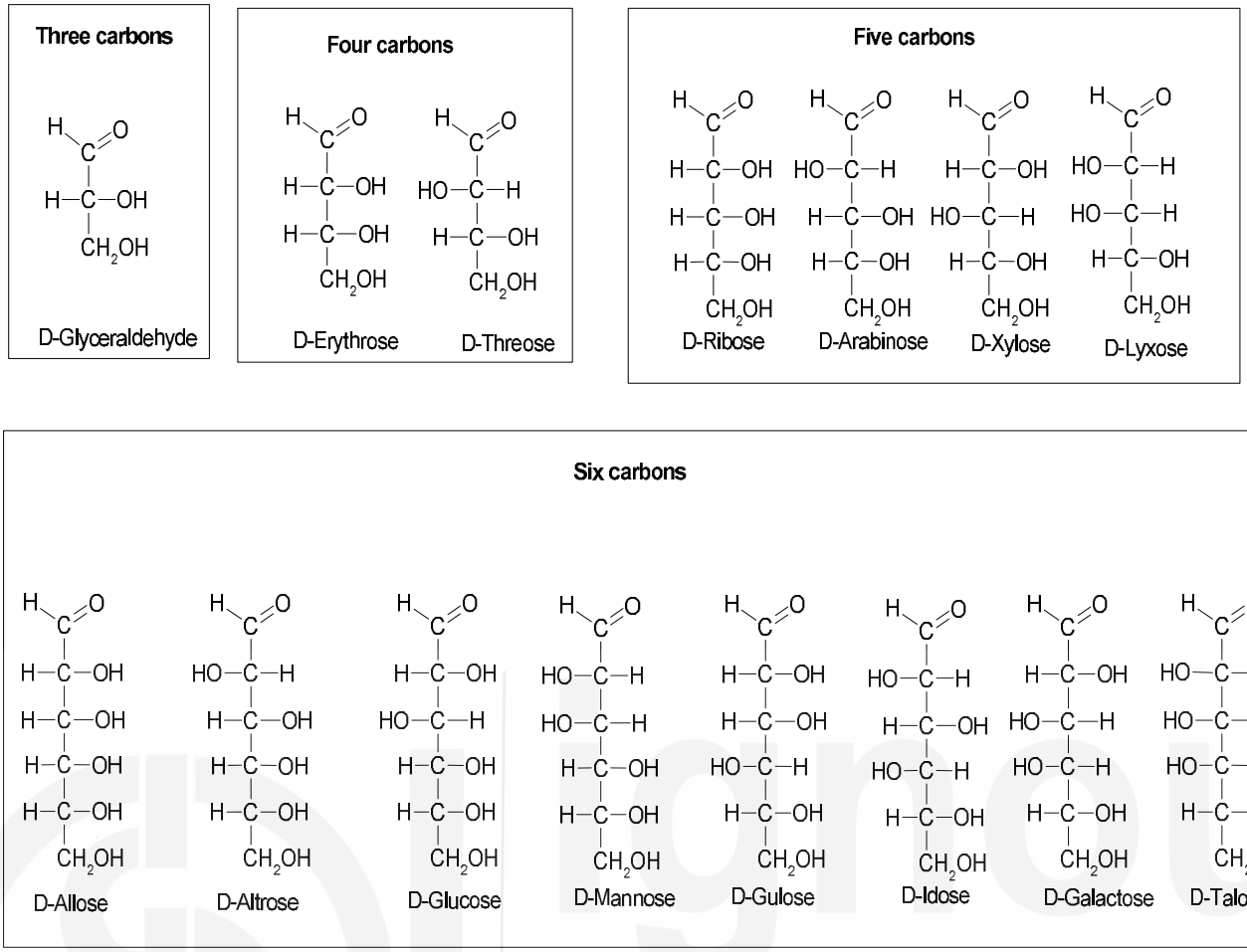


Fig. 9.1: D-Aldose Monosaccharides.

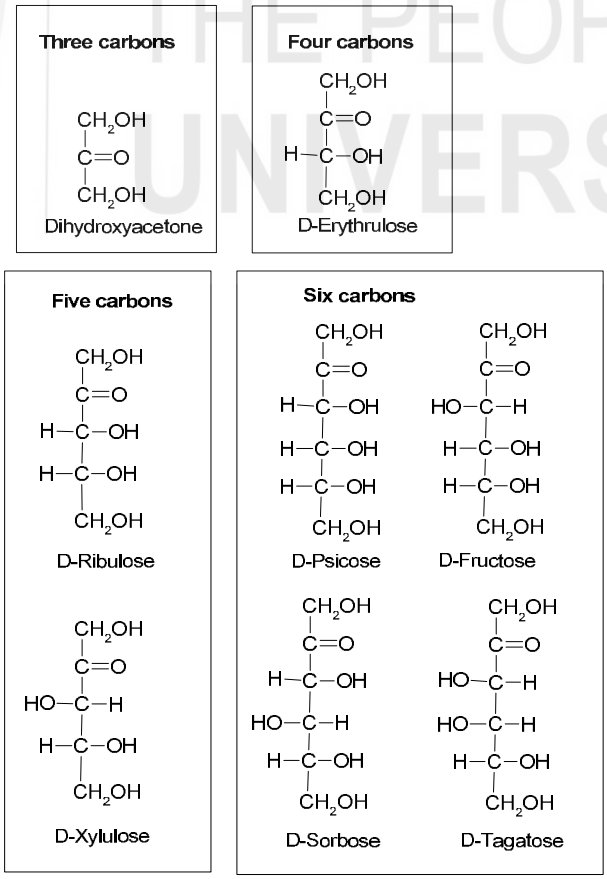


Fig. 9.2: D-Ketose Monosaccharides.

The existence of asymmetric carbon atom gives rise to isomers. Generally, a molecule with n chiral carbon atom can have 2^n stereoisomers. Molecules that are identical in composition but differ only in spatial configuration, are called stereoisomers. All the monosaccharides excluding dihydroxyacetone have one or more asymmetric (chiral) carbon atoms and therefore exist in optically active isomeric forms. Glyceraldehyde, an aldose carbohydrate has one chiral carbon atom (the middle carbon atom) and hence has two different optical isomers (D-Glyceraldehyde and L-Glyceraldehyde), or enantiomers (Fig. 9.3). Enantiomers are defined as pair of chiral molecules or steroisomers that are non-superimposable mirror images of one another. All the chiral carbons atoms in such molecules have opposite configuration. By convention, one of these two enantiomers is designated as the **D** isomer, the other as **L** isomer.

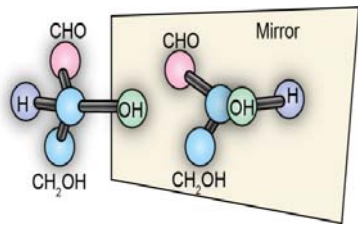


Fig. 9.3: Enantiomers of D-Glyceraldehyde.

You should note that while writing the chemical formula of a monosaccharide, the spatial configuration of –H and –OH groups assumes importance since they contain asymmetric carbon atom, e.g., D-and L-forms of glyceraldehydes (Fig. 9.4). The penultimate carbon serves as the reference carbon atom for naming the mirror images. The orientation of –H and –OH groups around the carbon atom just adjacent to the terminal primary alcohol carbon (penultimate carbon) determines as to which form the sugar belongs, the D-form or the L-form. If the –OH is on the right it belongs to D-form or D-series and if the –OH is to left it belongs to L-form or L-series. **In nature, D form of carbohydrates are more abundant than L form.** Stereo chemically all D- aldoses can be considered to be related to D- Glyceraldehyde and L- aldoses to L-Glyceraldehyde.

How will you represent these enantiomers on paper? The answer to the question was given by Fischer who puts forward Fischer projection formulas to represent three-dimensional sugar structures on paper. In the Fischer projection formulae, atoms joined to an asymmetric carbon atom by horizontal bonds project out of the plane of the paper, toward the reader (you); vertical bonds project behind the plane of the paper are represented away from the reader (you) (Fig. 9.4).

D-Glyceraldehyde L-Glyceraldehyde

Fischer projection formulas

D-Glyceraldehyde L-Glyceraldehyde

Perspective formulas

Fig. 9.4: Fischer projection formulas and perspective formula representation of the enantiomers of glyceraldehydes.

Stereochemistry (D/L form) does not represent the optical rotation of plane polarized light (+/-), for example; both D- Fructose and D- Glucose have same stereochemistry, i.e. -OH group at C-5 on the right side ; but behave differently in terms of optical activity. D- Fructose is levorotatory (-/l) while D-Glucose is dextrorotatory (+/d).

Exercise: Look at the glucose structure. How many carbon atoms are asymmetric in it? How many stereoisomeric forms are possible?

In glucose structure on careful observation, you will see that except two carbon atoms (C1 and C6) all other four carbon atoms are asymmetric, hence glucose is capable of having 2^4=16 stereoisomeric forms.

Look at the structures of D- Erythrose and D-Threose (4 carbon chain aldoses) in figure 9.1, these structures differ in orientation of –OH group only at one chiral center (C-2). These sterioisomers differ from one another only in the configuration with respect to a single carbon atom other than the reference carbon atom and are not mirror images of one another. Such a pair of stereoisomers is called **epimers**. Similarly, look again at figures carefully, you will find some more epimers such as D-Mannose and D-Glucose etc

While looking at the possible number of epimers, you will see stereoisomers that differ in the orientation at more than one chiral carbon; such stereoisomers are called **diastereoisomers** as for example D- Arabinose and D- Xylose. Look at their structures carefully, they are not related as epimers. Diastereoisomers have different physical and optical rotation properties. D-Ribose and D- Lyxose are also examples of another pair of diastereisomers.

SAQ 1

Do as directed.

a) D-Mannose is a ketotriose (True/ False).

b) Ribulose is ketopentose or aldopentose (Pick one option)

c) Generally, molecule with n chiral centers has how many stereoisomers?

d) D form of carbohydrates is more abundant than L form (True/ False).

e) Enantiomers are pair of chiral molecules with non superimposable mirror images (True/ False).

B) Cyclic structure of monosaccharides

We will now try to know the cyclical structure of monosaccharides. In the aqueous solutions, monosaccharides with five or more carbon atoms generally occur in ring structure. The ring structure is formed by an intramolecular (within the molecule) reaction between hydroxyl groups and aldehyde group of aldose or ketone group of ketose. A covalent bond is formed between the carbonyl group and the oxygen of a hydroxyl group along the chain. It leads to the formation of cyclic (ring) adducts (product formed due to addition of the reactants) that are known as hemiacetal and hemiketals, respectively. These cyclic structures now contain an additional asymmetric carbon atom and therefore, can exist in two steroisomeric forms. The asymmetric carbon atom is known as **anomeric carbon.** Anomeric carbon due to chirality can exist as

two stereoisomeric forms called anomers, α and β. Carbon, therefore, at position 1 in case of aldoses and at position 2 in ketoses is anomeric. The formation of two cyclic forms of D-glucose is shown in Fig. 9.5a. The six membered rings formed are known as pyranoses as they bear similarity to six membered ring compound pyran. The systematic names for these ring structures of D-glucose are α-D-glucopyranose and β-D- glucopyranose. Likewise, five membered structures are called furanoses on account of their resemblance to furan. The naturally predominant form of D-fructose is fructofuranose (Fig. 9.5b). However, aldopyranose ring is more stable than aldofurnaose ring.

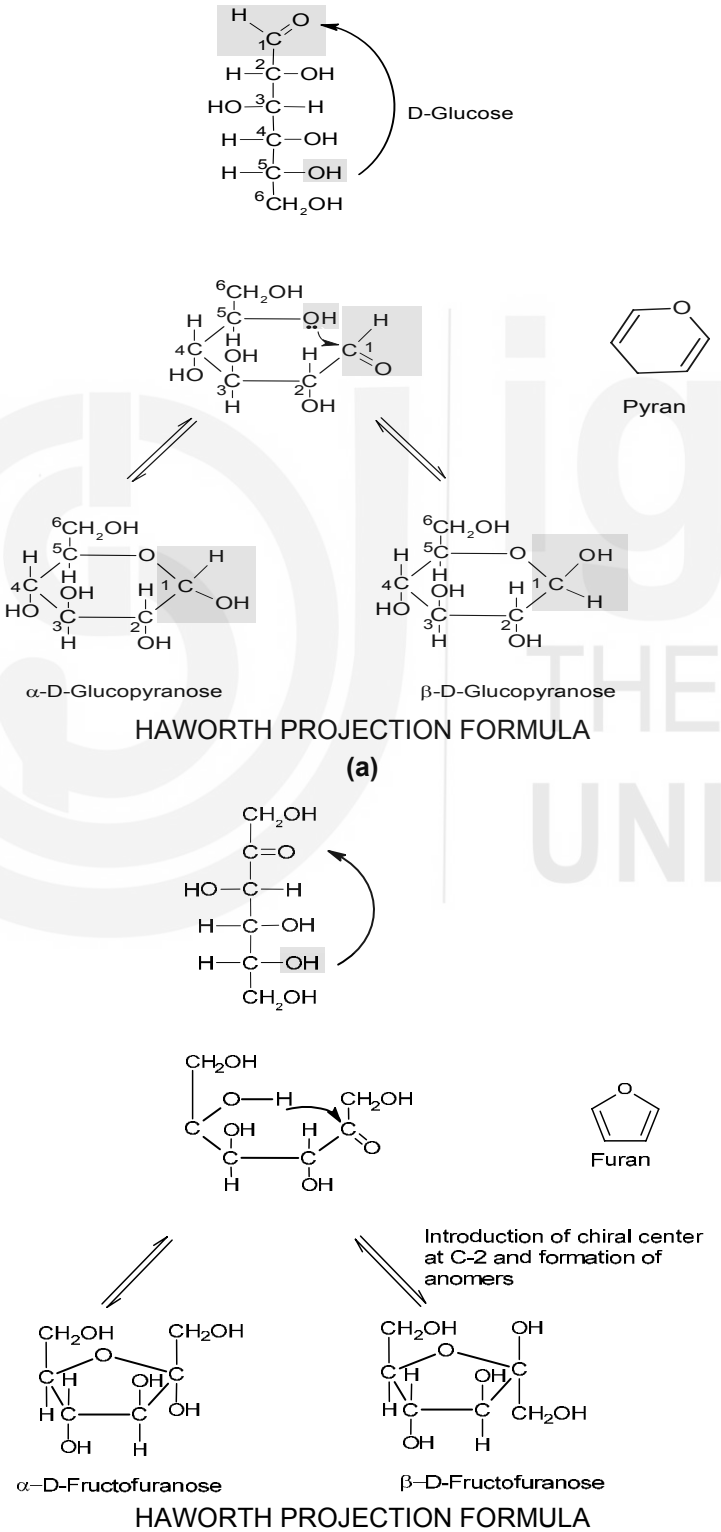


Fig. 9.5: (a) Cyclical forms of D-Glucose, (b) Cyclical forms of D-Fructose.

C) Haworth Projection

The cyclical structures of monosaccharide are represented by Haworth Projections (Fig. 9.5 a & b). A five- or six membered cyclical hemiacetal is represented as a planar pentagon or hexagon, respectively, lying perpendicular to the plane of the paper. Groups (OH) bonded to the carbons of the ring lie either above (those on left side) or below (those on right hand side) the plane of the ring. The last CH_2OH group is placed above the plane of the ring. The new chiral carbon in cyclic structure is called an *anomeric carbon***.** Stereoisomers that differ in configuration only at the anomeric carbon are known as *anomers*. In α form, OH group on the anomeric carbon is represented below the plane of the ring, while in β form it is above the plane of the ring structure.

The α and β anomeric forms of D-glucose interconvert in aqueous solution by **mutarotation**. Therefore, a solution of α-D glucopyranose (optical activity of +112.2°) and solution of β- D glucopyranose (optical activity of +19°) ultimately forms identical equilibrium mixtures (optical activity at +52.7°). The mixture solution consists of 36.4% α-D glucopyranose, 63.6% β- D glucopyranose and less than 0.3% of straight chain D-glucose.

α- D Glucopyranose (36.4%) ↔ D- Glucose (linear form) ↔ β- D Glucopyraose (63.6%)

D) Derivatives of Monosaccharides

1. **Amino sugars** are formed when one of the hydroxyl (–OH) of sugar gets replaced by an amino group ($–NH_2$), e.g. α- D-Glucosamine. An attachment of acetyl group to the amino group leads to the formation of α-D-N- acetylglucosamine. The two structures are shown in Fig. 9.6. These amino sugars are found in many oligo- and polysaccharides, including *chitin,* a polysaccharide in the exoskeletons of crustaceans and insects.

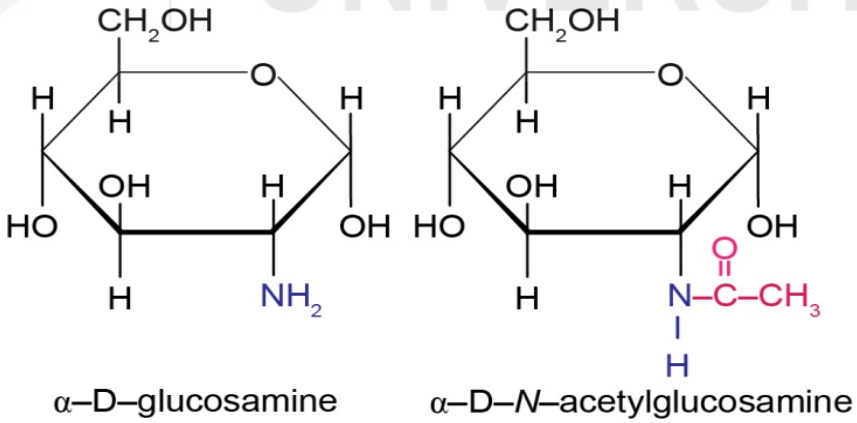
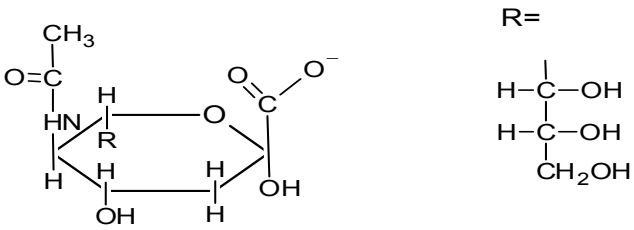


Fig. 9.6: Structures of Amino derivatives of α-D- Glucose: α-D- Glucosamine and α-D- N- Acetylglucosamine.

2. **Sialic acids:** *N*-acetylneuraminic acid is derived from N-acetylmannosamine and pyruvic acid. It is an important component of glycoproteins and glycolipids. The derivatives of *N*-acetylneuraminic are collectively known as **sialic acids** (Fig. 9.7) and are found widely in bacteria and animal systems.



N-Acetylneuraminic acid
(a sialic acid)

Fig. 9.7: N-Acetyl-D-neuraminic acid (Sialic acid).

3. **D-Ribose and 2-Deoxyribose:** Important derivatives of monosaccharide include D-Ribose and 2-deoxyribose. 2-Deoxyribose is derived from sugar ribose in which hydroxyl group at position 2 is replaced by hydrogen (Fig. 9.8). Deoxyribose and Ribose can also be further phosphorylated. These compounds form an important constituent of nucleic acids DNA and RNA, well known for the genetic information. Deoxy sugars also occur frequently in glycoproteins and polysaccharides.

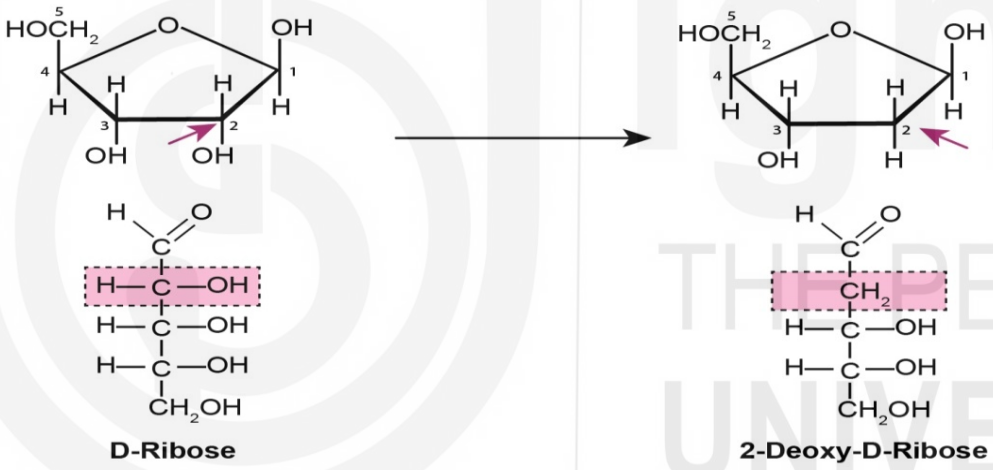


Fig. 9.8: Structures of D- Ribose and 2- Deoxy-D-Ribose.

E) Oxidation of Sugars

The carbonyl carbon of glucose gets oxidized to carboxyl group, resulting to the formation of gluconic acid; similarly aldoses on oxidation form aldonic acids.

Monosaccharides can be oxidized also at the other end of carbon chain at C-6 of glucose, galactose or mannose, yielding **uronic acids,** such as **D-glucuronic, galacturonic or mannuronic** acids. Monosaccharides with free anomeric carbon atoms are good reducing agents and reduce hydrogen peroxide, ferricyanide, certain metals and other oxidizing agents. Therefore, they are also known as reducing sugars.

9.2.2 Disaccharides

As the name implies (*Di- two, saccharide- sugar*) these molecules are made up of two monosaccharide units joined by a glycoside bond. The glycosidic bond is formed when a hydroxyl group of one sugar reacts with the anomeric

carbon of the other. The disaccharides commonly found in nature are sucrose, maltose, and lactose. Except sucrose, each of these structures possesses one free unsubstituted anomeric carbon atom, and thus each of these disaccharides is a reducing sugar. The end of the molecule containing the free anomeric carbon is called the **reducing end,** and the other end is called the **nonreducing end.** In the case of sucrose, both of the anomeric carbon atoms are substituted, that is, neither has a free -OH group. The substituted anomeric carbons cannot be converted to the aldehyde configuration and thus cannot participate in the oxidation–reduction reactions characteristic of reducing sugars. Thus, sucrose is *not* a reducing sugar.

A. Maltose

Maltose also known as malt sugar is an intermediate product of starch hydrolysis. Maltose can be hydrolyzed to its constituent glucose molecules by enzyme maltase present in the small intestine. It is made of two α-D Glucose units joined by (1→ 4) *O*-glycoside bond. It means that C-1 of α-D glucose forms a glycoside bond with C-4 of D-Glucose (Fig. 9.9). A systematic name for maltose is ***O-α-D-Glucopyranosyl-(1→4)-α-D-glucopyranose***.

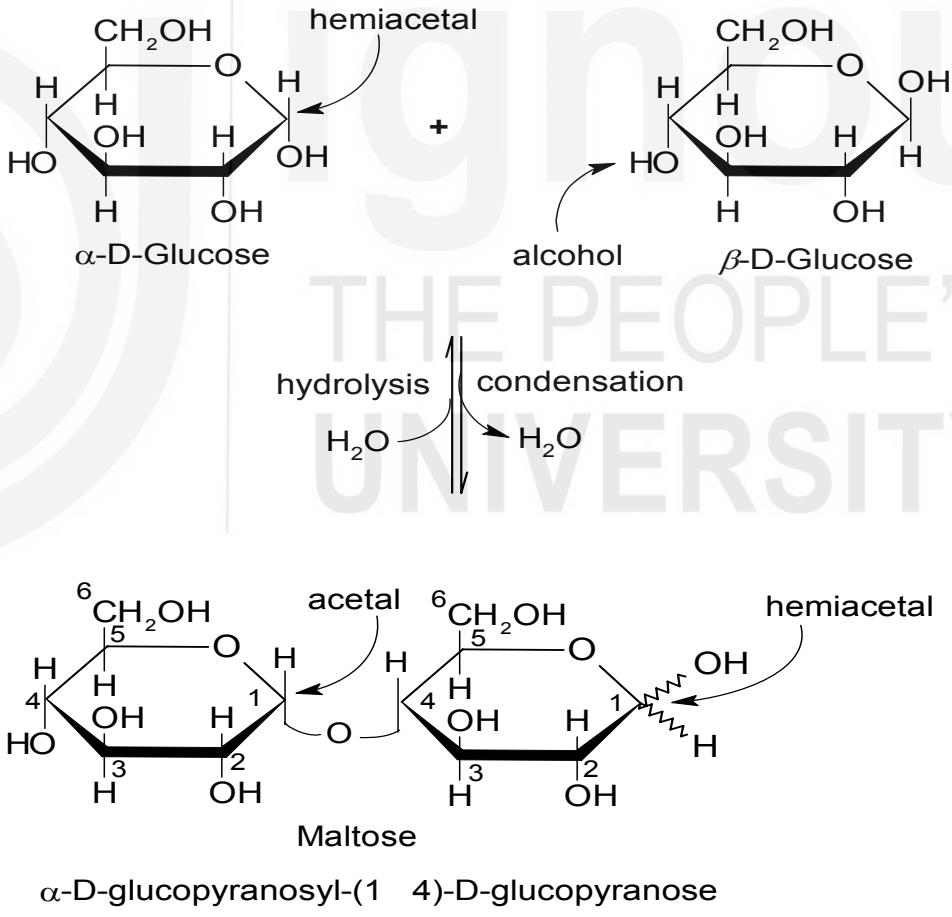


Fig. 9.9: Structure of Maltose.

B. Lactose

Lactose is the reducing sugar. It constitutes for 5 to 8% of human milk and 4-6% of cow's milk and has galactose and glucose as its monosaccharide units. Lactose is a disaccharide of β- D Galactose and D-Glucose joined by a β (1→ 4) O- glycoside bond (Fig. 9.10). The systematic name of lactose is ***O-β-D-Galactopyranosyl-(1→4)-D-glucopyranose.***

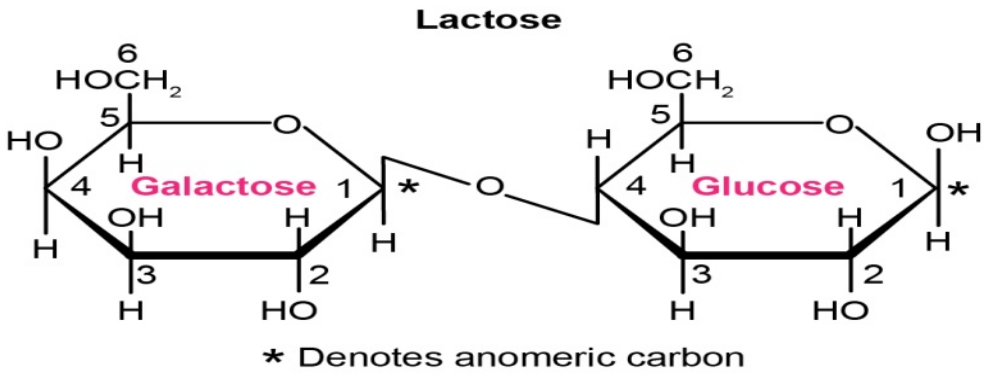


Fig. 9.10: Structure of Lactose.

C. Sucrose

Sucrose, is present in sugarcane and also known as cane sugar. It is a disaccharide of α-D Glucose and β- D Fructose joined by (1 → 2) glycoside linkage (Fig. 9.11). Therefore its systematic name is ***O-α-D-Glucopyranosyl-(1→2)-β-D-fructofuranoside***. Sucrose is a non-reducing sugar because anomeric carbons of both monosaccahrides are involved in glycoside bond formation. Sucrose is the sweetest among all sugars except fructose.

It is the main form in which carbohydrates are transported in plants. It is digested by intestinal enzyme sucrase to give glucose and fructose. Due to its sweet taste, it is incorporated in human diet mainly as sweetener.

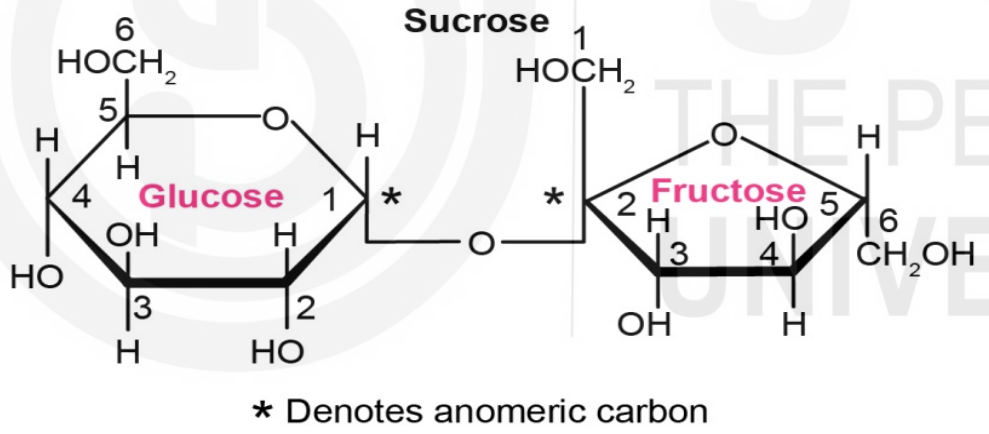


Fig. 9.11: Structure of Sucrose.

SAQ 2

Do as directed.

a) The asymmetric carbon atom is known as anomeric carbon (True/False).

b) The α and β anomeric forms of D-glucose get interconverted in aqueous solution by mutarotation (True/False).

c) Sucrose is a reducing sugar (True/False).

d) The systematic name of lactose is O-β-D-Galactopyranosyl-(1→4)-D-glucopyranose (True/False).

9.2.3 Oligosaccharides and Polysaccharides

In this subsection we will be discussing separately about oligosaccharides and polysaccharides.

Oligosaccharides

Oligosaccharides are short polymers, made up of 3-10 monosacharide units joined by glycosidic bonds. One of its well known examples is raffinose, also called melitose. Raffinose is a trisaccharide largely found in legumes and cruciferous vegetables, including beans, peas, cabbage, brussels sprouts, and broccoli. It consists of galactose connected to sucrose via α (1→6) glycosidic linkage.

Polysaccharides

Polysaccharides are polymers of the simple sugars and their derivatives containing more than 20 or so monosaccharide units, and some have hundreds or thousands of units. Their molecular weights range up to 1 million or more. Polysaccharides can be long straight chains or branched. They have been further divided into two classes:

A. Homopolysaccharides: Polysaccharides contain a single monomer or in other words molecules in which repeating monosaccharides are of same type are known as homopolysaccharides.

B. Heteropolysaccharides: These polysaccharides are made of different types of monosaccharides.

A. Homopolysaccharides

1. **Starch:** It is produced by green plants as major energy reserve, occuring intracellularly in the granular form. Starch is a homopolymer of α-D Glucose, composed of two types of polymers: α- amylose and amylopectin. The amylase content ranges from 10% to 30% and amylopectin ranges from 70% to 90% in the starch. α- Amylose contains long unbranched chains of several thousand α-D Glucose units linked by (1→ 4) glycoside bond. Amylopectin or β-amylose has a coiled structure. It contains straight chains of α-D Glucose units joined by (1→ 4) glycoside bond that branch out at every 24-30 glucose residues by forming (1→6) glycoside bonds. Amylopectin has high molecular weight ranging from 50,000 to 1,000,000 which indicates the presence of 300 to 5,500 glucose units per molecule. Amylose is more soluble in water compared to amylopectin which is sparingly soluble.

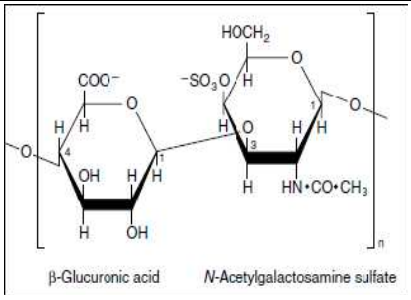
2. **Glycogen:** Glycogen is called as animal starch as it serves as the chief reserve of glucose in them. It is mainly stored in liver and muscles of animals in the form of small granules. Glycogen is a highly branched polysaccharide and structurally similar to amylopectin, but it has comparatively more number of glucose residues and branch points compared to amylopectin.

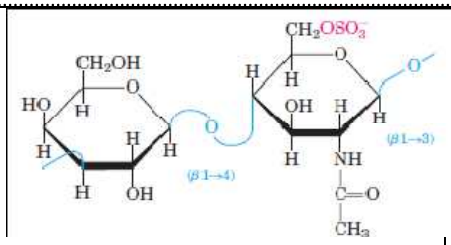
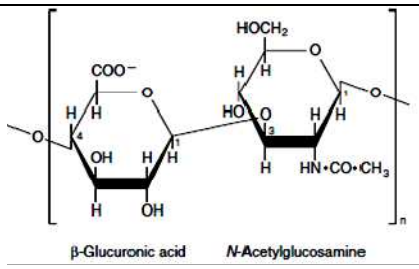
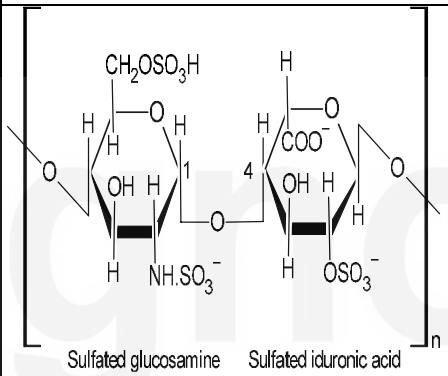
3. **Cellulose:** Cellulose is chief constituent of plant cell walls. It is a linear polymer composed of D-glucopyranose residues linked by β (1,4) glycosidic bonds. Cellulose molecules may contain as many as 12,000 glucose units. Structurally, it differs from α- amylose in the nature of glycoside bond (it has β (1→ 4) linkage instead of α (1→ 4) linkage). Therefore, it is fibrous, tough, white solid, insoluble in water. The digestive tracts of herbivores such as cow contain the enzyme **cellulase** which can hydrolyze β (1→ 4) linkage and digest the plants.

B. Heteropolysaccharides

1. **Glycosaminoglycans (GAGs):** These GAGs are also known as acid *mucopolysaccharides* as majority of these sugar residues are amino derivatives. They possess the ability to absorb lot of water and become viscous which helps in reducing friction. So these molecules can resist compression like a sponge which compresses to a certain extent on applying pressure and then regains its shape. These polysaccharides form main component of the extracellular spaces of connective tissues such as cartilage, tendon, skin. Walls of blood vessel consist of collagen and elastin fibers embedded in a gel-like matrix mainly composed of glycosaminoglycans. Classification of GAGs is made on the basis of the identity of the sugar residues they contain, the type of linkages between these residues and the presence and location of sulphate groups. Based on these criteria, GAGs are of different types: hyaluronic acid, chondroitin sulphate, dermatan sulphate, heparin and heparin sulphate, keratin sulphate (Table 9.1).

Table 9.1: Structure and functions of physiologically important GAGs.

Name of the GAG	Repeating Units Uronic acid: (type of *o-glycoside* linkage): amino sugar	Chemical Structure	Functions
Chondroitin-4-sulfate	D-Glucoronic acid: (β1→3): N-acetyl-D galactosamine-4-sulfate		Delivers nutrients t o the joint cartilage, helps to inhibit the enzymes that decompose the joint cartilage and speeds up the formation of a new joint cartilage.

