COER PAPER – XI PLANT BIOCHEMISTRY

UNIT- 3

Dr. M. PUGALENTHI,
Assistant Professor
Department of Botany,
Government Arts College (Autonomous),
Coimbatore - 641 018.
Mobile: 9894739777

Enzyme:

Nomenclature, Chemical Nature, and Mechanism

Enzyme: Nomenclature, Chemical Nature, and Mechanism!

One of the most important functions of proteins in living cells is to act as enzymes.

The word "enzyme" was first introduced by Kuhne in 1878. It is derived from the original Greek word enzyme (Gr. en-in, zyme-leaven), which means "in yeast".

In 1896, Buchner succeeded in extracting from the yeast cells a substance that was active in fermentation. This substance was later called zymase and represents a part of the enzyme system involved in fermentation. In 1926, Professor J.B. Sumner isolated from jack beans, by means of acetone, the enzyme urease in crystalline form.

Definition:

An enzyme may be defined as a complex biological catalyst that is produced by a living organism in its cells to regulate the various physiological processes of the body. Enzymes functional outside the living cells are called exoenzymes, e.g., enzymes present in digestive juices, lysozyme of tears. Enzymes functional inside living cells are known as endozymes, e.g., enzymes of Krebs cycle, enzymes of glycolysis, etc.

The substance on which an enzyme acts is called the “substrate” and generally speaking, the enzyme itself is named after the substrate by adding the suffix, ‘ase’ to that of substrate. Thus, for example, proteases are a group of enzymes acting upon proteins, lipases are a group of enzymes acting upon lipid substances and maltase is the name of enzyme acting upon maltose.

Sometimes the name of an enzyme indicates the nature of the reaction it brings about. For example, invertase which breaks sucrose into glucose and fructose, brings about inversion (this is a process in which the raw material showing one type of optical rotation gives out end products that shows the opposite type of optical rotation).

STRUCTURE OF ENZYMES

The active site of an enzyme is the region that binds substrates, co-factors and prosthetic groups and contains residue that helps to hold the substrate.
Active sites generally occupy less than 5% of the total surface area of enzyme.
Active site has a *specific shape* due to tertiary structure of protein.
A change in the shape of protein affects the shape of active site and function of the enzyme.

Nomenclature:

A scrutiny of the enzyme nomenclature reveals that in many cases, it is both inconsistent as well as misleading. Also instances are not lacking where different biochemists gave different names for the same enzyme. This anomaly has been removed by the International Commission on Enzymes in its report in 1961.

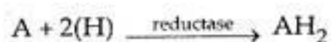
The Commission recognised that each enzyme should consist of: (1) name of the substrate and (2) a word ending in ‘ase’ specifying one kind of catalytic reaction as in succinic dehydrogenase, pyruvate transaminase. This nomenclature is precise and systematic, though in some cases, it is long and tongue-twisting. It is for this reason the trivial names are retained with official sanction but only with reference to their systematic names.

The modern system of enzyme classification was introduced by International Union of Biochemistry (IUB) in 1961. It groups enzymes into the following six categories.

1. Oxidoreductases:

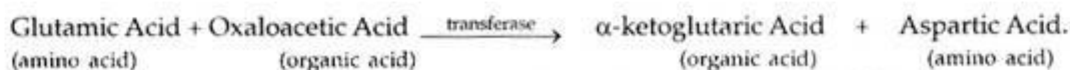
ADVERTISEMENTS:

They take part in oxidation and reduction reactions or transfer of electrons. Oxidoreductases are of three types—oxidases, dehydrogenases and reductases, e.g., cytochrome oxidase (oxidises cytochrome), succinate dehydrogenase, nitrate reductase.



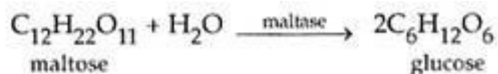
2. Transferases:

They transfer a group from one molecule to another e.g., glutamate-pyruvate transaminase (transfers amino group from glutamate to pyruvate during synthesis of alanine). The chemical group transfer does not occur in the Free State.



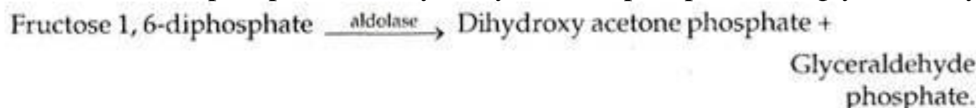
3. Hydrolases:

They break up large molecules into smaller ones with the help of hydrogen and hydroxyl groups of water molecules. The phenomenon is called hydrolysis. Digestive enzymes belong to this group, e.g., amylase (hydrolysis of starch), sucrase, and lactase.



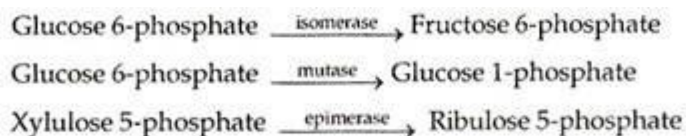
4. Lyases:

The enzymes cause cleavage, removal of groups without hydrolysis, addition of groups to double bonds or reverse, e.g., histidine decarboxylase (breaks histidine to histamine and CO_2), aldolase (fructose-1, 6-diphosphate to dihydroxy acetone phosphate and glyceraldehyde phosphate).



