الجامعة التكنولوجية

قسم الهندسة الكيمياوية

المرحلة الاولى

الكيمياء الاحيائية

م . علياء عصام

CHAPTER - 4

Amino Acids, Peptides & Proteins

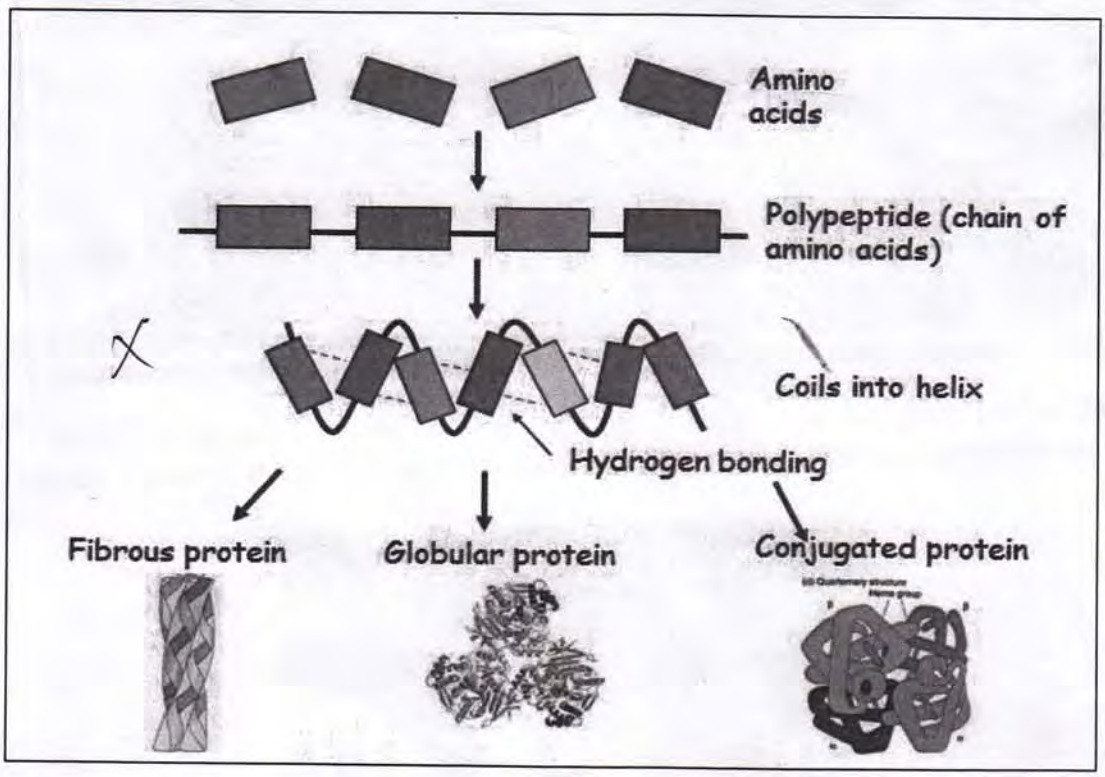
Introduction

Proteins are complex organic nitrogenous substances found in animal and plant tissues. The term protein is derived from Greek: Proteios means primary or holding first place.

Protein is the essential constituent of living cells.

Protein make up to 12% of the protoplasm. They are not only responsible for comprising the structure of the cell but are concerned with every function of the cell including those of respiration, catalysis of reactions by enzymes, transport of materials, regulation of metabolism, and defense actions.

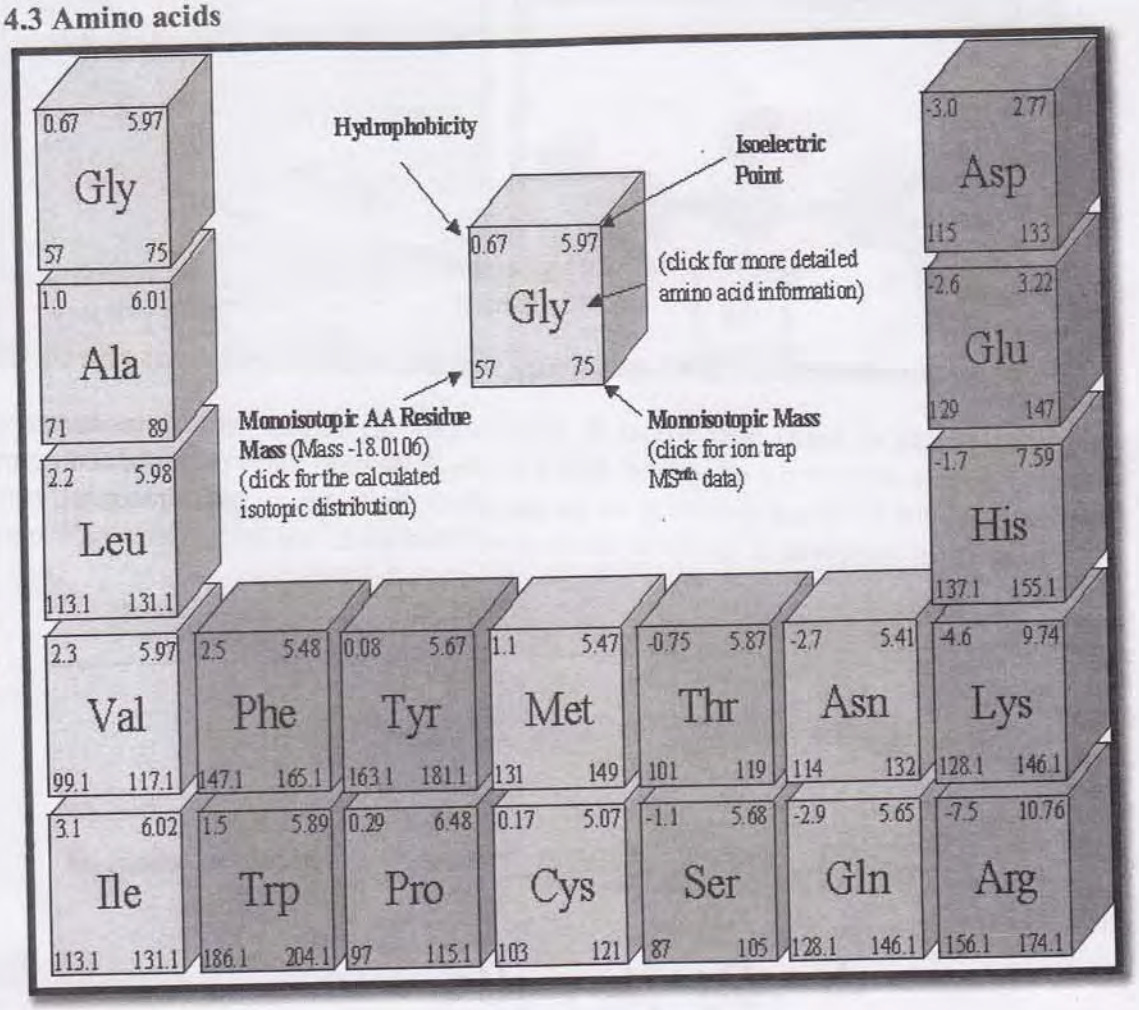
The foods rich in proteins are known as body building foods.



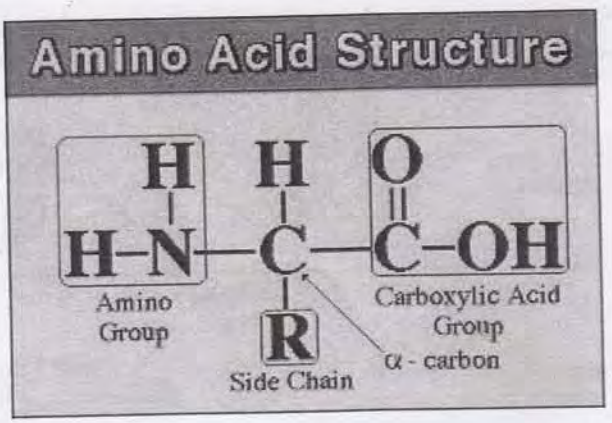
Sources of protein

Proteins are obtained from animal and plant sources. The animal sources of proteins include milk, egg, meat, fish, liver etc. Plant sources of proteins are pulses, nuts and cereals.

