BIOMOLECULE-II

NUCLEIC ACIDS

The other type of macromolecule that one would find in the acid insoluble fraction of any living tissue is the nucleic acid. These are polynucleotides. Together with polysaccharides and polypeptides these comprise the true macromolecular fraction of any living tissue or cell. For nucleic acids, the building block is a nucleotide. A nucleotide has three chemically distinct components. One is a heterocyclic compound, the second is a monosaccharide and the third a phosphoric acid or phosphate.

It is of two types (A) DNA (B) RNA

A. DNA (Deoxyribonucleic Acid)

Term was given by Zacharis. It is found in the cells of all living organisms except plant viruses, where RNA forms the genetic material and DNA is absent. In bacteriophages and viruses there is a single molecule of DNA, which remains coiled and is enclosed in the protein coat. In bacteria, mitochondria, plastids and other prokaryotes, DNA is circular and lies naked in the cytoplasm. In eukaryotes, it is found in nucleus and known as carrier of genetic information and capable of self-replication.

1. Chemical Composition

The chemical analysis has shown that DNA is composed of three different types of compounds.

i. Sugar molecule

Levene identified a five carbon sugar in nucleic acid in 1910. It is represented by a pentose sugar, the deoxyribose or 2-deoxyribose which is derived from ribose due to the deletion of oxygen from the second carbon.

ii. Phosphoric acid: H_3PO_4 that makes DNA acidic in nature.

iii. Nitrogenous base

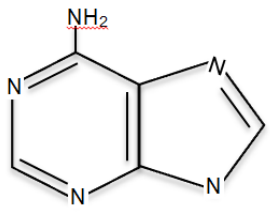
Kossel demonstrated the presence of two pyrimidines (cytosine and thymine) and two purines (adenine and guanine) in DNA and he was awarded Nobel Prize in 1910. These are nitrogen containing ring compounds**,** which are classified into two groups:

a. Purines: Two ring compounds namely Adenine and Guanine.

b. Pyrimidine: One ring compounds and include Cytosine and Thymine. In RNA, Uracil is present instead of Thymine.

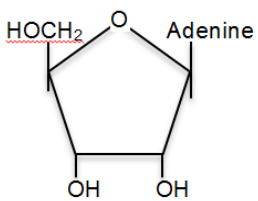
Nucleosides: Nucleosides are formed by a purine or pyrimidine nitrogenous base and pentose sugar. DNA nucleosides are known as deoxyribose nucleosides.

Nucleotides: In a nucleotide, purine or pyrimidine nitrogenous base is joined by deoxyribose pentose sugar (D), which is further linked with phosphate (P) group to form nucleotides.



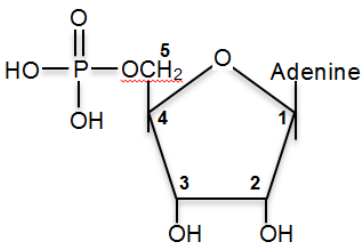
Adenine (Purine)

Nitrogenous base



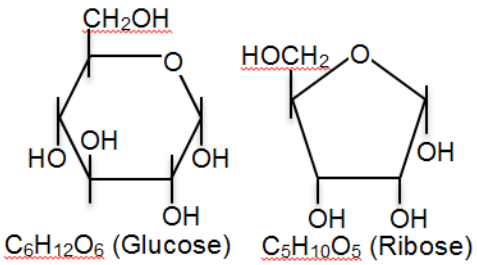
Adenosine

Nucleoside



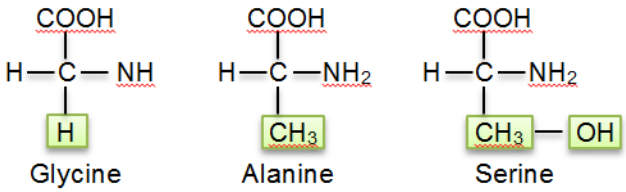
Adenylic acid

Nucleotide



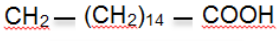
$C_6H_{12}O_6$ (Glucose) $C_5H_{10}O_5$ (Ribose)

Sugar (Carbohydrates)

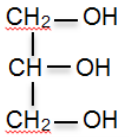


Glycine Alanine Serine

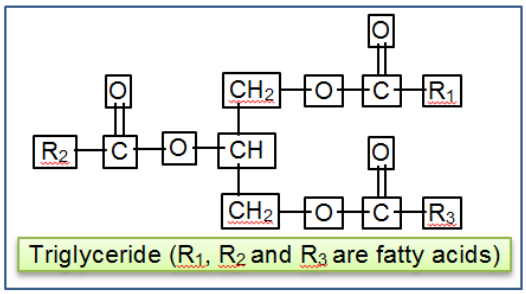
Amino Acids



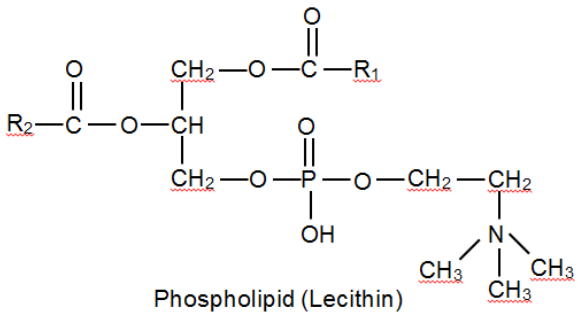
Fatty acid (Palmitic Acid)



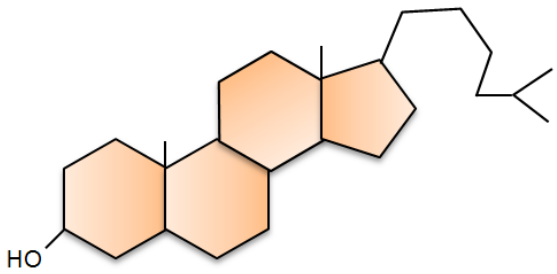
Glycerol



Triglyceride (R_1, R_2 and R_3 are fatty acids)

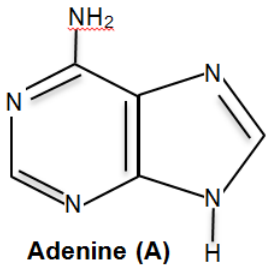


Phospholipid (Lecithin)

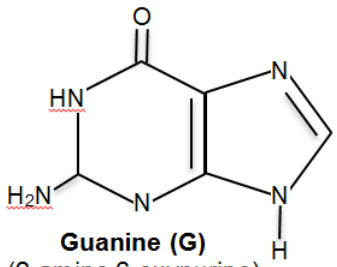


Cholesterol

Fats and Oils (Lipids)