Keratin sulfate	D-Galactose: (β1→4): N-acetyl-D-glucosamine-6-sulfate		Major components of the cornea, cartilage and bone
Hyaluronic acid	D-Glucoronic acid: (β1→3): N-acetyl-D Glucosamine		Retain water to keep tissues well lubricated and moist.
Heparin	L-Iduronate-2-sulfate: (α1→4): N-sulfo-D-glucosamine-6-sulfate		Prevents blood clotting.

2. **Proteoglycans:** These macromolecules contain chains of glycosaminoglycans (GAGs) and protein core having bottle brush like structure. The two components may be linked covalently or non-covalently. They are found in all connective tissues, extracellular matrix (ECM) and on the surfaces of many cell types.

3. **Glycoproteins:** These molecules contain oligosaccharide chains covalently attached to amino acid side-chains (proteins). These molecules are found on the outer surface of plasma membranes. They also perform many specialized functions as enzymes, hormones and immunoglobulins. The carbohydrate content of these molecules may vary from ≤1% to ≥ 90%. The sugars found in glycoproteins include glucose (Glc), galactose (Gal), mannose (Man), fucose (Fuc), *N*-acetylgalactosamine (GalNAc), *N*-acetylglucosamine (GlcNAc) and *N*-acetylneuraminic acid (NANA). The carbohydrates of glycoproteins are attached to the protein through *O*-glycosidic or *N*-glycosidic bonds.

4. **Glycolipids:** These molecules are membrane sphingolipids having oligosaccharides as hydrophilic head groups. Brain and neurons are particularly rich in glycolipids.

Functions of carbohydrates

Carbohydrates are the biomolecules that sustain life. Their oxidation is the central energy-yielding pathway in most of the cells. They play significant roles such as:

- Carbohydrates serve as *energy stores, fuels, and metabolic intermediates.* They are stored as starch in plants and glycogen in animals. Carbohydrates in the presence of oxygen decompose to CO_2 and H_2O, liberating energy which is utilized by the body.
- Ribose and deoxyribose sugars form part of the *structural framework of RNA and DNA.*
- Insoluble carbohydrate polymers are key *structural components in the cell walls of bacteria, plants* (such as cellulose in cell wall) and in the connective tissues of animals.
- Complex carbohydrate polymers are covalently *allied to several proteins and lipids* [Carbohydrates associated to lipid molecules, or glycolipids, are the main components of biological membranes]. Proteins that are covalently linked to carbohydrates are called glycoproteins. These two classes of biomolecules play key role as receptors and components of cell membranes. So they are involved in mediating interactions among cells by acting as intracellular signals regulating the metabolic activities in the cellular environment.

9.3 LIPIDS

In this section, you will be studying another biomolecule i.e, lipid about which you have read in your school text books. Lipids along with carbohydrates form an important constituent of your diet. You must have heard about fats and fatty acids, they are actually type of lipid molecules. Lipid molecules are heterogeneous groups of compounds and are generally termed as fats, oils, steroids, waxes or related compounds. German chemist Bloor in 1943 coined the term 'Lipid' (in Greek '*lipos*' means '*fat'*). It refers to a diverse class of biomolecules relatively insoluble in water but soluble in organic solvents such as chloroform. Chemically lipids are various types of esters of different alcohols. Lipids are classified into three major classes: Simple Lipids, Compound Lipids and Precursor and Derived lipids. They can also be classified on storage and structural basis. First of all let us now discuss simple lipids.

9.3.1 Simple Lipids

These molecules are esters of fatty acids with different alcohols.

R-OH + R'-COOH → R'CO-OR + H_2O

Alcohol Acid → Ester Water

These molecules are further subdivided in fats and waxes. Neutral fats or Triacylglycerols are esters of fatty acids with glycerol, whereas waxes are esters of fatty acids (saturated or unsaturated) with high molecular weight

monohydric alcohols (generally C_{16}- C_{36}). In vertebrates, cutaneous glands secrete wax and it functions as a protective coating to keep the skin lubricated and water-proof. Hair, wool and fur are also coated with wax. Waxes are the main storage form of energy in planktons. Marine organisms (whale, herring, salmon) consume planktons in large quantities, waxes serve as major food and storage lipids in them.

Let us begin with fatty acids that form the basic chemical constituent of lipid structure.

A) Fatty Acids

Fatty acids are long aliphatic carboxylic acids having a general formula R-COOH where R denotes a hydrocarbon chain and –COOH (carboxylic) is the functional group. Fatty acids are classified on the basis of- odd or even hydrocarbon chain, saturated or unsaturated hydrocarbon chain. As you know even hydrocarbon chain fatty acids have even number of carbon atoms whereas odd hydrocarbon chain fatty acids have odd number of carbon atoms. The hydrocarbon chain may be saturated (containing no double bonds) (Table-9.2) or unsaturated (containing one or more double bonds).

Table 9.2: General saturated fatty acids.

Systematic Name	Common Name	Source	Structure
Butanoic acid	Butyric acid	Butter	$CH_3(CH_2)_2COOH$
Hexanoic acid	Caproic acid	Butter	$CH_3(CH_2)_4COOH$
Octanoic acid	Caprylic acid	Coconut oil	$CH_3(CH_2)_6COOH$
Decanoic acid	Capric Acid	Coconut oil	$CH_3(CH_2)_8COOH$
n-Dodecanoic acid	Lauric acid	Coconut oil, Cinnamon, palm kernel, laurels, butter	$CH_3(CH_2)_{10}COOH$
n-Tetradecanoic acid	Myristic acid	Butter, Coconut Oil, Nutmeg, palm kernel, myrtles	$CH_3(CH_2)_{12}COOH$
n-Hexadecanoic acid	Palmitic acid	Animal and plant fats	$CH_3(CH_2)_{14}COOH$
n-Octadecanoic acid	Stearic acid	Animal and plant fats	$CH_3(CH_2)_{16}COOH$

The prefix n indicates normal unbranched structure.

The systematic name for naming a fatty acid chain is based on the hydrocarbon. Numbering starts from the first carbon (carboxy terminal). The carbon adjacent to carboxyl carbon (carbon 2, 3 and 4) are known as α, β and γ carbons respectively. Saturated fatty acid chain is assigned a suffix ‘anoic acid’/ ‘anoate’ while the unsaturated chain is assigned the suffix ‘enoic acid’

after the name of the hydrocarbon chain if it is monounsaturated. If there are more than one double bond as in case of polyunsaturated fatty acids, prefix *di*, *tri* etc are used before the word enoic acid. Unsaturated fatty acids contain one or more double bonds for example:

1. *Monoenoic acids*- unsaturated fatty acid having a single double bond.

   E.g., 18:1(Δ^9) Oleic acid ($C_{17}H_{33}$-COOH)

   CH_3-$(CH_2)_7$-CH=CH-$(CH_2)_7$-COOH

2. *Dienoic acids*- unsaturated fatty acids having two double bonds

   E.g., 18:2($\Delta^{9, 12}$) Linoleic acid ($C_{17}H_{31}$-COOH)

   CH_3-$(CH_2)_4$-CH=CH-CH_2-CH=CH-$(CH_2)_7$-COOH

3. *Trienoic acids*- unsaturated fatty acids having three double bonds

   E.g., 18:3($\Delta^{9, 12, 15}$) Linolenic acid ($C_{17}H_{29}$-COOH)

   CH_3-CH_2-CH=CH-CH_2-CH=CH-CH_2-CH=CH-$(CH_2)_7$-COOH

4. *Polyenoic acid*- unsaturated fatty acids having two or more double bonds, otherwise known as polyunsaturated fatty acids (PUFA)

   E.g. 20:4($\Delta^{5, 8, 11, 14}$) Arachidonic acid (C_{19}-H_{31}-COOH)

   CH_3-$(CH_2)_{14}$-$(CH=CH-CH_2)_3$-CH=CH-$(CH_2)_3$-COOH.

Several conventions use Δ for indicating the number and position of double bond in the hydrocarbon chain for example, 18:0 represents a fatty acid having 18 carbon long chain with no double bond, while18:1 (Δ^9) represents a fatty acid having 18 carbon chain length and one double bond at position 9 and 18:2 ($\Delta^{9, 12}$) represents a fatty acid having 18 carbon chain length and two double bonds, one at position 9 and the other at 12 starting from the carboxylic end.

B) Geometric isomerism of Fatty acids

Unsaturated fatty acids exhibit geometrical isomerism at the double bond in the hydrocarbon chain. The presence of a double bond produces a bend/kink of 120° in the straight chain of fatty acid. It leads to two possible arrangements and result in geometric isomers- *cis (means near: when the side chains or groups are on the same side)* and *trans (means far: when the side chains or groups are on the opposite sides of the double bond).* A well known example is represented by Oleic acid and Elaidic acid, both are 18 carbon fatty acids with one double bond at position 9. However, Oleic acid is *cis* isomer while elaidic acid is *trans*. Oleic acid assumes L- shape whereas elaidic acid remains straight. The two conformations are shown in Fig. 9.12.

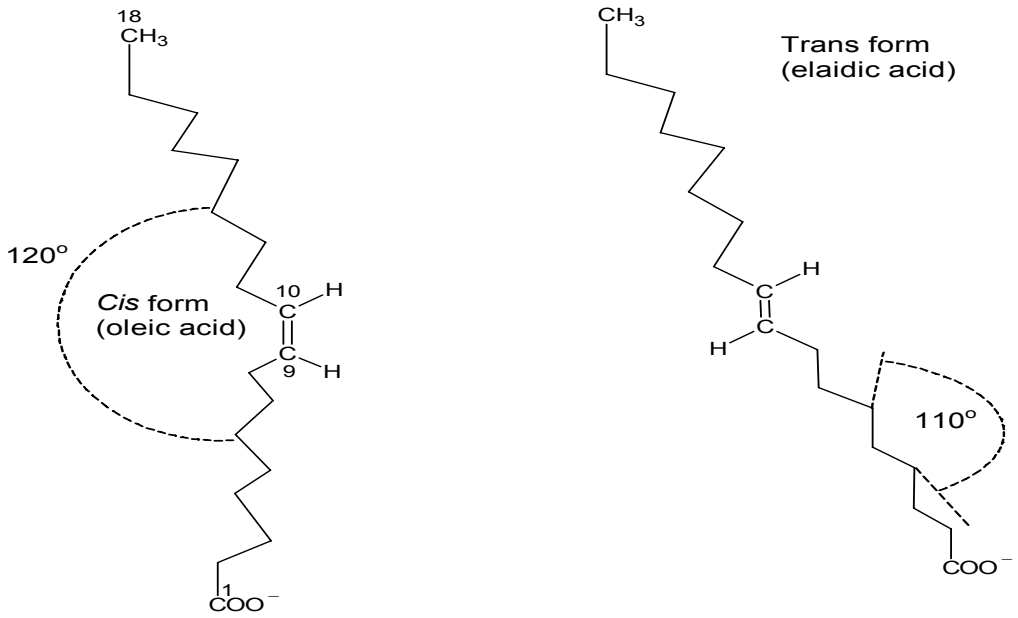


Fig. 9.12: Geometric isomerism of 18:1(Δ9) fatty acid (oleic and elaidic acids)

C) Properties of fatty acids

1. The long aliphatic hydrocarbon chains in fatty acids make them non polar; however carboxylic acid group at one end of the chain is polar. So, fatty acids are more soluble in organic/ non polar solvents such as chloroform and ether.

2. Short hydrocarbon chain fatty acids (C_4 to C_{10}) are liquids at room temperature whereas long chains (above C10) are solids. The melting point of fatty acids increases with chain length. The unsaturated fatty acids have lower melting point than saturated fatty acids with the same chain length.

3. **Saponification:** You might know that during a neutralization reaction, acid reacts with an alkali to form salt and water. Similarly, carboxylic acid group of a fatty acid reacts with an alkali (KOH or NaOH) to form salt of a fatty acid. Sodium and potassium salts of long chain fatty acids are called *soaps.* These soaps possess exceptional cleansing properties.

$$CH_3\text{-}COOH + NaOH \longrightarrow CH_3\text{-}COONa + H_2O$$

Fatty acids are assigned **Saponification number.** This number is the number of milligrams of KOH required to saponify 1 gm of fat. The saponification number gives information about the average chain length of the fatty acids in the fat. It varies inversely with the chain length of the fatty acids.

4. **Hydrogenation:** Hydrogenation is the process of addition of hydrogen. Unsaturated fatty acids are converted to their saturated forms by addition of hydrogen at the double bonds. Hydrogenation of oils can lead to their saturation and solidification, e.g., Vanaspathi.

5. **Iodine Number:** Similar to hydrogenation, halogen like iodine can add across the double bonds to form dihalogenated fatty acid. The process is known as iodination. Iodine number is defined as number of gram of iodine required to saturate 100 grams of lipid. It is used to determine degree of unsaturation in the lipids.

D) Neutral fat or Triglycerides (TG)

Triglycerides are the chief storage form of fatty acids in animals and plants. In general, fatty acids don't exist in free form. Saturated and unsaturated fatty acids can form esters with alcohol such as glycerol. Fatty acids can form mono-, di- or tri-esters with –OH group of alcohols. In triglycerides or triacyl glycerols, all the three hydroxyl groups of glycerol are esterified with same fatty acid (simple TG) or with different fatty acids (mixed TG). Tri-esters of glycerol are also known as *neutral fat*. Broad structure of triglycerides is shown in Fig. 9.13

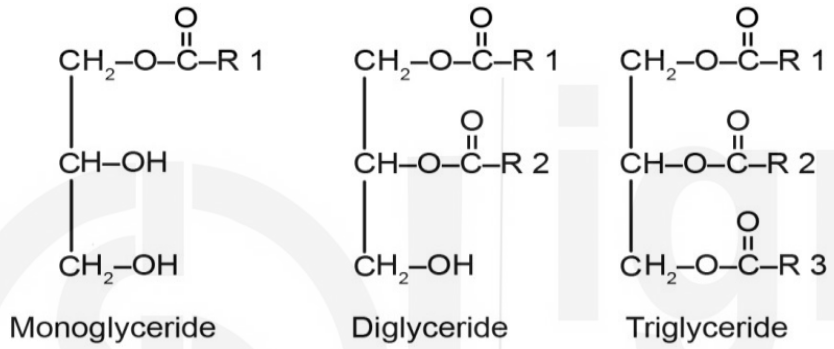


Fig. 9.13: Structures of Monoglyceride, Diglyceride and Triglycerides.

Natural fats, such as vegetable oils, dairy products and animal fat are complex mixtures of simple and mixed TG.

SAQ 3

Do as directed.

a) Waxes are esters of fatty acids with high molecular weight monohydric alcohols (True/False).

b) Linolenic acid is a dienoic fatty acid having two double bonds (True/False).

c) The long aliphatic hydrocarbon chains in fatty acids make them polar/non polar (Pick one option).

d) Sodium and potassium salts of long chain fatty acids are called …….. (Fill in the blank).

9.3.2 Compound Lipids

A) Phospholipids and their derivatives

Phospholipids or phosphatide lipids constitutes major component of all cell membranes. They are esters containing phosphoric acid, a nitrogeneous base, an amino acid or any other moiety apart from fatty acid and glycerol

(Fig. 9.14). Glycerol forms the backbone of phospholipids. Its two –OH groups (at positions 1 and 2) are esterified to the fatty acid chains. The third alcohol of the glycerol forms an ester bond with phosphoric acid. The phosphate group attached to the glycerol can form ester links with a variety of other molecules such as carbohydrates, choline, inositol and amino acids. Some of the major derivatives of phospholipids are phosphoglycerides, phosphoinositides, sphingomyelins and plasmalogens. Table 9.3 enlists different substituent groups found in phosphoglycerides.

The head of phospholipid molecule (glycerol and phosphate group) is hydrophilic, whereas the fatty acid tail is hydrophobic. Therefore, phospholipids unlike triglycerides are amphipathic or in other words, molecule with a polar-hydrophilic end and a hydrophobic end. In an aqueous solution, these molecules will self assemble into micelles or bilayers with polar ends facing water.

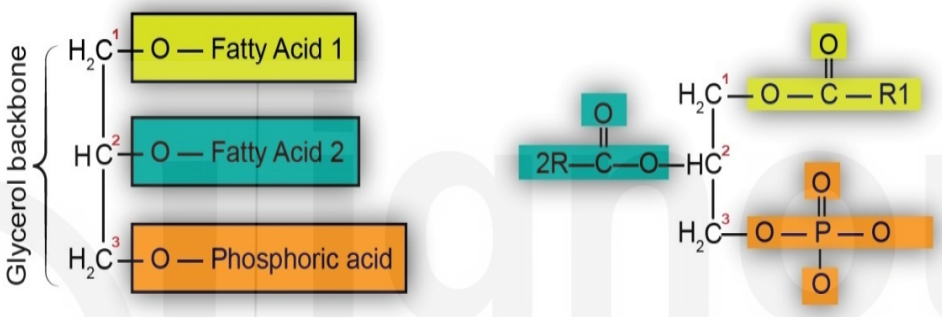


Fig. 9.14: Structure of Phospholipid.

Table 9.3: Substituent groups found in phosphoglycerides.

Name of the phospholipid	Substituent group	Structure of the substituent group
Phosphatidylcholine	Choline	$HO\text{-}CH_2\text{-}CH_2\text{-}N^+(CH_3)_3$
Phosphatidylethanolamine	Ethanolamine	$HO\text{-}CH_2\text{-}CH_2\text{-}NH_3$
Phosphatidylserine	Serine	$HO\text{-}CH_2\text{-}CH(COOH)\text{-}NH_3$

B) Phosphatidyl inositol or Lipositol

Lipositol contains a cyclic carbohydrate called inositol linked to phosphoric acid instead of nitrogen base present in phosphoglycerides (Fig. 9.15). Phosphatidyl inositol bi phosphate or PIP_2 is present in biomembranes. It plays a key role in the hormonal action on cell membranes.

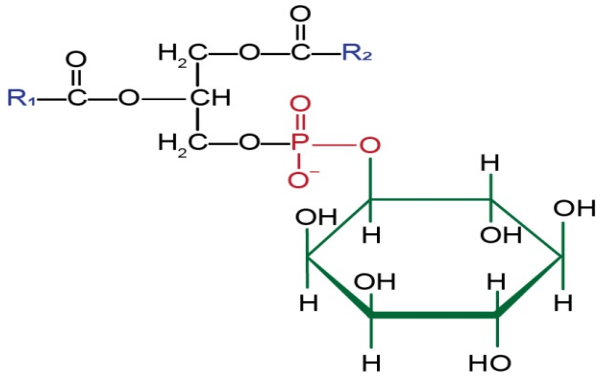


Fig. 9.15: Phosphatidylinositol.

C) Plasmalogens

Plasmalogens are found mainly in biomembranes of brain and muscle. Structurally, they are phospholipids similar to phosphatidyl ethanolamine except they contain ether linkage at C-1 with an unsaturated alcohol, not the ester linkage (Fig. 9.16).

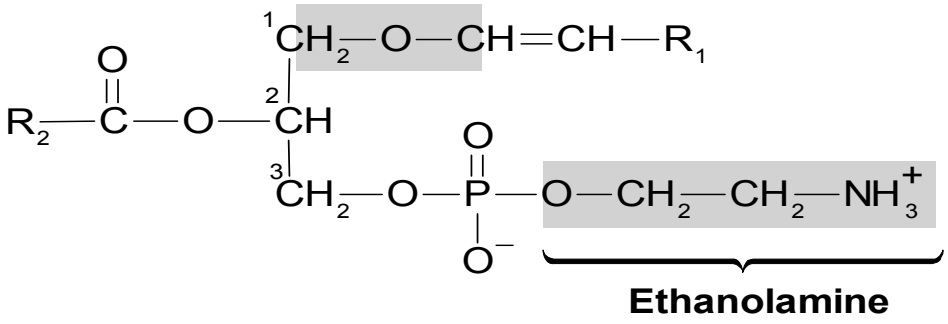


Fig. 9.16: Structure of Plasmogen.

D) Sphingolipids/Sphingomyelins

Have you heard about nerve cells? Do you know sphingomyelins are found in the animal cell membrane particularly in the myelin sheath of nerves? They do not contain glycerol. Sphingolipids usually consists of a complex 18-carbon unsaturated amino alcohol called '*sphingosine*' along with phosphoric acid, choline and fatty acid as shown in the Fig. 9.17. The association of sphingosine and fatty acids forms ceramide.

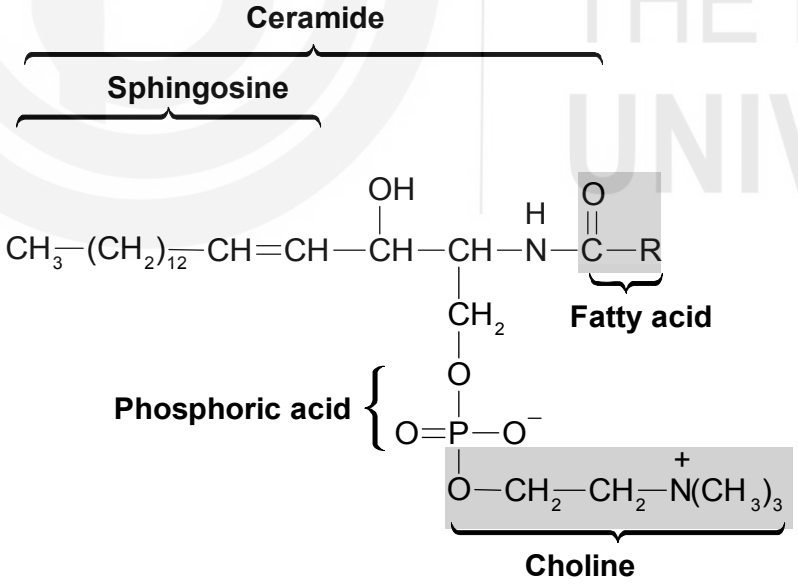


Fig. 9.17: Structure of Sphingomyelin.

E) Glycolipids

As the name suggests, glycolipids are molecules in which carbohydrate moiety is attached with lipids. They are distributed in every tissue of the body, mainly brain and nervous tissue. They are found on the surface of cell membranes. Lipids in this group constitute cerebrosides, gangliosides, sulfolipids and lipoproteins (proteins covalently linked to lipids).

9.3.3 Precursor and Derived Lipids

These molecules include fatty acids, glycerol, steroids, ketone bodies, lipid soluble vitamins and hormones.

A) Steroids

Steroids are cyclic lipids. They have a carbon skeleton consisting of a fused ring system known as 'cyclopentanoperhydrophenanthrene' nucleus (Fig. 9.18). These molecules have three cyclohexane rings (A, B and C) fused in nonlinear manner and a terminal cyclopentane ring (D).

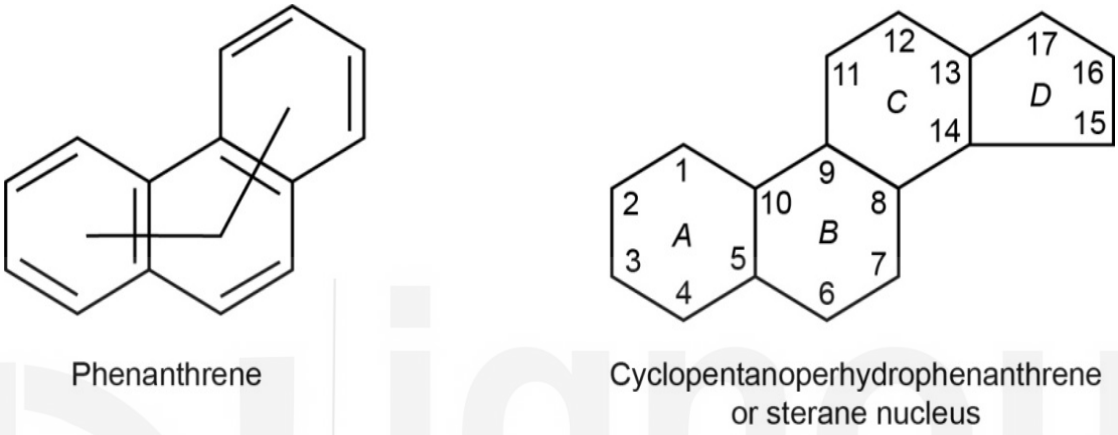


Fig. 9.18: Parent compounds of steroids- A: Phenanthrene; B: Sterane.

B) Cholesterol

Steroids having hydroxyl groups (hydroxy steroids) are known as **sterols**. Cholesterol is one of the well known sterol in the human body. Learner must have heard about how high levels of cholesterol is considered as one of the major reasons for causing heart attacks. Cholesterol is a 27 carbon compound with molecular formula $C_{27}H_{45}OH$. Structure of cholesterol is shown in Fig. 9.19. Cholesterol is insoluble in water, but soluble in solvents such as ether, chloroform, benzene etc. In its isolated form, cholesterol appears as white or faintly yellow, odourless, pearly leaflets or granules. Cholesterol plays a significant role in several functions as it serves as a precursor of bile acids, cortical hormones, sex hormones and Vitamin D_3.

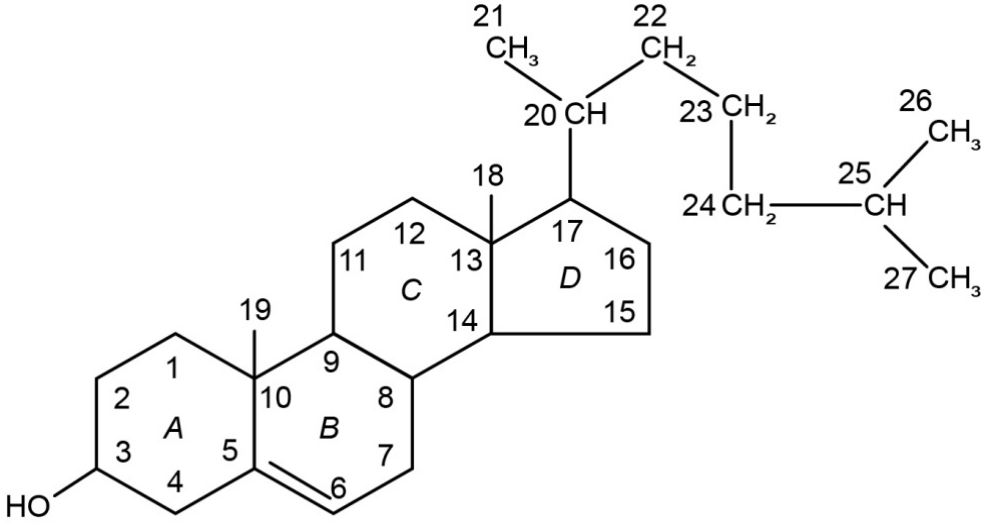


Fig. 9.19: Cholesterol.

C) Ergosterol

Ergosterol is a precursor for vitamin D. It is found in plants and yeasts. Structurally it differs from 7-dehydrocholesterol in its side chain. Irradiation of ergosterol with UV rays leads to Vitamin D_2; hence ergosterol is also known as Pro- Vitamin D_2.

9.3.4 Lipids as Signals, Cofactors and Pigments

Some types of lipids are present in fairly small quantities but play key roles as cofactors or signals. Phosphatidylinositol phosphate, prostaglandins, steroid hormones and vitamins D, A, E and K are some of such well known lipid molecules.

A) Prostaglandin

Prostaglandin is a 20 carbon skeleton and a five membered ring originating from a chain of arachidonic acid. They are derivatives of a C_{20} molecule called prostanoic acid. Since they were first isolated from human semen, they were named 'prostaglandins' (PGs). Prostaglandins and related compounds are now classified under eicosanoids. The eichosanoids include prostaglandins, thromboxanes and leukotriens.

B) Thromboxanes

Structurally, thromboxanes have six membered rings containing ether. Thromboxanes A_2 (TxA_2), the well-known member of this group is primarily produced by platelets. It promotes platelet aggregation and smooth muscle contraction.

C) Leukotrienes

They are found in leukocytes. Structurally, leukotrienes are hydroxyl-fatty acid derivatives of arachidonic acid. They are potent biological signals. Leukotrienes LTC_4, LTD_4 and LTE_4 have been identified as components of slow reacting substances of anaphylaxis.

SAQ 4

Do as directed.

a) Glycolipids are molecules in which protein moiety is combined with lipids (True/False).

b) Sphingomyelins contain glycerol (True/False).

c) Ergosterol is a precursor for …….. (Fill in the blank).

d) Thromboxanes are four membered rings containing ether (True/False).

9.4 AMINO ACIDS AND PROTEINS

9.4.1 Amino Acids

Another most important biomolecules are proteins that consist of one or more long chain of amino acid residues. Amino acids are composed of carbon, hydrogen, oxygen and nitrogen atoms. However, other atoms are also found in their side chains. Look at the Fig. 9.20. Amino acids consist of an amine group ($-NH_2$) and a carboxylic acid functional group (-COOH) along with a side chain (-R) specific to each amino acid. Carbon atom next to the carboxyl group is denoted as α-carbon. You will also see that around the α-carbon, other than carboxyl there are three more groups namely hydrogen, amino and R group. The R-group represents the side chains of amino acids which can be as simple as hydrogen atom (H) or a methyl group ($-CH_3$) or a complex structure.

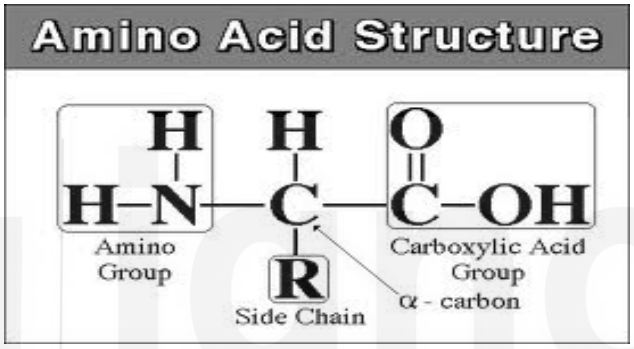
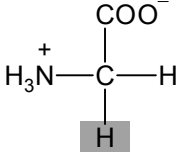
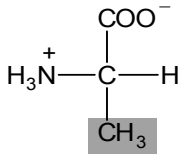
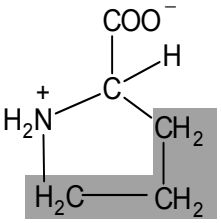
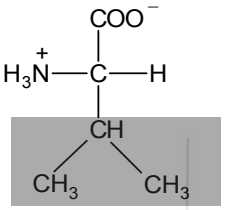
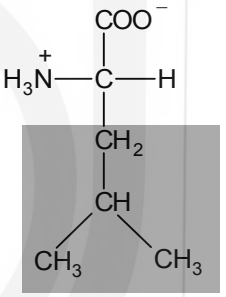
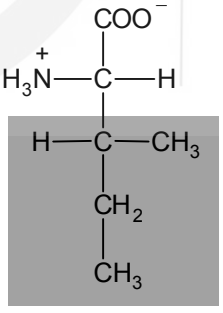
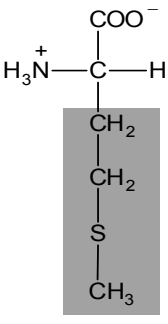


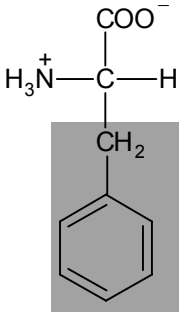
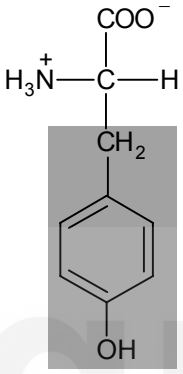
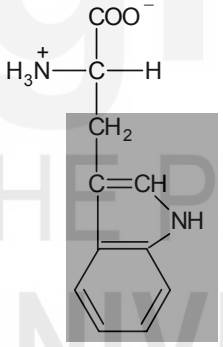
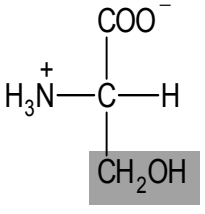
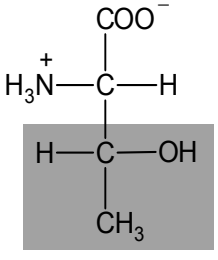
Fig. 9.20: Structure of an Amino Acid.

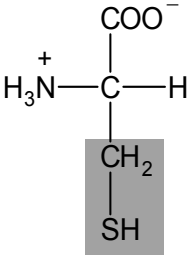
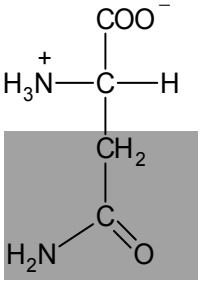
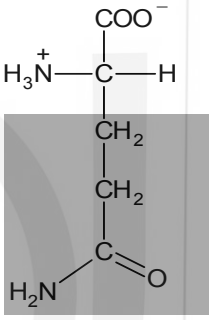
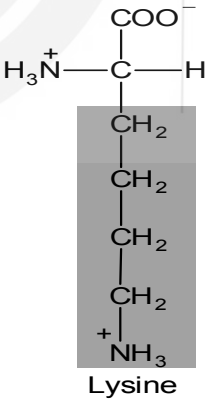
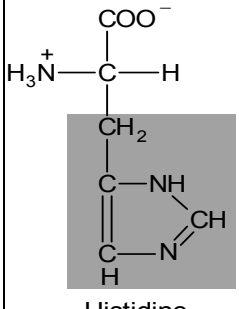
Out of 500 amino acids known so far, 23 are found in proteins. They are called proteinogenic (protein building) amino acids. These amino acids constitute the monomer units to form proteins. Twenty of the twenty three proteinogenic amino acids are encoded directly by the triplet codons in the genetic code. They are known as **standard amino acids.** Look at the Table 9.4 carefully. It enlists standard amino acids in several categories. Standard amino acids are distinguished from other amino acids that are modified after synthesis of proteins or other amino acids which are present in living organism but are not found in proteins (non-standard amino acids).