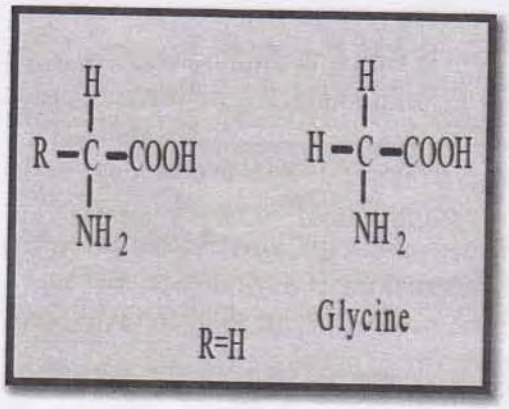
4.3 Amino acids

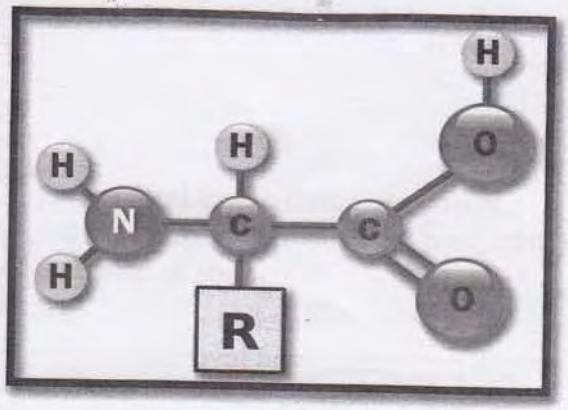


Amino acids are the simplest units of a protein molecule and they form the building blocks of protein structure. The general formula of an amino acid can be written as:



The general structure of α-amino acid in its un-ionized form.

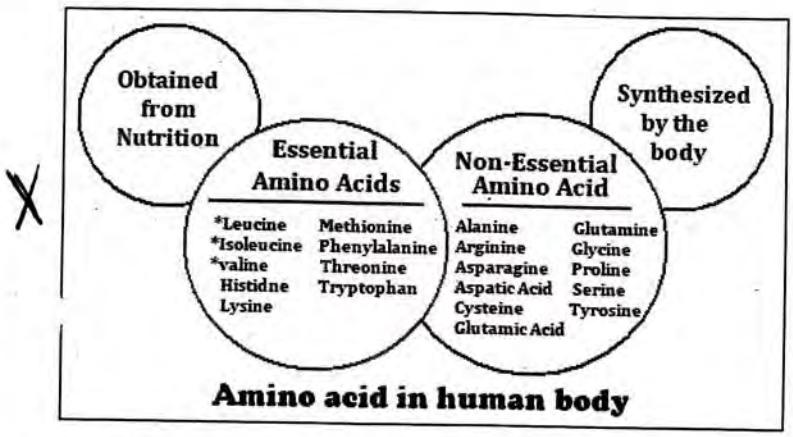




An amino acid is an amino carboxylic acid. R is the side chain or residue and it represents the group other than -NH_2 and -COOH. It may be a hydrogen atom (H) or a methyl group (-CH_3) or an aliphatic group or an aromatic group or a heterocyclic group. The amino acids are classified based on the nature of R groups.

Essential amino acids

Certain amino acids cannot be synthesized by the living organisms. They must be compulsorily included in the diet for normal health. These amino acids are called essential amino acids. For human being about 10 amino acids are considered as essential:



Amino acid in human body

Non-essential amino acids

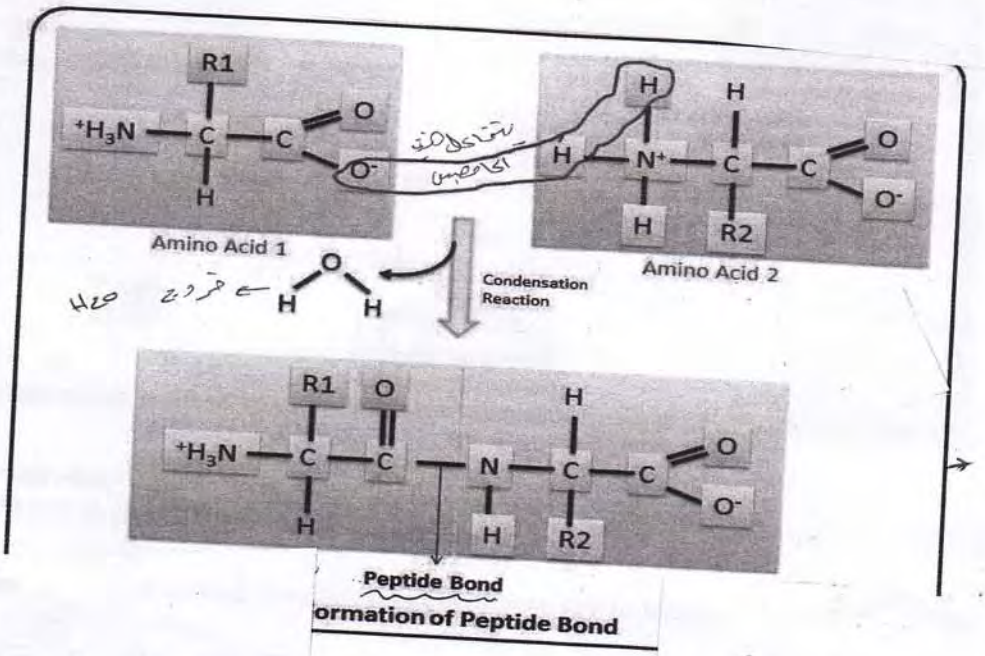
Certain amino acids can be synthesized in the cells from essential amino acids or from other compounds. So these amino acids need not be included in the diet. They are called non-essential amino acids.

Non protein amino acids

Certain amino acids which do not exist in proteins are called non protein amino acids eg. Ornithine and β-alanine etc..

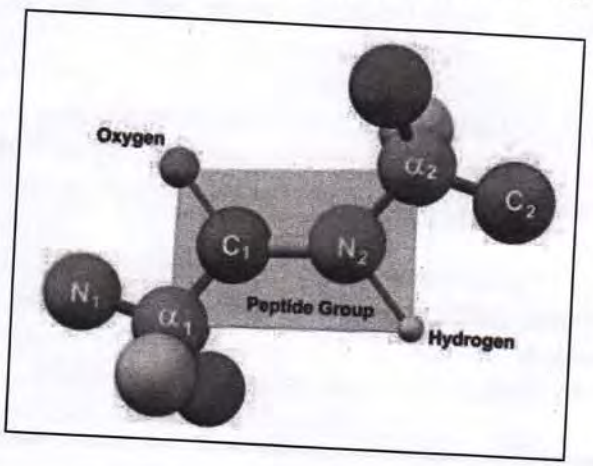
Peptide bonds

In proteins, amino acids are linked together by linkages called peptide bonds. The carboxyl group of one amino acid is joined to theα- amino group of another amino acid by a peptide bond.