Adenine (A)
(6-aminopurine)



Guanine (G)
(2-amino 6-oxypurine)

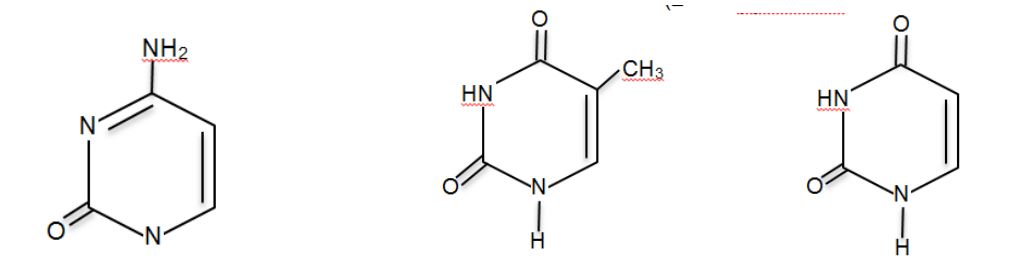


Figure: Structures of major purines (A, G) and pyrimidines (C, T, U) found in nucleic acids

2. Watson and Crick's model of DNA

In 1953 James Watson and Francis Crick suggested that in a DNA molecule there are two polynucleotide chains arranged antiparallal or in opposite directions *i.e.*, one polynucleotide chain runs in 5' to 3' direction, the other in 3'to 5' direction. It means the 3' end of one chain lies beside the 5' end of other in right handed manner.

Important features

Nucleic acids exhibit a wide variety of secondary structures. For example, one of the secondary structures exhibited by DNA is the famous Watson -Crick model. This model says that DNA exists as a double helix. The double helix comprises of two polynucleotide chains. The two strands (polynucleotide chains) of double helix are anti-parallel due to phosphodiester bond.

Each polynucleotide chain has a sugar-phosphate 'backbone' with nitrogenous bases directed inside the helix.

The nitrogenous bases of two antiparallel polynucleotide strands are linked through hydrogen bonds. There are two hydrogen bonds between A and T, and three between G and C. The hydrogen bonds are the only attractive forces between the two polynucleotides of double helix. These serve to hold the structure together.

The two polynucleotides in a double helix are complementary. The sequence of nitrogenous bases in one determines the sequence of the nitrogenous bases in the other. Complementary base pairing is of fundamental importance in molecular genetics.

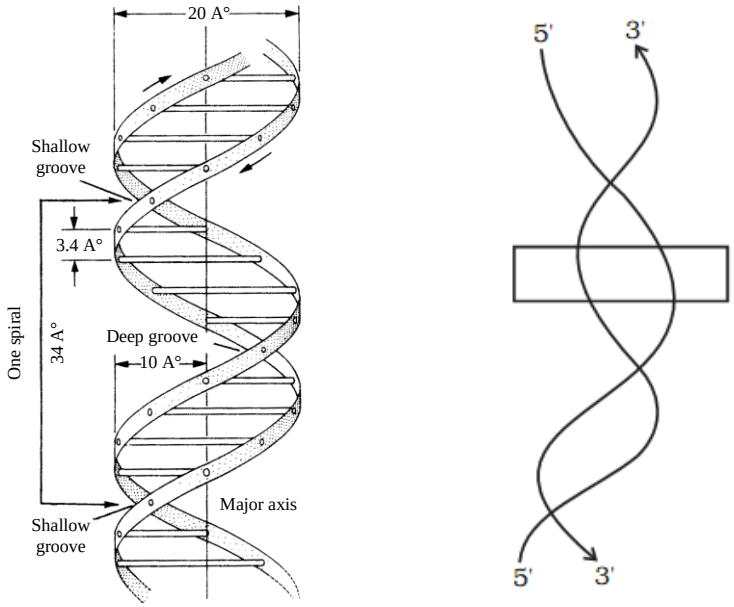


Fig. Double helical structure of DNA

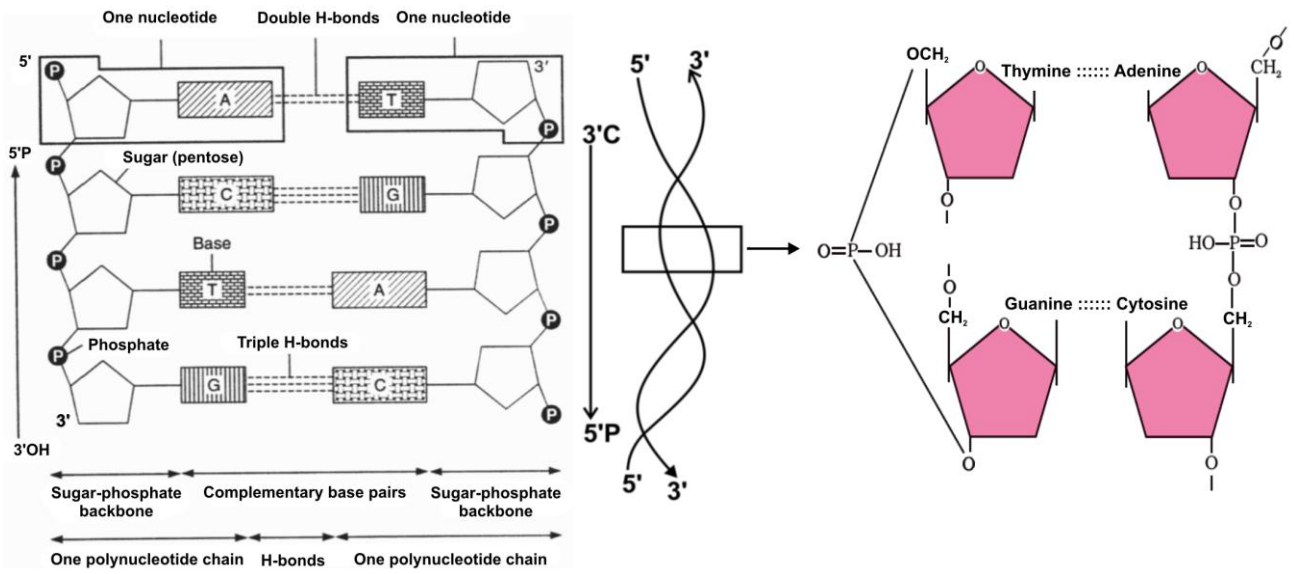


Figure: Diagrammatic representation of Watson and Crick's model of DNA

- **Erwin Chargaff** (1950) made quantitative analysis of DNA and proposed "base equivalence rule" stating that molar concentration of A = T & G = C or $\frac{A+G}{C+T}=1$ irrespective of species and $\frac{A+T}{G+C}$ is constant for a species. Sugar deoxyribose and phosphate occur in equimolar proportion.
- Ten base pairs occur per turn of helix (abbreviated 10bp). The distance between adjacent base pairs is approximately 3.4 Å. The helix is 20 Å (19.8 Å) in diameter.