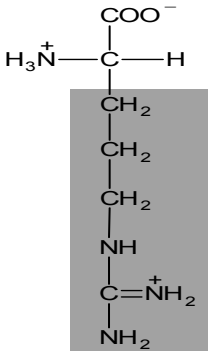
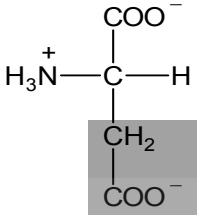
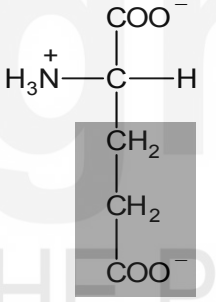
Table 9.4: Amino Acids present in Proteins.

							pK_a values
Amino acid	***Abbreviation/Symbol***	***M_r***	***Structure***	***pK_1 (-COOH)***	***pK_2 (-NH^+_3)***	***pK_2 (R group)***	***pI***
Nonpolar, aliphatic R groups							
Glycine	Gly G	75	 Glycine	2.34	9.60		5.97

Alanine	Ala A	89	 Alanine	2.34	9.69		6.01
Proline	Pro P	115	 Proline	1.99	10.96		6.48
Valine	Val V	117	 Valine	2.32	9.62		5.97
Leucine	Leu L	131	 Leucine	2.36	9.60		5.98
Isoleucine	Ile I	131	 Isoleucine	2.36	9.68		6.02
Methionine	Met M	149	 Methionine	2.28	9.21		5.74

Aromatic R groups							
Phenylalanine	Phe F	165	 Phenylalanine	1.83	9.13		5.48
Tyrosine	Tyr Y	181	 Tyrosine	2.20	9.11	10.07	5.66
Tryptophan	Trp W	204	 Tryptophan	2.38	9.39		5.89
Polar, uncharged R groups							
Serine	Ser S	105	 Serine	2.21	9.15		5.68
Threonine	Thr T	119	 Threonine	2.11	9.62		5.87

Cysteine	Cys C	121	 Cysteine	1.96	10.28	8.18	5.07
Asparagine	Asn N	132	 Asparagine	2.02	8.80		5.41
Glutamine	Gln Q	146	 Glutamine	2.17	9.13		5.65
Positively charged R groups							
Lysine	Lys K	146	 Lysine	2.18	8.95	10.53	9.74
Histidine	His H	155	 Histidine	1.82	9.17	6.00	7.59

Arginine	Arg R	174	 Arginine	2.17	9.04	12.48	10.76
Negatively charged R groups							
Aspartate	Asp D	133	 Aspartate	1.88	9.60	3.65	2.77
Glutamate	Glu E	147	 Glutamate	2.19	9.67	4.25	3.22

Key convention

If you notice second column in the Table 9.4, you will see that each of the twenty amino acids is assigned with three letter abbreviations (upper or lower case) and one letter code (upper case). The three letter abbreviations and one letter code act as shorthand to indicate the composition and sequence of large number of amino acids in proteins. The three letter code consists of first three letters of the name of amino acid. The abbreviations and one letter code is of immense usage in bioinformatics as it reduces the size of data files. It helps to make the purpose of notation easier, quicker and worth remembering.

Stereoisomerism in Amino acid

The α-carbon atom of amino acids except *glycine* is bonded to four different groups (a carboxyl group, amino group, R group and a hydrogen atom) in a tetrahedral arrangement. Therefore, α-carbon is a chiral centre and asymmetric in all of them except glycine. The difference in three dimensional orientation of bonding orbitals around α-carbon atom gives rise to two possible **stereoisomers** (Fig 9.21). Those amino acids having $–NH_3$ group to the right hand side are designated as D-forms and those having $–NH_3$ group to the left are designated as L-forms. The stereoisomers that are non-super imposable mirror images of each other are known as **enantiomers**.

Only L-α-Amino Acids occur in proteins. Several free L-α-Amino Acids play important roles in metabolic processes. D-amino acids are few and occur as small peptides in bacterial cell walls and antibiotics.

All amino acids except glycine have a chiral centre and are optically active (rotate the plane of polarized light). Some of the amino acids are ***dextrorotatory*** (rotating plane polarized light to the right, clockwise direction) and some ***levorotatory*** (rotating plane polarized light to the left). Dextrorotatory molecules are designated by prefix "(+)-" or "d-" signs while levorotatory molecules are designated by prefix "(-)-" or "l-" signs respectively. The "d" and "l" prefixes are distinct from the uppercase "D" and "L" prefixes that are based on actual configuration of enantiomer.

Fig. 9.21: Stereoisomerism in amino acid (geometric formulas).

Solid wedge shaped bonds project out of the plane of paper, the dashed bonds behind it.

Amino acid classification based on R-group

There are several ways to classify amino acids, but the simplest way of classification is based on structure and general chemical properties of their side chains or R groups. The chemical properties of the R groups are responsible for several characteristics of amino acids such as chemical reactivity, ionic charge and hydrophobicity. Amino acids are grouped into five main classes based on the polarity, size and shape of R-groups present in their side chains.

1) Non-polar aliphatic R groups: Glycine, Alanine, Proline, Valine, Leucine, Isoleucine and Methionine amino acids belong to this group.

2) Aromatic R groups: Phenylalanine, Tyrosine and Tryptophan.

3) Polar uncharged R groups: Serine, Threonine, Cysteine, Asparagine and Glutamine.

4) Positively charged R groups: Lysine, Histidine, Arginine.

5) Negatively charged R groups: Aspartate and Glutamate amino acids.

Essential Amino acids: Animals either lack the ability to synthesize certain amino acids or they make them in insufficient quantities than required during phases of active growth. These amino acids are classified as essential amino acids. Almost half the amino acids required for protein synthesis fall in this category and they have to be provided in the diet. They include leucine, isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, and histidine. Arginine is semi essential. Limitation of even one amino acid leads to a negative nitrogen balance.

Non-Standard Amino Acids

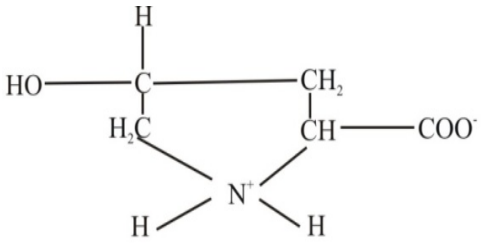
In addition to twenty common amino acids present in proteins several other amino acids exist in the living organisms. These amino acids occurs naturally in cells but do not participate in protein synthesis are called non-standard amino acids. They are either absent in proteins (carnitine, gamma amino butyric acid (GABA)) or are formed by modification of standard amino acids in a peptide molecule (hydroxyproline and selenomethionine). These modifications are often necessary for the function or regulatory role of a protein. Some of the examples include 4-hydroxyproline (derivative of proline) and 5-hydroxylysine (derivative of lysine) found in collagen of connective tissues (Fig. 9.22). Desmosine and γ-carboxyglutamate are some other examples. The addition of acetyl, methyl, adenyl or ADP-ribosyl groups to amino acids can increase or decrease a protein activity. Phosphorylated amino acids such as serine, threonine and tyrosine are often used to regulate the activity of several proteins.

There are several additional amino acids (~300) in cells which are not found in proteins (non-protein amino acids) but play a significant role in metabolism or exist as natural products. Amino acids such as ornithine and citrulline are important metabolic intermediates in the urea cycle. β-Alanine, an isomer of alanine is a constituent of vitamin pantothenic acid and coenzyme A. Quaternary amine creatine occurs naturally in vertebrates and helps to supply energy to all cells in the body primarily muscle. Gamma-aminobutyrate is found in free form in the brain.

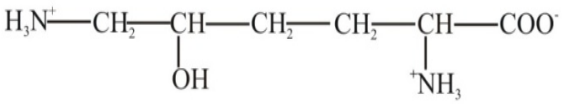
Amino acids as acids and bases

When an amino acid lacking ionizable R group is dissolved in water at neutral pH, it exists as the dipolar ion, a **zwitterion** and can act as acid as well as base (Fig. 9.23). It bears no net charge as it has equal number of oppositely charged ions. Such amino acids exhibiting acid-base character (amphoteric) are called **ampholytes**. The amino and carboxyl groups of amino acids as well as ionizable R groups of amino acids function as weak acids and bases.

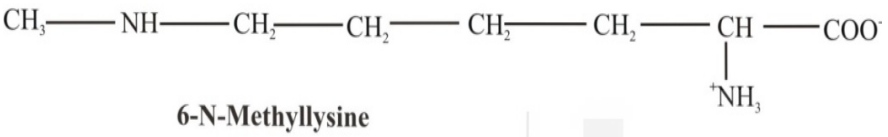
R-COOH and R-NH_3^+ are considerably weak acids. However, R-COOH is stronger acid than R—NH_3^+. At the physiological pH of 7.4, carboxyl groups and amino groups are completely ionized and are present as R-COO^- and R-NH_3^+respectively.



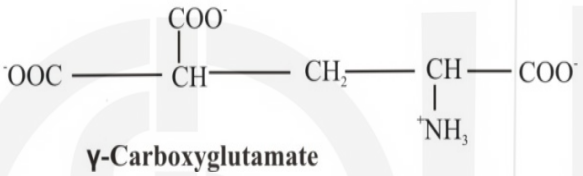
4-Hydroxyproline



5-Hydroxylysine

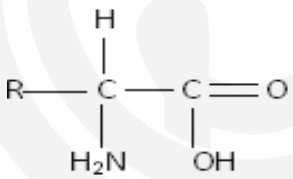


6-N-Methyllysine

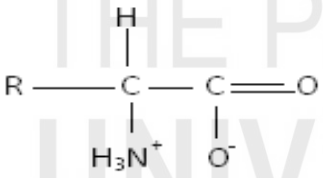


γ-Carboxyglutamate

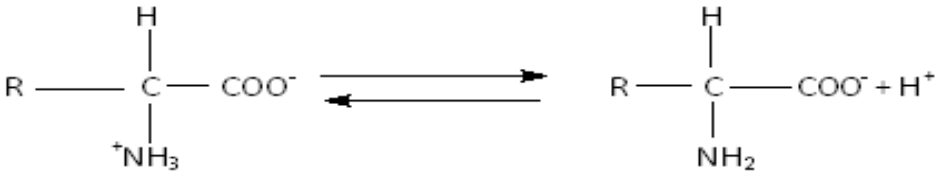
Fig. 9.22: Chemical structure of modified amino acids.



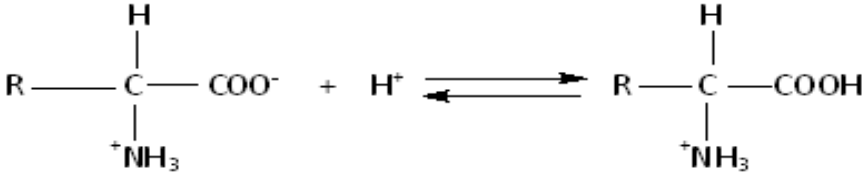
Nonionic Form



Zwitterionic Form



Zwitterion as acid



Zwitterion as base

Fig. 9.23: Nonionic and Zwitterionic forms of amino acids.

Physical Properties

Amino acids are colorless crystalline substances. Their shape varies from sharp needle shape (tyrosine) to hexagonal plates (cystine). Amino acids can be sweet (glycine and alanine) to bitter (arginine) or tasteless (tyrosine). Amino acids have high melting points and are soluble in the polar solvents (water and ethanol) because of their charged functional groups and insoluble in the non-polar solvents such as benzene, hexane and ether.

Amino acids do not absorb the visible light and are therefore colorless. Tyrosine and tryptophan absorb high wavelength ultraviolet light (250-290 nm). It helps in the detection and determination of protein quantity in different solutions. The light absorption property is extensively used to determine the concentration of biomolecules in the solution.

SAQ 5

Do as directed.

a) Glycine and serine are standard/non-standard amino acids (Pick one option).

b) Which amino acid is denoted by one letter code P?

c) Tyrosine and Tryptophan are aliphatic/aromatic amino acids (Pick one option).

d) The amino and carboxyl groups of amino acids as well as ionizable R groups of amino acids function as strong acids and strong bases (True/False).

Peptide Bond

The peptide bond is a covalent bond between α-amino group of one amino acid and α-carboxyl group of another forming an amide linkage (CO-NH). Two amino acids are joined covalently through an amide linkage termed as **peptide bond** (Fig. 9.24).

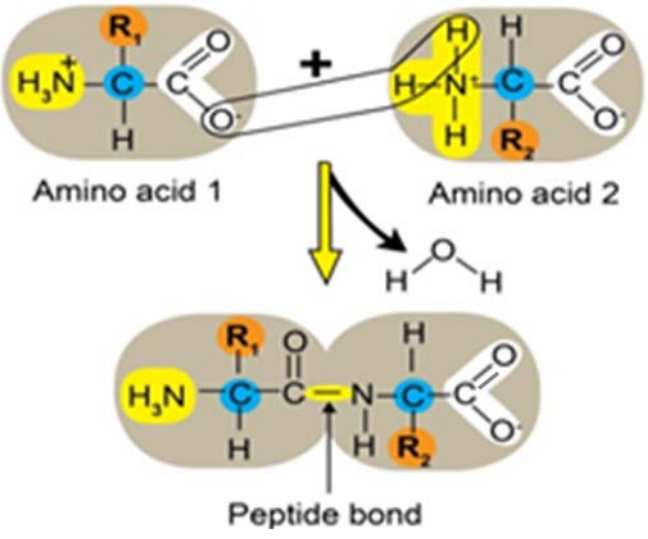


Fig. 9.24: Formation of a Peptide Bond.

When two amino acids molecules are linked, the product is called a dipeptide. On addition of more amino acids the chain length increases e.g. tripeptide contains three amino acid residues and a tetrapeptide; four and so on. When more amino acids are joined, the structure is called oligopeptide. Joining of many more amino acids leads to formation of a polypeptide. Amino acid polymers can also be classified on the basis of their molecular weight or the numbers of amino acid residues they contain. Molecules with molecular weight ranging from several thousand to several million daltons are called polypeptides. Molecules with low molecular weights, fewer than 50 amino acids are called as peptides. Proteins may have thousands of amino acid residues and consist of one or more polypeptide chains.

Each amino acid in a polypeptide chain is called residue. The terminal amino acid residue with free amino group is called as N-terminal residue and is written on the left hand side in an amino acid sequence. It refers to the start of a protein or polypeptide. Amino acid with free carboxyl group is known as C-terminal residue is written on the right hand side in an amino acid sequence. When the protein is translated from messenger RNA it is synthesized from N-terminus to C-terminus. The convention for writing polypeptide sequence is to put the N-terminus on the left and write the sequence from N-terminus to C-terminus. The names of intermediary amino acid residues are written in the same sequence as they are placed. For example, glutathione is a tripeptide of non-protein origin with glutamic acid (N-terminus), cysteine and glycine (C-terminus) residues. It is written as Glu-Cys-Gly.

9.4.2 Proteins

In this subsection, we will discuss the structure of protein and try to learn about simple, conjugated and derived proteins.

Protein Structure

Proteins are physically and functionally complex macromolecules performing multiple roles. What is it that makes one protein an enzyme, another a hormone or an antibody? How do they differ? What do you think? The answer to these questions lies in the protein structure. All the twenty amino acids do not occur in equal amounts in all the proteins. The amino acid composition of proteins is variable. Some of the amino acids may be present only once or absent from particular proteins while some of the amino acids may be repeated many times. The smaller amino acids predominate in most of the proteins. Some of the proteins contain a single polypeptide chain but many proteins are multisubunit. The multisubunit proteins are more complex in structure and contain two or more polypeptide chains. The individual polypeptide chains in the multisubunit proteins may be identical or different. If the subunits (individual polypeptide chain) are identical, the protein is known as **oligomeric** and the repeating subunit or individual polypeptide chain is called as **protomer**. Hemoglobin has four polypeptide chains (two identical α and two identical β). It can be considered as a tetramer of four subunits or dimer of αβ protomer. If the two polypeptides are linked covalently by disulphide bonds such as in insulin, they are generally called chains and not subunits.

Proteins do not have uniform regular structures. Proteins can be described in terms of levels of organization in primary, secondary, tertiary and quaternary structures (Fig. 9.25).

A. Primary Structure

The amino acid sequence of a protein is unique to that protein, and defines the structure and function of the protein. The primary structure of proteins refers to amino acid sequence of the polypeptide chain. It is held together by covalent peptide bonds, which are formed during the process of protein biosynthesis or translation. Post-translational modifications such as disulfide formation, phosphorylations and glycosylations are usually also considered a part of the primary structure, and cannot be read from the gene as they occur after translation is completed.

B. Secondary Structure

Secondary structure refers to highly regular local sub-structures. Two main types of secondary structure observed in proteins are, the alpha helix and the beta sheet. These were suggested in 1951 by Linus Pauling. These secondary structures are defined by patterns of hydrogen bonds between the main-chain peptide groups present in same chain or in different chains.

C. Tertiary Structure

It refers to three-dimensional structure of protein molecules. Primary sequence of amino acid folds into secondary structures and all secondary structural elements (helices, sheets, turns, and coils) come together through various non covalent interactions and get folded into the tertiary structure. This structure is functionally active as well as compact globular in nature. The folding of secondary structure into tertiary is driven by the hydrophobic interactions, but the structure is stable only when the parts of a protein domain (section of protein that can exist independently and sufficient to perform its function) are locked into place by *specific* tertiary interactions, such as salt bridges, hydrogen bonds, and the tight packing of side chains and disulfide bonds.

A variety of common and stable tertiary structures appear in a large number of proteins that are unrelated in both function and evolution– for example, many proteins are shaped like a TIM barrel. A TIM barrel fold is a tertiary structure which contains eight anti parallel β-strand in a circle, each connected with other through a α-helix in between.

D. Quaternary Structure

Quaternary structure refers to the arrangement of multiple folded protein in a multiple subunit complex. It reflects the fourth level of complexity in protein structure (Fig. 9.25). Large proteins having molecular weight of > 100 kD consist of two or more polypeptide chains. Each of the polypeptide unit is called as subunit. These subunits may have identical or different primary structure. The spatial arrangement or association of these subunits constitutes quaternary structure. Multi subunit proteins perform diverse functions in which one subunit may be regulatory in nature and other may be catalytic one. Hemoglobin, DNA polymerase and ion channels are well known examples of multiple subunit proteins with quaternary structure.

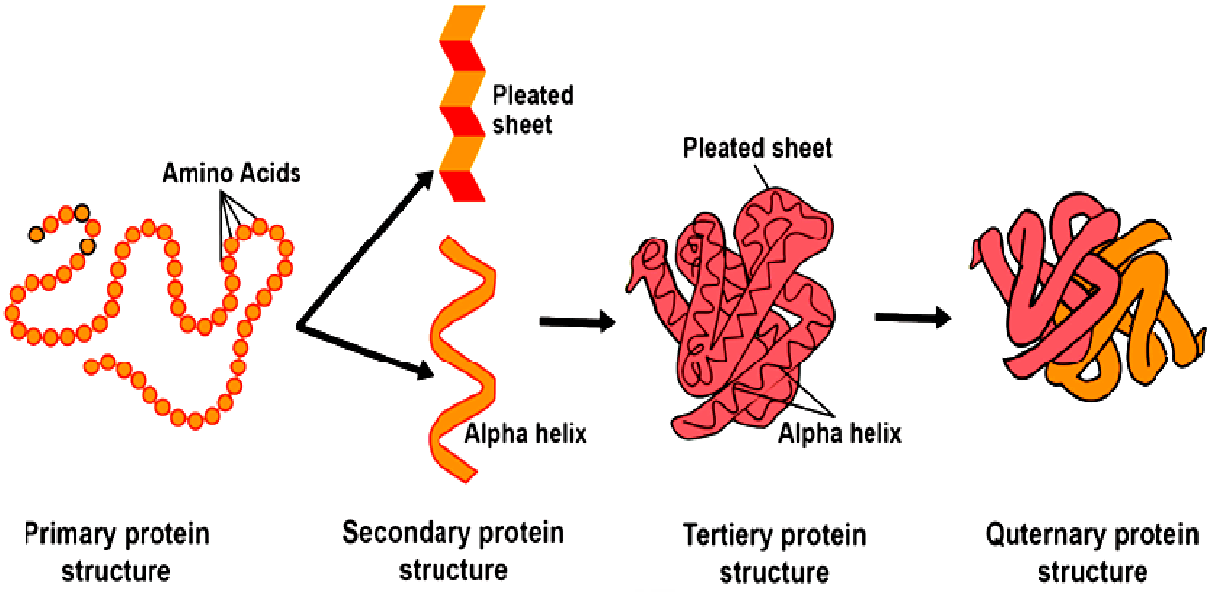


Fig. 9.25: Protein Architecture-Stages of Protein Folding.

Simple, Conjugated and Derived Proteins

Proteins are often bound to ligands such as cofactor and coenzymes or to other macromolecules. On the basis of their composition, proteins are classified in three categories.

A. **Simple proteins:** These proteins are made up of amino acids joined together by peptide bonds. On treatment with acids, proteins are hydrolyzed and liberate the constituent amino acids. These proteins are further divided on the basis of their solubility. Pancreatic ribonuclease A (RNase A) is an example of simple protein as it yields only amino acids upon hydrolysis.

B. **Conjugated proteins:** These proteins contain permanently associated chemical components along with amino acids. These chemical components or non-protein substances conjugated with simple proteins are called as prosthetic group. The prosthetic group may be either a metal or a compound. A protein without its prosthetic groups is called as apoprotein. A protein molecule combined with its prosthetic group is called holoprotein. Conjugated proteins are further divided into several categories on the basis of chemical nature of the prosthetic group for example metalloproteins, glycoproteins, phosphoproteins, lipoproteins and nucleoproteins. Enzymes perform their biological activity with the help of prosthetic groups.

C. **Derived proteins:** These are not naturally occurring proteins and are derived from simple proteins by the action of heat, enzymes and chemical agents. This group also includes artificially produced polypeptides. Peptones, peptides and proteoses are well known examples of derived proteins.

SAQ 6

Do as directed.

a) The convention for writing polypeptide sequence is to put the N-terminus on the left and write the sequence from N-terminus to C-terminus (True/False).

b) The peptide bond is a non-covalent bond between α-amino group of one amino acid and α-carboxyl group of another forming an amide linkage (CO-NH) (True/False).

c) Secondary/Tertiary structure of protein refers to three-dimensional structure of protein molecules (Pick one option).

d) Derived proteins are naturally occurring proteins (True/False).

9.5 SUMMARY

Let us summarize what we have learnt so far:

- A universal set of several hundred of molecules is found in living cells.
- Carbohydrates are the most abundant organic molecules on the earth. They are one of the main constituents of animal food. Carbohydrates can be broadly defined as polyhydroxy aldehydes or ketones and their derivatives.
- Carbohydrates are characterized by the type and number of monosaccharide residues present in their molecules. Glucose is the main carbohydrate.
- The maltose, sucrose and lactose are common examples of naturally occurring disaccharides. Starch and glycogen are polysaccharides of glucose in plants and animals.
- Lipids are a heterogeneous group of compounds that include fats, waxes, oils, steroids and several other derived molecules. Like carbohydrates they serve as energy reserve in the body. Lipids are classified into three major categories: simple, compound and derived lipids.
- Simple lipids are esters of long chain fatty acids and alcohol. They include fats, oils and waxes. Compound lipids are made up by further modification of simple lipids such as addition of phosphate, sulfate and carbohydrate or protein group. This class of lipids includes phospholipids, glycolipids and lipoproteins.
- Derived lipids are derived from hydrolysis of simple and compound lipids. Steroids, cholesterol, various lipid soluble hormones as well as fatty acids belong to this class of lipids.

- Proteins are linear polymers of amino acids whose sequence contain the information that gives each molecule its three dimensional structure and its biological functions. Interactions between biomolecules are stereospecific.
- There are about twenty amino acids that are used to form the proteins.
- Amino acids are joined together by peptide bonds to form the basic structure of proteins.
- Protein structures are organized hierarchically from primary, secondary, tertiary to quaternary structures. Proteins participate in almost every biochemical process in the body.

9.6 TERMINAL QUESTIONS

1. What are monosaccharides? Classify them.
2. Draw the structures of Lactose and Maltose.
3. Distinguish between Homopolysaccharides and Heteropolysaccharide? Give one example of each.
4. Discuss the properties of fatty acids.
5. What are phospholipids? Draw their structure.
6. Draw the structure of cholesterol.
7. How amino acids act as acid as well as base?
8. Describe the proteins in terms of levels of organization in primary, secondary, tertiary and quaternary structures.

9.7 ANSWERS

Self Assessments Questions

1. a) False, b) ketopentose, c) 2^n, d) True, e) True.
2. a) True, b) True, c) False, d) True.
3. a) True, b) False, c) non-polar, d) soap.
4. a) False, b) False, c) vitamin D, d) False.
5. a) Standard, b) proline, c) aromatic, d) False.
6. a) True, b) False, c) Tertiary, d) False.

Terminal Answers

1. Refer to Section 9.2.1.
2. Refer to Section 9.2.2 Subsection A and B.

3. Refer to Section 9.2.3 Polysaccharides, Subsection A and B.
4. Refer to Section 9.3.1 Subsection C.
5. Refer to Section 9.3.2 Subsection A.
6. Refer to Section 9.3.3 Subsection B.
7. Refer to Section 9.4.1.
8. Refer to Section 9.4.2

FURTHER READINGS

1. Albert L. Lehninger: Principles of Biochemistry, Worth Publishers, Inc. New York, 1984.
2. Harper's Illustrated Biochemistry, 29e. Robert K. Murray, David A Bender, Kathleen M. Botham, Peter J. Kennelly, Victor W. Rodwell, P. Anthony Weil, USA.
3. Donald J Voet Principles of Biochemistry, Jophn Wiley and Sons, Inc, USA.
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UNIT 10

ENZYMES: SPECIFICITY AND MECHANISM OF ACTION

Structure

10.1 INTRODUCTION

You have studied about the biomolecules in Unit 9 which include proteins, carbohydrates and lipids. Membrane proteins act as transporting molecules. The other important function of cellular proteins is to act as enzymes and catalyse biochemical reactions at a rate appropriate to the need of the cell.

In this unit you will study about the nature of enzymes and their functional aspects. Enzymes control the overall metabolic functions of the cell by speeding up the reaction rate. **They do not affect the equilibrium constant and remain unaffected at the end of the reaction.** The reactants bind to a specific site on the surface of enzyme molecule called active site. Enzymes show specificity for their substrate as well as for the reactions. Various factors such as temperature, pH, concentrations of enzymes and substrate affect the rate of enzyme-catalysed reactions. Some enzymes require additional factors for their normal activity. Allosteric enzymes have more than one active site which may be located on the same subunit or on different subunits. The detection and estimation of enzymes can be done by using various techniques which can measure their ability to convert the substrate into products.

Various enzyme-catalysed reactions characterise the metabolism of the cell. Regulation of these reactions is achieved by altering the enzyme activity. In the next unit you will read about the enzyme kinetics and regulation. Before you read this unit, you should refresh your memory about the structure of proteins.

Objectives

After studying this unit you should be able to:

- describe the role of enzymes in lowering the activation energy and in coupled reactions,
- list the type of enzymes and cofactors and discuss the mechanism of enzyme action,
- explain the effects of temperature, pH and enzyme concentration on the rate of enzyme action, and
- describe the essential features of allosteric enzymes, and iso enzymes, and assay of enzyme activity.

10.2 CHARACTERISTICS OF ENZYMES

A ribozyme is a RNA enzyme that catalyzes a chemical reaction. Ribozymes are found in the ribosome where they join amino acids together to form protein chains.

Enzymes act as catalysts and influence the rate of reaction in all the living organisms. In fact, essentially all chemical reactions in the living beings are catalysed by enzymes. All the enzymes known till now are proteins except ribozymes with a unique **three dimensional structure** that provides an **active site** for binding the other molecules, i.e. substrates to their surface. Each enzyme normally catalyses a few reactions but most often only one type of reaction. They are required in minute quantity/concentration to convert the substrates into products. The enzyme remains unaffected at the end of the reaction. **The enzymes molecule is much larger than the molecule of its substrate.** The molecular weights of enzymes range from thousands to millions, whereas the molecular weights of substrates are usually in few hundreds. Some enzymes are purely proteins, whereas others require non-protein assistant compounds for their catalytic activity.

Importance of Enzymes

Enzymes play an important role in **metabolism** as you know that all biochemical reactions are enzyme catalysed in the living organisms. They play an important role in **diagnosis**. Level of enzymes in blood are of diagnostic importance e.g. it is a good indicator in disease such as myocardial infarction in which creatine kinase enzyme level is very high. Enzymes can be used **therapeutically** such as digestive enzymes.

10.2.1 Activation Energy

All the chemical reactions in biological system have an energy barrier which prevents reactions from proceeding in an uncontrolled and spontaneous manner. **The input of energy required to break this energy barrier or to start a reaction is called the activation energy.** For example, a mixture of

hydrogen and oxygen will not react with each other until they receive enough energy from a heat source to achieve the activation energy. You may have noticed that a tin of petrol or kerosene oil kept exposed to air at room temperature,would not catch fire unless ignited by a spark. This tiny spark is adequate to supply the activation energy for few molecules to react. The amount of energy released as result of the conversion of the first few molecules of reactant to product is sufficient to activate other molecules. Similarly, chemical reactions taking place in living organisms also require activation energy. In biological systems, **enzymes** are important components which **enhance the chemical reactions by lowering the activation energy** and help the reactions to occur at a rate appropriate to the needs of the cell. **The enzymes speed up the reactions by lowering activation energy**. Enzymes combine with different types of substrates in such a way that they reduce the amount of energy required for a particular reaction (see Fig. 10.1). In the absence of enzymes in your gut, it would take many years instead of few hours for your last meal to be digested. You will read about the mechanism of enzyme action in the following sections.

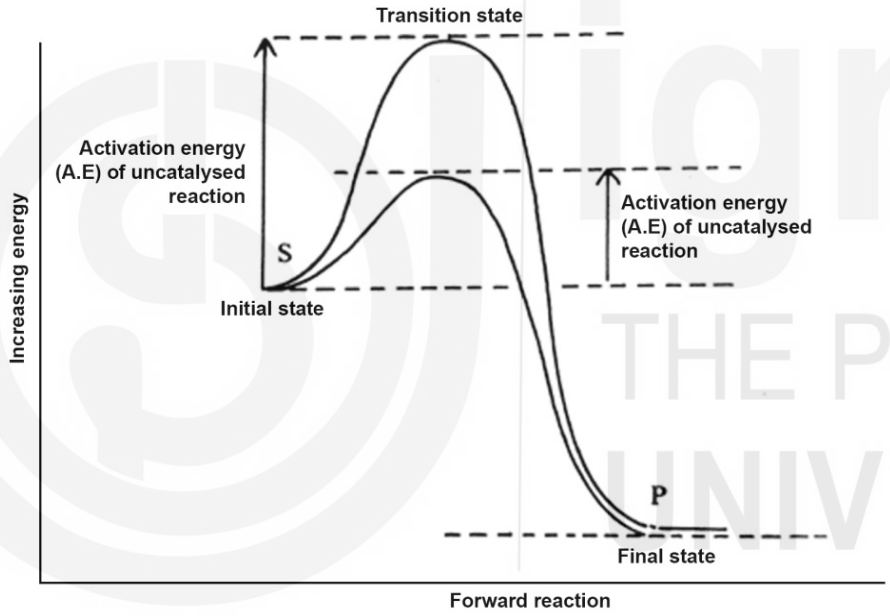
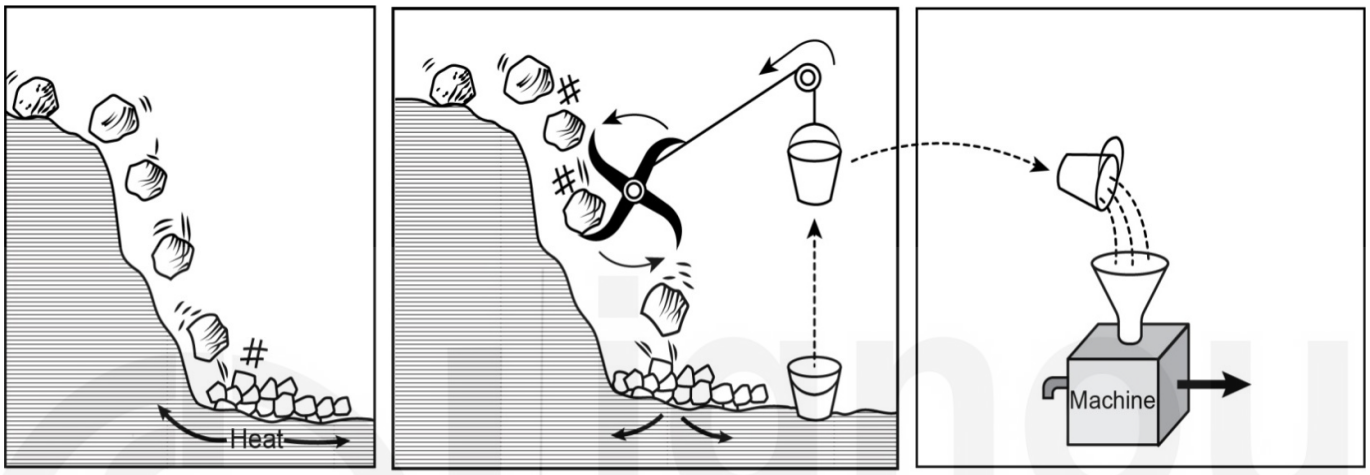


Fig. 10.1: Lowering of activation energy by an enzyme. The activation energy (A.E.) is the energy barrier which the reactants overcome as they react to form the product. The intermediate transition state of enzyme substrate complex lies at the peak of the activation energy barrier.

10.2.2 Coupled Reaction

The energy available to the cells is obtained by burning of food stuff. The breakdown of these food stuffs is carried out by series of reactions, i.e. catabolic reactions in a complex and controlled way. This energy is utilised by the cell for synthetic processes requiring energy, i.e. anabolic reactions which are endergonic in nature. Thus the catabolic reactions which are exergonic in nature and give energy, are coupled to anabolic chemical reactions. This process of coupling maintains a balance in the living systems. The coupling of these reactions is an important characteristic of living organism and is carried out with the help of enzyme-directed reaction pathways. This coupling process can be understood by examining a simple analogy where an energy releasing

catabolic chemical reaction is represented by stones falling from a hill top (see Fig. 10.2). The kinetic energy of falling stones would normally be wasted in the form of heat generated when they hit the ground (see Fig. 10.2 a). A part of this kinetic energy can, however, be trapped and used to drive a paddle wheel that lifts a bucket of water, as shown in Fig. 10.2 b. So we can say that the energy producing reaction of a stone falling is directly coupled to the energy requiring reaction of lifting the bucket of water. A part of the energy of falling stones is now used in lifting a bucket of water and the stones hit the ground with less velocity. So less energy is wasted as heat. The potential energy which is stored in the form of a bucket of water can be used for other purposes such as to drive hydraulic machines (Fig. 10.2 c).



(a) Kinetic energy transformed into heat energy only.

(b) Part of the kinetic energy is used to lift a bucket of water, and a correspondingly smaller amount is transformed into heat

(c) The potential Kinetic energy stored in the elevated bucket of water can be used to drive hydraulic machines.

Fig. 10.2: Schematic diagram of a mechanical model indicating the coupling of reactions. Stones falling from the hill top can be compared with the oxidation of food stuffs (a). Part of the energy of falling stones is conserved in the form of lifted bucket of water which can be compared to the synthesis of ATP(b). The conserved energy can be used for energy requiring reaction such as utilisation of ATP during endergonic reactions (c).

In the living organisms, enzymes play the role of paddle wheels and generate and trap energy in the form of high energy phosphate bonds of adenosine triphosphate (ATP). The ATP thus formed is utilised as a source of energy for many energy-dependent processes and is described as an energy currency of the cell. ATP is hydrolysed in neutral aqueous solutions accompanied by release of energy. The first cleavage of ATP to produce ADP and inorganic phosphate, releases 7.3 K. cals of energy per molecule (see Fig. 10.3).

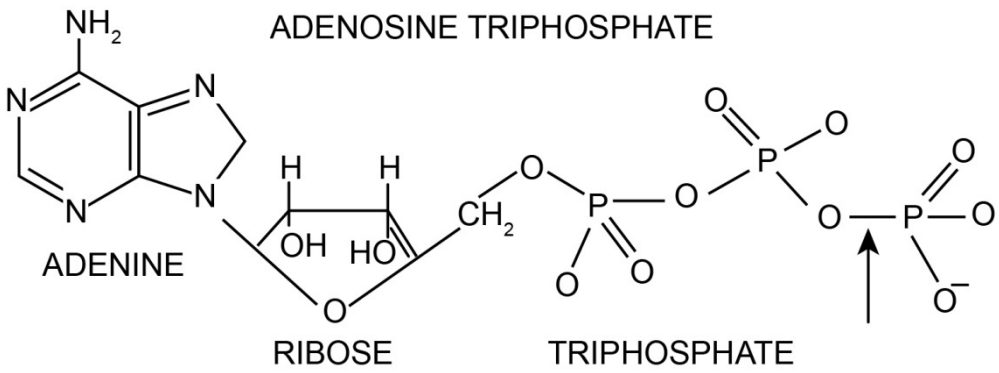


Fig. 10.3: Structural formula of ATP. The first cleavage indicated by an arrow releases 7.3 K. cals of energy per molecule.