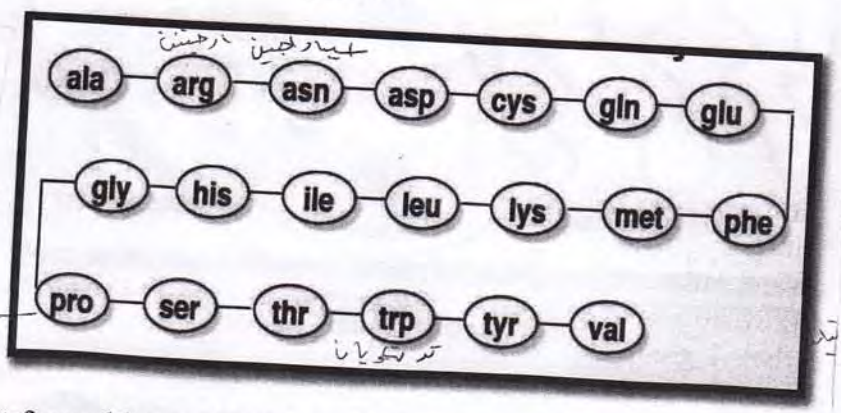


ormation of Peptide Bond

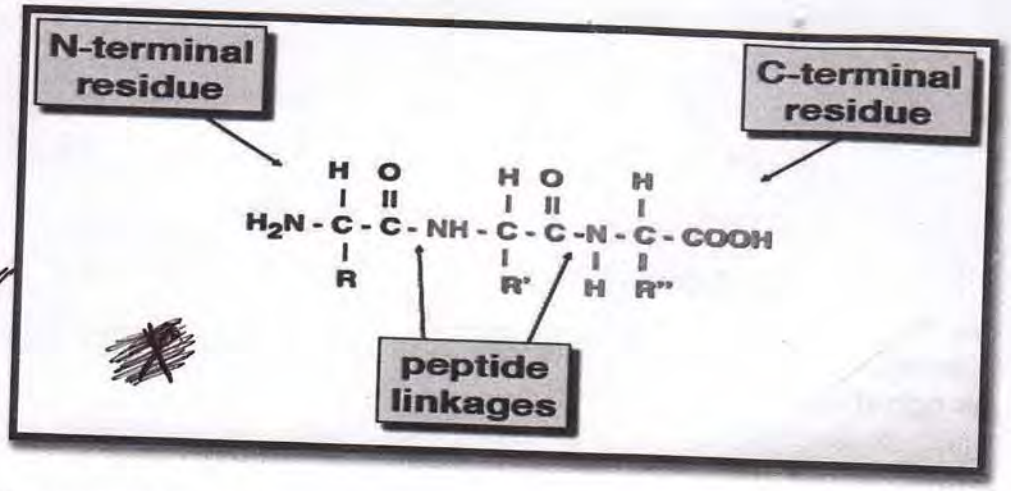
The peptide bond is also called as the amide bond. The two amino acids, joined by a peptide bond, constitute a dipeptide. The dipeptide is formed by simple condensation reaction.



Here is an example sequence of amino acids in a protein.It also shows the abbreviations of amino acids in a protein.



The product formed by a peptide bond is called a peptide. The compound formed by the linking of three amino acids is called as tripeptide.



4.4 Properties of proteins

4.4.1 Physical properties

1. Color and taste

Proteins are colorless and usually tasteless. These are homogeneous and crystalline.

2. Solubility

Solubility of proteins is influenced by pH. Solubility is lowest at isoelectric point and increased with increasing acidity of alkalinity.

3. Optical activity

All protein solutions rotate the plane polarized light to the left i.e. these are levorotatory.

4. Colloidal nature

Because of their giant size, the proteins exhibit many colloidal properties are:

a. Their diffusion rate is extremely low.

b. They may produce considerable light-scattering in solution, thus resulting in visible turbidity (Tyndall effect).

5. The comparatively week forces responsible for maintaining secondary, tertiary and quaternary structure of proteins are readily distrusted with resulting loss of biologic activity. This disruptions of native structure is termed denaturation. Physically, denaturation may be viewed as randomizing the conformation of a polypeptide chain without affecting its primary structure (Fig.4.3).

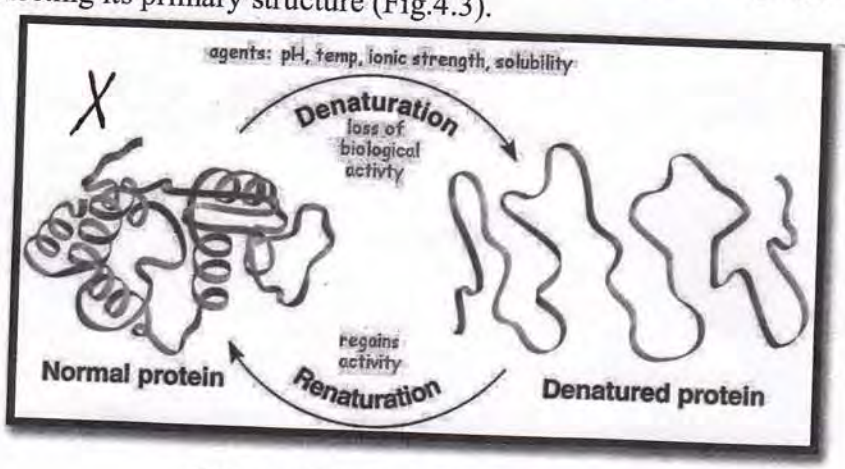


Fig. 4.3 Denaturation of protein.

The biological activity of most proteins is destroyed by exposure to strong mineral acids or bases, heat, urea, acetone, alcohol and ionic detergents etc. Denatured proteins are less soluble in water.

Enzymes & Kinetic enzymes

1 Introduction

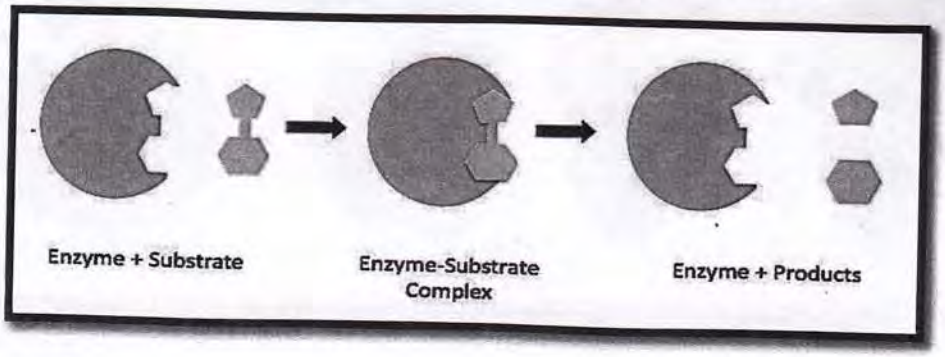
All the enzymes are proteins and they are produced by the living cells. They act as biological catalysts. Enzymes catalyzed and enhance the rate of biochemical reactions occurring in various vital processes like breathing, digestion, pumping of heart, formation of body tissues, contraction of muscles, transport of ions across the plasma membranes etc. So without enzymes there is no life. They are inactive at $0^{\circ}C$ and destroyed by moist heat at $100^{\circ}C$.

The substances on which the enzymes act are called as "Substrates".
Enzymes are highly specific in their action (i.e) an enzyme can act on a single or a small group of closely related substrates. During catalytic action, the enzymes do not undergo any permanent modification and regenerated at the end of the reaction. The general enzyme catalyzed reaction takes place as per the equation.

$$\left.\begin{array}{l} E + S \text{ ---------- } ES \\ ES \text{ ---------- } E + P \end{array}\right\}$$

E - Enzyme; S = Substrate; ES - Enzyme-Substrate complex.

P = product. ES complex is an unstable and highly energized complex.



For example:

α- amylase acts on starch and produce maltose units. In this reaction α-amylase is the enzyme, starch is the substrate and maltose is the product.

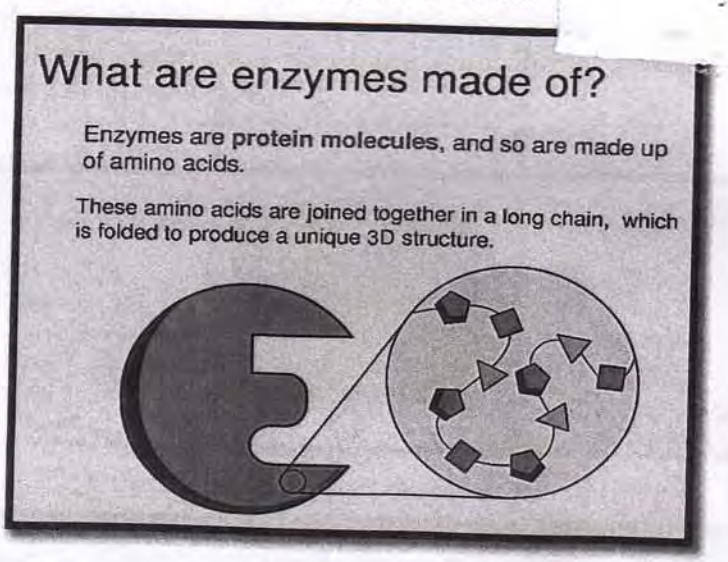
α- amylase + starch ---------- α- amylase (starch)
(E) (S) (ES)
α- amylase – starch ---------- α- amylase + maltose
(ES) (E) (P)

Most of the enzymes, synthesized by a living cell, function within that cell and hence are called as endo enzymes or intra cellular enzymes.