B. RNA (Ribonucleic Acid)

I. Structure of RNA

More commonly RNA is a single stranded structure consisting of an unbranched polynucleotide chain, but it is often folded back on itself forming helices. DNA is a double stranded structure and its two polynucleotide chains are bounded spirally around a main axis. RNA is made up of

i. Sugar: Ribose **ii. Phosphoric acid:** (H_3PO_4)

iii. Nitrogenous bases are two types:

a. Purines **b.** Pyrimidines

a. Purines are further divided into Adenine and Guanine.

b. Pyrimidines are divided into Cytosine and Uracil.

II. Types of RNA

i. Genetic RNA

Established by Conrat. In most of the plant viruses, some animal viruses and in many bacteriophages, DNA is not found and RNA acts as hereditary material. This RNA may be single stranded or double stranded.

ii. Nongenetic RNA

In the all other organisms where DNA is the hereditary material, different types of RNA are nongenetic. The nongenetic RNA is synthesized from DNA template.

In general, three types of RNAs have been distinguished.

a. Messenger RNA (mRNA)

Name mRNA was given by Jacob and Monod (1961) on the basis of his informative work.

It forms 5% of total RNA.

It is complementary strand to DNA template and carries genetic information to the cytoplasm for the synthesis of proteins.

It acts as a template for protein synthesis and has a short life span.

b. Ribosomal RNA (rRNA)

rRNA constitutes upto 80% of total RNA of the cell. It occurs in ribosomes, which are nucleoprotein molecules. Inside the ribosomes of eukaryotic cells rRNA occurs in the form of the particles of four different dimensions. These are designated 28S, 18S, 5.8S and 5S. The 28S, 5.8 S and 5S molecules occur in large subunit (60S subunit) of ribosome, whereas 18S molecule is present in the small subunit (40S subunit) of ribosome.

In prokaryotic cells only 23S, 16S and 5S rRNA are found which are synthesized in Nucleolus / SAT region.

c. Transfer RNA (tRNA)

It is about 10-15% of RNA of the cell.

tRNA molecules have been variously termed as soluble RNA or supernatant RNA or adapter RNA.

tRNA molecules are smallest, containing 75 to 80 nucleotides.

The transfer RNA is a family of about 60 small sized ribonucleic acids which can recognize the codons of mRNA and exhibit high affinity for 20 activated amino acids.

METABOLISM

Dynamic state of body constituents – Concept of metabolism

Basically, living organisms, like a simple bacterial cell, a protozoan, a plant or an animal, contain thousands of organic compounds.

These compounds or biomolecules are present in certain concentrations (expressed as mole/cell or mole/litre etc.).

All these biomolecules have a **turn over**. This means that they are constantly being changed into some other biomolecules and also made from some other biomolecules. This breaking and making is through chemical reactions constantly occuring in living organisms. Together all these chemical reactions are called metabolism.

Each of the metabolic reactions result in the transformation of biomolecules. A few examples for such metabolic transformations are: removal of CO_2 from amino acids making an amino acid into an amine, removal of amino group in a nucleotide base; hydrolysis of a glycosidic bond in a disaccharide, etc. In other words, metabolites are converted into each other in a series of linked reactions called metabolic pathways. These metabolic pathways are similar to the automobile traffic in a city. These pathways are either linear or circular.

These pathways criss- cross each other, i.e., there are traffic junctions. Flow of metabolites through metabolic pathway has a definite rate and direction like automobile traffic.

This metabolite flow is called the dynamic state of body constituents. Most important is that this interlinked metabolic traffic is very smooth and without a single reported mishap for healthy conditions.

Another feature of these metabolic reactions is that every chemical reaction is a catalysed reaction. There is no uncatalysed metabolic conversion in living systems. Even CO_2 dissolving in water, a physical process, is a catalysed reaction in living systems.

The catalysts which hasten the rate of a given metabolic conversion are also proteins. These proteins with catalytic power are named enzymes.

Metabolic basis for living

Metabolic pathways can lead to a more complex structure from a simpler structure (for example, acetic acid becomes cholesterol) or lead to a simpler structure from a complex structure (for example, glucose becomes lactic acid in our skeletal muscle).

The former cases are called biosynthetic pathways or **anabolic** pathways. The latter constitute degradation and hence are called catabolic pathways.

Anabolic pathways, as expected, consume energy. Assembly of a protein from amino acids requires energy input.

On the other hand, **catabolic** pathways lead to the release of energy. For example, when glucose is degraded to lactic acid in our skeletal muscle, energy is liberated. This metabolic pathway from glucose to lactic acid which occurs in 10 metabolic steps is called glycolysis.

Living organisms have learnt to trap this energy liberated during degradation and store it in the form of chemical bonds. As and when needed, this bond energy is utilised for biosynthetic, osmotic and mechanical work that we perform.

The most important form of energy currency in living systems is the bond energy in a chemical called adenosine triphosphate (ATP).

The living state

The most important fact of biological systems is that all living organisms exist in a steady-state characterised by specific concentrations of each of these biomolecules. These biomolecules are in a metabolic flux.

Any chemical or physical process moves spontaneously to equilibrium.

The steady state is a non-equilibrium state. One should remember from physics that systems at equilibrium cannot perform work. As living organisms work continuously, they cannot afford to reach equilibrium. Hence the living state is a non-equilibrium steady-state to be able to perform work. Living process is a constant effort to prevent falling into equilibrium. This is achieved by energy input.

Metabolism provides a mechanism for the production of energy. Hence the living state and metabolism are synonymous. Without metabolism there cannot be a living state.

ENZYMES

History of cellular enzymes

Enzymes (Gk. *en* = in; *zyme* = yeast) are proteinaceous substances which are capable of catalysing chemical reactions of biological origin without themselves undergoing any change.

Enzymes are **biocatalysts**. An enzyme may be defined as “a biomolecule that enhances the rate of biochemical reactions but does not affect the nature of final product”. They are produced by living cells only.

Enzymes are also called ‘biological middle man’.

The study of the composition and function of the enzyme is known as **enzymology**.

The term enzyme (meaning-in yeast) was used by Willy Kuhne (1878), while working on fermentation. At that time living cells of yeast were thought to be essential for fermentation of sugar.