SAQ 1

Read the following statements and write True (T) or False (F).

i) Substrate is the reactant of an enzyme catalysed reaction. [ ]

ii) Enzymes are proteins with an ability to catalyse specific reaction. [ ]

iii) All enzyme catalysed reactions produce energy for ATP synthesis. [ ]

iv) Exergonic and endergonic reactions are coupled in all living organisms. [ ]

v) All the enzyme catalysed reactions have higher activation energy. [ ]

10.3 TYPES OF ENZYMES

The Enzyme Commission of the **International Union of Biochemists (IUB)** has classified enzymes into **six categories** on the basis of their functional specificity. Each category is further sub-divided into particular groups consisting of number of enzymes (Table 10.1). Most of the enzymes end as – 'ase'. The first part of the name usually indicates the substrate on which the enzyme acts as for example, amylase enzyme acts on the substrate amylose or starch. A few enzymes are still called by their old names, e.g., pepsin, trypsin, chymotrypsin etc.

Rennin is an enzyme secreted by kidneys to regulate the blood pressure. It is digestive enzyme in gastric juice. **Renin** is a hormone produced by the kidney to control blood sodium levels.

Table 10.1: Classification of Enzymes (IUB system).

Sl. No.	Enzyme Category	Type of reaction	Examples
1.	Oxidoreducases	Oxidation reduction reactions	***Oxidases:*** transfer hydrogen to oxygen, e.g. cytochrome oxidase $Cyt\ H_2 + 1/2O_2 \rightleftharpoons Cyt + H_2O$ reduced cytochrome oxidized cytochrome ***Dehydrogenases*****:** transfer hydrogen to a molecule other than oxygen. e.g. lactatedehydrogenase $C_3H_6O_3 + NAD \rightleftharpoons C_3H_4O_3 + NADH_3$ lactate pyruvate This reaction occurs in liver following heavy exercise.

2.	Transferases	Transfer a functionally important group from one molecule to another	***Transaminases*:** transfer amino groups, making new amino from existing ones e.g. asparatate transaminase aspartate + α-ketoglutarate (amino acid) ⇅ (carboxylic acid) glutamate oxaloacetate (amino acid) (carboxylic acid) This occurs in all cells. ***Kinases*:** transfer phosphate, usually from ATP to another substance e.g. hexokinase ATP + glucose → ADP + glucose-6-phosphate This reaction activates glucose prior to its breakdown in respiration. **Phosphorylases** which add inorganic phosphate without using ATP belong to this category.
3.	Hydrolases	Split molecules in two by the action of water	*All digestive enzymes* fall into this category: pepsin, trypsin etc. e.g. amylase $starch_n + H_2O \rightarrow starch_{n-1}$ + maltose These reactions occur in the gut and transform large insoluble food molecules into smaller soluble ones so as to be absorbed. Similar reactions also occur in the lysosomes of cells. *Phosphatases* which remove phosphate groups from organic molecules by hydrolysis also fall in this category.
4.	Lyases	Add or remove groups without involving water	***Carboxylases*:** add CO_2 e.g. ribulosediphosphate carboxylase ribulosediphosphate + CO_2 → phosphoglyceric acid This occurs in the stroma of chloroplasts and is the principal mechanism by which atmospheric CO_2 is turned into organic material. ***Decarboxylases*:** remove CO_2

			e.g. pyruvate decarboxylase pyruvic acid → acetaldehyde + CO_2 released (It is an Intermediate step in the conversion of sugars to ethanol during alcoholic fermentation).
5.	Isomerases	Convert one isomer of a compound into a different isomer by redistributing the atoms	***Mutases:*** e.g. phosphoglucomutase glucose-1-phosphate ⇌ glucose-6-phosphate This reaction occurs at early steps in the respiration of sugars.
6.	Ligases	Link together two molecules at the expense of ATP	***Synthetases:*** e.g. aminoacylsynthetases Join amino acids to tRNA during protein synthesis.

SAQ 2

According to the IUB system enzymes are classified into six categories. Name these categories and give one example for each.

10.4 COFACTORS

As you have read above, all the enzymes are proteinaceous in nature except ribozymes. Most of the enzymes consist of only polypeptide chains. Some enzymes, however, require an additional chemical component for carrying out their catalytic function. These constituents are non-protein and are called **cofactors**. A complete, catalytically active enzyme together with its cofactor is called a **holoenzyme**. The protein part of the holoenzyme is called **apoenzyme,** which is inactive. The cofactors are of three kinds; prosthetic groups, coenzymes and metal ions.

Prosthetic groupsare organic compounds and are permanently bound to apoenzymes by covalent bonds. For example, in the enzymes peroxidase and catalase, which catalyse the break down of hydrogen peroxide to water and oxygen, heme is the prosthetic group and is a permanent part of the enzyme's active site.

Coenzymes are also organic compounds, but their binding with apoenzyme is transient by non covalent bond, and occurs only during the catalysis. The same coenzyme molecule may act as cofactor in different enzyme-catalysed reactions. Apart from helping enzymes in their catalytic activity these coenzymes also function as transient carrier, i.e. temporary acceptors for specific atoms or functional groups. **Vitamins are essential components of many coenzymes.** For example, the coenzymes

nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) contain vitamin niacin and are electron carriers. You will study about Vitamins in detail in Unit 12 of this course. Coenzyme A (CoA) contains vitamin pantothenic acid and carries (transfers) functional groups such as, an acetyl group.

Metal ions are required by certain enzymes for their catalytic activity. Metal ions such as Mg^{2+}, Mn^{2+} or Zn^{2+} bind with specific side chains at active site and at the same time with the substrate. The binding of metal ions with the substrate helps to break it down into products. For example, zinc is a cofactor for proteolytic enzyme carboxypeptidase. It binds with side chains of amino acid residues in the active site of the enzyme and with a -carboxyl group of substrate amino acid. It is here that the peptide bonds of substrate are broken by the enzyme. Table 10.2 summarises various type of cofactors.

Table 10.2: Some cofactors and their related enzymes.

Cofactor	Enzyme	Reaction
Prosthetic groups		
Heme	Catalase	$2H_2O_2 \dashrightarrow 2H_2O+O_2$
Heme	Peroxidase	$2H_2O_2 \dashrightarrow 2H_2O+O_2$
Coeznymes		
Flavin mononucleotide (FMN)	Some dehydrogenases	Removal of hydrogen atoms
Thiamine pyrophosphate (TPP)	Some decarboxylases	Removal of CO_2
Metal ions		
Zn^{2+}	Carboxypeptidase	Hydrolysis of proteins
Cu^{2+}	Ascorbic acid oxidase	Ascorbate $\rightleftharpoons$ dehydroascorbate
Mg^{2+}	Hexokinase	Glucose + ATP $\dashrightarrow$ glucose phosphate

SAQ 3

Differentiate between coenzymes and prosthetic group.

10.5 MECHANISM OF ENZYME ACTION

In spite of the excellent information that has accumulated over the last several years on the physical, chemical and structural aspects of enzymes, much remains to be understood about their mechanism of action. Significant information on the mechanisms by which ribonuclease, chymotrypsin, lysozyme and some other enzyme catalyse reaction is, however, available.

The general mechanism of enzyme action can be viewed as having three stages. The enzyme first binds to the substrate followed by the formation of enzyme substrate coupled (ES) which finally alters the substrate and forms the product. The events in enzyme catalysed reaction are represented by the equation.

Binding catalysis Release

$$E + S \rightleftharpoons ES \rightleftharpoons EP \rightarrow E + P$$

where E is enzyme S is substrate

P is product ES is enzyme substrate complex

As you know an enzyme speeds up a reaction by lowering its activation energy. Enzymes have specific regions on their surface to which the substrate binds and catalysis takes place. This region is called as the active site of the enzyme. The enzyme recognizes and binds the substrate to its active site and forms an activated enzyme substrate complex. The enzyme acts upon the specific bonds within the substrate which resultsin the formation of the product. Thus the enzymes lower the energy barrier and allow the organisms to carry out reactions at a faster speed.

As enzyme lowers the energy barrier, i.e. activation energy of both the forward and reverse reactions to the same extent. It does not alter a reaction's equilibrium state but merely increases the speed with which the reaction approaches an equilibrium. Furthermore, since a catalyst such as, an enzyme is not permanently changed by participation in a reaction, it comes out exactly as it started, and is ready to catalyse the reaction again. One of the factors responsible for enzyme action is their specificity about which you will read in the following subsection.

10.5.1 Enzyme Specificity

One of the most significant features of enzymes is their specificity with respect to the nature of the reaction they catalyse and also in the actual reactant, i.e. substrate molecule. Enzymes have varying degrees of specificity for substrates. They may recognize and calatyse **a single substrate, a group of substrates or a particular type of bond. You can understand it better by Table 10.3.**

Table 10.3: Types of Enzyme specificity.

Type of Specificity	Reaction Type	Example
Absolute	Catalyze one type of reaction for a single substrate	Urease catalyzes only the hydrolysis of urea
Group	Catalyze one type of reaction for similar substrate	Hexokinase adds a phosphate group to hexoses
Linkage	Catalyze one type of reaction for a specific type of bond	Chymotrypsin catalyzes the hydrolysis of peptide bonds

For example, proteolytic enzymes catalyse the reactions involving protein digestion, whereas urease and succinic dehydrogenase act exclusively on urea and succinate respectively. Enzyme specificity varies even within a particular group of enzymes. Let us take the example of proteolytic enzymes. The reaction catalysed by these enzymes is the hydrolysis of peptide bond (Fig.10.4a). Subtilisin cleaves any peptide bond joining the amino acids of the substrate, whereas enzyme thrombin catalyses the hydrolysis of peptide bonds only between arginine and glycine (Fig. 10.4b). Still some other proteolytic enzymes have different degree of specificity, for example trypsin breaks peptide bonds only on the carboxyl side, i.e. Cterminal of amino acids lysine and arginine (Fig. 10.4c). The specificity differences are due to the biological functions of various proteolytic enzymes. Since bacteria use any protein as a source of carbon and nitrogen, subtilisin enzyme found in them can act upon any type of proteins. Enzymes trypsin and chymotrypsin have limited specificity as they break down the proteins into 5 to 20 amino acids long fragments in the mammalian digestive system.

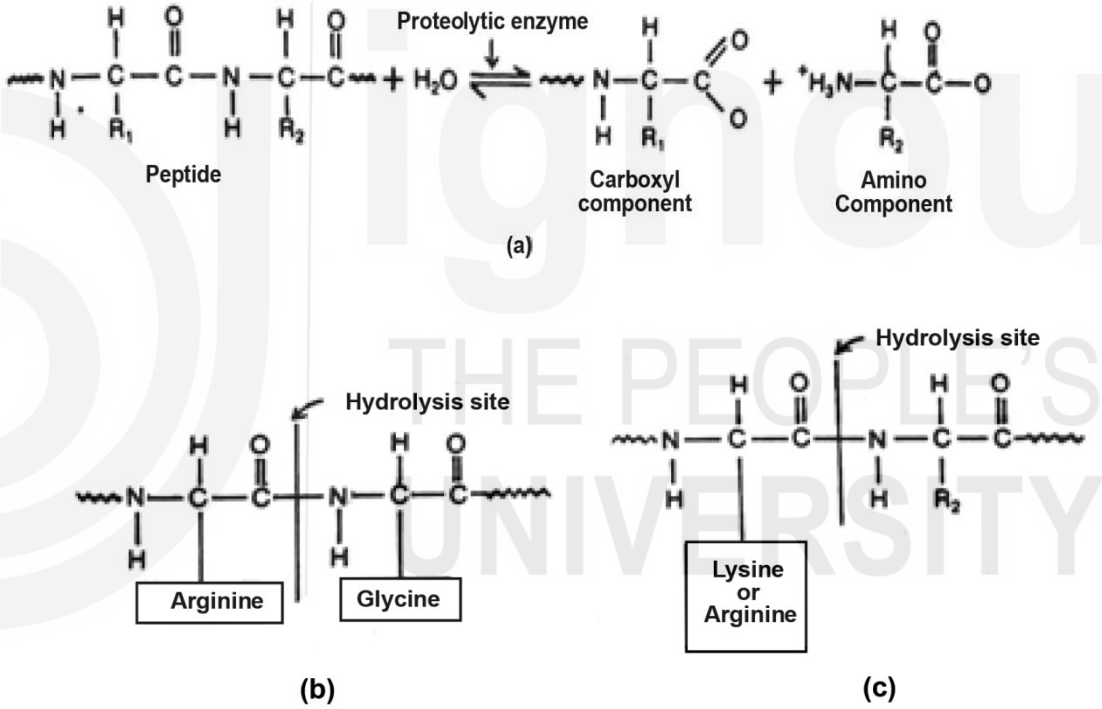


Fig. 10.4: a) Proteolytic enzymes carry out hydrolysis of peptide bonds; b) Specificity of enzyme thrombin; c) Specificity of enzyme trypsin.

Thrombin participates in blood clotting and shows absolute specificity as it splits only a single peptide bond in fibrinogen releasing the fibrin monomer which helps in the formation of fibrin clot. The least specific group of enzymes is hydrolases.

Some enzymes exhibit stereospecificity acting exclusively on different stereoisomers of the same compound e.g., the kidney D-amino acid-oxidase D-isomers of amino acids. However, a small group of enzymes such as racemases catalyse the interconverstion of D and L forms. Some enzymes exhibit geometrical specificity acting only on cis or trans isomers. Cis and trans isomers are geometrical isomers and have different arrangement of their constituent atoms and groups with respect to the double bonds joining the two

carbon atoms. For example, maleic acid and fumaricacid have the same molecular formula but differ in the arrangement of the group the C = C bond. As shown in Figure10.5 Maleic acid, with both the carboxylic group on the same side, is the cis form of fumaric acid, the trans isomer in which these groups are on the opposite side.

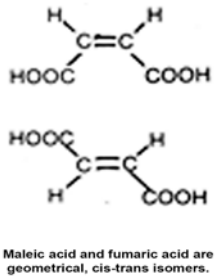


Fig. 10.5: Maleic acid and fumaric acid are geometrical, cis-trans isomers.

In view of the specificity exhibited by the enzymes in binding with the substrates, Emil Fischer in 1984 put forward the **lock and key hypothesis.** According to this hypothesis the substrate molecules fit into the active sites of the enzymes like a key fits into the lock. This results in the formation of transient ES-complex which breaks down into enzymes and product (Fig. 10.6a). The enzyme substrate complex can be isolated from the enzymes that work slowly thus giving the direct evidence for the formation of these ES-complexes.

Although the lock and key model accounts for much of the enzyme substrate specificity, certain observations about enzyme behaviour cannot be explained by this model. For example, sometimes compounds other than the actual substrate bind to the enzyme and fail to form the reaction products. In the 1960's Daniel Koshland proposed the **induced-fit hypothesis** according to which the active site of the enzyme does not initially exist in a shape complementary to the substrate but is induced by the substrate to assume the complementary shape to bind the substrate molecule. Active site of an enzyme is thus regarded as flexible in nature (see Fig. 10.6b). However, a flexible active site is not necessary for all enzyme catalysed reactions as some are adequately explained by the lock and key model.

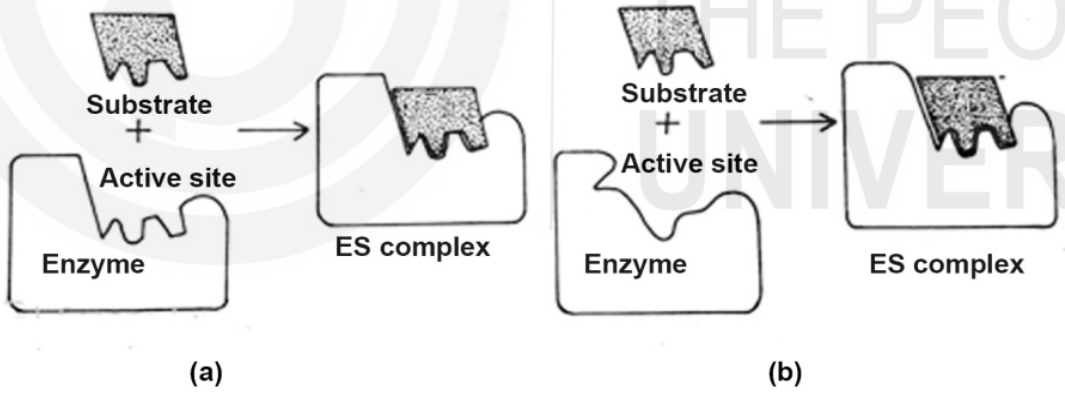


Fig. 10.6: a) Lock and key model: the active site of the enzyme is complementary in shape to that of the substrate. Substrate fits in the active site like a key fits in the lock. b) Induced fit model: the enzyme changes its shape upon binding the substrate. The active site acquires complementary shape to that of substrate only after the substrate is bound.

10.5.2 Molecular Basis of Enzyme Action

The molecular basis of enzyme action depends on its three dimensional structure. The making .and breaking of chemical bonds by an enzyme in a reaction are preceded by the formation of an enzyme substrate complex. As you have read earlier, the substrate is bound to a specific region of enzyme called active site. The properties and positions of side chains of the enzymes

exposed at the active site determine which substrates will bind to it. The specific amino acid side chains at the active site are not necessarily close to each other in the linear protein chain. Folding of the protein chain, however, brings these groups together. For instance, negatively charged side chain groups, like those of asparatate and glutamate, can be forced together, due to the folding of the enzyme protein molecules in spite of their tendency to repulse each other. This leads to an increase in affinity for the positively charged groups of the substrate. Similarly, some amino acid side chains forced together as a result of folding of peptide chains, may interact through hydrogen bonding, for example in trypsin, a normally non-reactive serine molecule (CH_2OH) becomes highly reactive by acquiring two negative charges (Fig. 10.7).

When an enzyme is denatured, the native three dimensional structure is disrupted. Amino acid side chains of the active site unfold and separate from the site. This prevents their participation in a chemical reaction thus inactivating the enzyme. Enzyme inactivation is also caused even when only a functional side group is displaced or substituted in the enzyme.

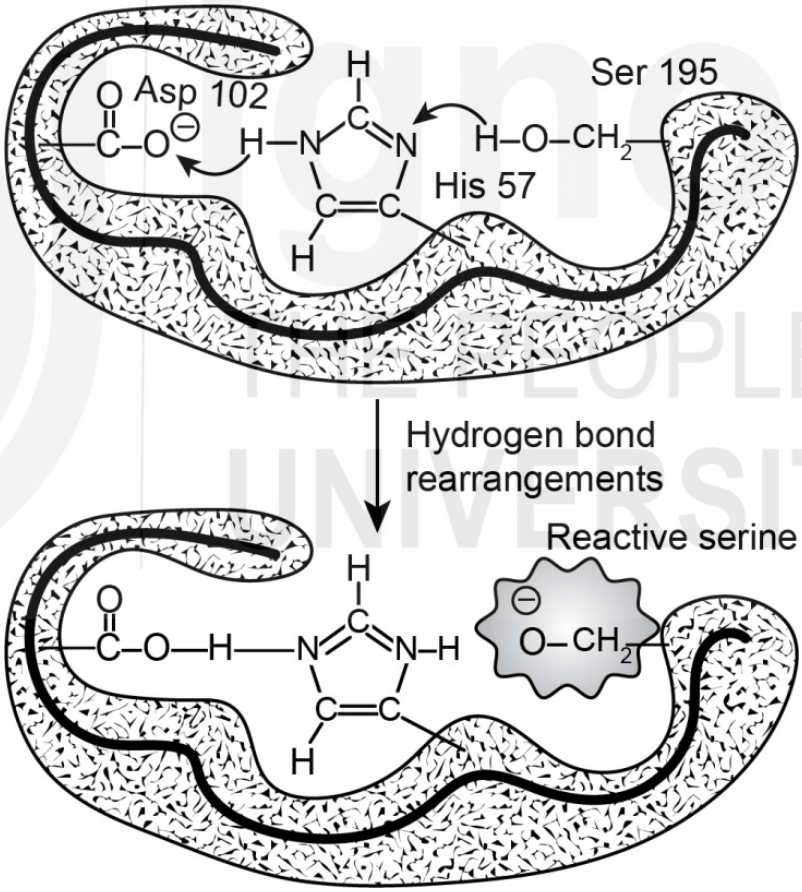


Fig. 10.7: Rearrangements of hydrogen bond makes serine molecule highly reactive in the active site of the enzyme.

Although enzymes differ widely in structure, specificity and mode of catalysis, various mechanisms operate at the active site of enzymes, each contributing to the lowering of activation energy. These mechanisms can be generalised as follows:

- The binding of reacting molecules with each other brings them in close proximity, thus increasing the chance of a reaction.

- The substrates are bound to the enzyme in such a manner that the formation of several types of temporary non-covalent bonds between them force a redistribution of charges within substrates. This redistribution imposes a strain on specific covalent bonds in the substrates resulting in the breaking of the bond.
- Hydrophobic amino acids eliminate water molecules from the active site to create a water free zone so that non polar groups react more easily.
- Acidic and basic amino acids in the active site of the enzyme transfer the protons to and from the reactants for catalysis of reaction. This is known as acid-base catalysis.
- Enzymes react with the substrate to form transient, high energy unstable covalently linked enzymes substrate-complex which undergoes further reactions to form products.

Although these factors are believed to contribute to the acceleration of reaction rate by various enzymes, the exact mechanism involved is yet to be known.

SAQ 4

Complete the following statements with appropriate words.

i) The substrate binds on the …….. of the enzyme and forms…….. .

ii) Enzymes do not alter the …….. of the reactions.

iii) …….. acts upon any peptide bond between the amino acids.

iv) Thrombin has …….. specificity.

v) According to …….. active site assumes the complementary shape after the …….. bound.

vi) …….. of the polypeptide chains bring the side chains together in the active site region.

vii) Transfer of protons to and from the reactants is …….. catalysis.

10.6 FACTORS AFFECTING THE RATE OF ENZYME ACTION

Enzymes are capable of enhancing the reaction rate enormously, sometimes a million times compared to uncatalysed reactions. The rates of enzymes catalysed reactions are expressed as the **"turnover number"**. The turnover number is the number of substrate molecules transformed into product in unit time by a single enzyme molecules transformed into product in unit time by a single catalytic site, and depends upon the enzyme concentration. So if the enzyme concentration is known, the turnover number can be calculated.

$$\text{Turnover number} = \frac{\text{moles of product formed per unit time}}{\text{moles of enzymes}}$$

Turnover numbers of several enzymes are listed in Table 10.4. The highest turnover number is of carbonic anhydrase which converts 3.6×10^6 CO_2 molecules to H_2CO_3 per minute. International Union of Biochemistry has now recommended a new unit for reaction rates called **Katal (kat)**. One Kat is the amount of enzyme that converts one mole of substrate into product per second.

Table 10.4: Turnover Number of Some Enzymes.

Enzyme	Turnover Number
Carbonic anhydrase	3.6×10^6
Acetylcholinesterase	1.5×10^6
Urease	1.0×10^6
Amylase	1.0×10^5
Lactic dehydrogenase	6.0×10^4
Chymotrypsin	6.0×10^3
Lysozyme	3.0×10^1

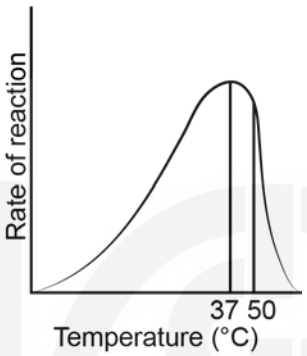


Fig. 10.8: Effect of temperature on the rate of enzyme catalysed reactions. Optimum temperature varies for different enzymes and different species.

Rates of enzyme catalysed reactions depend on several factors like temperature, pH, concentration of enzyme and substrate etc.

10.6.1 Effect of Temperature

Within certain limits temperature influences the enzyme catalysed reactions in the same way as it affects ordinary uncatalysed chemical reactions. As the temperature increases, the rate of a chemical reaction increases owing to an increase in the number of activated molecules. But when the temperature rises above a certain limit, it destroys the tertiary structure of the enzyme causing the loss of its activity. Similarly low temperature, such as freezing temperatures, generally inactivate the enzyme. It therefore, follows the rule thefor every enzyme under a given set of conditions, there is a temperature at which the activity of the enzyme is at its maximum. This is known as optimum temperature (see Fig. 10.8). Most enzymes are denatured above 50°C. Optimum temperature for mammals and birds is about 35° - 40°C whereas in plants and in other animalss it is between 20° - 35°C. Some procaryotes and certain fishes found in Antartica region are exceptional in that they are adapted to life in hot spring and at freezing cold temperatures. They have optimum temperature at over 80°C and below freezing point respectively.

10.6.2 Effect of pH

Characteristically, each enzyme has a pH value at which the reaction rate is optimum usually within the pH range 7.0 ± 1.5. This is known as optimum pH at which a certain enzyme causes a reaction to progress most rapidly. On each side of this pH value the reaction is decreased and at certain pH values on both sides an enzyme may be inactivated or even denatured. Therefore, while studying enzymes, buffers are used to keep the enzyme at an optimum pH.

Buffers are the combination of substances that resist changes in pH when acid or bases are added. It consists of a weak acid and its conjugate base or a weak base and its conjugate acid, for example acetic acid (CH_3COOH) and acetate (CH_3COO^-) can serve as a buffer when pH of the solution has to be adjusted around pH 1.

While majority of enzymes function optimally around neutral pH values, some enzymes like pepsin in stomach has an unusually low optimum pH (Fig. 10.9).

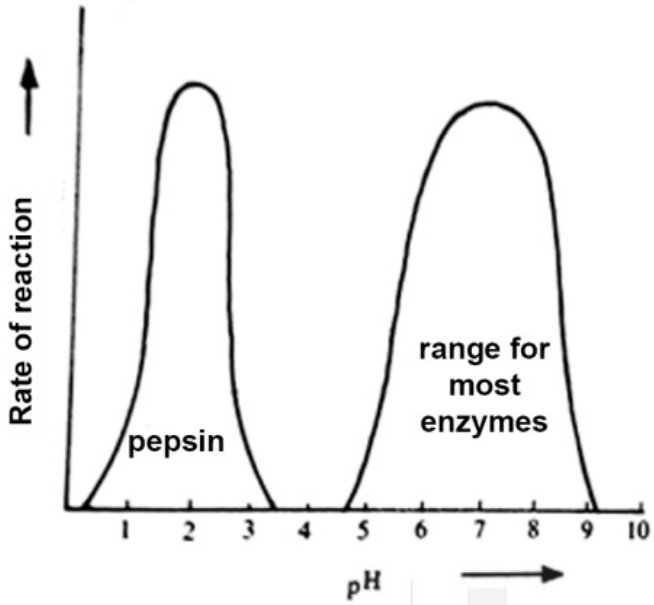


Fig. 10.9: Effect of pH on the rate of enzyme catalysed reactions. Proteolytic enzyme pepsin has low optimum pH.

10.6.3 Effect of Enzyme Concentration

As you know, enzymes increase the reaction rate through catalysis. Under constant conditions of temperature, pH and substrate concentrationthe velocity of the reaction is directly proportional to the amount of enzymes present, i.e., the reaction rate is increased with the increase in enzyme concentration. However, the equilibrium constant for the reaction is not affected by the presence of enzymes.

SAQ 5

Match the statements in column I with those in column II.

Column I		Column II	
i) Temperature influences enzyme catalysed reactions	[ ]	a) It is active only in acidic medium.	
ii) Mammals and birds also have optimum temperature.	[ ]	b) Enzyme concentration has its effect on reaction rate.	
iii) Enzyme pepsin acts on peptide bonds in the stomach.	[ ]	c) Enzymes are inactivated at freezing temperature.	
iv) Reaction rate is directly proportional to the amount of enzyme.	[ ]	d) It is between 35° C to 40°C	

Enzymes not only speed up the reactions, they also have a marked effect on the kinetics of the reaction. Substrate concentration plays an important role in enzymes kinetics about which you will read in the next Unit.

10.7 ALLOSTERIC ENZYMES

Some enzymes may have two functionally different binding sites. One of the site, the active site, binds the substrate and catalyses the reaction. The other type of site, known as allosteric or **regulatory site** binds another molecule which is called **effector or modulator.** Such enzymes are known as allosteric enzymes. These enzymes are oligomeric in nature, i.e. they have more than one subunit. The active site and allosteric site may be located on the same subunit or on different subunits of the enzyme and the conformational changes caused by binding are transmitted between subunits. Effector molecules are of two types: positive effectors, i.e. **activators** that enhance enzyme activity and negative effectors or **inhibitors** which inhibit enzyme activity. Binding of effectors causes conformational changes in the allosteric enzymes which influence their catalytic activity (see Fig. 10.10). We can also say that allosteric enzyme action is regulated by effector molecules. You will study about allosteric enzyme regulation in detail in Unit 11. These effector molecules can bind at the same allosteric site or at different allosteric sites.

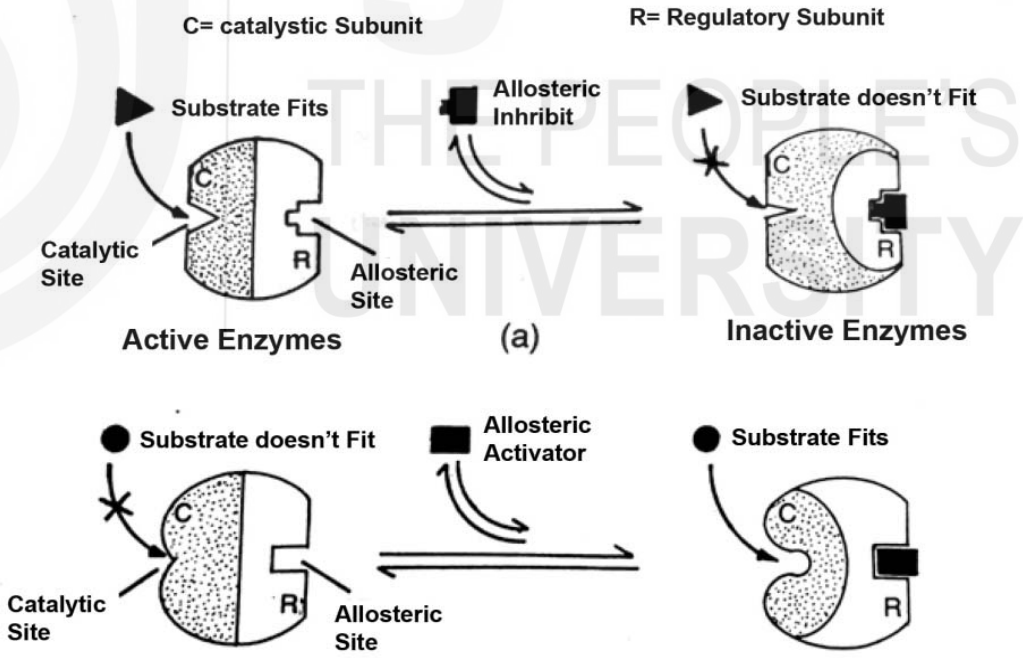


Fig. 10.10: Schematic diagram showing the functioning of allosteric enzymes.

Binding of a substrate to one active site may influence subsequent bindings to other active sites of an enzyme with more than one active sites. This behaviour of enzymes is known as **cooperativity.** When binding of the first substrate molecule promotes binding of subsequent substrate molecules, it is called **positive cooperativity. Negative cooperativity** is when, after the binding of the first substrate molecule subsequent substrate binding occurs less readily.

10.8 ISOENZYMES

Several enzymes exist in their multiple forms termed **isoenzymes** or **isozymes.** These isoenzymes are not isomers. All isoenzyme forms of a given enzyme catalyse the same reaction but are chemically distinct molecules. They may differ in amino acid composition, amino acid sequence, charge, molecular weight, etc. They usually differ in one or more kinetic properties such as Km, Vmax for their substrate.

Isoenzymes are found in all vertebrates, insects, plants and unicellular organisms. Different tissues may contain different isoenzymes which may differ in their affinity for the substrates. One of the first and best known example of universally occurring isoenzymes is lactate dehydrogenase that exists in five different forms: M_4, M_3H, M_2H_2, MH_3 and H_4. Each of these isozymes is a tetramer made up of four polypeptide subunits. These subunits are of two types and occur in different combinations in the isozymes. These two types of subunits are named as M and H types depending upon their location; M in muscles and H in heart (Fig. 10.11).

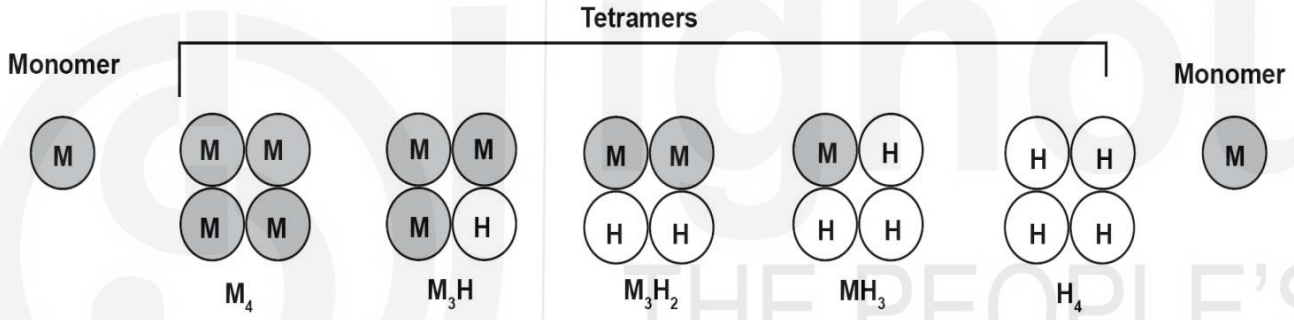


Fig. 10.11: Lactate dehydrogenase enzyme is a tetramer, has five isozymic forms. Each tetramer has M and/or H types of monomers in different numbers.

All the forms of lactate dehydrogenase isoenzymes do not necessarily occur in equal proportion in all the cells as M_4 dominates in muscles and H_4 is predominantly present in the heart. Other isoenzyme forms include oxidases, dehydrogenases. Several regulatory enzymes also exist in isoenzyme forms. The molecular difference of isoenzyme molecules is due to the difference in their locations such as malate dehydrogenase, which occurs in different forms in cystosol and in mitochondria. Analysis of isozymes is frequently used in medical diagnosis.

10.8 ASSAY OF ENZYME ACTIVITY

All enzymes so far known are proteins, yet the specific tests for protein can not be used for the detection and/or quantification of enzymes. Evidently such tests cannot make distinction between enzymes and non-enzymes proteins and between various enzymes. The amount of enzymes in a given solution or tissue extract can be conveniently measured or assayed quantitatively by using techniques that can measure their ability to convert the substrate into product.