Some enzymes are liberated from the living cells after synthesis, secreted to the environment and function in extra cellular regions. These enzymes are called as exo enzymes or extra cellular enzymes

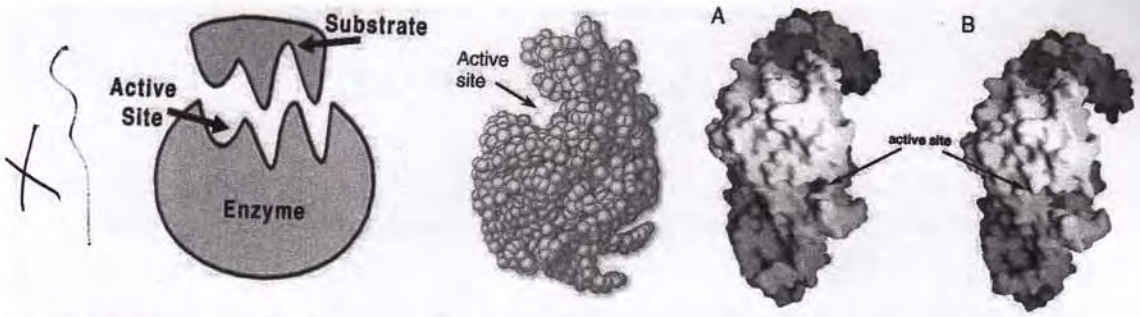
5.2 Chemical nature of enzymes

All the enzymes are proteins with no exception. Some enzymes are simple proteins i.e. their molecules consist of only amino acids.



Many enzymes are conjugated proteins and their molecules consist of Amino acids and a non-protein part (not made up of amino acids). The non-protein part of the enzyme is known as coenzyme or prosthetic group, without which the enzyme is inactive. The protein part of the enzyme (in conjugated type) is known as the apoenzyme. The coenzyme and the apoenzyme complex is called as holoenzyme.

Coenzyme + Apoenzyme---------- Holoenzyme



The active site and the other part of the enzyme undergo conformational modification when they come in contact with the substrate. Koshland's induced fit hypothesis of enzyme-substrate interaction postulates that the active site of the enzyme consists of a number of 'active' contact amino acids which permit the substrate to come close to the reactive groups of the enzyme which thereupon undergoes a conformational change, binding the substrate firmly to the enzyme and promoting catalytic activity.

Naming of enzymes

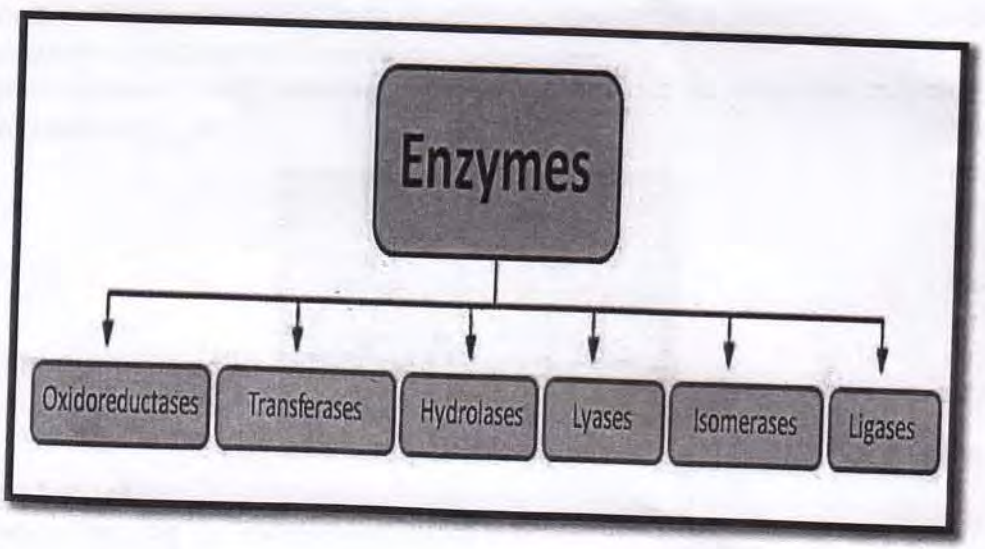
Except the enzymes ptyalin, pepsin, trypsin and renin, all the other enzymes are usually named by adding suffix - ase to the main part of the name of the substrate on which they act.

Examples:

Maltase acts on maltose
Lactase acts on lactose
Lipases act on lipids
Proteases act on proteins
Amylases act on starch (amylum)

Classification of enzymes

The most comprehensive system for the classification of enzymes was devised in 1961 by the Enzyme Commission of International Union of Biochemistry (IUB). The 6 major classes of enzymes are:

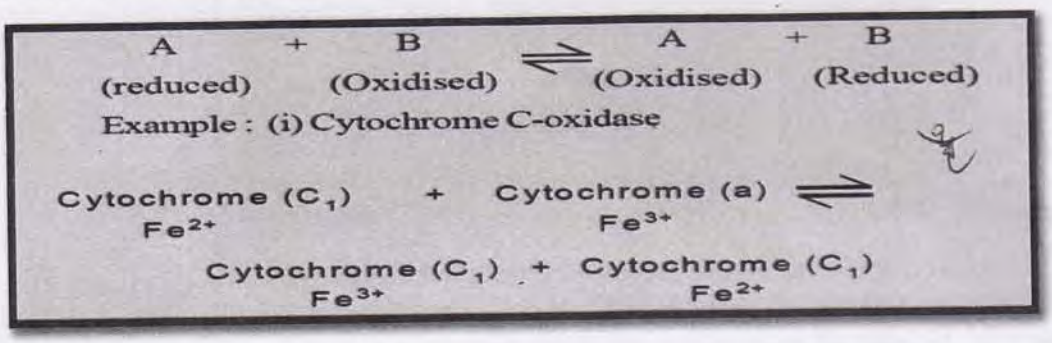


Enzymatic Action

- Oxidoreductases (EC 1): oxidation–reduction reactions, which entail the transfer of electrons from a substrate to another substrat
- Transferases (EC 2): move a chemical group from one compound to another compound
- **Hydrolases (EC 3):** Hydrolysis of a chemical bond
- Lyases (EC 4): formation of a double bond
- Isomerases (EC 5): catalyzes substrate to an isomeric form
- Ligases (EC 6): catalyzes a reaction that joins two substrates

1. Oxidoreductases

Enzymes catalyzing oxido-reduction reactions between two substrates A and B are called as oxido-reductases.



In this reaction cytochrome C1 is oxidized and cytochrome is reduced simultaneously by the action of cytochrome C-oxidase.