Edward Buchner (1897), a German chemist proved that extract zymase, obtained from yeast cells, has the power of fermenting sugar (alcoholic fermentation).

There are some nucleic acids that behave like enzymes. These are called ribozymes. **E.g., RNA enzymes (RNase).**

Northrop and Kunitz prepared crystals of pepsin, trypsin and chymotrypsin.

Arber and Nathans got noble prize in 1978 for the discovery of restriction endonucleases which break both strands of DNA at specific sites and produce sticky ends. These enzymes are used as microscissors in genetic engineering.

Resonate the Concept

Zymogen: These are precursor of enzymes known as zymogens.
Examples

1. Pepsinogen → Pepsin
2. Prorennin → Rennin
3. Trypsinogen → Trypsin
4. Chymotrypsinogen → Chymotrypsin

Types of enzymes

Two types of enzymes are

i. Simple enzyme-Made up of only protein. E.g., Urease, Amylase, Trypsin, Pepsin
ii. Conjugated enzyme- Made up of protein and nonprotein part. Non protein part is called cofactor Protein part is called Apoenzyme. Both together called as Holoenzyme.

Holoenzyme = Apoenzyme + co-factor

Activity of enzyme is due to co-factor, which can be separated by dialysis. Co-factor is small, heat stable and may be organic or inorganic in nature.

Three types of cofactors may be identified. Prosthetic group, coenzyme and metal ions.

i. **Prosthetic group (Non-dialyzable)**

Prosthetic groups are organic compounds distinguished from other cofactors in that they are permanently bound to the apoenzyme, *e.g.*, in peroxisomal enzymes peroxidase and catalase which catalyzes breakdown of hydrogen peroxide to water and oxygen, haem is the prosthetic group and it is a part of the active site of the enzyme.

ii. **Coenzymes (Dialyzable)**

Lipmann discovered coenzymes. Coenzymes are also organic compounds but their association with the apoenzyme is transient, usually occurring only during the course of catalysis. In general coenzymes not only assist enzymes in the cleavage of the substrate but also serve as temporary acceptor for one of the product of the reaction. The essential chemical component of many coenzymes are vitamins, *e.g.,* coenzyme nicotinamide adenine dinucleotide (NAD), nicotinamide adenine dinucleotide phosphate (NADP) contains the vitamin niacin.

iii. Metal ions

A number of enzymes require metal ions for their activity. The metal ions form coordination bonds with specific side chains at the active site and at the same time form one or more coordination bonds with the substrate. The latter assist in the polarization of substrate bonds to be cleaved by the enzyme. The common metal ions are Zn^{++}, Cu^{++}, Mg^{++}. Inorganic part of enzyme acts as prosthetic group in few enzymes which are called activators. These activators are generally metals. Hence these enzymes are called Metalloenzymes such as

Enzymes Activators	
Activators	**Enzymes**
Iron (Fe)	Acotinase, Catalase and Cytochrome oxidase
Zinc (Zn)	Dehydrogenase, Carbonic anhydrase
Copper (Cu)	Tyrosinase, Cytochrome oxidase
Magnesium (Mg)	Hexokinase, Phosphotransferase
Manganese(Mn)	Peptidase, Decarboxylase
Molybdenum (Mo)	Nitrate reductase
Nickel (Ni)	Urease
Boron (B)	Enolase

Classification and Nomenclature of enzymes

Thousands of enzymes have been discovered, isolated and studied. Most of these enzymes have been classified into different groups based on the type of reactions they catalyse.

Enzymes are divided into 6 classes each with 4-13 subclasses and named accordingly by a four-digit number.

The first digit denotes the class, the second sub-class, the third sub-sub-class and the fourth one is for the particular enzyme name. Thus, E.C. 2.7.1.1 denotes class 2 (Transferases)-subclass 7 (transfer of phosphate) sub-sub-class 1 (an alcohol functions as phosphate acceptor). The 4th digit indicates hexokinase.

i. Oxidoreductases/dehydrogenases

These enzymes catalyse **oxidation reduction** reactions, usually involving the transfer of hydrogen atoms or ions from one molecule to another. Eg., Alcohol dehydrogenase, cytochrome oxidase

$$S \text{ reduced} + S' \text{ oxidised} \longrightarrow S \text{ oxidised} + S' \text{ reduced}.$$

ii. Transferases

These enzyme catalyse the transfer of a specific group (*e.g.,* amino, methyl, acyl, phosphate) from one kind of molecule to another. **e.g.:** $S - G + S' \longrightarrow S + S' - G$

Ex. Phosphotransferase, Peptidyl transferase etc.

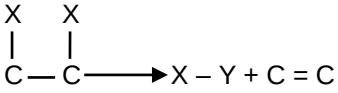
iii. Hydrolases

These enzyme catalyse the hydrolysis of organic foods *i.e.*, the breakdown of large molecules by addition of water. Most of the hydrolysing (digestive) enzymes are located in lysosomes. *e.g.*, all digestive enzymes such as lipases (digest the stored food material of castor seeds).

iv. Lyases :

These enzymes catalyse the breakage of specific covalent bonds and removal of groups without hydrolysis *e.g.,* fumerases, carboxylases, aminases, histidine decarboxylase

(splits *C–C*-bond of histidine, forming CO_2 and histamine).



v. Isomerases

These enzymes catalyse the rearrangement of molecular structure to form isomers. *e.g.*, phosphohexose isomerase (phosphoglucomutase) acts on glucose 6-phosphate to form fructose 6-phosphate (both C_6 compounds)

vi. Ligases or Synthetases

These enzymes form bonds and join two molecules together, using energy supplied from the breakdown of ATP, *e.g.*, DNA ligase is used to repair breaks in DNA molecules.

OLYMPIAD AND AIIMS CORNER

NOMENCLATURE AND CLASSIFICATION

Dauclax (1883), introduced the nomenclature of enzymes. Usually enzyme names end in suffix**-ase** to the name of substrate *e.g.*, Lactase acts on lactose, maltase act on maltose, amylase on amylose, sucrase on sucrose, protease on proteins, lipase on lipids and cellulase on cellulose. Sometimes arbitrary names are also popular *e.g.*, Pepsin, Trypsin and Ptylin etc. Few names have been assigned as the basis of the source from which they are extracted *e.g.*, Papain from papaya, bromelain from pineapple (family Bromeliaceae).

Enzymes can also be named by adding suffix–ase to the nature of chemical reaction also *e.g.*, Oxidase, dehydrogenase, catalase, DNA polymerase.