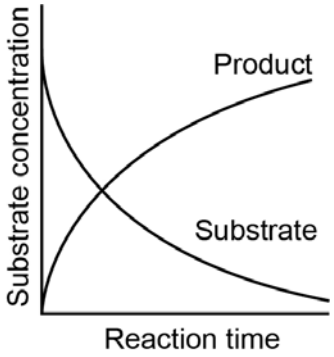


Fig. 10.12: Concentrations of Substrate and product vary during the course of enzyme-catalysed reactions. As the reaction proceeds, substrate concentration decreases and product concentration increases.

One can detect the presence of an enzyme by using a specific quantification procedure either for the substrate or for product. Under optimal conditions the velocity of enzyme catalysed reaction is directly proportional to the concentration of the enzyme. One can therefore determine the concentration of enzyme from the velocity of the disappearance of the substrate or that of the formation of product under optimal conditions. As shown in Fig. 10.12, during the enzyme action the concentration of substrate decreases, while that of product increases. **This quantitative estimation of enzyme activity is called as the assay of an enzyme. Activity of enzyme is represented in enzyme units**.

By international convention one unit of enzyme activity is defined as that amount which transforms one micro mole substrate to product in one minute under optimal conditions. **Enzyme assays are carried out at their optimal pH, temperature and with a near saturating concentration of substrates**.

Concentration of different substrates or products can be determined using methods such as colorimetric, spectrophotometric, fluorometric or isotopic labelling procedures. The choice of the procedure is determined by the nature of the substrate or product. For example, spectrophotometric assays are used when either the substrate or product absorbs light of some specific wavelength. Highly sensitive assay procedures are usually based on fluorescence or radioactive measurements.

In many instances substrates are modified by introducing specific groups for the estimation of their products. Such modification of the substrate should not, however, result in decrease in the rate of its transformation to product by the enzyme. For instance the introduction of p-nitrophenyl phosphate compound in substrate makes the substrate useful for the assay of phosphatases. The product of enzyme action is an ionised form having pnitrophenylate ion which is of yellow colour and the intensity of colour indicates the activity of the enzyme.

We finish this unit on characteristics and mechanism of enzymes. We will continue with the enzyme kinetics and regulation of enzyme activity in the next unit.

10.9 SUMMARY

In this unit you have studied that:

- Enzymes are proteins that catalyse the biochemical reactions by lowering the activation energy. Enzymes increase the rate of the reactions but do not alter the equilibrium point.
- Enzymes facilitate the coupling of energy releasing exergonic reactions to energy requiring endergonic reactions.
- Enzymes are highly specific with respect to the nature of reaction as well as to their substrate. Enzymes substrate specificity ha been explained by lock and key and induced fit hypothesis.

- Reactions by the enzymes are facilitated on the basis of proximity and orientation of side chains of the substrate and enzyme, acid-base catalysis by the side chain of charged amino acids of active site and by covalent interactions between enzymes and substrates.
- Changes in the concentration of enzymes, substrates, pH and temperature influence the rate of *enzyme* catalysed reaction.
- Some enzymes require additional factors called cofactors for their catalytic activity. Prosthetic groups are organic cofactors and arepermanently bound to the enzymes. Coenzymes are also organic compounds but their association is transient. Some enzymes require metal ions for their activity.
- Allosteric enzymes are oligomeric and have more than one active site where the allosteric effector molecules influence their enzyme activity. Influence of one substrate binding on subsequent substrate bindings is known as cooperatively.
- Enzyme activity can be estimated by different procdures such as spectophotometric, fluorometric and isotopic labeling methods.

10.10 TERMINAL QUESTIONS

1) What is activation energy?
2) Discuss briefly the variations in the degree of enzyme specificity.
3) How temperature and pH affect the rate of enzyme action?.
4) Explain briefly the functioning of allosteric enzymes.

10.11 ANSWERS

Self Assessment Questions

1) i) T, ii) T, iii) F, iv) T, v) F.
2) i) Oxidoreductases-Cytochrome oxidase
   ii) Transferase-hexokinase
   iii) Hydrolases-amylase
   iv) Lyases-Decarboxylase
   v) Isomerases-Phosphoglucomutase
   vi) Ligases-Aminoacylsynthetases
3) Coenzymes are the organic cofactors which are transiently bound to enzymes for their catalytic activity.
4) i) active site, enzyme-substrate complex
   ii) equilibrium state

iii) subtilisin

iv) absolute

v) induced fit hypothesis, substrate

vi) folding

vii) hydrophobic, non-polar

viii) acid-base

5) i) c, ii) d, iii)a, iv) b.

Terminal Questions

1) The energy required to start a reaction is called the activation energy. In biological systems activation energy is lowered with the help of enzymes.

2) Enzymes exhibit specificity for the substrate as well for the reactions they catalyse. There is also variation in specificity within the particular groups of enzymes. Example of proteolytic enzymes can be given.

3) Refer to Sub-Sections 10.6.1 and 10.6.2.

4) Allosteric enzymes are regulated by other molecules called as effectors. These effectors bind to the allosteric or regulatory site of the enzyme and may act as activator or inhibitor of enzyme action.

UNIT 11

ENZYME KINETICS AND REGULATION

Structure

11.1 INTRODUCTION

Kinetics is the study of reaction rates, their quantitative measurement and a systematic study of the factors influencing the activity of enzymes. In this unit, you will gain an insight of the enzymatic mechanisms as well as role played by enzyme activity in regulating metabolic pathways. Enzymes convert substrates to products through a series of steps (enzymatic mechanism). Therefore the effect of substrate concentration on enzyme activity is one of key concepts in enzyme kinetics. Several models have been proposed to explain the kinetics of enzyme catalyzed reactions. Classical experimental work for single enzyme catalyzed reactions is Henri-Michaelis-Menten plot, Briggs Haldane equation, Lineweaver-Burk plot, etc.

The catalytic efficiency of enzymes needs to be effectively controlled to ensure proper homeostasis. Several different mechanisms are prevalent in the cell to regulate the activity of enzymes. The effector molecules regulating the activity of enzymes act as vital elements in the integration of metabolic pathways. The necessity for regulation of enzyme arises from the fact that the complex system of biological transformations has to be regulated so that concentrations of key metabolites are controlled both in terms of space and time to direct metabolism in a desired direction and not allow drifting. Any abnormalities in the metabolic pools of enzymes or their regulation will lead to severe disorders or diseases such as cancer, diabetes or neurodegeneration.

Objectives

After studying this unit, you should be able to:

- derive Michaelis-Menten equation,
- explain the mechanisms of enzyme catalysis,
- draw Lineweaver-Burk plot,
- describe K_m and V_{max}, and
- determine different regulatory mechanisms for directionality of metabolic pathways.

11.2 ENZYME KINETICS

Kinetic analysis helps to disclose the number and order of the individual steps involved in the transformation of substrates to products. In the past, data generated from the experiments of enzyme catalyzed reactions was collected and analyzed to determine the rate of a reaction. It was found out that at low concentrations of substrate, the reaction was of first-order with respect to the substrate. However, at the higher concentrations of substrate, the reaction became zero-order. Please recall from your chemistry books regarding the zero order or first order enzyme catalyzed reactions. Generally all single substrate enzyme catalyzed reactions and even multi-substrate reactions where concentrations of all but one were kept constant follows the same order. At constant enzyme concentration, graph of initial velocity [v_o] (on y-axis) against substrate [S] concentration (on x-axis) was found to exhibit a hyperbolic curve (Fig. 11.1).

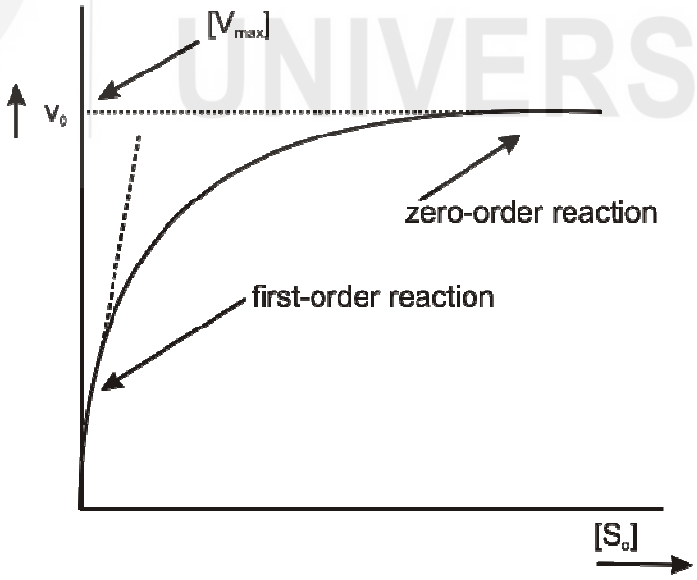


Fig. 11.1: Graph of initial velocity against substrate concentration for a single substrate enzyme catalyzed reaction.

The general equation from the graph is

$$v_o = \frac{V_{max}[S_o]}{[S_o] + b}$$

V_{max} = Maximum velocity = maximum value of v_o

b = constant = value of [S_o] where $v_o = ½\ V_{max}$

In general terms, in a mono substrate enzyme catalyzed reaction and considering just one substrate binding site per enzyme molecule, substrate [*S*] comes in physical contact with enzyme [*E*] to form an enzyme substrate complex [*ES*] complex which eventually undergoes a further reaction and leads to the formation of product [*P*]. It can be represented as:

rate constant k_1 rate constant k_2

$$E + S \rightleftharpoons ES \longrightarrow E + P$$

rate constant k_{-1}

k_1 = rate constant for the association of substrate and enzyme

k_2 = rate constant for the breakdown of enzyme and product

k_{-1} = rate constant for the dissociation of [*ES*] complex to form free enzyme and substrate

The overall rate of reaction is limited by two factors:

1) The amount of concentration of enzyme

2) The breakdown of enzyme-substrate complex

At low substrate concentrations, the overall rate of reaction will be limited by the rate at which enzyme and substrate molecules react to form enzyme-substrate complex. At constant enzyme concentration, the rate of reaction will be proportional to the substrate concentration (first-order reaction). However, at high substrate concentration enzyme will be saturated with the substrate and therefore no free enzyme will be available. So the overall rate of reaction will be independent of the substrate concentration. The maximum initial velocity possible will be

$V_{max} = k_2 [E_o]$

SAQ 1

How does substrate concentration affect the rate of enzymatic reactions?

11.2.1 Michaelis-Menten Equation

Kinetic models used to explain above mentioned findings were proposed by Michaelis and Menten (1913). The Michaelis-Menten equation demonstrates the relationship between initial reaction velocity and substrate concentration. Derivation of this equation begins with the generalized scheme of events considering a single substrate enzyme catalyzed reaction as stated earlier; Substrate [*S*] interacts with enzyme [*E*] to form an enzyme substrate complex [*ES*] complex which eventually breaks down to free enzyme [*E*] and the formation of product [*P*]. The Michaelis and Menten set out the following scheme:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

The term k_1 denotes the rate constant for the formation of *ES* complex. *ES* complex has two fates, it can dissociate back to enzyme and substrate with rate constant k_{-1}, or proceed to form product and release the free enzyme with a rate constant k_2. In any enzyme catalyzed reaction, the concentration of substrate should be five or six orders higher than that of enzyme. The above model also assumes that $k_2 << k_1$. There is every possibility that reaction can go backward but if we consider only the initial rate of a reaction, we can ignore the backward reaction.

The overall rate of reaction is termed as initial velocity (v_o) and it will depend on two factors – the rate of formation of product (k_2) and the concentration of enzyme bound with the substrate i.e [*ES*]

So $v_o = k_2[ES]$ ……equation 1

Michaelis and Menten made two assumptions in their model. First, the availability of excess substrate [*S*] >> [*E*]. Secondly a rapid equilibrium is established between the reactants ([*E*] + [*S*]) and [*ES*] complex. Moreover, the breakdown of enzyme-substrate complex is too slow to cause any change in equilibrium. Hence Michaelis and Menten model is also known as "Rapid equilibrium model". Thus at the equilibrium state:

$k_1[E][S] = k_{-1}[ES]$ ……equation 2

$$\frac{[E][S]}{[ES]} = \frac{k_{-1}}{k_1} = K_s$$ ……equation 3

K_s is the dissociation constant. If E_o is the total enzyme concentration, it is equal to the sum of free enzyme [*E*] and enzyme bound to the substrate [*ES*] as represented in the following equation:

$E_o = [E] + [ES]$ ……equation 4

$E = [E_o] - [ES]$

Substituting the value of *E* in equation 3

$$\frac{([E_o] - [ES])[S]}{[ES]} = K_s$$

$([E_o] - [ES])[S] = K_s[ES]$

$[E_o][S] - [ES][S] = K_s[ES]$

$[E_o][S] = K_s[ES] + [ES][S]$

$$[E_o][S] = [ES](K_s + [S])$$

$$[ES] = \frac{[E_o][S]}{(K_s + [S])}$$

Substituting the value of [*ES*] in equation 1 we get

So $v_o = k_2 \frac{[E_o][S]}{(K_s + [S])}$ ……..equation 5

Maximum rate of enzyme reaction will be achieved when all the enzyme molecules are bound to the substrate molecules.

So $V_{max} = k_2 [E_o]$ …….equation 6

Substituting this value in equation 5 we get

$$v_o = \frac{V_{max}[S]}{(K_s + [S])}$$

Michaelis and Menten also made the supposition that initial substrate concentration [S_o] is much higher than the initial enzyme concentration [E_o], in such a scenario the formation of enzyme-substrate complex will have no such big change in free substrate concentration. The expression for v_o will be:

$$v_o = \frac{V_{max}[S_o]}{(K_s + [S_o])}$$ …….equation 7

The above equation equation 7 is well known as Michaelis-Menten equation

Briggs Haldane modified Michaelis-Menten Plot:

The Michaelis-Menten model is dependent on the assumption of rapid equilibrium approach in any enzyme catalyzed reaction. It limits the applicability of the equation to only rapid kinetic reactions which might not be the case with many of the other enzyme reactions. Most of enzyme catalyzed reactions assume a constant concentration of enzyme-substrate complex [*ES*].

Generally if an enzyme is mixed with high concentration of a substrate there is an initial period expressed as pre-steady state (lasts in micro seconds) where concentration of [*ES*] slowly builds up. Eventually the concentration of [*ES*] builds up and attains a steady state, remains constant over time. The **steady-state concept** was introduced by Briggs and Haldane in 1925, a modified Michaelis-Menten method. This concept was considered more valid assumption than the earlier ones. The Michaelis-Menten model gave importance to the formation of [*ES*] while Briggs-Haldane method focuses on the consistency of [*ES*] complex, its maintenance at constant concentration and breakdown to products.

Considering the single substrate enzyme catalyzed reaction one more time. In this reaction at steady state, rate of formation of [*ES*] will be equal to the rate of its decomposition to products. Therefore

$k_1[E][S] = k_{-1}[ES] + k_2[ES]$ ---------- equation 8

Separating the constant from variables:

$$\frac{[E][S]}{[ES]} = \frac{k_{-1} + k_2[ES]}{k_1} = K_m$$ ---------- equation 9

Where K_m = Michaelis constant

Substituting K_s with K_m in the above equations (4-7)

$$E = [E_o] - [ES]$$

Substituting the value of *E* in equation 9

$$\frac{([E_o] - [ES])[S]}{[ES]} = K_m$$

$$([E_o] - [ES])[S] = K_m[ES]$$

$$[E_o][S] - [ES][S] = K_m[ES]$$

$$[E_o][S] = K_m[ES] + [ES][S]$$

$$[E_o][S] = [ES](K_m + [S])$$

$$[ES] = \frac{[E_o][S]}{(K_m + [S])}$$

Substituting the value of [*ES*] in equation 1 we get

So $v_o = k_2 \frac{[E_o][S]}{(K_m + [S])}$

Since $V_{max} = k_2 [E_o]$

Substituting this value in above equation

$$v_o = \frac{V_{max}[S]}{(K_m + [S])}$$

Since substrate concentration is much higher than the enzyme concentration [*S*]~ $[S_o]$ so

$$v_o = \frac{V_{max}[S_o]}{(K_m + [S_o])}$$ ……..equation 10

The above equation equation 10 is similar to well known Michaelis-Menten equation. The only change is the K_m instead of K_s in the denominator. So the equation retains its previous name of Michaelis-Menten equation and constant K_m is known as Michaelis-Menten constant.

A graph of v_o at y-axis vs substrate concentration [*S*] at x-axis will be in the form of a hyperbolic curve (Fig. 11.2).

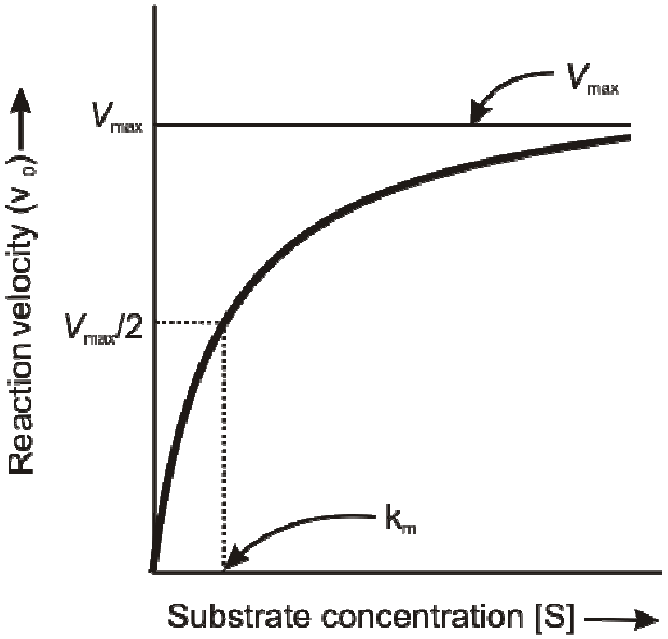


Fig. 11.2: Michaelis-Menten plot of a single substrate catalyzed enzyme reaction.

SAQ 2

Explain how the rate of an enzyme-catalyzed reaction reaches a maximum value at high substrate in a Michaelis-Menten equation?

11.2.2 Lineweaver Burke Plot

If you look at the Michaelis-Menten plot, you will observe that v_o approaches V_{max} in a tangential manner at higher substrate concentrations. So if you want to determine V_{max} and K_m from the plot, it will be difficult and unsatisfactory. A hyperbolic curve nature of the graph makes it difficult to determine the accurate value of V_{max} and K_m. Therefore, to overcome this difficulty, Lineweaver and Burk (1934) suggested a straight line graph for enzyme catalyzed reactions obeying Michaelis-Menten equation. They did not made any new assumptions and derive the Lineweaver-Burk plot, which is also known as double reciprocal plot. Lineweaver and Burk took the Michaelis-Menten equation and inverted it.

$$v_o = \frac{V_{max}[S_o]}{(K_m + [S_o])}$$

$$\frac{1}{v_o} = \frac{(K_m + [S_o])}{V_{max}[S_o]}$$

$$\frac{1}{v_o} = \frac{[S_o]}{V_{max}[S_o]} + \frac{K_m}{V_{max}[S_o]}$$

$$\frac{1}{v_o} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}[S_o]}$$

This equation is known as Lineweaver-Burk equation. It is in the form of y = mx+c …….equation 11

which is the equation of a straight line graph. Plot of $1/v$ against $1/S_o$ is linear and obeys Michaelis-Menten equation (Figure 11.3). This is known as Lineweaver-Burk plot or double reciprocal plot.

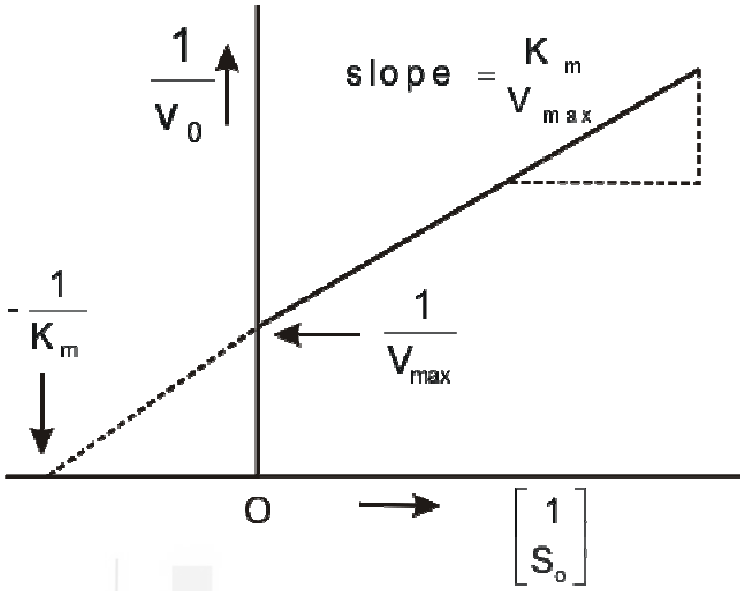


Fig. 11.3: Double reciprocal plot of the Michaelis-Menten equation.

11.2.3 Significance of K_m and V_{max}

K_m or Michaelis constant is defined as the substrate concentration which allows the enzyme to achieve half of V_{max} (maximal velocity) [refer Figure 11.2 and 11.3]. Moreover, K_m has the units of concentration but it is independent of the enzyme and substrate concentration. K_m measures inverse of affinity. The substrate concentration (K_m) needed to transfer half of the enzyme molecules into *ES* complex denotes the affinity of the enzyme for the substrate. Low values of K_m denote high affinity of enzyme for the substrate. On the other hand, high values of K_m suggest that enzyme needs relatively high levels of substrate concentration for its saturation thereby implying poor affinity of the enzyme for substrate.

Both K_m and V_{max} are kinetic parameters. The rate of reaction becomes maximum when the enzyme is fully saturated with substrate molecules and is denoted as V_{max}.

SAQ 3

Tick [√] mark the correct option:

a) Michaelis-Menten equation relates the rate of an enzyme-catalyzed reaction to substrate concentration/product concentration (pick one).

b) A hyperbolic curve gives an accurate value of V_{max} and K_{max} (True/False).

c) Lineweaver-Burk plot is linear/hyperbolic (Choose one option).

d) K_m is equal/more to the substrate concentration at ½ V_{max} (Choose one option).

11.2.4 k_{cat} and Turnover Number

To determine the enzyme efficiency in enzyme kinetics, we are interested to know how many maximum molecules of substrate can be converted into product per catalytic site of a given concentration of enzyme per unit time.

$k_{cat} = V_{max}/E_t$

where

k_{cat} = Turnover number,

V_{max} = Maximum rate of reaction when enzyme catalytic site is saturated with substrate E_t =Total enzyme concentration or concentration of total enzyme catalytic sites.

The k_{cat} is a direct measure of catalytic production of product under optimal conditions. The units of **Turn over number (**k_{cat}) = (moles of product/sec)/ (moles of enzyme) or sec^{-1}.

Enzyme carbonic anhydrase catalyzes conversion of carbon dioxide to carbonic acid and bicarbonate ions. Its turnover number of 400,000 to 600,000 s^{-1} suggests that each enzyme molecule can produce up to 600,000 molecules of product (bicarbonate ions) per second.

11.3 REGULATION OF ENZYME ACTIVITY

Have you thought of ways by which enzyme activity can be regulated? There are various mechanisms of enzyme regulation as listed below:

1. Enzyme Quantity
2. Inhibition
   a) Reversible Inhibition
   b) Irreversible Inhibition
3. Allosteric Regulation
4. Feedback Regulation or Inhibition
5. Co-valent Modification
6. Proteolytic degradation

1. Enzyme Quantity

The quantity of enzymes or turnover number is determined by the overall rate of synthesis and rate of degradation of the enzyme. Any change in its quantity can be affected by a change in rate constant for the overall synthesis and degradation processes or both. The concentration of proteins or enzymes remained essentially constant in a state of 'dynamic equilibrium'. It gets influenced by a wide range of physiologic, hormonal or dietary factors. The turnover number of the enzymes can vary from minutes to hours to days for different enzymes.

1.1 Genetic Control: Enzyme synthesis at the gene level

Genes involved in the synthesis of enzymes can be induced or repressed. Induction of enzymes can be done at the gene expression, RNA translation or at the level of post-translational modifications. Hormones or growth factors signal cascade may lead to an increase in the expression or translation of enzyme not present before the signal. Inducers induce the synthesis of enzymes while repressors decrease the production of enzyme. Inducers are generally substrates or structurally similar molecules that initiate the synthesis of enzymes. Inducible enzymes in humans include tryptophan pyrrolase, threonine dehydratase, HMG-CoA reductase and cytochrome P-450. On the other hand, a metabolite or repressor when produced in excess inhibits the synthesis of enzymes involved in its formation. These regulatory molecules block the transcription of mRNA by binding to a part of DNA termed as operator. Repressors are allosteric proteins to which specific molecules can bind to alter their shape and ability to bind DNA. Inducer molecules bind to the repressor molecule and prevent its binding to the operator region of DNA. It allows the transcription of the coding sequences for the enzymes. For example, ***lac*** operon in *E.coli*, lactose acts an inducer to transcribe the synthesis of three enzymes (β-galactosidase, permease and transacetylase) involved in its degradation if it is present in the surrounding environment.

2. Inhibition

Molecules that bind to the enzymes and cause a decrease in their activity are called enzyme inhibitors. These molecules either bind at the active site of enzyme thereby preventing the substrate molecule to bind to the enzyme or they may inhibit the catalytic activity of enzyme. You should know that many of these molecules perform several regulatory roles in the metabolism. Some of them are used as herbicides or pesticides. Most of the drug molecules also act as enzyme inhibitors. Natural enzymes inhibitors e.g. poison are a part of the defense mechanisms in wild life animals.

Enzyme inhibitions are mainly classified into two types:

a) Reversible Inhibition

b) Irreversible Inhibition

a) Reversible Enzyme inhibition: In reversible enzyme inhibition, loss of the enzyme activity due to inhibitory molecule is reversible. Enzyme activity gets restored on the removal of inhibitor. These inhibitors bind non-covalently and give rise to different kinds of inhibition. Multiple weak bonds between the inhibitor and the enzyme combine to give strong binding which prevents the formation of product. They can be easily removed by dilution or dialysis to restore full enzyme activity. Reversible inhibitors tend to form equilibrium with an enzyme leading to certain level of inhibition.

b) Irreversible Enzyme Inhibition: Irreversible inhibition is different from temporary enzyme inactivation by the reversible inhibitor. The enzyme activity is lost on the binding of inhibitor molecule to enzyme and its activity cannot be recovered afterwards. These inhibitory molecules are

highly specific and can modify enzyme 3D structure. The enzyme gets inactive or there is time dependent loss of enzyme concentration. Inhibition cannot be removed by dilution or dialysis without losing enzyme activity. They generally form or break covalent bonds with the amino acid residues essential for substrate binding, catalysis or maintenance of enzyme conformation.

Examples: Heavy metal ions such as mercury, lead, aldehydes and haloalkanes. Alkylating agents such as iodoacetate and iodoacetamide forms covalent linkages with –*SH* groups of the enzyme.

$$E\text{-}SH + ICH_2.CO^-_2 \text{ ----------} \rightarrow E\text{-}S\text{-}CH_2.CO^-_2 + HI$$

Several important drugs are well known examples of irreversible inhibitors. Widely used drugs such as penicillin act by covalently inhibiting the enzyme transpeptidase, thereby preventing the synthesis of bacterial cell walls and thus killing the bacteria. You must have heard of tablet aspirin. The drug aspirin inhibits the enzyme cyclooxygenase thereby reducing the synthesis of inflammatory signals. Enzyme monoamine oxidase deaminates neurotransmitters such as dopamine and serotonin, and lowers the levels of these hormones in the brain. A neurodegenerative disease such as Parkinson's disease is linked with low levels of dopamine, while depression is related with low levels of serotonin. Suicide inhibitor such as drug (-) deprenyl, is widely used to treat Parkinson disease and depression.

3. Allosteric Regulation

Allosteric means 'other structure or different site'. Allosteric enzymes are the enzymes whose catalytic activity can be modulated by the effector molecules. These molecules do not participate in catalysis directly. They bind at a site other than the active site on the enzyme and can cause activation or inhibition of the enzyme. The binding is reversible, non-covalent and brings about the conformational change in the active site. These conformational changes occur at the tertiary and quaternary levels of protein organization. Allosteric enzymes are generally bigger and composed of multiple subunits having distinct active site and allosteric site. Several studies on X-ray crystallography and site directed mutagenesis have confirmed the existence of two separate sites on variety of enzymes.

Allosteric enzymes exhibit a characteristic sigmoidal saturation curve rather than hyperbolic curve [Michaelis-Menten curve] when v_o is plotted versus [S] on account of cooperativity of structural changes between enzyme subunits (Fig. 11.4). Allosteric enzymes behave in a cooperative system manner with both the substrate as well as modulator. A small change in substrate, inhibitor or activator concentrations brings about a huge change in the rate of reaction. The effectors that increase the catalytic activity are called positive effectors and those that reduce or inhibit the catalytic activity are called negative effectors.

For example, Phosphofructokinase (PFK) is regulated by

a) Negative effectors: High levels of ATP and citrate

b) Positive effectors: High levels of ADP and AMP

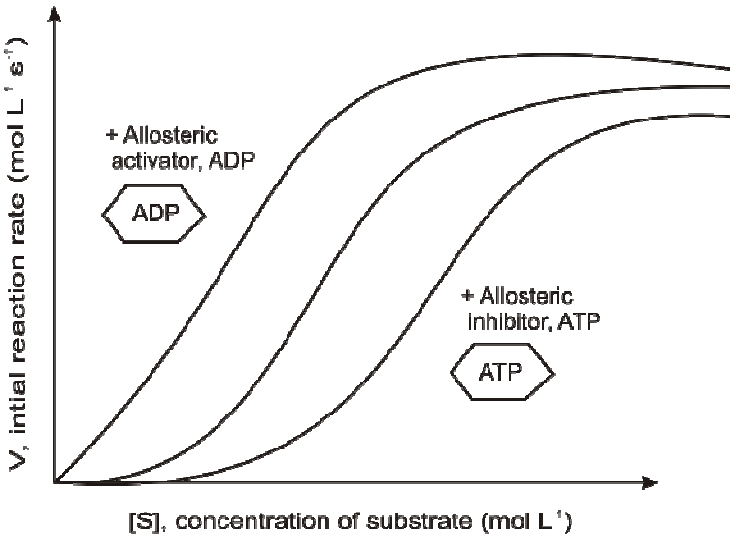


Fig. 11.4: Sigmoidal kinetics of allosteric enzymes.

4. Feedback Regulation or Inhibition

Another interesting aspect of enzyme regulation is the feedback regulation; inhibition of an enzyme in the biosynthetic pathway by the end product of the pathway. This type of inhibition is also termed as feedback inhibition and it develops when metabolic demand for the end product of the pathway gets declined. The end product binds to the regulatory site of the enzyme at the start of metabolic pathway and suppresses its activity. Feedback inhibitors do not bear any structural similarity to the substrates of the enzymes. For example, consider the following reaction:

Enz 1 Enz 2 Enz 3

A----------→ B---------→ C ---------→ D

High concentrations of end product D will act as feedback inhibitor of enzyme Enz 1. Small molecules such as amino acids or nucleotides act as feedback inhibitors in several biosynthetic pathways. In the bacterial enzyme system, L-threonine is converted to L-isoleucine in a sequence of five steps metabolic pathway. Isoleucine binds to the first enzyme in the pathway threonine dehydratase in a non-covalent manner and inhibits its own production.

SAQ 4

Do as directed:

a) The concentration of proteins or enzymes remained essentially constant in a state of '_____equilibrium' (Fill in the Blank).

b) Repressors are _____ proteins to which specific molecules can bind to alter their shape and ability to bind DNA (Fill in the Blank).

c) Feedback inhibitors do not bear any structural similarity to the substrates of the enzymes (True/False).

d) High/Low concentrations of end product will act as feedback inhibitor (Pick one option).

5. Covalent Modification

Covalent modification is also a means of regulating enzyme activity. The reversible covalent modification process of enzyme regulation involves many target proteins and membrane channels. Modified groups are attached to the enzyme by covalent bond. The covalent modification activates some enzymes as well as inactivates others. Most modifications are reversible. Phosphorylation and dephosphorylation are the most common but not the only means of covalent modification. Enzyme regulation by phosphorylation-dephosphorylation plays a key role in cell signaling. It allows the cell to respond to a signal at its surface and transmits its effect to intracellular enzymes. Phosphorylation cascade is highly selective. Seryl, Threonyl or Tyrosyl residues on the regulatory enzymes are phosphorylated by specific protein kinases. The very nature of protein folding determines whether protein kinase has access to the substrate undergoing phosphorylation. The removal of phosphoryl groups is catalyzed by protein phosphatases. Mammalian cell possesses contain many phosphorylated proteins, several protein kinases and phosphatases that catalyses their interconversion for regulatory control. Phosphorylation influences the functional properties of affected enzyme by altering its three-dimensional structure. Phosphorylation of one enzyme can lead to phosphorylation of a different enzyme which in turn acts on another enzyme, and so on.

Other covalent modifications such as glycosylation, hydroxylation, fatty acid acylation, palmitoylation and prenylation are unique changes that shape structure and localization of enzyme for its lifetime. Hydrophobic acylations can cause the target protein to be associated with a membrane rather than the cytosol.

6. Proteolytic enzymes

Certain enzymes are secreted as precursors in an inactive form and are known as proenzymes or zymogens. Proenzymes help the proteins to be transported or stored in inactive forms that can be converted in active forms at the particular site. Precursor of pepsin is pepsinogen, trypsin is synthesized as trypsinogen and procarboxypeptidase is zymogen of carboxypeptidase. Several other examples include blood clotting enzymes, procollagen and proinsulin. Zymogens underlie the mechanism whereby the levels of enzymes can be readily increased at the post translational level by proteolytic cleavage, an irreversible modification. Generally, cellular and bacterial proteolytic enzymes are synthesized as inactive precursor (zymogen) to prevent undesired protein degradation. Conversion of zymogens to active form either requires accessory molecules or the autocatalytic processes in response to drop in pH.

Some examples of enzymes and their zymogens are as given below:

Enzyme	Precursor	Function
Trypsin	Trypsinogen	pancreatic secretion
Chymotrypsin	Chymotrypsinogen	pancreatic secretion
Carboxypeptidase	procarboxypeptidase	pancreatic secretion

Elastase	proelastase	pancreatic secretion
Phosphalipase A_2	prophospholipase A_2	pancreatic secretion
Pepsin	Pepsinogen	Secreted in gastric juice (Most active in pH range 1-5)

11.4 SUMMARY

- Enzyme kinetics is the basis for enzyme catalyzed biochemical reactions. Therefore, understanding of enzyme kinetics is important to understand these biological processes.
- Kinetics provides a rationale for the complex behavior of enzymes. The rational is based on simple chemical principles.
- For single substrate reactions, at constant *E*, increasing *S* results in increased product formation to a point where product formation no longer increases. This saturation is presumed to reflect the fact that all *E* is now in the form of *ES*.
- Kinetic model proposed by Michaelis-Menten used equilibrium assumption for deriving Michaelis-Menten equation.
- The equilibrium assumption was later modified to introduce a more valid steady state assumption. The equation remains the same except the definition of Michaelis constant (K_m). The substrate concentration at which velocity is half of V_{max} (maximal velocity) is known as Michaelis constant (K_m). This constant gives an idea about the affinity of the enzyme for its substrate.
- The hyperbolic graph of *v* versus *S* obtained by Michaelis-Menten equation proved inadequate for determining the accurate value of V_{max} and K_m. Lineweaver and Burk gave the solution by inverting the Michaelis-Menten equation to give double reciprocal plot.
- Tight integration of metabolic pathways is possible using regulatory enzymes. Regulatory enzymes generally catalyzed the slowest step in the metabolic pathway. These enzymes tend to maintain the homeostasis in spite of numerous changes in the external environment of the cell.
- Enzyme can be regulated by the end product of a biosynthetic pathway. The final end product inhibits the first enzyme of that pathway by binding to an allosteric site which is distinct from the active site. The inhibition results from a conformational change in the oligomeric structure of the first enzyme.
- In another type of regulation, amino acid chains of the enzyme are reversibly modified, mainly by phosphorylation and dephosphorylation. This produces an active or inactive enzyme.
- Regulation of enzyme activity by inhibitors, allosteric and covalent modifiers has wide range of applications in the fields of medicine and agriculture.