This class includes several subclasses based on the group on which the enzymes act. The enzymes acting on:

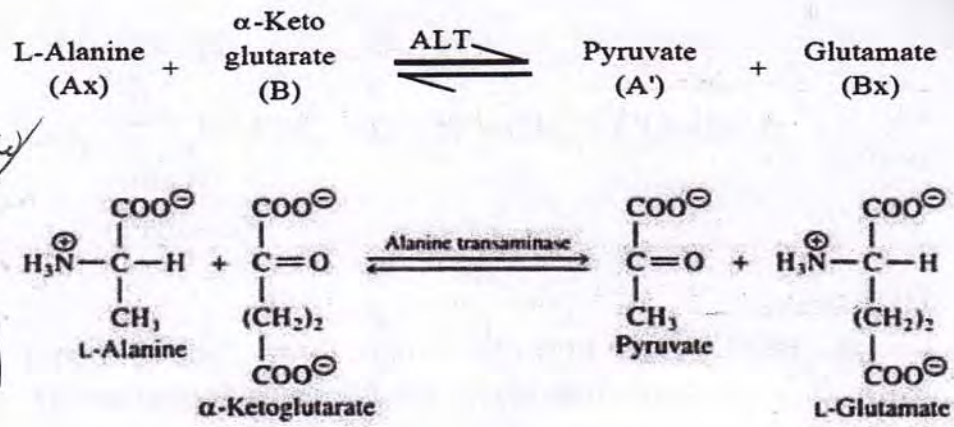
-CH -OH	(1.1)
-C=O	(1.2)
-C=CH	(1.3)
$-CH-NH_2$	(1.4)
-CH-NH	(1.5)

2. Transferases

Enzymes catalyzing the transfer of a group (x) from one substrate (AX) to another (B) are known as transferases.

$$AX + B \longleftrightarrow A' + BX$$

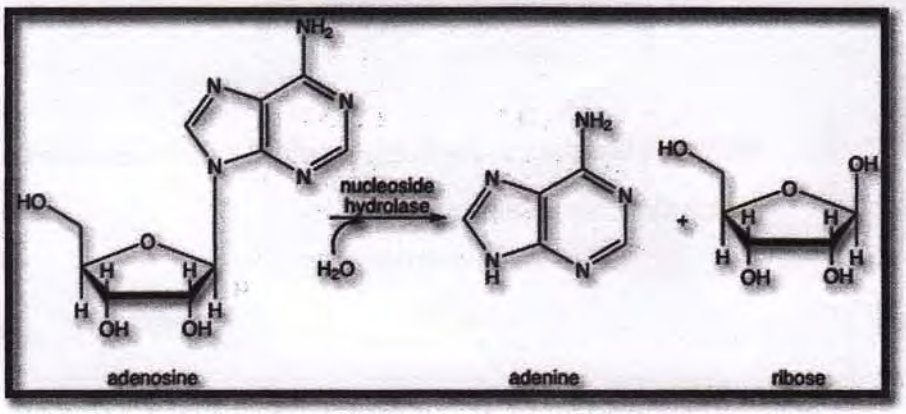
Example:
The reaction catalyzed by alanine transaminase (ALT) is:

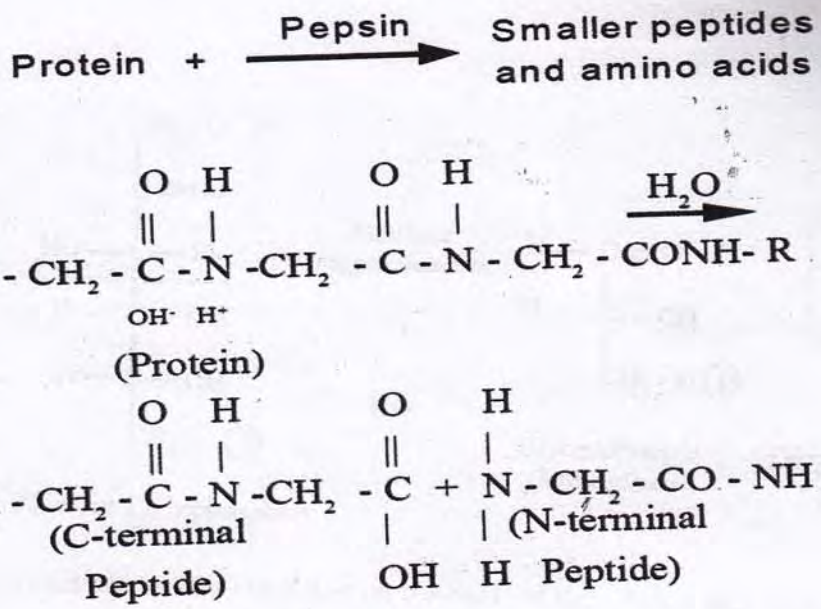


In this reaction the amino group from alanine is transferred to α-ketoglutarate to form glutamate.
These enzymes are further divided into subclasses on the basis of nature of the group transferred.

Transfer of	(a) one carbon compounds	(2.1)
	(b) aldehyde or ketonic groups	(2.2)
	(c) acyl groups	(2.3)
	(d) glycosyl groups	(2.4)
	(e) Phosphate groups	(2.7)
	(f) Sulphur containing groups	(2.8)

3. Hydrolases
The hydrolases are those enzymes which catalyze hydrolysis reactions i.e the direct addition of water molecule (s) across the bond, which is to be cleaved. The substrate for these enzymes are esters, ethers, peptides and glycosides. Example: Pepsin. This enzyme is a gastro intestinal enzyme which is proteolytic in nature and involve in the hydrolysis of proteins present in the food.



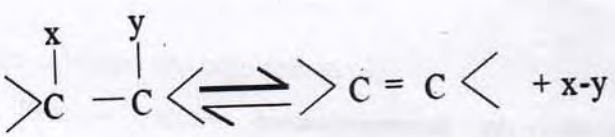


The hydrolases are divided into several subclasses, depending on the nature of the group or bond being hydrolyzed viz.

(a) esterases etc. - hydrolyse ester bonds	(3.1)
(b) glycosidases - hydrolyse glycosidic bonds	(3.2)
(c) peptidases - hydrolyse peptide bonds	(3.4)

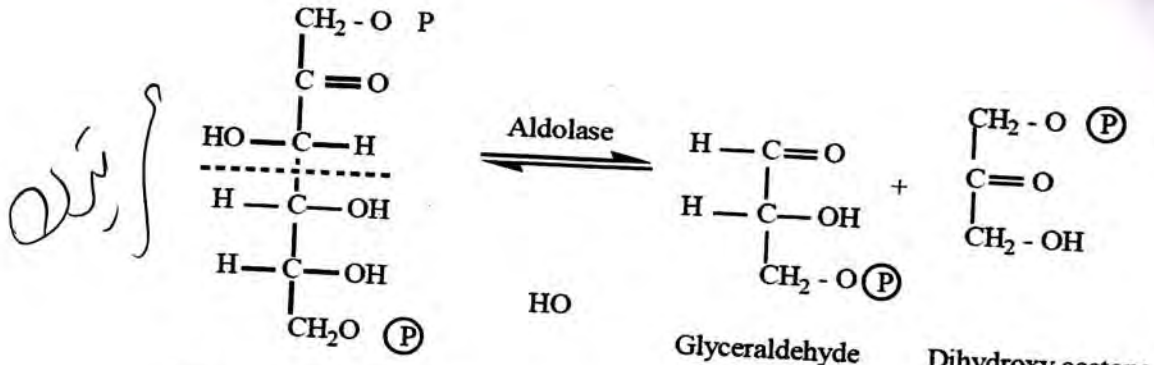
4. Lyases

The lyases are a smaller class of enzymes that catalyze the removal of a small molecule from a larger substrate molecule. Since the reactions are reversible, lyases may also be considered to catalyze the addition of small molecules to the substrate molecule.



Example:

Fructose-1-6-diphosphate ⇌ Dihydroxy acetone phosphate + glyceraldehyde 3-phosphate



The lyases are further classified on the basis of the linkage they attack viz., acting on:

a. C-C bond	(4.1)
b. C-O bond	(4.2)
c. C-N bond	(4.3)
d. C-S bond	(4.4)
e. C-halide bond	(4.5)

5. Isomerases

This class includes all enzymes which catalyze isomerization reactions i.e. interconversion of optical, geometrical or position isomers.

Example:

Retinene isomerase catalysis the conversion of:

Trans-retinene ⟷ cis-retinene.