Modern names are given after chemical action. They are more systematic, informative but slightly longer. *e.g.*, ATP : D-glucose phosphotransferase.

Common simpler names used at the place of systematic names called **trivial names**.

Older classification

The older classification of enzymes is based on the basis of reactions which they catalyse. Many earlier authors have classified enzymes into two groups

1. Hydrolysing enzyme

The hydrolysing enzymes of hydrolases catalyse reactions in which complex organic compounds are broken into simpler compounds with the addition of water. Depending upon the substrate hydrolysing enzymes are :

Carbohydrases

Most of the polysaccharides, disaccharides or small oligosaccharides are hydrolysed to simpler compounds. e.g., Lactase on lactose to form glucose and galactose, sucrase/invertase on sucrose to form glucose and fructose, amylase or diastase on starch to form maltose, maltase on maltose to form glucose, cellulase on cellulose to produce glucose.

Esterases

These enzymes catalyse the hydrolysis of substances containing ester linkage, *e.g*., fat, pectin, etc. into an alcoholic and an acidic compound.

Proteolytic enzymes

The hydrolysis of proteins into peptones, polypeptides and amino acids is catalysed by these enzymes

Amidases

They hydrolyse amides into ammonia and acids.

2. Desmolysing enzymes

Most of the desmolysing enzymes are the enzymes of respiration *e.g.,* oxidases, dehydrogenases, (concerned with transfer of electrons), transaminases, carboxylases etc.

Mechanism of Enzyme Action

Energy is required to bring the inert molecules into the activated state. The amount of energy required to raise the energy of molecules at which chemical reaction can occur is called **activation energy.** Enzymes act by decreasing the activation energy so that the number of activated molecules is increased at lower energy levels. If the activation energy required for the formation of the enzyme-substrate complex is low, many more molecules can participate in the reaction than would be the case if the enzyme were absent.

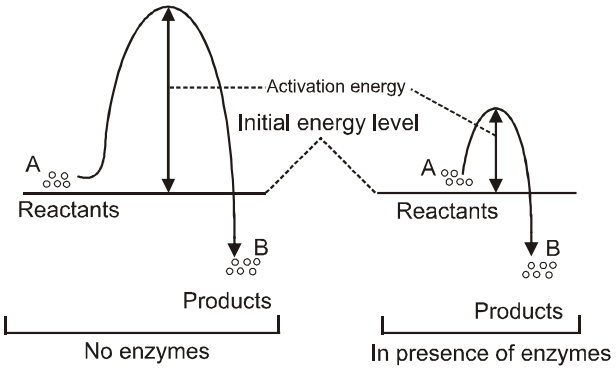
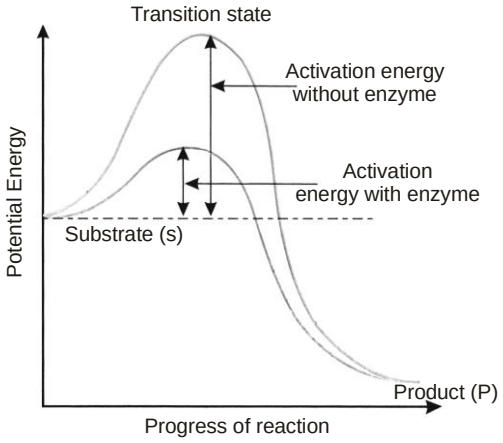


Fig : Showing that activation energy of an enzyme catalysed reaction is lower than that of an uncatalysed reaction



Concept of activation energy

Nature of Enzyme Action

Each enzyme (E)has a substrate (S)binding site in its molecule so that a highly reactive enzyme-substrate complex (ES) is produced. This complex is short-lived and dissociates into its product (P) and the unchanged enzyme with an intermediate formation of the enzyme-product complex (EP). The formation of the ES complex is essential for catalysis.

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

The catalytic cycle of an enzyme action can be described in the following steps:

1. First, the substrate binds to the active site of the enzyme, fitting into the active site.
2. The binding of the substrate induces the enzyme to alter its shape, fitting more tightly around the substrate.
3. The active site of the enzyme, now in close proximity of the substrate breaks the chemical bonds of the substrate and the new enzyme- product complex is formed.
4. The enzyme releases the products of the reaction and the free enzyme is ready to bind to another molecule of the substrate and run through the catalytic cycle once again.

There are two theories to explain the mode of action of enzymes.

LOCK AND KEY HYPOTHESIS

The hypothesis was put forward by Emil Fisher (1894). According to this hypothesis the enzyme and its substrate have a complementary shape. The specific substrate molecules are bound to a specific site of the enzyme molecule.

The theory can be explained easily by the fact that a particular lock can be opened by a particular key specially designed to open it. Similarly enzymes have specific sites where only a particular substrate can be attached. The lock and key model accounts for enzyme specificity.

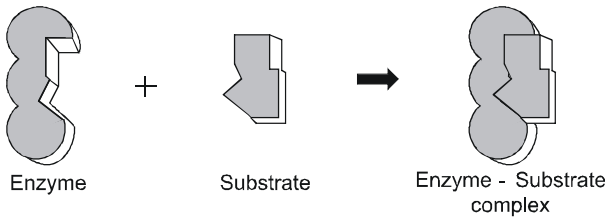


Fig : Lock and key model of enzyme action

Induced fit hypothesis: This hypothesis was proposed by Daniel E. Koshland (1959).

According to this view, active site is not rigid but static and it has two groups– buttressing group and catalytic group. Initially substrate bind to the buttressing group which induces the catalytic group to fit the substrate and catalytic group weakenes the bonds of reactant or substrate by electrophilic and nucleophilic forces.

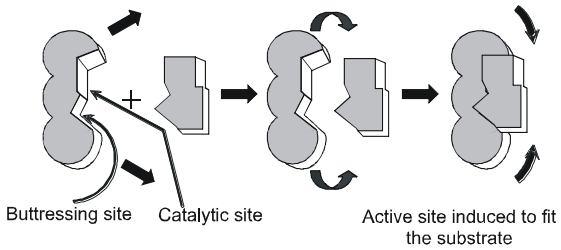


Fig : Induced fit model of enzyme action

OLYMPIAD AND AIIMS CORNER

Site of Enzyme Action

All enzymes are produced in the living cells. About 3,000 enzymes have been recorded. These are of two types with regard to the site where they act.

Intracellular Enzymes

Most of the enzymes remain and function inside the cells, they are called the intracellular enzymes or endoenzymes. Some of these enzymes are found in cytoplasmic matrix. Certain enzymes are bound to ribosomes, mitochondria and chloroplast etc.