11.5 TERMINAL QUESTIONS

1. Why is the rate of an enzyme-catalyzed reaction proportional to the amount of *ES* complex?
2. Explain the maximal velocity V_{max} in the v_o vs *S* graph?
3. Derive double-reciprocal equation from Michaelis-Menten equation and give its importance.
4. How a value for K_m can be obtained from the v_o vs *S* graph when v_o = 1/2 V_{max}?
5. Describe allosteric regulation of the enzyme activity?
6. What are proteolytic enzymes? Give examples.

11.6 ANSWERS

Self Assessment Questions

1. At low substrate concentrations, the overall rate of reaction will be limited by the rate at which enzyme and substrate molecules react to form enzyme-substrate complex. At constant enzyme concentration the rate of reaction will be proportional to the substrate concentration (first-order reaction). However, at high substrate concentration enzyme will be saturated with the substrate and therefore no free enzyme will be available. So the overall rate of reaction will be independent of the substrate concentration.
2. At high substrate concentration S_o, $Km <<<< S_o$ (numerically), so the term $K_m + S_o$ in the Michaelis-Menten equation becomes equal to S_o. $v_o = (V_{max}\ S_o)/S_o$, and S_o cancels. Therefore, at high S_o, $v_o = V_{max}$.
3. a) Substrate concentration, b) False, c) Linear, d) Equal
4. a) dynamic, d) allosteric, c) True, d) High

Terminal Questions

1. The product formation takes place after *ES* complex formation in an enzyme catalyzed reaction. Enzyme *E* must bind to the substrate *S* before the product is formed. Therefore, the rate of an enzyme-catalyzed reaction is proportional to the amount of *ES*.
2. At high substrate concentrations, enzyme *E* will be bound to substrate *S*. So the maximum amount of *E.S* is formed under these conditions. Since the rate is proportional to the amount of *ES*, the rate is at a maximum value under these conditions.
3. Refer to section 11.2.1 and 11.2.2
4. When $v_o = V_{max}/2$, then $V_{max}/2 = V_{max}.S/K_m + S$

   cancelling V_{max},

$1/2 = S/(K_m + S)$

$K_m + S = 2S$

or $K_m = S$ at $v_o = V_{max}/2$

5. Allosteric control is one of the important mechanisms of enzyme regulation. In an allosteric enzyme, activity of the enzyme is controlled by the binding of molecule at a site other than the active site. This other site is known as allosteric site. The binding of an allosteric modulator induces conformational changes which results in the alterations in the catalytic activity of the enzymes. There are two types of allosteric modulation-positive allosteric modulation when binding of modulator to enzyme increases the rate of reaction. On the other hand negative allosteric modulator decreases the activity of enzyme (for details refer to section 11.3, subsection-3).

6. Refer to section 11.3, subsection-6.

FURTHER READINGS

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UNIT 12
VITAMINS

Structure

Dietary source of vitamins

12.1 INTRODUCTION

In the preceding units, you have studied the structure and function of proteins, nucleic acids, carbohydrates and lipids which form major organic constituents of cells and tissue of the human body. **There is another class of organic compounds called vitamins that are required in very small quantities but are very significant for the growth and proper functioning of the body**. As you know enzymes are proteins that employ organic (coenzymes) and inorganic (e.g. magnesium ion) molecules as a cofactor to assist them in catalytic reactions.

In simple words, vitamins are defined as essential organic nutrients that cannot be produced in the human body. These vitamins are obtained from different types of food that we eat. If certain vitamin is inadequate or low in the diet, a vitamin deficiency may occur such as scurvy (Vitamin C deficiency) disease. You may be familiar with the term **vitamins** when physician generally recommends patients who have vitamins deficiency to take foods rich in vitamins, or suggests vitamin supplements available in the market in the form of syrup or tablets. However, green vegetables, grains, fruits, animal products and nuts are rich sources of vitamins.

Therefore, in this Unit we will discuss about vitamins. This unit begins with biological significance of vitamins and their classification and you will learn the structure, function, deficiency diseases and dietary sources of water-soluble vitamins (vitamin B complex and vitamin C) and fat-soluble vitamins (A,D, E and K).

Objectives

After studying this unit, you should be able to:

- define the vitamins and their classification;
- describe the structure and function of the water-soluble vitamins;
- list the active form of the B-complex vitamins;
- explain the biological role of vitamin C;
- explain the structure and physiological function of fat-soluble vitamins (A, D, E and K);
- differentiate between water-soluble vitamins and fat-soluble vitamins;
- explain the role of vitamins as antioxidants.
- describe the dietary source and dietary requirement of vitamins; and
- discuss the vitamin deficiency diseases and their symptoms.

12.2 BIOLOGICAL SIGNIFICANCE OF VITAMINS

Vitamins are indispensable molecules for the food metabolism, growth and development of the body. They keep body healthy. Most of the vitamins or their derivatives function as coenzymes or prosthetic groups in the enzyme catalysed reactions. Some vitamins like A, C and E act as antioxidants that fight against free radicals (i.e. superoxide anion and hydroxyl radicals) and inflammation. If these free radicals are not neutralised, they may prove to be harmful to the body. Many enzymes do not work unless they bind optimally to other specific prosthetic group. Vitamins are also the major source of coenzymes. Water-soluble vitamins (B-complex vitamins) serve as precursors for coenzymes for example; niacin and riboflavin are precursors of the NAD and FAD coenzymes respectively and vitamin C can act directly as a coenzyme for the hydroxylation of amino acids required for the synthesis of collagen.

Other functions of vitamin are equally important. **Fat-soluble vitamins** play diverse physiological functions such as vitamin A is required for vision and cell growth, vitamin D for calcium and phosphate metabolism; vitamin K for blood clotting process and vitamin E serves as an antioxidant and boosts up the immunity of the body.

Vitamin deficiencies can cause serious health problem such as a deficiency of vitamin A causes night blindness and other vitamin deficiencies diseases are beriberi (thiamine); pellagra (niacin); pernicious anemia (B_{12} and folic acid) and scurvy (vitamin C). The biochemical functions, deficiency diseases, dietary sources and recommended dietary allowances of vitamins are summarized in Table 12.1.

Table 12.1: Summary of Vitamins.

Water soluble Vitamins	Biochemical Functions	Deficiency diseases	Dietary sources	Recommended Dietary Allowances (RDAs)
Vitamin B-complex (1-8)				
1. Thiamin	Decarboxylation reactions	Beriberi	cereals, groundnuts, soybean and capsicum.	1.1- 1.4 mg/day for human
2.Riboflavin	Electron carrier (Hydrogen atom transfer)	Extremely rare	Yeast, milk, eggs and green vegetables	1.3 -1.6 mg/day for male and female.
3.Niacin		Pellagra	Peanuts, legumes, meat, eggs and milk	14-18 mg/day for male and female.
4.Pantothenic acid	Acyl group transfer	Periferal nerve damage	Whole grains, vegetables	5 mg/day
5.Pyridoxin	Amino group Transfer in protein metabolism	Disorders in amino metabolism	eggs, liver, yeast, cereals, legumes and milk	2.0 mg/day for male and female
6.Biotin	Carboxyl group transfer	Impaired lipid and carbohydrate metabolism	Liver, kidney, yeast, nuts, tomatoes and egg yolk	150-300 µg/day for male and female
7.Folic acid	One carbon group transfer	Megaloblastic anemia	Green vegetables, whole grains, cereals,	200 µg/day for male and female, 500 µg/day for pregnant women
8.Vitamin B_{12}	Methyl group transfer	Pernicious anemia	animal foods, meat, milk, egg and fish.	2 µg/day for male or female and 1.2 µg/day for pregnant women

Vitamin C	Helps in proline hydroxylation for collagen synthesis and serves as an antioxidant	Scurvy	Citrus fruits, peanuts, tomatoes and green vegetables	40-60mg/day for male and female
Fat Soluble vitamins				
Vitamin A	Normal vision and regulate cell growth	Night blindness, xerophthalmia and keratomalacia	Carrot, papaya and fish liver oils	400 µg/day for child, 600-800 µg/day for male and female
Vitamin D	Absorption of calcium and bone formation	Rickets in child and osteomalesia in adults	Sunlight exposure and cod liver oil	15 µg/day for male and female.
Vitamin E	Antioxidant and anti-fertility factor	Deficiency extremely rare in human	Wheat and germ oil	8-10 mg/day
Vitamin K	Promotes blood clotting	Impaired blood clotting (hemorrhagic)	Spinach, cabbage and green leafy vegetables	70-140 µg/day

Recommended Dietary Allowances (RDAs): It is the average daily intake amount of essential nutrients to meet the requirement of all healthy people. (Source: ICMR report, RDA for vitamins for Indians, 2010).

RDAs relate to vitamin requirements for the normal functioning of the body. Therefore, the decreased and increased level of vitamins in the body is generally termed as.

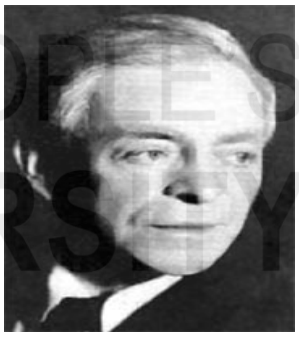
1. **Hypovitaminosis** means vitamin deficiency in the body. A vitamin deficiency can occur when usual intake is lower than the RDA and
2. **Hypervitaminosis** refers to excess intake of vitamins than the RDA. It is a condition of abnormal accumulation of vitamins (particularly fat-soluble vitamins) in the body which can cause toxicity to adults. Hypervitaminosis can occur due to overconsumption of vitamin-supplements.

SAQ 1

Match the column I with that in column II

Column I	Column II
a) Pernicious anemia	i. Thiamin
b) Beri-beri	ii. Vitamin C
c) Night blindness	iii. vitamin B_{12}
d) Scurvy	iv. Folic acid
e) Megaloblastic anemia	v. Niacin
f) Hemorrhagic	vi. Vitamin D
g) Rickets	vii. Vitamin K
h) Pellagra	viii. Vitamin A

12.3 VITAMINS: CLASSIFICATION



Casimir Funk

The scientist **Casimir Funk** (1884 –1967) coined the term **vitamin which** means **'vital amines'** as one of the earlier compounds thiamine (vitamin B_1) was discovered as an amine to prevent beriberi disease. However, all vitamins are not amines. He isolated the first vitamin (B_1) from rice bran. In the beginning, many vitamins were discovered and designated by letters of the alphabet (A, B_1 and B_2, C, D, E and K) as their chemical structure were not known.

Vitamins are traditionally classified into two groups on the basis of solubility as fat-soluble or water-soluble (Fig. 12.1). This classification defines where they function in the body. **Water-soluble vitamins** act in the cytosol of cells or in extracellular fluids such as blood and **fat-soluble vitamins** are primarily responsible for protecting cell membranes from free radical damage. There are total thirteen vitamins. Four are Fat-soluble: viz vitamins A, D,E and K and nine are water soluble vitamins i.e.- thiamine, riboflavin, niacin, pyridoxin, biotin, folic acid, vitamin B_{12} and pantothenic acid (collectively known as B-complex vitamins)and vitamin C. Water soluble vitamins are easily excreted out via urine. Hence, they are not stored in the body while fat-soluble vitamins are stored in the body and their excess amount can lead to metabolic abnormality.

Now chemical structure, function and deficiency diseases of vitamins have been well established about which you will learn in the next sections.

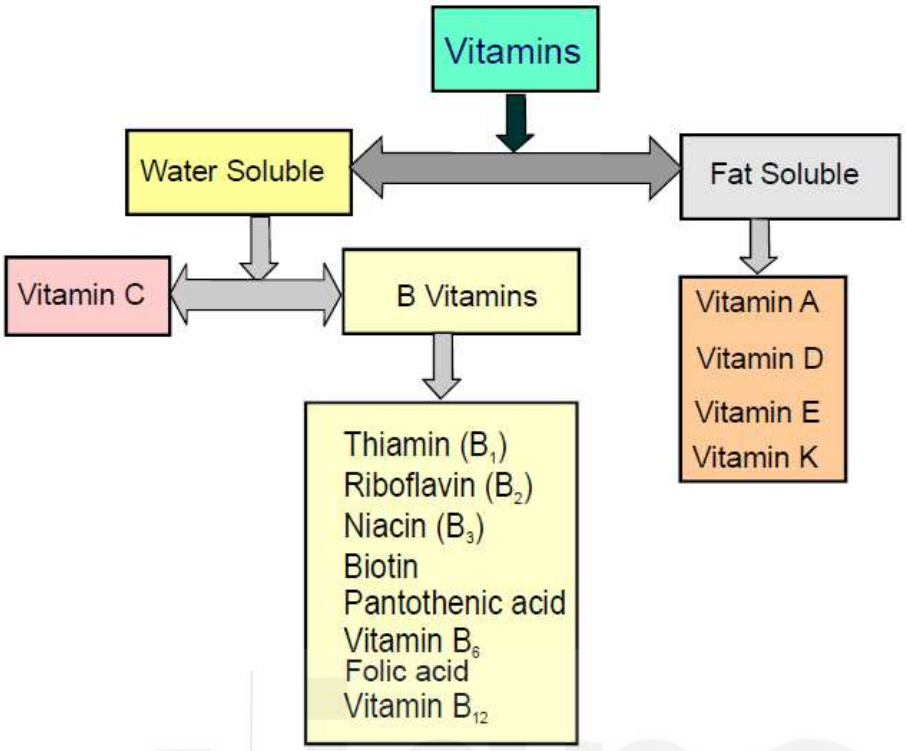


Fig. 12.1: Classification of Vitamins.

SAQ 2

a) Classify vitamins.

b) Explain why water-soluble vitamins are not stored in the body.

12.4 WATER SOLUBLE VITAMINS: STRUCTURE AND FUNCTION

Water-soluble vitamins are soluble in water. They are often absorbed by the intestine and then transported to the liver via the bloodstream. When water-soluble-vitamins are taken in excess, they are excreted out in urine and thus, they are not stored in the body.

There are two types of water-soluble vitamins:

I. Vitamin B (a complex of vitamins) and

II. Vitamin C.

The B-complex vitamins are so called because it comprises as many as eight vitamins that include thiamine (vitamin B_1), riboflavin (vitamin B_2), niacin, pantothenic acid (vitamin B_5), pyridoxine (vitamin B_6) and cobalamin (vitamin B_{12}).These vitamins are precursors to coenzymes that help in specific enzymes activities while vitamin C can participate directly as a coenzyme in biochemical reactions. Vitamin C is also known as antioxidant vitamin.

Let us discuss water soluble vitamins (B-complex vitamins and vitamin C) one by one.

12.4.1 B-Complex Vitamins

As mentioned above the vitamin B-complex contains altogether eight vitamins that are soluble in water. We shall discuss each of them separately.

i. Thiamine (Vitamin B_1)

Thiamine was first vitamin (B_1) to be discovered in the rice bran. The structure of thiamine consists of a pyrimidine and a thiazole that are joined by a methylene group (-CH_2-) and its active form is the **Thiamine pyrophosphate** (TPP) (Fig. 12.2). Thiamine is synthesized in bacteria, fungi and plants.

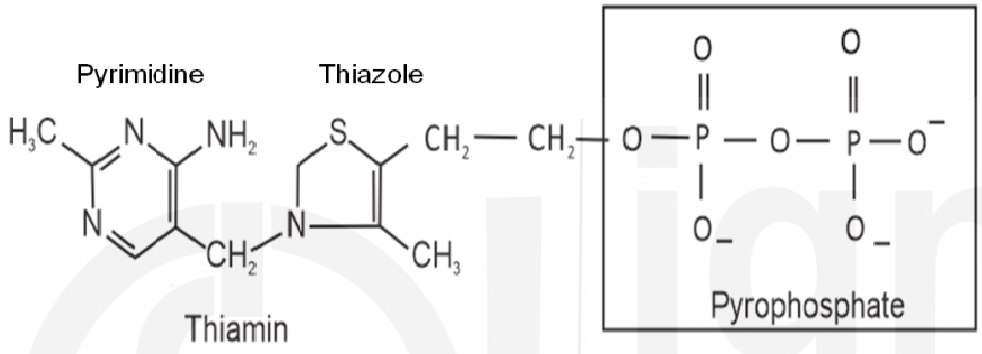


Fig. 12.2: Chemical structure of thiamine and TPP.

Function

Vitamin B_1 is a sulfur containing vitamin that participates in energy metabolism of carbohydrates, lipids and proteins. **Thiamine pyrophosphate** (TPP) is actively involved as a coenzyme in the dehydrogenation and decaroboxylation reactions of carbohydrate metabolism. TPP dependent enzymes are transketolase, pyruvate dehydrogenase complex and α – ketoglutarate dehydrogenase.

Deficiency disease and dietary food sources

An inadequate intake of diet deficient in Vitamin B_1 causes a beriberi disease in child and adults. The primary sources of vitamin B_1 are whole grain cereals, leafy green vegetables, asparagus, egg, fruits, legumes (beans and lentils), and soymilk.

ii. Riboflavin (Vitamin B_2)

Riboflavin was discovered just after vitamin B_1, hence it is known as vitamin B_2. The term riboflavin is derived from Ribose (sugar) and the Flavin (the ring moiety). The flavin group is bound to ribitol, a derivative of ribose (a sugar alcohol) by a carbon-nitrogen bond (Fig. 12.3). Scientists Kuhn, Gyorgy and Wagner (1933) isolated riboflavin from eggs. Riboflavin has intense yellow colour and is widely used as a food additive.

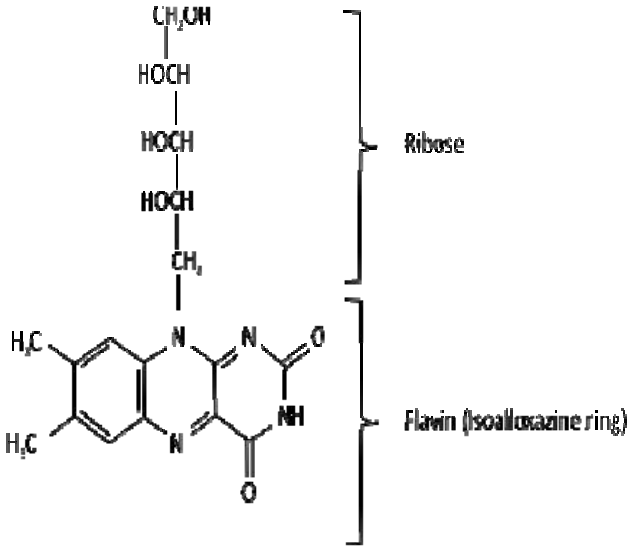


Fig. 12.3: Structure of Riboflavin.

Function

Riboflavin occurs in two forms, Flavin mononucleotide (FMN) and Flavin adenine dinucleotide (FAD) in the human body. Both FMN and FAD actas coenzymes which participate in redox reaction by accepting and donating the hydrogen atoms. Enzymes that require FAD or FMN are called flavoproteins and they are referred as dehydrogenase enzymes. They primarily serve as electron carriers in the mitochondrial electron transport system which is necessary for releasing energy from carbohydrates, fats and proteins.

Deficiency diseases and dietary food sources

The symptoms of riboflavin deficiency are fatigue, slow growth; cheilosis, (swollen, cracked lips) glossitis (inflammation of tongue) dry and scaly skin (seborrhic dermatitis), abnormal vision and corneal inflammation. Milk, liver, eggs and leafy vegetables are rich source to meet the riboflavin body's requirement.

iii. Niacin (Vitamin B_3)

The term ‘Niacin’ is referred collectively to nicotinamide and nicotinic acid. Niacin is unusualamong vitamins as it was discovered as a chemical compound, nicotinic acid which is a derivative of nicotine. Niacin is pyridine 3-carboxylic acid and occurs as nicotinamide in tissues. It is unique vitamin as it can also be synthesized from the amino acid tryptophan.

Functions

Niacin occurs in two forms Nicotinamide adenine dinucleotide (NAD) and Nicotinamide adenine dinucleotide phosphate (NADP). Structurally NADP differs from the NAD as it has additional phosphate group on 2' position of the Ribose ring (Fig. 12.4).

NAD is primarily involved in catabolic reactions (glucose catabolism) and NADP in anabolic reactions (*i.e.* fatty acid synthesis). Niacin is necessary for energy-transfer reactions during food metabolism. The function of niacin is similar to the riboflavin coenzymes in that it carries hydrogen molecules (and

their electrons) during metabolic reactions of glucose and fats. Enzymes that are required for NAD are dehydrogenase enzymes. These include lactate dehydrogenase, pyruvate dehydrogenase complex, α-ketoglutarate dehydrogenase and malate dehydrogenase.

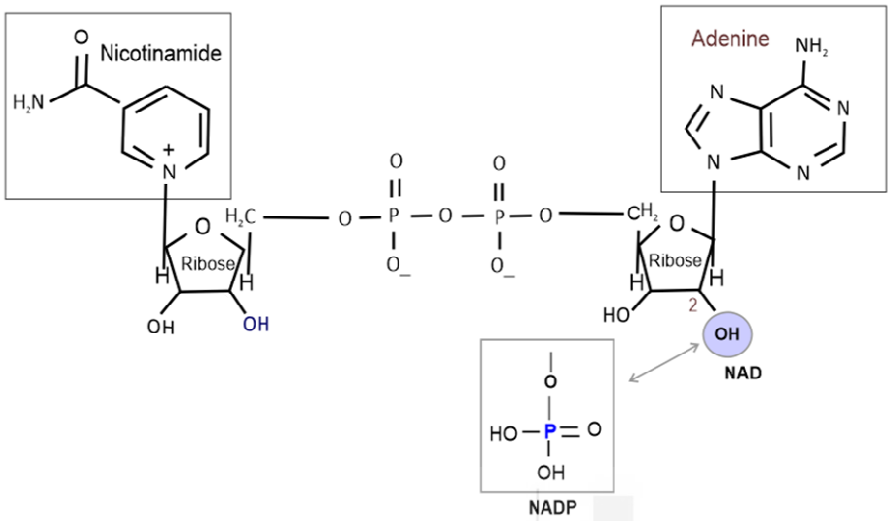


Fig. 12.4: Structure of NAD^+ and $NADP^+$.

Deficiency disease and dietary food sources

Nutritional significance of Niacin was known when pellagra disease occurred in people. Pellagra (pellis -skin; agra – rough) was diagnosed due to the niacin deficiency in the diet. Peanuts, legumes, meat, eggs and milk are good sources of this vitamin.

iv. Pantothenic acid (Vitamin B_5)

Pantothenic acid is also a member of the vitamin B complex family. The name of this vitamin comes from the Greek word pantos, meaning "everywhere". It was identified in the meat extract by Roger J. Williams in 1931. It consists of β alanine and pentoic acid that are joined by the peptide linkage (Fig. 12.5). Pantothenic acid is an essential nutrient for animals as well as microorganisms. This vitamin helps to release energy from the carbohydrates and fats.

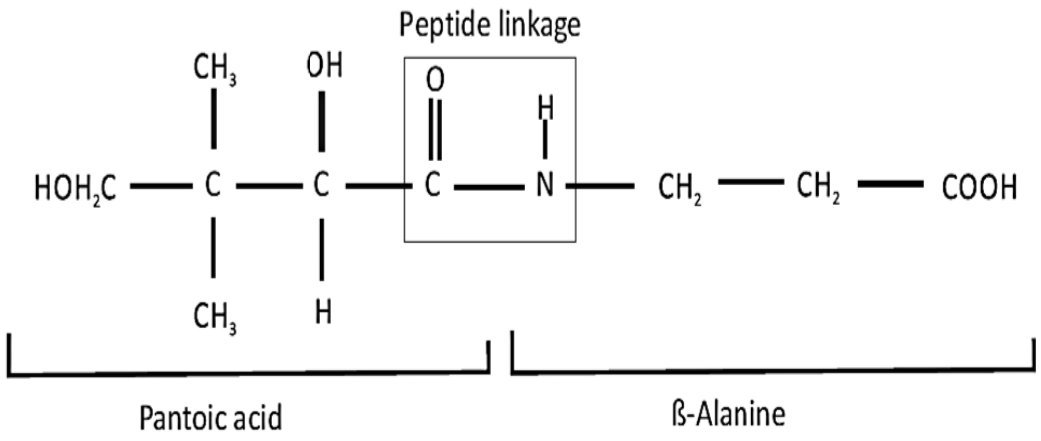


Fig. 12.5: Structure of Pantothenic acid.

Vitamin B_5 is needed for the synthesis of coenzyme A. Coenzyme A serves as an acyl group carrier for the intermediate compound called Acetyl-CoA. It participates in several metabolic pathways, and is required for the synthesis of lipids, neurotransmitters, steroid hormones, and hemoglobin. It helps in maintenance and repair of tissues and cells of the body. Pantothenic acid is involved in the formation of Coenzyme A (A for acyl). It is also important for signal transduction and enzyme activation and deactivation respectively.

Paul Gyorgy

Deficiency disease and dietary food sources

Pantothenic acid deficiency is rarely found in people as it is widely found in animals products and plants.

v. Vitamin B_6 (Pyridoxine)

Vitamin B_6 occurs in three forms: pyridoxine, pyridoxamine and pyridoxal (Fig. 12.6). Paul Gyorgy identified this vitamin. The pyridine ring is common in all three of vitamin B_6.

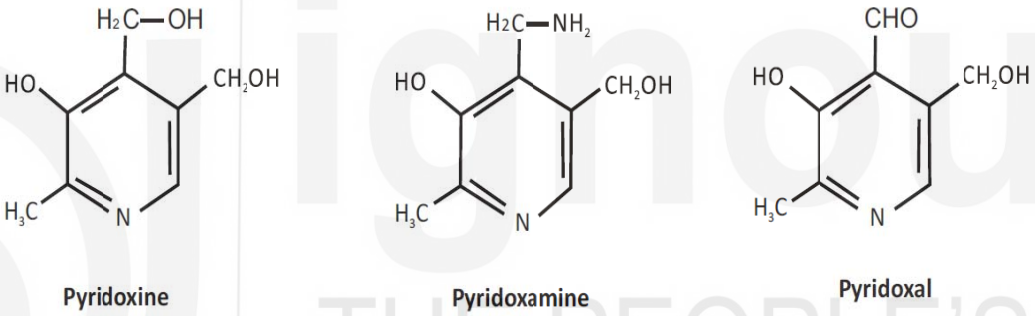


Fig.12.6: Structure of vitamin B_6.

Function

All three from of vitamin B_6 can be converted to the active form-**Pyridoxal-5-phosphate**. The primary function of vitamin B_6 is to transfer amino group from the amino acids for amino acid synthesis. The PLP-dependent enzymes are called transaminases such as asparate transaminases and alanine transaminase. It is involved in the metabolism of neurotransmitters e.g. dopamine, serotonin, γ-aminobutyric acid (GABA), epinephrine, histamine.

Deficiency disease and dietary food sources

Vitamin B_6 deficiency usually is not common as it is found in all vegetables, animal products, cereals and fruits. Its biological activity can be diminished due to overheating of food. Therefore, the clinical symptoms can be weakness, stomach pain, nausea and vomiting, dermatitis and peripheral neuropathy.

vi. Biotin

In 1935, Dutch biochemist Fritz Kogl, isolated biotin in crystalline form from the egg yolk. Biotin structure is composed of an imidazole group, thiophene ring and a fatty acid side chain (Fig. 12.7). This vitamin is necessary for cell growth and metabolism of fats and amino acids.

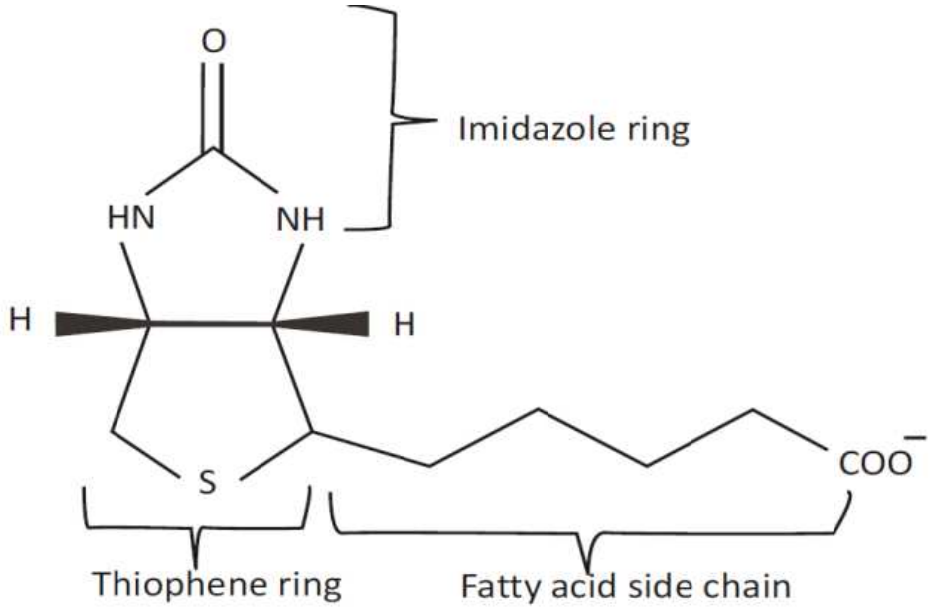


Fig. 12.7: Structure of Biotin.

Function

Biotin as a coenzyme transfers carbon dioxide in the metabolism. It is the coenzyme of pyruvate carboxylase, acetyl CoA caboxylase, propionyl CoA carboxylase. It plays critical role in the breakdown of food (carbohydrates, fats and proteins) and transform into energy. In addition, it is involved in many biological reactions, particularly in fat and protein metabolism.

Deficiency disease and dietary food sources

Biotin is widely distributed in many food sources as biocytin, Hence its deficiency does not occur usually in the human body. Liver, kidney, yeast, nuts, tomatoes and egg yolk are some of the well known sources of biotin.

Biocytin is a conjugated form of the vitamin biotin and the amino acid Lysine. The biotinidase enzyme breakdown biocytin into biotin which serves as cofactor for carboxylase enzymes.

vii. Folic acid

The term, folic acid is derived from Latin word 'folium (leaf)' It is also known as pteroylglutamic acid or folacin. Plants are a rich source of folic acid especially, spinach leaves. Folic acidconsists of a ***pteridine*** ring, ***para-amino benzoic acid*** **(PABA)**, and ***glutamic acid*** *(Fig. 12.8)*. Folic acid converts into folate in the body. The active form of folic acid is the tetrahydrofolate (THF).

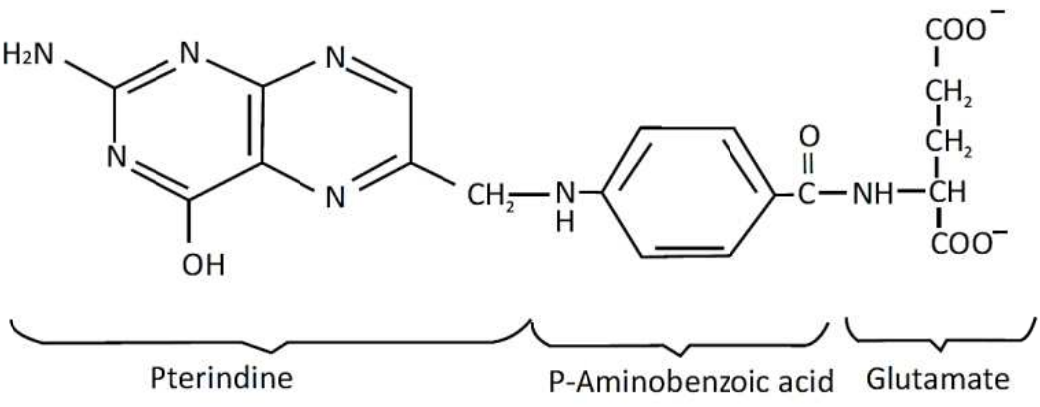


Fig. 12.8: Structure of Folic acid.

Function

Folic acid coenzyme transfers single-carbon compounds in biochemical reactions involved in the synthesis of DNA (deoxyribonucleic acid) and amino acids. Both folate and vitamin B_{12} are interconnected in their capacity to transfer and accept single-carbon compounds, which are called methyl groups. For example, THF with a methyl group donates its carbon compound to vitamin B_{12}. This action transforms vitamin B_{12} into an active coenzyme, which will in turn catalyse the conversion of homocysteine to methionine. Without vitamin B_{12}, folate in its methyl form becomes trapped inside cells and this is unavailable to support cell growth. In addition, folate is needed for the formation of healthy red blood cells (RBCs) and it prevents anemia especially in infants and pregnant women. Folate is essential for brain development and function. Human body needs folate to synthesize and methylate DNA.