Triose phosphate isomerase catalyses the conversion of:

D - Glyceraldehyde 3- phosphate ⟷ Dihydroxy acetone phosphate

The isomerases are further divided into the subclasses:

A. racemases	**(5.1)**
B. epimerases	**(5.2)**
C. cis-trans isomerases	**(5.3)**

6. Ligases

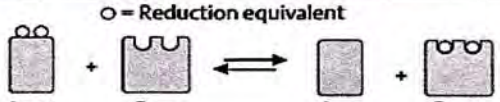
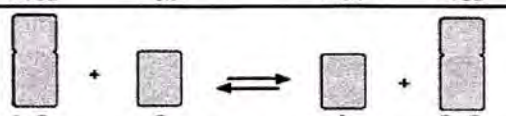
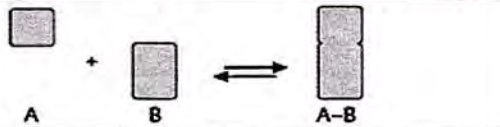
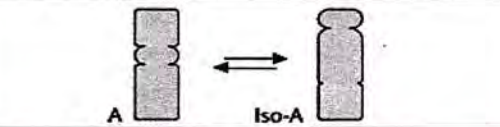
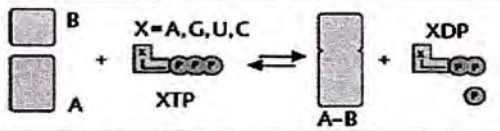
These enzymes are otherwise known as synthetases. They catalyze synthesis reactions by joining two molecules, coupled with the breakdown of a phosphate bond of adenosine triphosphate. ATP cleavage provides energy for the new bond formation.

Example:

Formation of malonyl CoA from acetyl CoA in the presence of acetyl CoA carboxylase.

The subclasses of ligases are based on the nature of bond forme in the product. Formation of:

a. C-O bond (6.1)
b. C-S bond (6.2)
c. C-N bond (6.3)
d. C-C bond (6.4)

Class	Reaction type	Important subclasses
1 Oxidoreductases	O = Reduction equivalent 	Dehydrogenases Oxidases, peroxidases Reductases Monooxygenases Dioxygenases
2 Transferases		C_1-Transferases Glycosyltransferases Aminotransferases Phosphotransferases
3 Hydrolases		Esterases Glycosidases Peptidases Amidases
4 Lyases ("synthases")		C-C-Lyases C-O-Lyases C-N-Lyases C-S-Lyases
5 Isomerases		Epimerases *cis trans* Isomerases Intramolecular transferases
6 Ligases ("synthetases")		C-C-Ligases C-O-Ligases C-N-Ligases C-S-Ligases

Factors influencing enzyme activity

The activity of enzymes is markedly affected by several factors. These factors are:

1. Temperature
2. pH
3. Substrate concentration
4. Metal ions (activators)
5. Inhibitors
6. ~~Enzyme concentration etc.~~

1 pH

All the enzymes have a particular pH at which their activity is maximal; above or below this pH the activity is low. The pH at which the enzyme shows maximum activity is known as optimum pH. Some of the enzymes and their optimum pH are:

(a) Pepsin - 2.0
(b) Amylase - 7.0
(c) Trypsin- 8.5
(d) Alkaline phosphatase - 9.9

Only in this optimum pH, ionization of active amino acids in enzymes and substrate are favored for ES complex formation. The pH activity relationship is shown in the (Fig. 5.1).

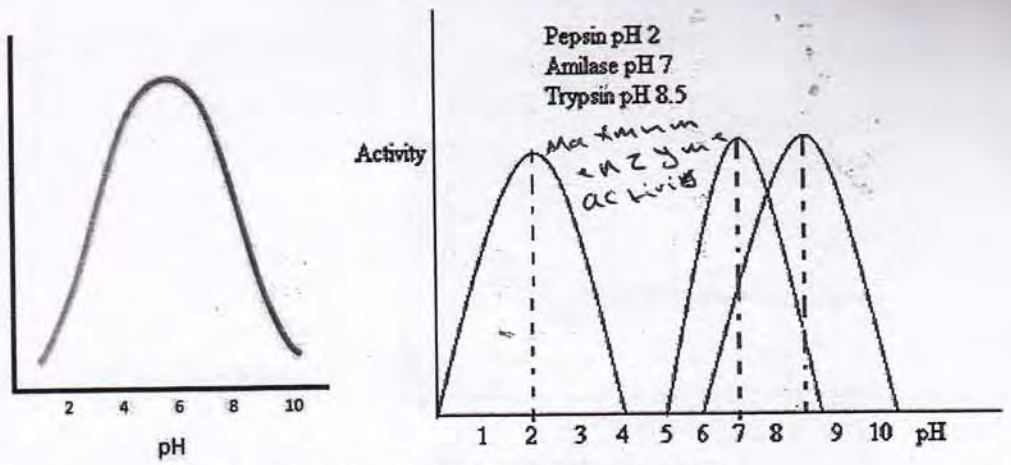


Fig. 5.1 The pH activity relationship.

.2 Temperature

Rise in temperature causes increase in the rate of enzyme catalyzed reactions up to a certain temperature i.e. about 45°C. Above which the activity declines due to denaturation of enzymes (due to their protein nature). As the enzyme is denatured and inactivated, the reaction which it catalyzes slows down and ultimately stops. So the temperature at which the enzyme shows maximum activity is known as optimum temperature. The optimum temperature of most of the enzymes is found to be 37°C. The relationship of enzyme activity to temperature is shown below in Fig. 5.2:

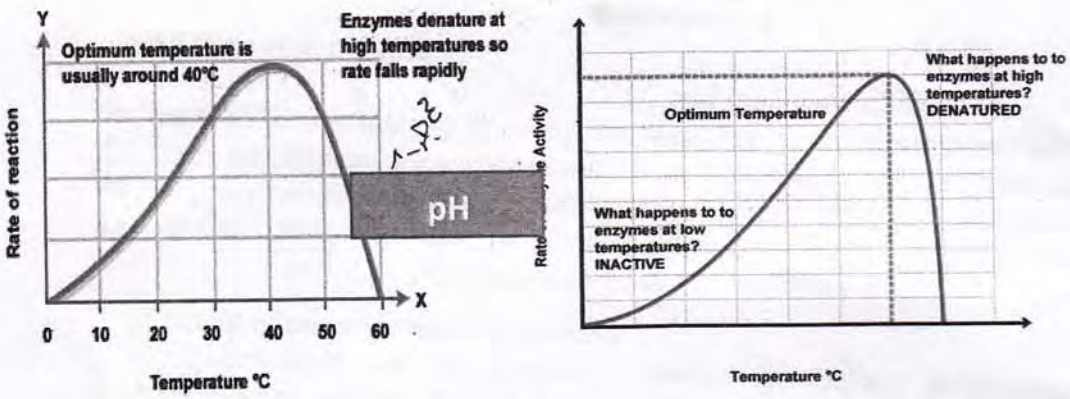
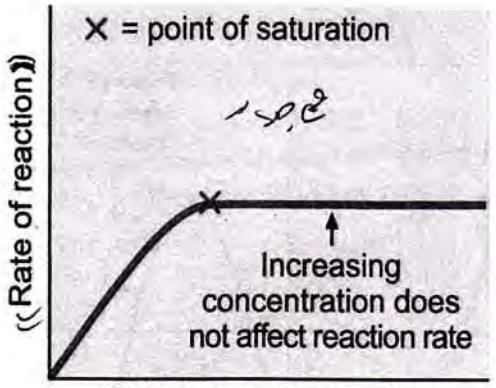


Fig. 5.2 The temperature activity relationship.

3 Substrate concentration

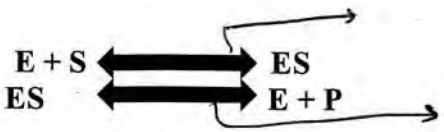
With a fixed amount of enzyme, the reaction rate is proportional to the concentration of substrate. But this is true up to a certain concentration after which the increase in concentration of substrate does not further increase the velocity of the reaction.

Since the number of active sites on an enzyme molecule are limited, a stage will come when all of them have filled with the substrate molecules. This is known as saturation of enzyme. Now, since none of the active sites of the enzyme is free, further addition of the substrate molecule will not increase the product formation (Fig.5.3).



Substrate concentration - activity relationship.

It was Michaelis and Menten in 1913, who proposed a successful explanation for the effect of substrate concentration on the enzyme activity. According to them the enzyme 'E', and the substrate 'S' combine rapidly to form a complex, the enzyme substrate complex 'ES'. The complex then breaks down relatively, slowly to form the product of the reaction. The enzyme regenerated can involve in another round of catalysis.