Extracellular enzymes

Certain enzymes leave the cells and function outside them. They are called the extracellular enzymes or exoenzymes. They mainly include the digestive enzymes. *e.g.*, salivary amylase, gastric pepsin, lysozyme present in tears and nasal secretion.

Rennet tablets with enzyme rennin from calf's stomach are widely used to coagulate protein caseinogen for cheese (casein) formation.

Properties of enzymes

1. Chemical reactions

Chemical compounds undergo two types of changes. A physical change simply refers to a change in shape without breaking of bonds. This is a physical process. Another physical process is a change in state of matter when ice melts into water, or when water becomes a vapour. These are physical processes. However, when bonds are broken and new bonds are formed during transformation, this will be called a chemical reaction. For example

$$Ba(OH)_2 + H_2SO_4 \rightarrow BaSO_4 + 2H_2O$$

is an inorganic chemical reaction. Similarly, hydrolysis of starch into glucose is an organic chemical reaction. Rate of a physical or chemical process refers to the amount of product formed per unit time. It can be expressed as rate = $\frac{\delta p}{\delta t}$

Rate can also be called velocity if the direction is specified. Rates of physical and chemical processes are influenced by temperature among other factors, A general rule of thumb is that rate doubles or decreases by half for every 10ºC change in either direction. Catalysed reactions proceed at rates vastly higher than that of uncatalysed ones. When enzyme catalysed reactions are observed, the rate would be vastly higher than the same but uncatalysed reaction. For example.

$$CO_2 + H_2O \; H_2CO_3$$

$$\text{Carbon dioxide water} \xrightleftharpoons{\text{Carbonic anhydrase}} \text{carbonic acid}$$

In the absence of any enzyme this reaction is very slow, with about 200 molecules of H_2CO_3 being formed in an hour. However, by using the enzyme present within the cytoplasm called carbonic anhydrase, the reaction speeds dramatically with about 600,000 molecules being formed every second. The enzyme has accelerated the reaction rate by about 10 million times. The power of enzymes is incredible indeed

There are thousands of types of enzymes each catalysing a unique chemical or metabolic reaction. A multistep chemical reaction, when each of the steps is catalysed by the same enzyme complex or different enzymes, is called a metabolic pathway. For example.

$$\text{Glucose} \rightarrow \text{Pyruvic acid}$$

$$C_6H_{12}O_6 + O_2 \rightarrow 2C_3H_4O_3 + 2H_2O$$

2. Colloidal nature

All enzymes are colloidal in nature and thus provide large surface area for reaction to take place. They posses extremely low rates of diffusion and form colloidal system in water.

3. Catalytic properties

Enzymes are active in extremely small amounts, *e.g.*, one molecule of invertase can effectively hydrolyze 1,000,000 times its own weight of sucrose. One molecule of catalase is able to catalyze conversion of 5,000,000 molecules of hydrogen peroxide.

4. High efficiency

The effectiveness of an enzymatic reaction is expressed in terms of its turn over number or catalytic centre activity, means number of substrate molecules on which one enzyme molecule acts in one minute.

5. Molecular weight

Enzymatic proteins are substances of high molecular weight. Bacterial ferredoxin one of the smaller enzymes has molecular weight of 6,000, where as pyruvic dehydrogenase one of the largest-has a molecular weight of 4600000.

6. Specificity of enzyme

Most of the enzymes are highly specific in their action. A single enzyme will generally catalyze only a single substrate or a group of closely related substrates. The active site possesses a particular binding site which complexes only with specific substrate. Thus, only a suitable substrate fulfils the requirements of active site and closely fixes with it.

7. Reversibility of reaction

The enzyme-controlled reactions are reversible. The enzymes affect only the rate of biochemical reactions, not the direction. *e.g.*, Lipase can catalyse splitting of fat into fatty acids and glycerol as well as synthesis of fatty acids and glycerol into fats.

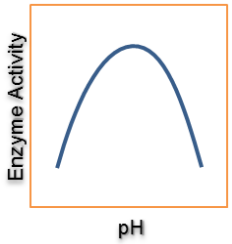
Resonate the Concept

Highest turnover number is of carbonic anhydrase (36 million/min or 600000 per second) and lowest is of lysozymes (30/min or 0.5 per second). So carbonic anhydrase is fastest enzyme and lysozyme is slowest enzyme.

Factors Affecting the Enzyme Activity

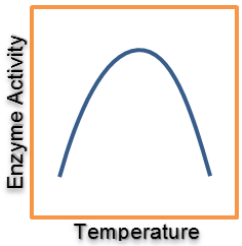
Hydrogen ion concentration (*pH*)

Some enzymes act best in an acid medium, other in an alkaline medium. For every enzyme there is an optimum *pH* where its action is maximum *e.g.*, 2 for pepsin, 6.8 for salivary amylase, 8.5 for trypsin. Most enzyme show maximum activity in a *pH* range of about 6.0 to 7.5 *i.e.*, near neutral *pH* (endoenzymes).



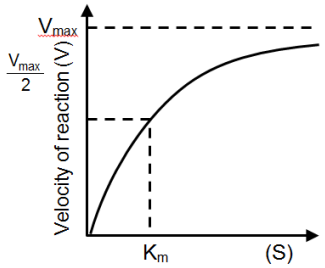
Temperature

Within certain limits (5-40°*C*) the rate of an enzyme catalyzed reaction increases as the temperature increases. The Q_{10} of most enzymatic reactions is 2, *i.e.*, every 10°*C* rise in temperature doubles the rate of reaction. Most enzymes show maximum activity in a temperature range of 25 to 40°*C*. Beyond this temperature, enzymes becomes denatured but the enzymes are not destroyed by freezing, and regain their lost activity if the temperature is raised to normal.



Substrate concentration

If there are more enzyme molecules than substrate molecules, a progressive increase in the substrate molecules increases the velocity of their conversion to products. However, eventually the rate of reaction reaches the maximum. At this stage the active sites of all the available enzyme molecules are occupied by the substrate molecules. Therefore, the substrate molecules occupy the active sites vacated by the products and cannot increase the rate of reaction further.



Effect of concentration of substrate on enzyme activity

Michaelis Constant

Michaelis and Menten (1913) introduced a constant K_m (Michaelis constant). It is a mathematical derivative or constant which indicates the substrate concentration at which the chemical reaction catalysed by an enzyme attains half its maximum velocity (V_{max}). K_m indicates affinity of the enzyme for its substrate. K_m value differs from substrate to substrate because different enzymes differ in their affinity towards different substrates. A high K_m indicates low affinity while a low K_m shows strong affinity. Protease acts on different proteins, so it's K_m value will differ from protein to protein.