Deficiency disease and dietary food sources

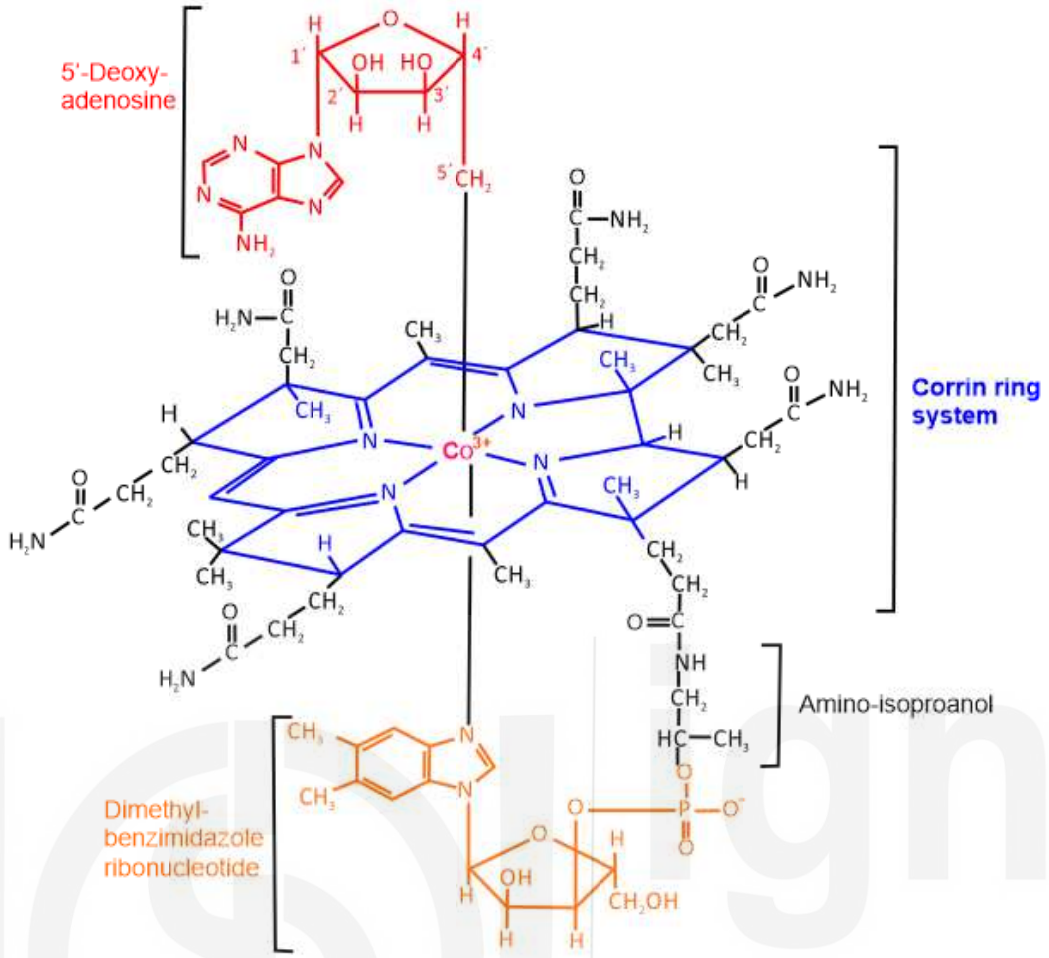
The deficiency of folic acid affects the synthesis of nucleic acid and amino acids. The deficiency of folic acid is believed to be the most common form of vitamin in the pregnant females. The lack of this vitamin causes defect in the RBC formation and in the development of the nervous system of the newborn babies. Hence, folic acid deficiency in pregnant women can cause neural tube birth defect in newborn. In the body, folic acid deficiency are not produced proper RBCs and result in megaloblastic anemia disease. This disease is characterised by larger red blood cells than normal. The symptoms of these diseases resemble with the pernicious anemia (vitamin B_{12} deficiency).The clinical symptoms are weakness, fatigue, depression and breathing problem.

The folic acid is abundant in plants and animals products. Green leafy vegetables, whole grains, cereals, yeast, soybean and eggs are some of the important sources of this vitamin. Folic acid is also available in tablet form to meet the vitamin requirement and recommended especially to pregnant women

viii. Vitamin B_{12}

Vitamin B_{12} is the largest (molecular weight = 1355.4) and most complex of all the vitamins. It consists of a class of chemically related compounds known as vitamers (exhibiting vitamin activity). Neither plants nor animals are capable of synthesizing vitamin B_{12}. Some bacteria such as *Escherichia Coli* found in the human gut that are capable synthesizing this vitamin. The basic structure of vitamin B_{12} as synthesized by bacteria is the hydroxocobalamin but it is converted into other forms such as methylcobalamin and 5'-deoxyadenosyl-cobalamin in the human body.

Vitamin B_{12} is the only vitamin which contains a cobalt metal bound to a corrin ring. Hence it is also called cobalamin. The chemical structure of cobalamin was determined by Dorothy Crowfoot Hodgkin in 1956. The structure of vitamin consists of Corrin ring, dimethylbenzimidazole (DMB) and 5-deoxy adenosine (Fig. 12.9).Vitamin B_{12} occurs in two active forms: methylcobalamin and deoxyadenosylcobalamin.



Dorothy Crowfoot Hodgkin

Fig. 12.9: Structure of Vitamin B_{12}.

Function

Vitamin B_{12} coenzyme serves as a methyl group carrier, accepting the carbon from tetrahydrofolate derivatives. It functions as a coenzyme in the conversion of homocysteine to methionine, in the metabolism of fatty acids and amino acids, and in the production of neurotransmitters.

The function of folate and vitamin B_{12} are quite close to each other because folate gives up methyl group to B_{12}.

Vitamin B_{12} is required for the normal functioning of the brain, nervous system and formation of red blood cells (RBCs).

Deficiency disease and dietary food source

The deficiency of vitamin B_{12} refers to pernicious anemia which is caused by an absence of the intrinsic factor. The intrinsic factor present in the intestine is required for vitamin B12 absorption. Pernicious anemia occurs in those people who have genetically deficit the intrinsic factor. Due to lack of vitamin B_{12} RBCs are not properly synthesized. The primary features include weakness, fatigue, impaired nervous system functioning and skin allergy. The vegetarian people generally have the vitamin B_{12} deficiency. The best dietary sources of vitamin B_{12} are animal meat, egg, fish and probiotics.

12.4.2 Vitamin C

Unlike B-complex vitamins (coenzyme derivative), vitamin C acts directly as a coenzyme as well as an antioxidant. It is also the water-soluble vitamin. It is chemically known as ascorbic acid. It is also called antiscorbutic vitamin as it cures the scurvy disease.

Free radicals are atoms or substance bearing unpaired electrons. Hence, they are considered unstable molecules. They are generally reactive oxygen species (i.e. $O_2^{\bullet}$, $OH^{\bullet}$). Overproduction of free radical leads to oxidative stress and causes damage to lipids, proteins and DNA.

Oxidative stress refers to an imbalance between antioxidants and free radicals in the body.

It is a ketolactone ($C_6H_6O_8$) containing six carbon atoms with hydroxyl groups that resembles glucose to some extent. It is acidic in nature owing to ionization of two hydroxyl groups separated by the double bonds. It is very strong reducing agent thus it is a water soluble antioxidant. Vitamin C is a heat sensitive as it easily breaks down at high temperature. However, it is an odorless, crystalline compound and soluble in water.

Vitamin C has no derivative or coenzyme form but exits in two forms. L-ascorbic acid (reduced) and L-dehydroascorbic acid (oxidized) (Fig. 12.10).

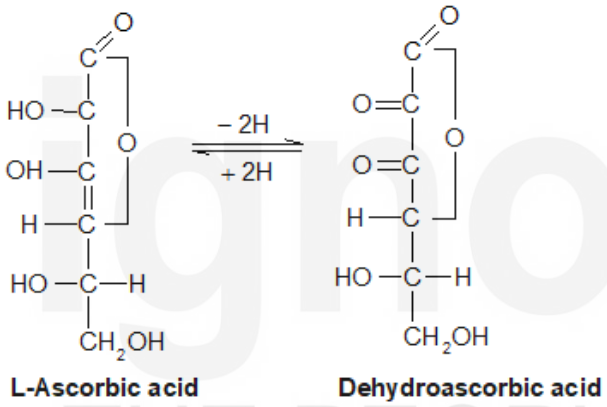


Fig. 12.10: Structure of vitamin C (Ascorbic acid).

Vitamin C is not synthesized in the human body and therefore, we must take citrus foods that are rich source of vitamin C.

Functions: Vitamin C plays multiple biochemical roles -

It has a special role in the synthesis of collagen. It serves as a cofactor for the prolyl hydroxylases and lysyl hydroxylases enzyme which are required for the hydroxylation of proline and lysine in the collagen synthesis. Collagen is the main structural protein of the connective tissue of the human body. It is also found in the skin, bones, and hair.

Vitamin C helps in wound healing, bones, and teeth formation. It enhances the iron absorption in the body. It supports the immune system of the body to fight against infections. Vitamin C neutralizes free radicals in the cells thus preventing free radicals damage.

Deficiency diseases and dietary food source

Lack of vitamin C in diet can cause scurvy disease (Fig. 12.11). Fragility of blood capillaries, bleeding from the gums and tooth are common symptoms of vitamin C deficiency. Vitamin C is present in higher concentration in the citrus fruits, spinach and gooseberry (Amla) (Fig. 12.12). Which are recommended to patient who has the scurvy disease Vitamin C supplements are also available in tablet or syrup (liquid) form.

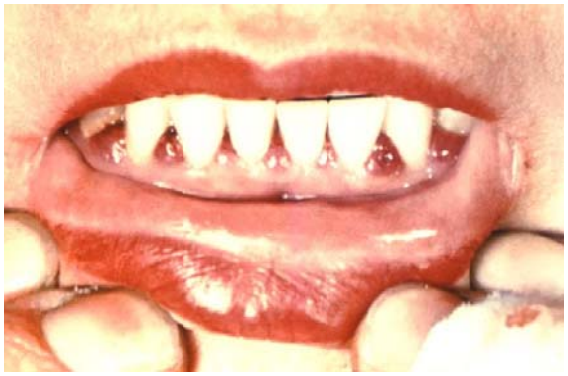


Fig. 12.11: Teeth gums affected by scurvy Centre for Disease control and Preventions.

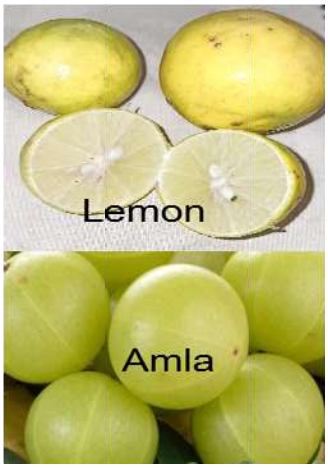


Fig. 12.12: Dietary source of Vitamin C.

SAQ 3

a) Indicate whether the following statements are true (T) or false (F):

i. Vitamins are inorganic compounds. [ ]

ii. TPP participates in the carboxylation reactions. [ ]

iii. Thiazole ring is present in the niacin vitamin. [ ]

iv. Pellagra disease is caused by the niacin deficiency. [ ]

v. Riboflavin is used as a food additive. [ ]

vi. Vitamin B_1 is a sulfur containing vitamin. [ ]

vii. Thiamine deficiency causes Beriberi disease. [ ]

b) Fill in the blanks:

i. The tryptophan amino acid can synthesize.....vitamin.

ii. The coenzymes …….. serve as electron carriers in mitochondrial electron transport system.

iii. Neural defect in newborn is caused by deficiency of ……..... vitamin.

iv. The Cobalt metal containing vitamin is ……. .

v. The water soluble antioxidant vitamin is …… .

c) Match the column I with that in column II

Column I	Column II
i. Electron carrier	a) Pyridoxine
ii. Methyl group transfer	b) Vitamin C
iii. Amino group transfer	c) Biotin
iv. Carbon dioxide group transfer	d) Vitamin B_{12}
v. Hydroxylation of amino acids in collagen synthesis	e) Niacin

12.5 FAT-SOLUBLE VITAMINS

Unlike the water soluble vitamins, fat-soluble vitamins are soluble in fat. Vitamins A, D, E and K are collectively known as fat soluble vitamins. Fat-soluble vitamins are often absorbed along with dietary fat. These fat soluble vitamins are stored in liver and adipose tissue and their requirement is something like “demand and supply” *i.e.*, whenever there is a demand from cells, release/utilization of these vitamins occur. The fat-soluble vitamins can be harmful when they are stored in the body in excess over prolonged periods.

However, human body needs fat soluble vitamins to keep healthy skin, hair, eyes, heart and bone. Let us study fat soluble vitamins one by one.

12.5.1 Vitamin A

Vitamin A is the generic name for those similar compounds that are called retinoids.

Retinal and Retinoic acid are the biologically active form of Vitamin A. Plant derived carotinoids including β-Carotene are called retinoids which are converted to retinal in the human body. It is another source of vitamin A. Hence it is called provitamin A.

Vitamins A has three forms: retinol, retinal, retinoic acid which all are composed of β-ionone cyclic ring and an isoprenoid side-chain (Fig.12.13).

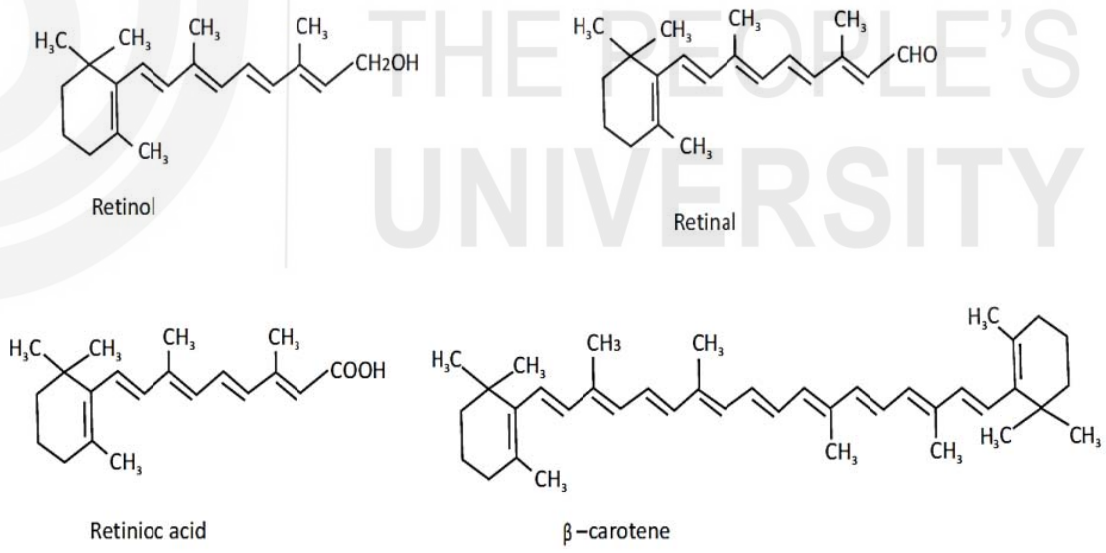


Fig. 12.13: Structure of forms of the Vitamin A.

Function

Vitamin A plays a central role in our vision. Retinol is transported via blood to the retina, where it is converted to the retinal. Retinal combines with the opsin protein to form the rhodopsin, a light-absorbing molecule. Rhodopsin is necessary for both color identification and low light vision. When light strikes on the rhodopsin, the 11-cis-retinal changes to all-trans retinal. As a result, a nerve signal reaches to the visual centre of the brain and thereby produces a visual image (Fig.12.14).

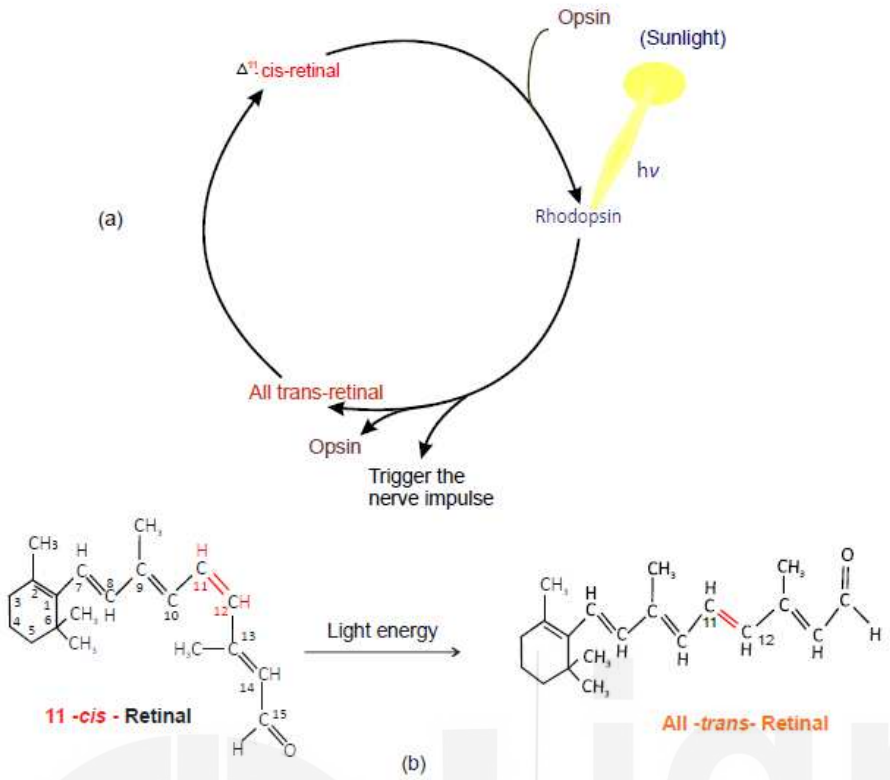


Fig.12.14: a) Visual Cycle b) Photoisomerization of 11-cis retinal to all-trans retinal in eye retina.

Retinoic acid acts like a hormone which regulates cell differentiation, growth, and embryonic development.

Vitamin A has an antioxidant activity in neutralising free radicals in the body. Vitamin A not only assists the vision function of eyes but also maintains the coverings and linings of the eyes.

Deficiency diseases and dietary food source

Deficiency of vitamin A in the nutritional diet produces night-blindness. It results in less rhodopsin and a decrease in the detection of low-level light. The night blindness is the most definitive sign of vitamin A deficiency.

Insufficient intake of dietary vitamin A over period of time leads to complete vision loss. In fact, vitamin A deficiency disease xerophthalmia (dryness of cornea and loss of tears) in children is the one of the key health issue worldwide. Papaya, carrots, spinach, pumpkins red palm oiland animal products are good sources to meet vitamin A requirement.

12.5.2 Vitamin D

Vitamin D is known as sunlight vitamin as it is synthesized with the help of sunlight in the body. Ultraviolet B (UV-B) radiation in sunlight synthesizes vitamin D from 7-dehydrocholesterol in the skin. The 7-dydrocholestrol is a precursor of cholesterol. When the sunlight exposure is inadequate to the body, dietary intake of vitamin D is required. *Cod* liver oil is the rich source of this vitamin. The active from of vitamin D is the calcitriol (vitamin D_3) which acts as a hormone (Fig.12.15).

Antioxidants are substance or molecule that inhibit free radical formation. They protect cells from harmful effect of free radicals. Antioxidants are capable of donating electrons to the free radicals thus neutralising their highly reactive nature.

Some antioxidants are enzymes such as catalase, superoxide dismutase, glutathione and; some are natural substance (*i.e.* vitamins A, C,E and phytochemicals that are present in plants.

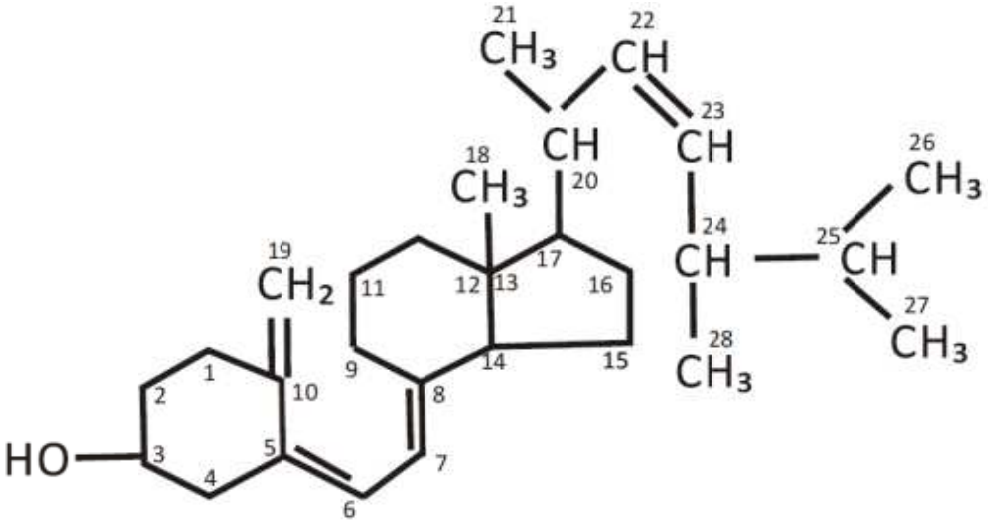


Fig. 12.15: Structure of Vitamin D.

Functions

Vitamin D assists in the absorption of calcium and phosphorus, helping bones to grow denser and stronger. It is necessary for growing children. It is also responsible for absorption of calcium and phosphorus in the human intestine.

Deficiency disease and dietary food source

Vitamin D deficiency leads to poor bone mineralization in the body resulting in osteomalacia disease in old people and rickets in children. The common symptoms are muscle weakness, softening of tissues and fragility of the bones. Vitamin D is used to treat rickets diseases hence it is also called **anti-rachitic factor**.

12.5.3 Vitamin E (Tocopherols)

Vitamin E is known astocopherol. It is a group of four natural compounds: tocopherols are designated as α (alpha), β (beta), γ (gamma) and δ (delta) based on number and position of methyl groups on the chromane ring. Among these, α-tocopherol is the most active form of tocopherol (Fig.12.16). It is synthesized in plants and photosynthetic organisms. Vitamin E is a derivative of 6-hydroxy chromane ring with phytyl side chain.

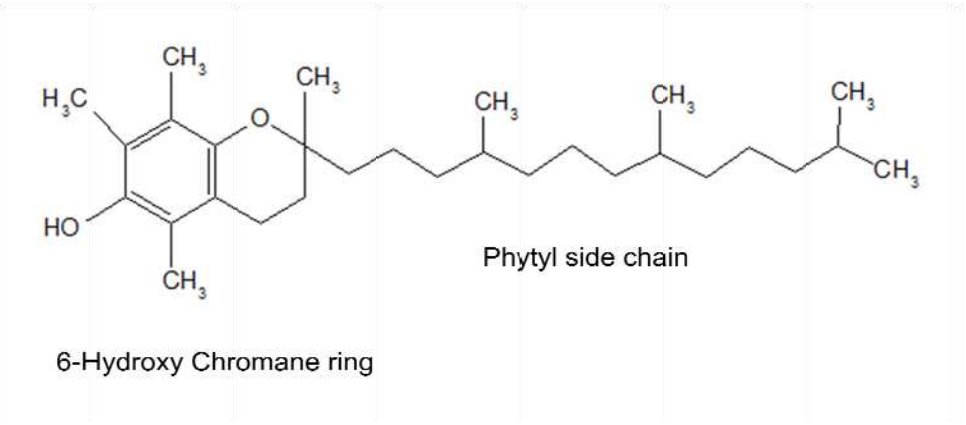


Fig. 12.16: Structure of α-tocopherol.

Function

Vitamin E acts as an antioxidant in the cell membrane and inhibits the chain reaction of free radicals. It protects cell membranes, proteins, and DNA from free radical damage. It stops oxidation of the polyunsaturated fatty acids and lipids in the skin cells thereby help to maintain healthy skin.

Deficiency disease and dietary food source

Diet with lack of vitamin E causes infertility in humans. The other symptoms of vitamin-E deficiency are muscular dystrophy, degeneration of the kidneys and necrosis of the liver. The rich source of vitamin E is corn oil, soyabean oil and nuts.

12.5.4 Vitamin K

Vitamin K is known as anti-hemorrhagic factor as it is necessary vitamin for blood clot formation in the body. Scientist H. Dam discovered vitamin K in 1929. The structure of vitamin K consists of a *quinine ring and isoprenoid side chain (Fig.12.17).* Vitamin K is found in plant foods as phylloquinone (K_1). Bacteria present in the human intestine can synthesize vitamin K as a menaquinone (K_2).

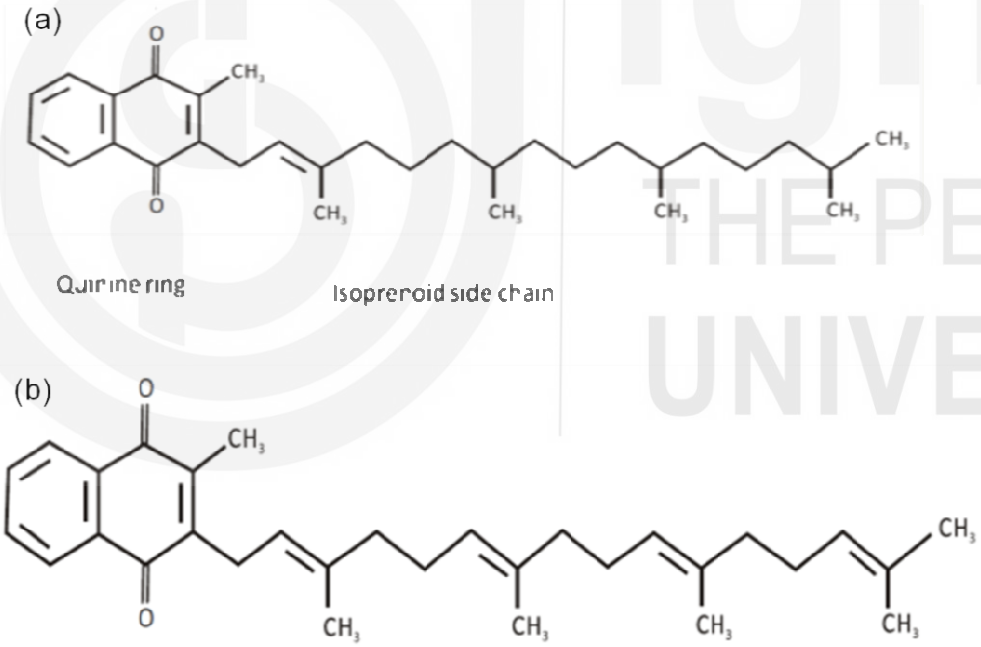


Fig. 12.17: Structure of Vitamin K_1 (a) and K_2 (b).

Functions

Vitamin K is essential for blood clotting process where it is required for formation of γ-carboxy glutamic acid from the glutamic acid which plays a critical role in blood clotting cascade.

Deficiency disease and dietary food sources

Blood coagulation process is affected due to an inadequate amount of vitamin K in the diet. Its deficiency delays the blood clotting process. Spinach, cabbage and green leafy vegetables are the best sources of this vitamin.

Some antioxidant vitamins and their related functions are given in Table 12.2.You will learn about Free radicals and Antioxidants Unit 13 of this course.

Table 12.2: Antioxidant vitamins and their biochemical functions.

Antioxidant vitamins	Functions
Vitamin A	Protects cells from free radical damage, regulates glutathione level (an intracellular antioxidant) and reduces inflammation.
Vitamin E	Inhibits the lipid peroxidation process caused by free radicals.
Vitamin C	Protects biomolecules (DNA, RNA, proteins) from the harmful effect of free radicals in cytosol. It also promotes the cell regeneration.

SAQ 4

a) State the important role of the following vitamins.

1. Vitamin A.....
2. Vitamin E.....
3. Vitamin D .....
4. Vitamin K.....

b) Fill in the blanks with correct answer(s).

i. β-Carotene is a source of …….. .

ii. Active form of vitamin A that is required for vision is …… .

iii. The absence of vitamin ..... causes infertility in human.

iv. Vitamin ..... helps in wound healing.

v. Natural source of Vitamin D is ……. .

vi. Vitamin ……. is involved in the hemorrhagic condition.

vii. The cell membrane damage is protected by vitamin ……..…. .

12.6 SUMMARY

In this unit so far, you have learned that:

- Vitamins are important dietary components for keeping body healthy with normal function. Their insufficient amount in the human body results in serious health problems.Different foods such as green vegetables, nuts, seeds, legumes, meats, milk, liver, fish and egg are good source of vitamins which can reduce the symptoms of vitamin deficiency diseases.

- B-complex vitamins primarily serve as coenzymes of metabolic enzymes. They assist in transferring of functional groups during biochemical reactions. They also help in digestion of food.
- Niacin (NAD and NADP) and riboflavin (FAD)act as an electron carrier in various redox reactions. Niacindeficiency causes pellagra disease.
- Pyridoxal-5-phosphate (PLP) is the most active form of vitamin B_6needed for amino group transferin the amino acid metabolism.
- Vitamin B_{12} is a metal (Cobalt ion)-containing vitamin. It assists in transferring of hydrogen atom and methyl group in the metabolic reaction. The lack of vitamin B_{12} leads to pernicious anemia. It works closely with folic acid.
- Folic acid (active form: tetrahydrofolate) is necessary for the development of red blood cells in human beings. It is essential in preventing serious birth defects during fetus development. Its deficiency causes the megaloblasticanemia.
- Vitamin C is an important natural anti-oxidant molecule required for the healthy tissues. It is essential for collagen synthesis, healing of wounds and immune system. Its deficiency causes scurvy disease.
- Vitamin A is important for normal vision, regulation of gene expression and cell differentiation. Its deficiency leads to poor vision and night blindness.
- Vitamin D acts as hormone and helps in calciumabsorption, bone and teeth formation. Its deficiency causes rickets disease in children and osteomalacia in adults. Sunlight is the natural source of this vitamin.
- Vitamin E is an intracellular antioxidant and protects cell membrane and DNA from the oxidative damage.
- Vitamin K helps in blood clot formation.

12.7 TERMINAL QUESTIONS

1. What do you mean by vitamins?
2. Explain coenzymes and their roles in metabolism.
3. Write the dietary sources of each of the water-soluble vitamins (niacin, pyridoxin, folic acid and cobalamin).
4. Differentiate between water-soluble vitamins and fat-soluble vitamins.
5. Define the term hypovitaminosis and hypervitaminosis.
6. Write the clinical symptoms of folic acid and vitamin C deficiencies.
7. List the Antioxidant vitamins and their roles.
8. Explain the visual cycle of the eye.
9. Explain how water-soluble and fat-soluble vitamins absorbed into the body.

12.8 ANSWERS

Self Assessment Questions

1. a) iii, b) i, c) viii, d) ii, e) iv, f) vii, g) vi, h) v.

2. a). Vitamins are classified into two major categories on the basis of their solubility in water or in fat. Water-soluble vitamins (B-complex vitamins and vitamin C are soluble in water while fat-soluble vitamins A, D, E and K in fat.

   b). Being hydrophilic nature, water soluble vitamins are readily soluble in water and thus execrated out easily via urine thereby preventing toxicity. Hence, they are not stored in the body.

3. a). i) False, ii) True, iii) False, iv) True, v) True, vi) True, vii) True.

   b.) i) Niacin, ii) NAD and FAD, iii) Pellegra, iv) Vitamin B_{12}, v) Vitamin C.

   c). i) e, ii) d, iii) a, iv) c, v) b.

4. a). i) helps in maintaining the normal vision.

   ii) an excellent antioxidant that protect the cell membrane and DNA from free radical damage.

   iii) helps in calcium and phosphorus absorption that build bones and teeth.

   iv) helps in blood clot formation.

   b). i) Vitamin A, ii) Retinal, iii) Vitamin E, iv) Vitamin C.

   v) Sunlight, vi) vitamin K, vii) Vitamin E.

TERMINAL QUESTIONS

1. Vitamins are organic compounds that are required in small quantities for proper functioning of the human body. Refer to section 12.1.

2. Refer to Section 12.4

3. Refer to Table 12.1

4. Refer to Section 12.3

5. Refer to Section 12.2

6. Refer to B-complex vitamin subsection (vii) for folic acid and the section 12.4.2 for vitamin C.

7. Refer to table 12.2.

8. Refer to Subsection 12.5.1.

9. Water-soluble vitamins are absorbed easily into the small intestine and then transport into the liver via blood circulation while fat-soluble vitamins are absorbed along with the dietary fat by the small intestine and then transported to the liver through blood circulation within the body.

FURTHER READINGS

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Acknowledgement of Figures

Fig. 12.1: Lippincott's Illustrated Review: Biochemistry

Fig. 12.9: Principle of Biochemistry, Lehninger and COX

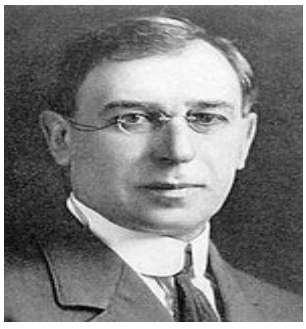
Fig. 12.11: https://phil.cdc.gov/Details.aspx?pid=19449

UNIT 13

FREE RADICALS AND ANTIOXIDANTS

Structure

Moses Gomberg (1866-1974) is the founder of radical chemistry. He was born in Elizabetgrad, Russian Empire. In 1886, Moses entered the University of Michigan, where he obtained his B.Sc in 1890 and his Doctorate in 1894.



13.1 INTRODUCTION

In the previous Unit you have studied about vitamins and their function in the body. This Unit aims to describe the nature, structure and biochemistry of free radicals (FRs) and antioxidants (AOs). The present Unit also explains the involvement of FRs and AOs in various normal and abnormal (patho-physiological) conditions. **FRs are free entities, produced as byproducts of some normal physiological functions like phagocytosis and electron transport chain (ETC) whereas AOs are the substances that protect cells from any kind of oxidation**. AOs operate as a kind of scavenging system to eliminate or nullify the actions of FRs, by virtue of which normal cells and tissues are being protected.

In the second half of the Unit you will study about o*xidative stress* (OS). OS is a condition developed due to the "**imbalance between the levels of FRs and AOs**" and is an extensively studied area of research. It is considered to be the most prominent cause for many popular disorders. In addition to this, sources of AOs and their mode of action will be discussed. Alzheimer's normally caused by degeneration of neurons is one of the common disease due to OS. At the end of the Unit, you will wonder to know the causes and symptoms of aging (senescence) with special emphasis vis-a-vis its biochemical background.

Objectives

After studying this Unit, you should be able to:

- define terms like FRs, AOs and oxidative stress,
- list out the sources of FRs and AOs,
- discuss the common sites of FRs generation,
- explain the damage and protection exhibited by both FRs and AOs, and
- explain the process of aging (senescence).

"An atom or a group of atoms having unpaired electrons are called free radicals"

13.2 FREE RADICALS AND THEIR SOURCES IN THE BODY

Oxygen containing compounds give rise to molecules known as "free radicals" (FRs), which can be defined as atoms or molecules or ions species capable of independent existence and contain one or more unpaired electrons. These molecules are highly reactive. These radicals tend to attack nearest compounds in the process of searching for the needed electron, to obtain stability. Hydroxyl atom ($HO^{\cdot}$), oxygen molecule (O_2), Nitric Oxide ($NO^{\cdot}$), Ferryl ion (FeO_2^{+}) and superoxide molecules ($O_2^{-\cdot}$) are some of the examples of FRs (Table 13.1). Most of the FRs are generated in our body by virtue of normal biochemical reactions and physiological processes. Some of the important reactions are discussed below.

Table 13.1: Important Free radical species in pathology

Species	Molecular formula
Superoxide	$O_2^{-\cdot}$
Hydroxyl	$HO.$
Nitric oxide	$NO.$
Ferryl ion	$FeO.^{2+}$

13.2.1 Respiratory Burst

The host defense system against harmful pathogens includes immune cells like neutrophils, monocytes, eosinophils and macrophages. They perform their activity through phagocytosis. Neutrophils, in their defense system protect against the pathogens like bacteria, where they inject oxidizing agents like hydrogen peroxide, hypochlorous and other oxygen radicals into the phagocytic vacuole to kill pathogens. NADPH oxidase plays a key role in this process (Fig 13.1). This process is referred to as respiratory burst. Reactive Oxygen Species (ROS) are generated during normal aerobic metabolism (example: electron transport chain), where constant production of oxygen derived radicals occurs. If the production of such ROS and FRs is uncontrolled, it may cause damage to the surrounding bio molecules and healthy tissue.

ROS is a generic collective term indicating a number of active and reactive partially reduced O_2 metabolites. Some of them, such as $O_2^{-\cdot}$ and $HO^{\cdot}$, can be defined as true free radicals, which are reactive molecular species with an impaired electron in their outer orbital.