.4 Effect of activators

Ions, like Mg^{2+} , Cu^{2+} , Mn^{2+} , Zn^{2+} and monovalent ions such as Na^{+} and K^{+} are required for the activity of many enzymes. For example, amylases need Cl^{-} ions, Zn^{2+} ions are required for carbonic anhydrase action, Fe^{2+} and Cu^{2+} ions are required for enzymes involved in redox reactions. Several peptidases are activated by Mn^{2+}, Zn^{2+} or Co^{2+}. Enzymes requiring metal ions or enzymes which contain metal ions in their structure are called as metalloenzymes.

.5 Effect of concentration of enzyme

The velocity of an enzymatic reaction is directly proportional to the concentration of enzyme. In case the enzyme concentration is doubled then as much as twice active site become available to combine with the substrate, provided an excess of substrate is present and so the maximum velocity is also doubled. At a fixed concentration of the substrate a level is reached when all the substrate molecules are utilized and no more change in velocity of the reaction takes place (Fig. 5.4).

Enzyme Kinetics

For all enzymatic processes the rate of the reaction depends upon the concentration of the enzyme and its substrates, other conditions like temperature and pH being constant. Figure 6.1 shows the relationship between the substrate and product concentrations.

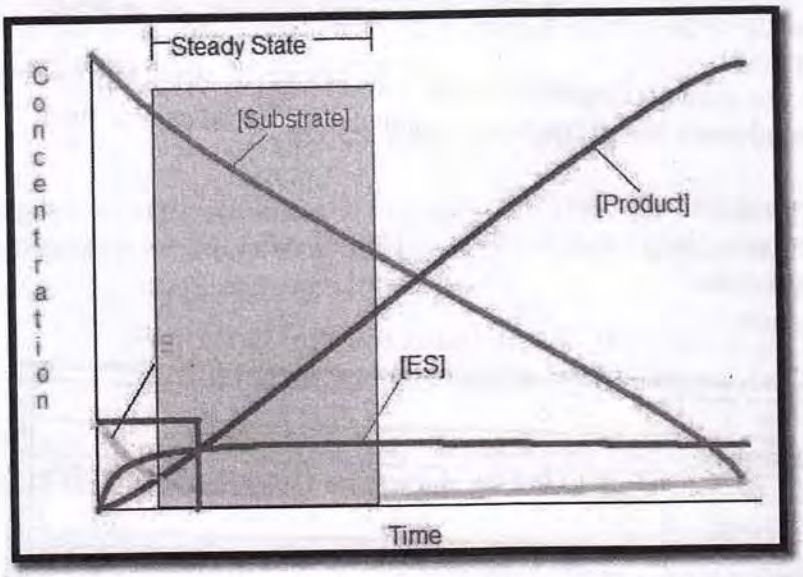


Fig .6.1 Relationship between substrate and product concentrations.

Although the velocity increases linearly with enzyme concentration, at constant enzyme concentration it increases hyperbolically as the substrate concentration increases. This indicates that the enzyme has a definite number of sites to combine with substrate. When all sites are occupied, no further rate enhancement occurs and the enzyme is saturated with the substrate.

Derivation of M - M Equation

Leonor Michaelis and Mand L. Menton in 1913 proposed a successful explanation for the effect of substrate concentration on the enzyme activity.
According to them the enzyme E, and the substrate S combines rapidly to form a complex, the enzyme substrate complex ES. This complex then breaks down relatively and slowly to form the product P of the reaction .These sequence of reactions can be represented in the following equations.

$$E + S \underset{K_2}{\overset{K_1}{\rightleftharpoons}} ES \qquad \text{Step1}$$

$$ES \underset{K_4}{\overset{K_3}{\rightleftharpoons}} E + P \qquad \text{Step2}$$

Step 1: K_1 & K2 are the rate constants of the forward and backward reactions.

Step 2: K3 & k4 are the rate constants of the forward and backward reactions respectively.

This is true only for the enzyme reactions which fulfill the following conditions:
a. Only a single substrate and a single product are involved.
b. The reaction proceeds essentially to completion.

c. The concentration of the substrate is much greater than that of the enzyme in the system.
d. An intermediate enzyme substrate complex is formed.
e. The rate of decomposition of the substrate is proportional to the concentration of the enzyme substrate complex.

It is assumed that the concentration of S is much greater than that of E and that only initial velocities are measured, where only a small fraction of S has been converted. Under these conditions, concentration of:

P------ ES can be ignored.

Applying law of mass action to the first step of the reaction in which K_1 and K_2 are the rate constants for the forward and backward reaction respectively:

The rate of forward reaction = K_1 [E] [S]..... (1)
The rate of backward reaction = K_2 [ES]..... (2)

Applying law of mass action to the second step of the reaction in which k3 and k4are the rate constants for the forward and backward reaction respectively,

The rate of forward reaction = K_3 [ES]..... (3)

The rate of backward reaction can be neglected.The total enzyme in the system can be represented as:

[E_t] = [E] + [ES]..... (4)

Where [E] is the uncombined free enzyme concentration, [ES] the enzyme substrate concentration and [E_t] the total enzyme concentration.
The velocity of the overall reaction is:

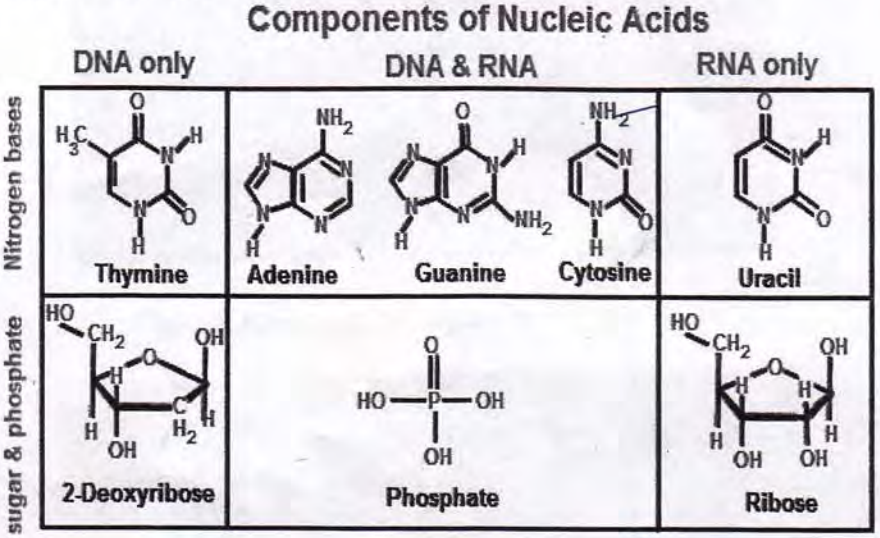
V = K_3 [ES]..... (5)

This is the actual rate equation for the overall reaction but it is not useful since neither K_3 nor [ES] can be measured directly. It is assumed that the reaction proceeds at steady state where the rate of formation of [ES] equals to the rate of degradation of [ES]. The rate of formation of ES, V_f is proportional to E and S as in any second order reaction.

NUCLEIC ACIDS

1 Introduction

Nucleic acids are colorless, complex, amorphous compounds made up of three units: purine and pyrimidine bases, sugar and phosphoric acid. The nucleic acids are of two types DNA and RNA. It is very important to know the structure of nucleic acids and their components.