Enzyme Inhibition

Enzyme inhibitor is defined as a substance which bind with the enzyme and bring about a decrease in catalytic activity of that enzyme. The inhibitor may be organic or inorganic in nature. There are three broad categories of enzyme inhibition.

1. Reversible inhibition
2. Irreversible inhibition
3. Allosteric inhibition

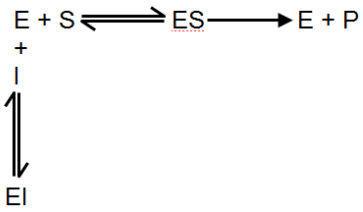
1. Reversible Inhibition

The inhibitor binds non-covalently with enzyme and the enzyme inhibtion can be reversed if the inhibitior is removed. The reversible inhibition is further sub-divided into

- **I.** Competitive inhibition
- **II.** Non-competitive inhibition

I. Competitive inhibition

The inhibitor (I) which closely resembles the real substrate (S) is regarded as a **substrate analogue**. The inhibitor competes with substrate and binds at the active site of the enzyme but does not undergo any catalysis. As long as the competitive inhibitor holds the active site, the enzyme is not available for the substrate to bind. During the reaction, ES and EI complexes are formed as shown below.



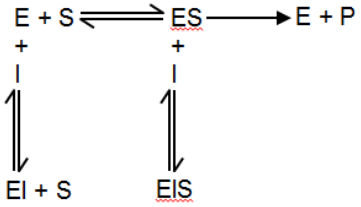
The relative concentration of the substrate and inhibitor and their respective affinity with the enzyme determines the degree of competitive inhibition. The inhibition could be overcome by high substrate concentration. In competitive inhibition, the **K_m value increases** whereas **V_{max}** remains **unchanged**

The enzyme succinate dehydrogenase (SDH) is a classical example of competitive inhibition with succinic acid as its substrate. The compounds, namely, malonic acid, glutaric acid and oxalic acid have structural similarity with succinic acid and compete with the substrate for binding at the active site of SDH.

II. Non-competitive inhibition

The inhibitor binds at a site other than the active site on the enzyme surface. This binding impairs the enzyme function. The inhibitor has no structural resemblance with the substrate. The catalysis is prevented, possibly due to a distortion in the enzyme conformation.

The inhibitor generally binds with the enzyme as well as the ES complex. The overall relation in non-competitive inhibition is represented below.

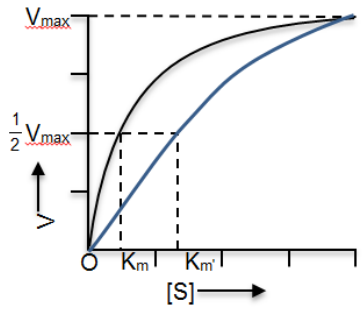


For non-competitive inhibition, the **K_m value is unchanged** while **V_{max} is lowered.**

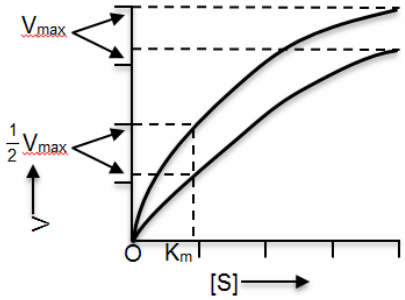
e.g., cyanide inhibits the mitochondrial enzyme cytochrome oxidase which is essential for cellular respiration. This kills the animals.

AMP is a non-competitive inhibitor of fructose biphosphate phosphatase, the enzyme that catalyzes the conversion of fructose 1, 6 biphosphate to fructose 6 phosphate.

Heavy metal ions (Ag^+, Pb^{2+}, Hg^{2+} etc.) can non-competitively inhibit the enzymes by binding with cysteinyl sulfhydryl groups.



Effect of competitive inhibitor on enzyme velocity



Effect of non-competitive inhibitor on enzyme velocity

2. Irreversible inhibition

The inhibitors bind covalently with the enzymes and inactivate them, which is irreversible. These inhibitors are usually toxic substances.

Iodoacetate is an irreversible inhibitor of the enzymes like papain and glyceraldehyde 3-phosphate dehydrogenase. Iodoacetate combines with sulfhydryl (–SH) groups at the active site of these enzymes and makes them inactive.

Diisopropyl fluorophophate (DFP) is a ***nerve gas*** developed by the Germans during Second World War. DFP irreversibly binds with enzymes containing serine at the active site, e.g. ***serine proteases, acetylcholine esterase.***

3. Allosteric inhibition (Modulation)

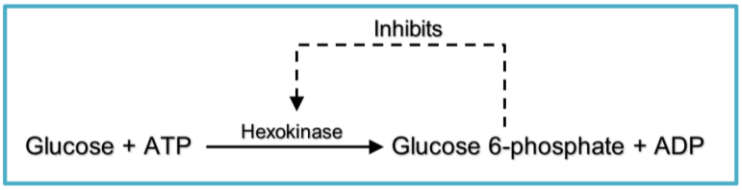
Allosteric literally means 'another place'. Some inhibitors join an enzyme at a specific site and change the form of the active site meant for the substrate.

These inhibitors are known as modifiers or modulators and the sites where they fit in are called allosteric sites. Modulators are of two types-positive (activators) and negative (inhibitors).

Change of active site form prevent the binding of substrate to the enzyme and stops the reaction. The process is called allostery or allosteric inhibition,

The enzyme with allosteric sites are called allosteric enzymes. Jacob and Monod have termed this phenomenon as allosteric transition.

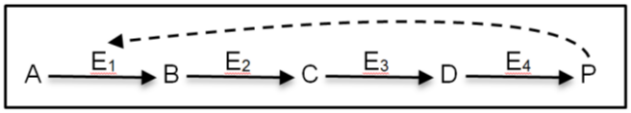
An example of allosteric enzyme inhibition is hexokinase that converts glucose to glucose 6-phosphate. Glucose 6-phosphate causes allosteric inhibition of hexokinase. This is called feedback allosteric inhibition.



Feedback inhibition

Feedback inhibition or ***end product inhibition*** is a specialized type of allosteric inhibition necessary to control metabolic pathways for efficient cellular function.

In number of cases, accumulation of the final product of the reaction is capable of inhibiting the first step of reaction. The product P checks the activity of enzyme which converts A into B. It is quite useful mechanism because it checks the accumulation of products.



The phenomenon in which the end product of a metabolic pathway can regulate its own production by inhibition of the enzymes of its own pathway is called **feed back inhibition** or negative feed back inhibition.