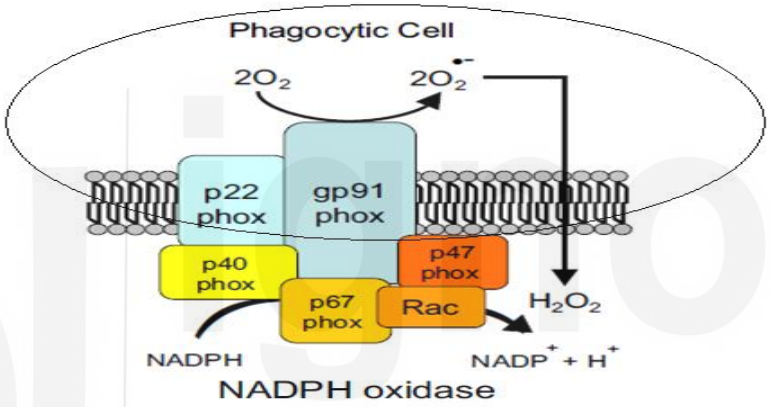


Fig. 13.1: Generation of H_2O_2 inside phagocytic cell.

13.2.2 Abnormal Environmental Conditions

Cells exposed to hypoxia (diminished oxygen levels) or hyperoxia (increased oxygen levels) generate abundant amount of ROS, which will cause damage to the sub-cellular compartments of the cell, leading to cell leakage and cell shrinking. Similarly, cells exposed to radiation in aerobic conditions are more prone to generate ROS, that's why regular exposure to X-rays is considered to be dangerous. However, high altitudes and deep seas are the common sites for environmental hypoxic conditions. This is the reason why people wear oxygen cylinders.

Phagocytosis is the process where, outer layer of the cell preferably plasma membrane is involved in engulf or uptake the nearby particles (like prey). This phenomenon is popularly studied while discussing food intake process by amoeba and even observed in immune cells like macrophages or neutrophils while attacking pathogens.

13.2.3 Endothelium Derived Free Radicals

Nitric oxide (NO) produced by endothelial cells of vascular system, is considered as Endothelium Derived Relaxing Factor (EDRF). NO is an important vasodilator and responsible to maintain vascular tone. In addition to this, NO plays a significant role in anti-platelet aggregation. By virtue of above said functions, NO is referred to as EDRF. Superoxide is continuously produced by endothelial surface, which reacts with NO (both are FRs) to form nitrate (non-FRs). Hence, impaired (unpaired) endothelial function may alter the vascular tone and lead to uncontrolled FRs generation.

$$NO + O_2 \rightarrow \text{Intermediates} \rightarrow NO^{3-}$$

13.2.4 Electron Transport Chain (ETC)

ETC is considered to be a constant producer of FRs, as oxygen acts as the terminal electron acceptor of ETC and produces highly reactive superoxide radical. This is a non-enzymatic reaction involving intervention of redox components such as the semi-ubiquinone compound of the mitochondrial ETC (Fig. 13.2). In general, electrons escape from the ETC at the level of ubiqunone-cytochrome-c and react with molecular oxygen to produce $O_2^{-\cdot}$ molecule. In addition to above discussed reactions, many compounds will interact with molecular oxygen to produce FRs, e.g adrenaline. FRs are also generated during detoxification process of various drugs like paracetamol, alcohol at the site of cytochrome P450.

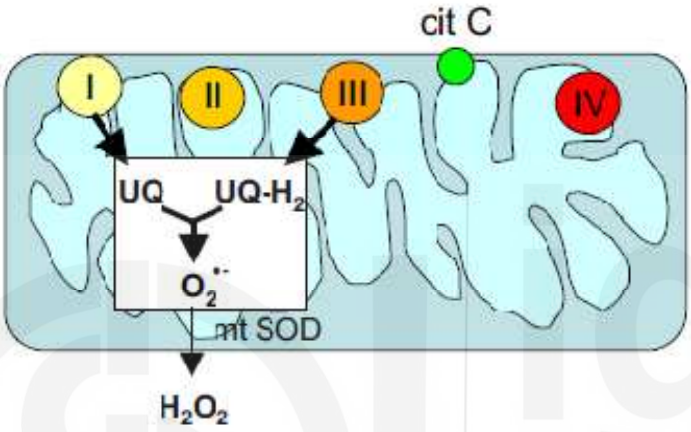


Fig. 13.2: Generation of superoxide molecule in Electron transport chain.

SAQ 1

Fill in the blanks:

i) …….. is the founder of radical chemistry.

ii) …….. is the constant producer of FRs in our body.

iii) EDRF is …….. .

iv) Semi-ubiquinone is present in …….. .

13.3 FREE RADICALS INTERACTIONS WITH PROTEINS, LIPIDS AND NUCLEIC ACIDS

FRs being highly reactive and unstable, try to steal the electron from the nearby macromolecules (proteins, lipids and nucleic acids), during which they create a new FR centre within the macromolecule. This newly generated FR disrupts the whole molecule as well as other components of the cell in a cascade like mechanism. This is how uncontrolled production of FRs can lead to cell injury and sometimes even death. In Unit 9 on Biomolecules of BZYCT-135, you have studied how essential are all these macromolecules in the maintenance of normal growth and function. In this context, we are going to

study the impact of FRs on these vital molecules. Moreover, diseases such as cardiovascular (atherosclerosis (hardening of arteries), stroke), diabetes, cancer, and major disorders like Alzheimer's are the consequences of FRs pathophysiology (Fig. 13.3). Scientific research carried out over the past few decades revealed that interaction of FRs with biomolecules produces nitro derivatives of proteins, oxidized products of lipids and thymine dimers from nucleic acids. In the following subsection we will be studying more details about them.

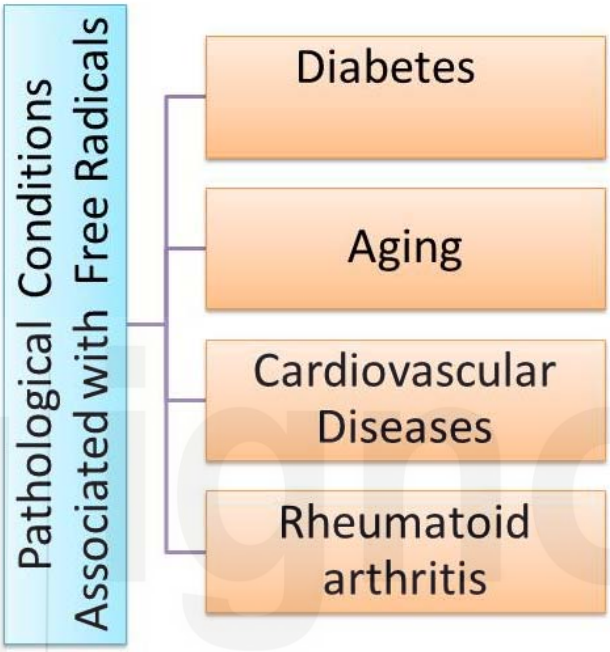


Fig. 13.3 Diseases involving free radical interaction.

13.3.1 Proteins

FRs mediated oxidation of proteins leads to aggregation and fragmentation, owing to which proteins lose their native structure and biological activity. Proteins upon oxidation with FRs form carbonyls, viz oxo acids and/ or aldehydes with the same or less carbon numbers than that of parent amino acid being targeted by the FRs (Table 13.2). These reactions can alter intrinsic membrane properties like fluidity, ion transport, loss of enzyme activity, protein cross-linking and inhibition of protein synthesis.

Table 13.2: Amino acid derivatives after oxidation with FRs.

Amino acid	Oxidized product
Glycine	Glyoxal, Glyoxylic acid; Formaldehyde and Formic acid
Lysine	Lysine hydroperoxides
Alanine	Acetaldehyde, Acetic acid
Tyrosine	Dityrosine, Tryptophan
Histidine	Oxohistidine

13.3.2 Lipids

If you recall the important functions of lipids as discussed in Unit-9 of BZYCT-135, most of the sub-cellular components of the cell are made up of lipid bilayer (plasma membrane). These bilayers are abundant in polyunsaturated fatty acids (PUFA), that have more double bonds. These are the potential sites from where FRs prefers to steal electrons. In general, FRs steal the electron from the hydrogen paired with the carbon at the double bond. This in turn, produces a carbon with an unpaired electron, to stabilize this newly formed carbon-centered FRs, molecular rearrangements will occur (Fig. 13.4). By virtue of molecular rearrangements, a new molecule called conjugated diene (CD) will arise. CD easily reacts with oxygen radical to form a peroxy radical. This peroxy radical can attack another lipid membrane and carries forward the process in a cascade like mechanism. This attack of FRs over lipids is known as “lipid peroxidation” also known as auto-oxidation. Increased levels of lipid peroxidation products and lipofuscin pigments result in inactivation of prostacycline and NO which will affect normal function of the cell.

Cascade

“An inevitable chain of events due to an act affecting a system”.

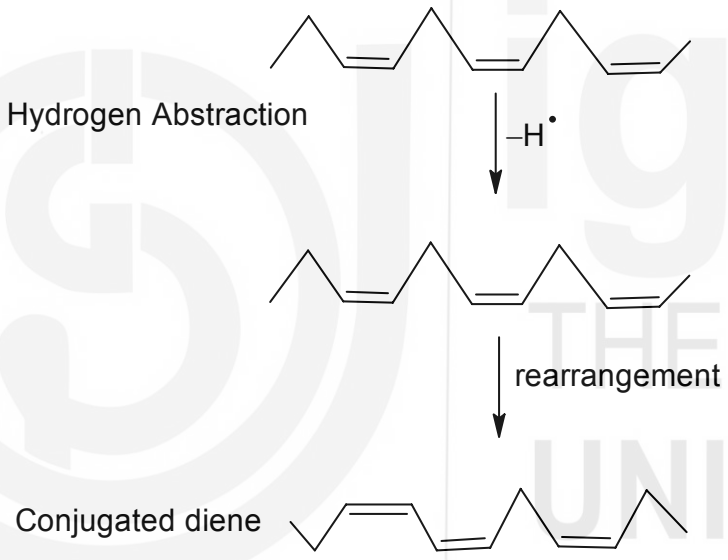


Fig.13.4: Formation of conjugated diene.

13.3.3 Nucleic Acids

So far, you have read how FRs affect structure and functions of proteins and lipids. Let us now discuss how both DNA and RNA get affected by the FRs. As we have discussed, FRs tend to remove electrons from the double bonds existing in the surrounding molecules, in the same way DNA and RNA can be targeted. DNA and RNA possess more double bonds (A=T/U), where H atom from the methyl group of thymine and each of the C-H bonds of 2'-deoxyribose will be targeted. The conjugate structure of nitrogen bases will give an added advantage to FRs to target nucleic acids. As a result of this thymine dimers originate which will influence replication, transcription and translation functions of DNA. In later stages DNA may get cross linked leading to Alzheimer’s and certain specific cancers. However, in Section 13.6 of this Unit you will know more about aging and its associated issues.

Before proceeding further, try to attempt the following SAQ.

SAQ 2

i) Define free radical.

ii) List out the important biochemical reactions and physiological processes where free radicals are generated.

iii) Match the following:

Column A	Column B
a) Proteins	i) lipid peroxidation
b) Lipids	ii) thymine dimmers
c) Nucleic acids	iii) oxo acids

13.4 ANTIOXIDANTS: SOURCES AND BIOLOGICAL SIGNIFICANCE

When free radicals exceed the capacity to be scavenged by the cell, the condition is referred to as *oxidative stress*.

The term “antioxidant” (AOs) was used for the first time in 1920s to denote any *substance that fights against rust or any other forms of oxidation*. While discussing about AOs in a biological system, they are assumed as “*scavengers*” due to their ability to remove FRs. AOs can interact and terminate the cascade mechanism (as discussed above) to minimize the damage caused by FRs. In this aspect AOs and its associated system can be considered as a first line of defense mechanism against cellular oxidative stress.

Prior to having the knowledge of cellular defense mechanisms, it is essential to know the biologically significant components involved in this mechanism. Antioxidant system is composed of both AOs molecules (dietary and metabolic) and antioxidant enzymes (Fig. 13.5). Here it is important to note that, these enzymes are located within cells, whereas AOs are available both as extra and intracellular material. In this context, let us discuss in detail about some of the well-known AOs and antioxidant enzymes.

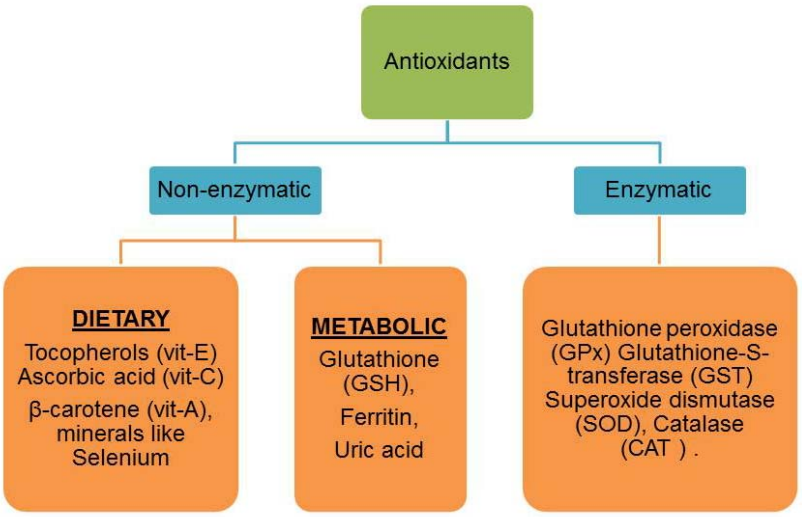


Fig. 13.5: Types of Antioxidant system.

13.4.1 Glutathione (GSH)

Glutathione (γ-glutamylcysteinylglycine) is a tripeptide consisting of glutamic acid, cysteine and glycine (Fig.13.6). It is commonly known as reduced glutathione. It is important to note that out of the total glutathione content, 95%is available as GSH and remaining 5% as GSSG i.e., glutathione oxidized. Glutathione reductase and transhydrogenases are the enzymes responsible for the continuous production, conversion and regeneration of these redox states. GSH is a potential cofactor for antioxidant enzymes and protects mitochondria from its endogenous oxygen radicals. Antioxidant property of GSH is exerted by its –SH group through donating its electron. GSH is abundantly found in liver and plays a vital role in phase-II reaction of cytochrome P450 where fat soluble substances are converted into water soluble ones. Tremendous amount of research work has been carried on how to replete the endogenous GSH levels. The best way suggested is to take diet rich in sulphur containing amino acids like cysteine and methionine. On the other hand, it is also stated that consuming green leafy vegetables may reduce the rapid depletion of GSH from circulation.

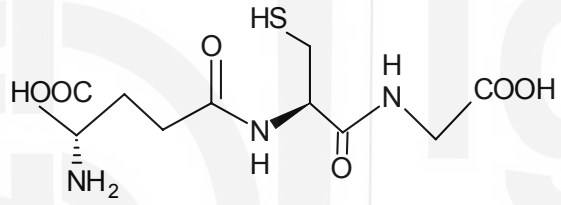


Fig. 13.6: Structure of glutathione.

13.4.2 Tocopherols

The word tocopherol is derived from Greek, where *tokos*-child birth; *pherin*- to bear or carry, believing that necessary for fertility. One of the well-known tocopherol is Vitamine-E. Tocopherols a group of lipophlic compounds involved in peroxy radical scavenging and terminating FRs cascade reaction. These tocopherols are abundantly found in biological membranes. Till date eight well recognized tocopherols have been identified, which are biologically active. Among these α-, β-, γ- and σ- are the important ones. Tocopherol consists of a chromane ring with a polyisoprenoid side chain of varying length (Fig 13.7). The hydroxyl group present on the ring donates hydrogen atom to reduce FRs and side chain involves in binding with the biological membranes. α- tocopherol is the most abundant and potent tocopherol. Vegetable oils, nuts, seeds and whole grains are the rich sources of tocopherols.

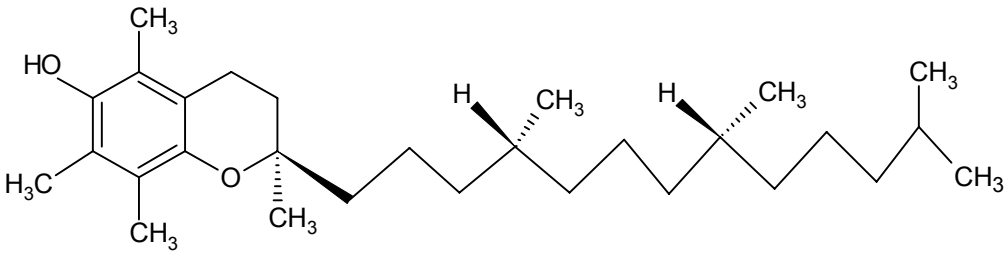
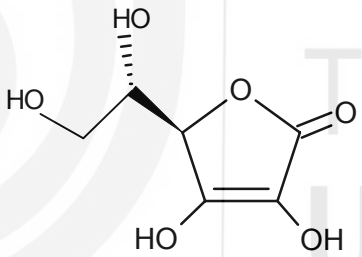


Fig. 13.7: α- tocopherol (vitamin-E).

It is well recognized as a natural antioxidant, protecting biological membranes from FRs attack. It is amazing to know that, it acts as a first line of defense mechanism against FRs mediated oxidative stress in cell and sub cellular components (mitochondria).

13.4.3 Ascorbic Acid (vitamin-C)

Ascorbic acid is popularly known as vitamin-C. You have already studied about this vitamin as water soluble vitamin in the Unit-12 of this course. You're aware of coenzyme nature, sources and recommended dietary allowances. But here we are discussing the same vitamin with a different view as an "antioxidant". Ascorbic acid is hexose derivative and acidic in nature, because of its *enolic hydroxyl* groups (Fig. 13.8). Coming to its antioxidant property it is considered to be as a strong reducing agent. Many of its biological activities are associated with its ability to undergo reversible oxidation and reduction i.e., interconversion of ascorbic acid to dehydroascorbic acid. L-ascorbic acid and dehydroascorbic acid (oxidized product of L-ascorbic acid) are the two biologically active forms of ascorbic acid. Reduced form of ascorbic acid is abundantly found in plasma and tissues. Vitamin C not only eliminates FRs from circulation but also promotes the regeneration of α-tocopherol by which it efficiently prevents lipid peroxidation.



(a)

(b)

Fig. 13.8: a) structure of vitamin C. b) r*ich sources of vitamin-C.*

13.4.4 β-carotene (Vitamin-A)

Vitamin-A belongs to the category of naturally occurring carotenoids (Fig. 13.9) which are lipophilic in nature due to which they can easily integrate and lipid membranes offer stability and protection to them. Carotenes are popular in the ability to scavenge FRs although their concentration is 50-fold less than α- tocopherol in the plasma. So far, about 500 carotenoids have been identified, among these carotenoids ***β-carotene*** is the most significant and responsible for red-orange colour in carrot. Uniqueness of β-carotene lies in ***β-rings*** at both ends of the molecule. Chemically it is classified as a *terpenoid.* ***Lycopene is*** a plant pigment (responsible for colouring) that is also a carotenoid found in some fruits (e.g. tomato). In addition to colouring agent it also acts as a protective antioxidant. You have already studied about recommended dietary allowances values, and functions of this vitamin under fat soluble vitamins in the Unit-12 of this course. The alternative double bonds present in the structure of β-carotene play a vital role in removing FRs.

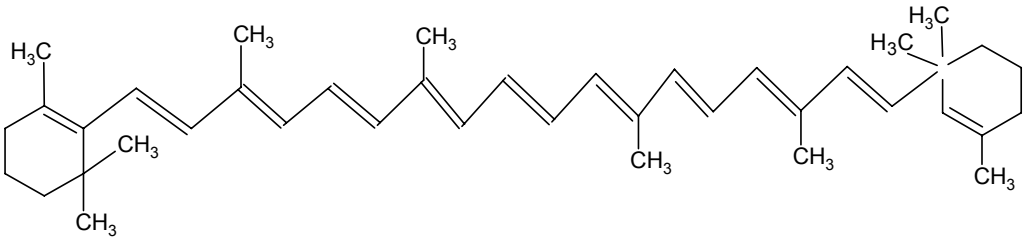


Fig.13.9: β-carotene.

In addition to the above studied antioxidant substances there are few other substances which either directly or indirectly are involved in scavenging activity. For example, thiol groups also known as sulfhydryl groups (-SH) found in cysteine amino acid act as FRs scavengers. Selenium, an essential trace element acts as co-factor for glutathione peroxidase enzyme. α-lipoic acid, a vitamin like compound produced by the body plays key role in the recycling of antioxidants. After studying this topic it can be adduced that consuming green leafy vegetables may enhance anti oxidant status of the cell.

13.5 MECHANISMS OF PROTECTION AGAINST RADICAL DAMAGE

So far, we have studied about nature and chemistry of free radicals and antioxidant molecules. While studying these we have come across the formation of free radicals and the unwanted consequences taking place upon their interaction with biomolecules.

Every biologically active cell has certain defense mechanism to minimize the interaction of free radicals with biomolecules. This intra cellular mechanism prevents the destructive changes in the structure and function of biomolecules like proteins, lipids and nucleic acids. This mechanism is mainly composed of *antioxidant molecules* and certain *cytoprotective* enzymes (Fig.13.5). Hence, this mechanism is referred to as "*scavenging system"*,that works as a cascade. In the coming sub sections we are going to learn more about how this scavenging system functions and its significance in protecting the cell from free radical damage.

13.5.1 Cytoprotective Enzymes

These are the group of enzymes which catalyse the scavenging reactions inside a living cell. By virtue of these enzymes cell is able to overcome the oxidative stress and becomes self-defensive. Hence, these enzymes are known as cytoprotective. This group consists of three major enzymes, which are as under.

I **Superoxide dismutase (SOD)** is considered as first line of defense to protect cells from stress induced by FRs). SOD is available either as a zinc/copper containing enzyme in the cytosol or as manganese containing metalloprotein in mitochondria of mammalian cells. It removes the O^{-}_{2} by combining it with protons ($2H^{+}$) to form hydrogen peroxide (H_2O_2) and oxygen (O_2).

(A) $O_2^{\bullet -} + O_2 + 2H^+ \xrightarrow{\text{Superoxide dismutase}} H_2O_2 + O_2$

II **Catalase (CAT)** is found in all living organism to interact with oxygen. It is located within the peroxisome and cytosol of the cell and decomposes H_2O_2 formed by SOD to water (H_2O) and oxygen (O_2). CAT has the highest *turnover number* (refer to Units-10 &11 of this course) of all enzymes.

$$(B)\quad 2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

III **Glutahione peroxidase (GPx)** is a selenium-containing metalloenzyme, partially located within cellular membranes. The main function of the GPx is to protect cell from oxidative damage. To perform this, it removes H_2O_2 by decomposing it to H2O, while removing reduced glutathione it gets converted into oxidized glutathione (Fig. 13.10).

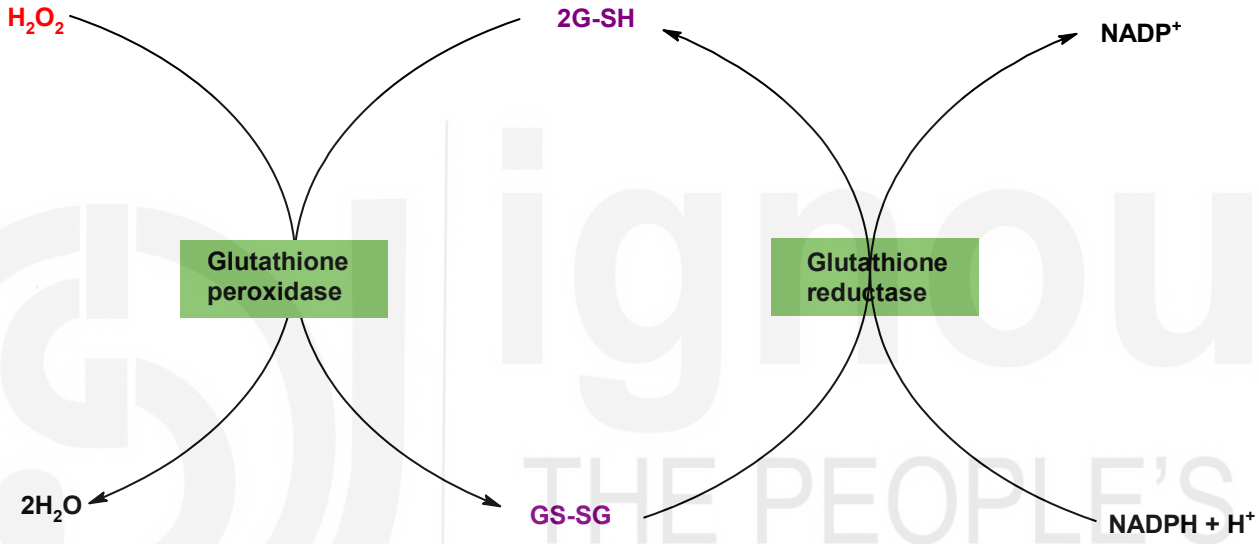


Fig. 13.10: Decomposition of H_2O_2 by glutathione peroxidase.

GPx can also terminate the chain reaction of lipid peroxidation by converting lipid hydroperoxides to their respective alcohols in the cell membrane. Regeneration of the reduced glutathione is done by NADPH-dependent *glutathione reductase*.

SAQ 3

i) Define the term antioxidant.

ii) List out the antioxidant enzymes.

13.6 BIOCHEMISTRY OF AGING (SENESCENCE)

The word “Senescence” means *“Loss of a cell’s power of divisional growth”.* Aging or senescence is a normal physiological process and though it cannot be stopped, owing to its incomplete understanding. However, hypothetically it is considered that, aging can be slowed down by appropriate measures

starting at an early age. Physiologically, it affects the cells and systems by virtue of its impairing the ability to maintain homeostasis that occurs during *stress*. Hence, the elderly people are more vulnerable to disease processes as the aging progresses. Life expectancy across the globe has increased from 47 years in 19th century to 75 years in 21st century, due to improved medication, treatment and prevention of infectious diseases. Some *gerontologists* like to distinguish between young old (55 to 74) and old-old (75+) (however, 60 years of age and above are aged).

13.6.1 Theories of Aging

There are various theories (Fig. 13.11) to explain the mechanism behind aging, among those well established are as follows:

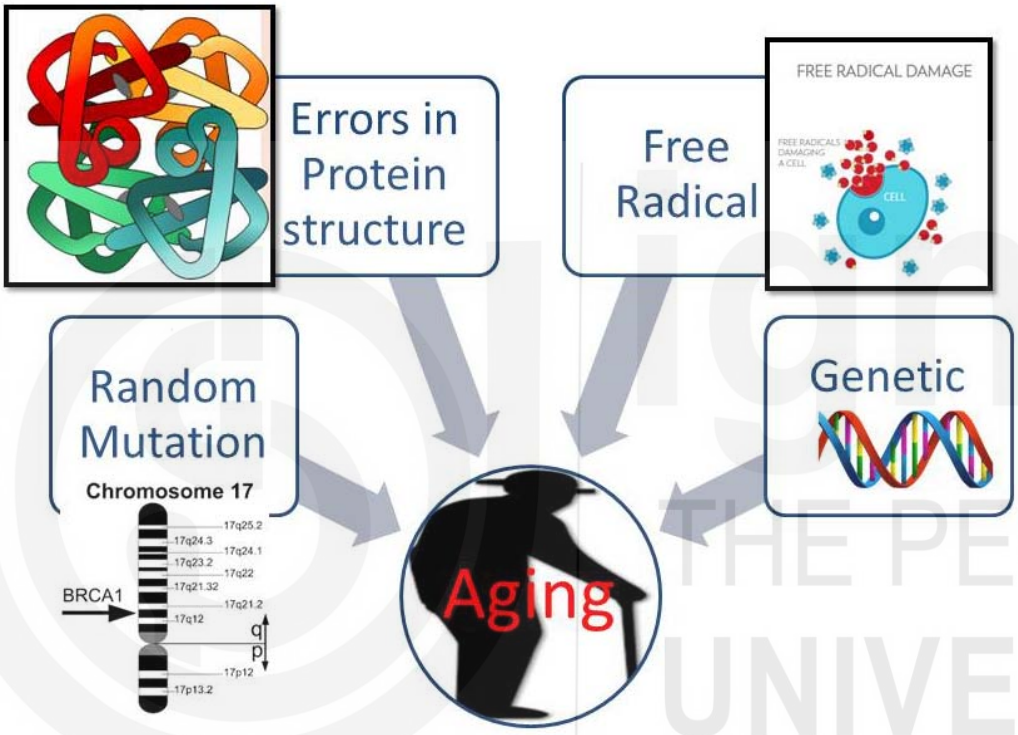


Fig. 13.11: Theories of Aging.

1. **Random Mutation Theory:** According to this theory random mutations occur in DNA of somatic cells, which introduces abnormalities by causing destruction of collagen and cell shrinkage that leads to cell death.

2. **Errors in Protein Structure:** In this theory, abnormalities are produced by increased cross-linkage of collagen and other proteins as a result of non enzymatic combination of glucose with amino groups on these proteins.

3. **Free Radical Theory:** This theory has been proposed by Dr. Denham Harmon in 1956. As we have studied above, free radicals interact with bio molecules to form altered products with impaired biological activity. According to this theory, this is found to be the major cause of aging. It is interesting to note that, species which can produce more superoxide dismutase shows longer life span, indicating some significant relationship between aging and antioxidant status.

Werner's** syndrome* commonly known as ***progeria,is one of the evidence for the cumulative DNA abnormalities in humans. It is a rare autosomal recessive progeroid syndrome. In the year 1904, **Otto Werner** a German scientist identified this syndrome, hence named after him. It has a global incidence rate of 1 in 1, 00, 000 live births, with higher rates in Japan and Sardinia 1 in 20, 000 to 40,000 and 1 in 50, 000 respectively. Progeria occurs due to the genetic abnormality in gene coding for **DNA helicase**, which is causing chromosomal damage. The person with this syndrome suffers with accelerated rate of early aging. To know more about this syndrome you can visit the following web link https://en.wikipedia.org/wiki/Progeria.

4. **Genetic Theory;** According to this theory aging process is dependent on the nature of genes and the proteins they encode. That's why aging varies from species to species. It is believed that decline in the body's physiological function is genetically programmed and can't be prevented.

So far you have studied various theories associated with aging, now let us know in brief about the changes observed in various vital systems of the body with growing age. Figure 13.12 shows the age associated changes**,** as age advances distinguishable changes can be noticed both in physiology and structure of the cell, which in turn affect the whole organism. These changes include digestive, respiratory, circulatory, excretory and reproductive systems as well. Communicating systems like nervous, endocrine and immune system are also affected by increasing age; both in their functioning and performance. **Regular exercise, healthy dietary habits and stress free life style may delay aging process but can't prevent aging**.

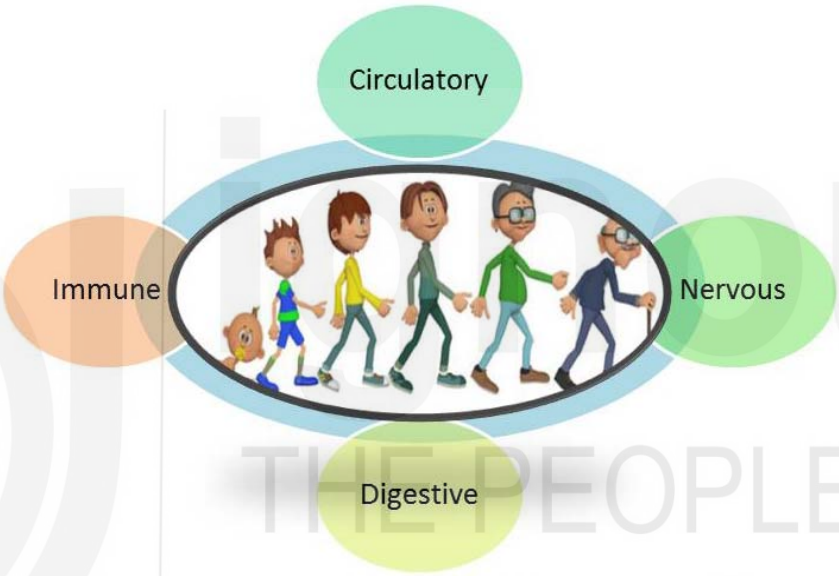


Fig. 13.12: Vital systems affected by growing age.

13.7 SUMMARY

In this Unit you have studied:

- The chemical nature of free radicals, their origins in biological systems along with the patho physiological consequences occur upon their interaction with macromolecules.
- You have also studied about various physiological conditions that are responsible for the production of FRs and ROS.
- Composition of *scavenging* system along with individual roles of antioxidant molecules and enzymes. In the second half of the Unit you came across the role and importance of cytoprotective enzymes in protecting the cell from free radical damage.
- In this unit you have been sensitised about mode of action of these enzymes in minimizing the effects of FRs and ROS.
- At the end of the Unit you have studied about different theories of aging and its associated changes.

13.8 TERMINAL QUESTIONS

1. Explain the biochemical reactions and processes where free radicals are generated.
2. Discuss the consequences of free radical interaction with macromolecules.
3. Write the significance of cytoprotective enzymes in a cell.
4. What is antioxidant? Explain the different types of antioxidants with suitable examples.
5. Write a short note on theories of aging and list the changes associated with aging.

13.9 ANSWERS

Self Assessment Questions

1. i) Moses Gomberg
   ii) Electron transport chain
   iii) Nitric oxide
   iv) Mitochondria
2. i) Free radical is an atom or molecule or ion capable of independent existence and contains one or more unpaired electrons.
   ii) Respiratory burst, abnormal environmental conditions, endothelium derived and ETC
   iii) a) iii, b) i, c) ii.
3. i) Antioxidants are substances that protect against any kind of oxidation.
   ii) SOD, CAT, GPx, GST.

Terminal Questions

1. Free radicals are generated in the biochemical reactions and processes like respiratory burst, abnormal environmental conditions, endothelium derived and ETC. ROS are generated during normal phagocytosis in neutrophils.
2. Once free radicals attack the bio molecules they leave reaction center later continues to steal the electrons form nearby atom or molecule in a cascade manner. Proteins, lipids and nucleic acids are the major targets of free radicals. These molecules upon oxidation produce oxo acids, conjugated dienes and thymine dimers respectively. This further impairs the normal functioning of molecules.

3. Cytoprotective enzymes are group of enzymes that protect cell from free radical damage. Lack or insufficiency of these enzymes leads to a state called oxidative stress, which is considered to be major reason for aging or senescence.

4. Antioxidants are composed of both enzymes and molecules that are endo and exo genous in nature. Refer to Section 13.4 for further details.

5. There are four major theories that largely influence the process of aging. Refer to Section 13.6.1 for further details.

FURTHER READINGS

1. Review of Medical Physiology by William F. Ganong. 22nd edition. LANGE publishers

2. Textbook of Medical Physiology by Veena Mehta Ahuja. 2nd edition. ANE publishers.

3. Biochemistry by U. Satyanarayana and U. Chakrapani. 3rd edition. Books and Allied (P) Ltd.

4. Redox mechanisms in hepatic chronic wound healing and fibrogenesis. Erica Novo and Maurizio Parola. Journal of fibrogenesis and tissue repair, 2008, 1:5, 1-58.

Acknowledgement of Figures

Fig. 13.1: modified from Parola *et al*.,

Fig. 13.2: modified from Parola *et al*.,

Fig. 13.8: http://www.winstudent.com/the-top-10-sources-of-vitamin-c/