Nucleic acids

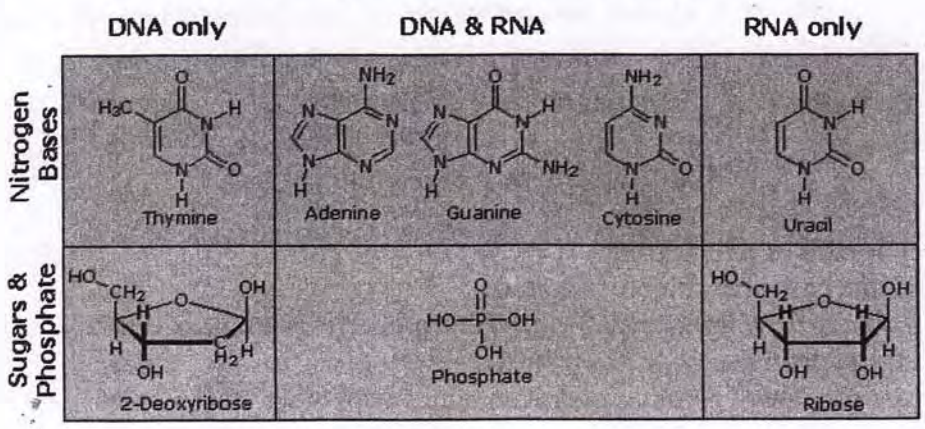
Two types of nucleic acids are present in all mammalian cells.

They are DNA - deoxy ribonucleic acid and RNA- ribonucleic acid.

DNA is present in the nucleus and mitochondria. RNA is present in the nucleus, ribosome and cytoplasm.

Nucleic acids are acidic substances containing nitrogenous bases, pentose sugar and phosphoric acid. Both DNA and RNA are polynucleotides. They are polymers of mononucleotides.

In nucleic acids, nucleotides are joined together by phosphodiester linkages.



Nucleosides

A nucleoside is composed of purine or pyrimidine base and a pentose sugar. Two types of pentose sugar are present in nucleoside, they are ribose and deoxy ribose (Fig. .3). In the case of purine nucleosides, the sugar is attached to N-9 of the purine ring, whereas in pyrimidine nucleosides, the sugar is attached to N-1 of the pyrimidine ring. The type of linkage is N-glycosidic linkage (Fig. .4).

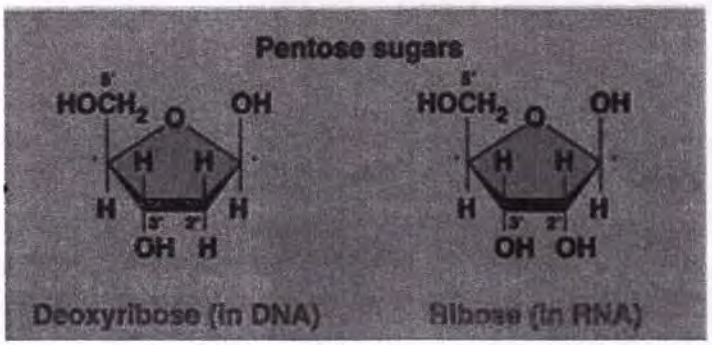


Fig. 3 Structure of sugars in nucleic acids

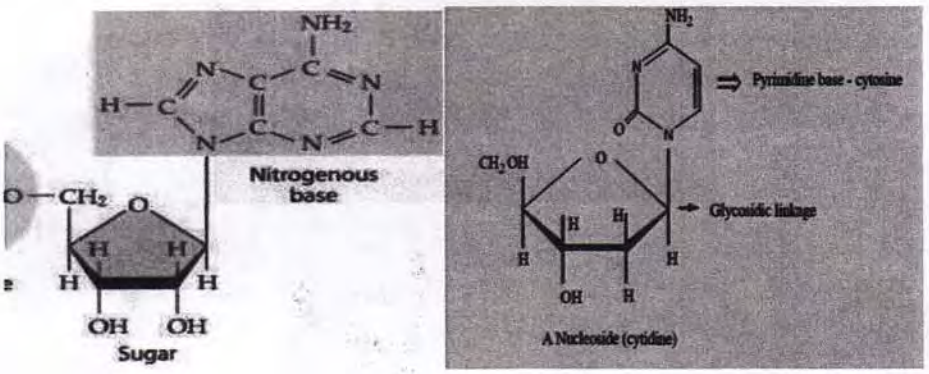
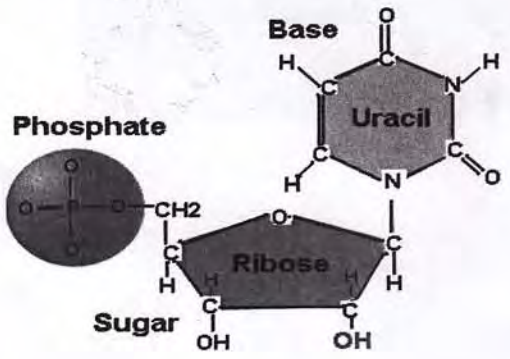


Fig. 4 Structure of a nucleoside

Nucleotides

Nucleotides are phosphorylated nucleosides usually one or two of hydroxyl groups of ribose (or) deoxyribose are phosphorylated. Thus a nucleotide has three structural components. They are nitrogenous base, sugar and phosphate. Phosphate is attached to ribose (or) deoxy ribose through an ester linkage (Fig. .5).



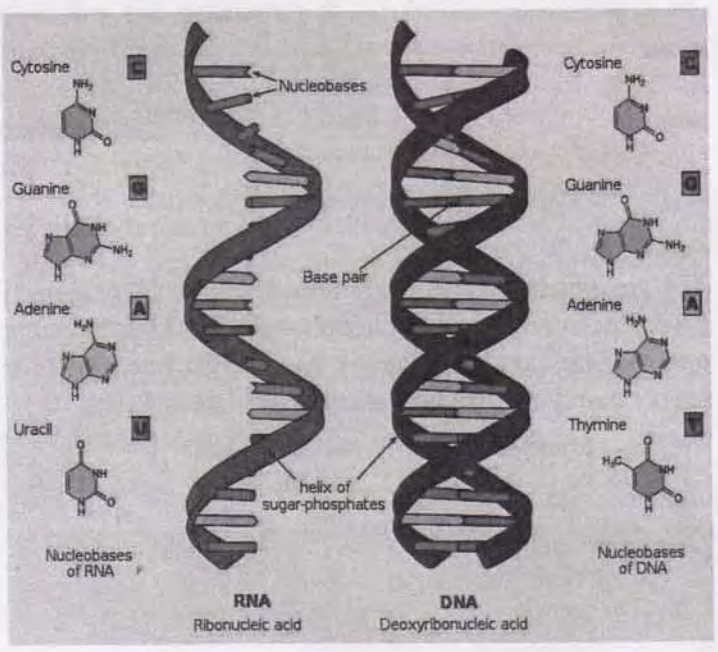
Ncleotide

Fig. .5 Structure of a nucleotide.

Structure of DNA

.1 Primary structure

Nucleotide sequence of a nucleic acid is known as its primary structure which confers individuality to the polynucleotide chain. Polynucleotide chain has direction. They are represented in 5'---> 3' and 3'----> directions. Each polynucleotide chain has 2 ends. The 5' end carrying a phosphate group and 3' end carrying an unreacted hydroxyl group (Fig 7.6).



Functions of DNA

1. DNA is the genetic material of living organisms. It is the greatest super chip ever made by man.
2. DNA contain all the information required for the information of an individual organism.
3. The genetic information in DNA is converted to characteristic features of living organisms like color of the skin and eye, height, intelligence, ability to metabolize particular substance, ability to withstand stress, susceptibility to disease and ability to produce or synthesize certain substances.
4. DNA is the source of information for the synthesis of all cellular proteins. The segment of DNA that contain information for a protein is known as gene.
5. DNA is transmitted from parents to off springs and hence transmit genetic information from one generation to another.
6. The amount of DNA in any given species or cell is constant and is not affected by nutritional and metabolic states.

Structure of RNA

RNAs are present in the nucleus, ribosomes and cytoplasm of eukaryotic cells. They are involved in the transfer and expression of genetic information. They act as primer for DNA formation. Some act as enzymes and as coenzymes. RNA also function as genetic material for viruses.

RNAs are also polynucleotides. In RNA polymer, purine and pyrimidine nucleotides are linked together through phosphodiester linkages. The sugar present is ribose. The nitrogenous bases present in RNA are adenine and guanine (purine bases), uracil and cytosine pyrimidine bases). The nucleotides present in RNA are adenylic acid,guanidylic acid, cytidylic acid and uridylic acid.

Purine → A, G

Pyrimidine → U, C