This type of inhibition can be shown in *Escherichia coli* bacterium which synthesises the amino acid isoleucine from a substrate threonine by a series of intermediate reactions (*i.e.*, α ketobutyrate threonine deaminase, α Aceto hydroxy butyrate, α keto β methyl valerate etc).

When isoleucine accumulates in amounts more than required, it stops its own production by inhibiting the activity of the enzyme. Threonine deaminase which catalyzes the first reaction of the series.

This type of metabolic control in which the first enzyme of a series is inhibited by the end product, is known as end product inhibition.

Test your Resonance with concept

1. A ribose (but not deoxyribose) nucleotide is

(1) Cytosine — pentose sugar — phosphate (2) Guanine — pentose sugar — phosphate

(3) Thymine — pentose sugar — phosphate (4) Uracil — pentose sugar — phosphate

2. Which one of the following pairs is not correctly matched?

(1) Recombinant DNA→ DNA formed by the joining of segments of DNA from different sources

(2) Purine →Nitrogenous bases Cytosine, thymine and uracil

(3) ATP→ The principal energy carrying compound in the cell

(4) r-RNA→ RNA molecules found in ribosomes

3. Which of the following is not an attribute of enzymes?

(1) They are proteinaceous in nature (2) They speed up the rate of biochemical reactions

(3) They are specific in nature (4) They are used up in reactions

4. What is correct about enzymes?

(1) They are most active at pH 7.0 (2) They are all amino acids

(3) They are all proteins (4) They are most active at a temperature of 0°C

5. Enzymes are different from inorganic catalysts

(1) Not being used up in reactions (2) Being proteinaceous in nature

(3) Having high diffusion rate (4) Working at high temperature

Answer Key

1. (4) **2.** (2) **3.** (4) **4.** (3) **5.** (2)

Hormones

The word hormone has been derived from a Greek word (*hormõn*) meaning to arouse to activity'. By the classic definition, a hormone is a substance that is synthesised in minute quantities in one tissue and transported by circulatory system to another organ. The tissue or organ where they are produced are called **effectors** and those where they exert their influence are called **targets**. Based on their site of action hormones are of two types: local and general. The local hormones have specific local effects, for example, cholecystokinin. On the other hand, the general hormones are secreted by various endocrine glands and are transported through the blood to cause physiological action at points away from their place of origin, e.g, growth hormones, thyroid hormone, adrenocorticotropin, etc.

The hormones are required in extremely small quantities and perform a variety of regulatory functions ranging from growth, vegetative and sexual development, cellular oxidation to thermal regulation and the metabolism of carbohydrates, proteins and fats. Hormone action at the cellular level begins by its association with specific receptor.

The plant hormones are known as 'phytohormones'. These are organic compounds produced naturally in higher plants and controlling growth or other physiological function either at the site of their origin or far remote from their place of production. Auxins, gibberellins, cytokinins, abscisic acid (ABA), and ethylene are the four major types of hormones found in plants.

Vitamins

These are organic molecules in food that are required in minute quantities for normal metabolism but cannot be synthesised in adequate amounts by humans and animals. A dietary or physiologic deficiency of anyone of them leads to a specific set of disease symptoms that can be corrected by administration of that vitamin alone. The vitamins are synthesised by plants and bacteria. These are classified on the basis of solubility as:

a. **Water soluble vitamins** which include the B-complex group of vitamins, and vitamin C (ascorbic acid). B-complex vitamins are found in whole grain cereals, legumes, leafy green vegetables, meat and dairy products. Citrus fruits are good source of vitamin C.

b. **Fat soluble vitamins** are soluble in fats, e.g. vitamin A, D, E and K. These are present in food fats, yolks, vegetable seed oils, etc. The vitamins function as coenzyme or cofactor and are required in very small quantities for normal metabolism of animals including us.

Additional Points

- Most of the vitamins of B complex group act as coenzyme.
- Myosin is a structural component of muscle. It has *ATPase* activity also.
- Smallest enzyme oxidase and largest enzyme catalase, which are found in peroxysome.
- Synthesis of enzymes occur in polysome (aggregation of ribosomes).
- Competitive inhibitor increases Michaelis constant (K_m) but it has no effect on $V_{max.}$
- Regulators of metabolism are enzymes, vitamins and hormones.
- RNA polymerase enzyme form RNA from DNA and DNA polymerase is responsible for synthesis of DNA from DNA
- Enzyme that catalyses the conversion of soluble proteins into insoluble ones in called coagulase and the process is called enzyme coagulation.
- Albinism is caused by the deficiency of tyrosinase.
- Nitrogenase enzyme is inactivated by oxygen.
- Some enzymes are active at very high temperature (70–80°C) called extremozymes *e.g.*, Taq polymerase.
- In a polypeptide or a protein, amino acids are linked by a peptide bond which is formed when the carboxyl (-COOH) group of one amino acid reacts with the amino ($–NH_2$) group of the next amino acid with the elimination of a water moiety (the process is called dehydration).
- In a polysaccharide the individual monosaccharides are linked by a glycosidic bond. This bond is also formed by dehydration. This bond is formed between two carbon atoms of two adjacent monosaccharides. In a nucleic acid a phosphate moiety links the 3'-carbon of one sugar of one nucleotide to the 5' -carbon of the sugar of the succeeding nucleotide.
- The bond between the phosphate and hydroxyl group of sugar is an ester bond. As there is one such ester bond on either side, it is called phosphodiester bond.
- Nucleic acids exhibit a wide variety of secondary structures.
- For example, one of the secondary structures exhibited by DNA is the famous Watson - Crick model. This model says that DNA exists as a double helix. The two strands of polynucleotides are antiparallel i.e., run in the opposite direction. The backbone is formed by the sugar-phosphate-sugar chain. The nitrogen bases are projected more or less perpendicular to this backbone but face inside.
- A and G of one strand compulsorily base pairs T and C, respectively, on the other strand.
- There are two hydrogen bonds between A and T. There are three hydrogen bonds between G and C.
- Each strand appears like a helical staircase.
- Each step of ascent is represented by a pair of bases. At each step of ascent, the strand turns 36°. One full turn of the helical strand would involve ten steps or ten base pairs.
- The pitch would be 34Å. The rise per base pair would be 3.4A. This form of DNA with the above mentioned salient features is called B-DNA.
- RNA is found in the cytoplasm and nucleolus. Inside the cytoplasm it occurs freely as well as in the ribosomes.
- RNA can also be detected from mitochondria, chloroplasts and associated with the eukaryotic chromosomes. In some plant viruses RNA acts as hereditary material.