5. Isomerases:

The enzymes cause rearrangement of molecule structure to effect isomeric changes. They are of three types, isomerases (aldose to ketose group of vice-versa like glucose 6-phosphate to fructose 6-phosphate), epimerases (change in position of one constituent or carbon group like xylulose phosphate to ribulose phosphate) and mutases (shifting the position of side group like glucoses-phosphate to glucose-l-phosphate).



6. Ligases:

(Synthetases). The enzymes catalyse bonding of two chemicals with the help of energy obtained from ATP, e.g, phosphenol pyruvate PEP carboxylase (combines phosphenol pyruvate with carbon dioxide forming oxaloacetate accompanied by hydrolysis of ATP.)

The modern system of enzyme nomenclature introduced by International Union of Biochemistry (IUB) envisages a method of giving four numbers to any given enzyme, the first number indicating the main class into which the enzyme falls, the second and third indicates the subclass and subclasses respectively and the fourth is the serial number of the enzyme in its particular sub-class; the four numbers are separated by points.

Thus malic dehydrogenase is given the enzyme commission number (Ec. No. 1) 1.1.1.37. The first 1 indicates that the enzyme is an Oxidoreductase, the second 1 indicates that the enzyme acts on CH-OH group of donors and third 1 indicates that in the reaction which the enzyme promotes, NAD or NADP functions as an acceptor molecule, 37 which is the last number in the serial number given to this particular enzyme is the group characterised by properties that 1.1.1 indicate.

Chemical Nature of Enzymes:

All enzymes are proteinaceous in nature (Sumner, 1926) with the exception of recently discovered RNA enzymes. Some enzymes may additionally contain a non-protein group.

On the basis of differences in chemical nature, the enzymes may be described as follows:

(i) Simple Enzymes:

Some enzymes are simple proteins, i.e., on hydrolysis, they yield amino acids only. Digestive enzymes such as pepsin, trypsin and chymotrypsin are of this nature.

(ii) Conjugate Enzymes:

It is an enzyme which is formed of two parts – a protein part called apoenzyme (e.g., flavoprotein) and a non-protein part named cofactor. The complete conjugate enzyme, consisting of an apoenzyme and a cofactor, is called holoenzyme.

There can be an enzymatic activity only when both components (apoenzyme and cofactor) are present together. The cofactor is sometimes a simple divalent metallic ion (e.£.,Ca, Mg, Zn, Co, etc), and sometimes a nonprotein organic compound. However, some enzymes require both kinds of cofactors. If the cofactor is firmly bound to the apoenzyme, it is called prosthetic group.

For example, cytochromes are the enzymes that possess porphyrins as their prosthetic groups. If, instead of being more or less permanently bound to the apoenzyme the cofactor attaches itself to the apoenzyme only at the time of reaction, it is called a coenzyme.

(iii) Metallo-enzymes:

The metal cofactors involved in enzymic reactions are both monovalent (K^+) and divalent cations (Mg^{++}, Mn^{++}, Cu^{++}). These may be loosely held by the enzyme, or as in some cases, go into the composition of the molecule itself. If the metal forms part of the molecule, as iron of haemoglobin or cytochrome is, the enzymes are called metallo-enzymes.

(iv) Isoenzymes (Isozymes):

At one time it was believed that an organism has only a single enzyme for a given step of a metabolic reaction. It was later discovered that a substrate may be acted upon by a number of variants of an enzyme producing the same product.

The multiple molecular forms of an enzyme occurring in the same organism and having a similar substrate activity are called isoenzymes or isozymes. Over 100 enzymes are known to have isoenzymes. Thus a-amylase of wheat endosperm has 16 isozymes, lactic dehydrogenase has 5 isoenzymes in man, while alcohol dehydrogenase has 4 isozymes in maize. Isoenzymes differ in activity optima and inhibition.

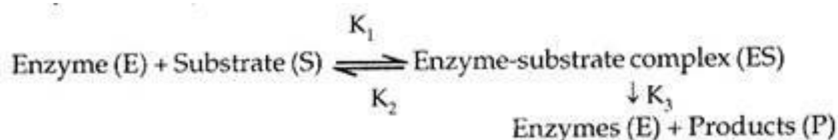
The most thoroughly studied isozyme is lactic dehydrogenase (LDH) which occurs in five possible forms in organs of most vertebrates as observed by starch gel electrophoretic separation. Two basically different types of LDH occur. One type, which is inhibited strongly by relatively low concentrations of pyruvate, predominates in the heart and is called heart LDH.

The other type, less easily inhibited by pyruvate, occurs in many skeletal muscles and is thus called muscle LDH. The heart LDH consists of 4 identical subunits, which are called H subunits. The muscle LDH consists of 4 identical M subunits. The two types of subunits, H and M, have different amino acid compositions, enzyme kinetics and immunological properties. These subunits in different combinations produce 5 isoenzymes.

They are thus useful to organism in adapting to varied environmental conditions.

Mechanism of Enzyme Action:

The enzyme promotes a given reaction, but itself remains unchanged at the end of the reaction. In 1913, Michaelis and Menten proposed that an intermediate enzyme-substrate complex is formed during the enzymic activity. The following scheme may be written to illustrate concept:



Enzymes are biological catalysts which accelerate the rate of reaction by altering the kinetic properties. Thus, the enzyme (E) exercises its catalytic role on the substrate (S) by forming an enzyme- substrate complex (ES) by a reversible reaction where K_1 is the rate constant for the formation of ES, and K_2 is the rate constant for the dissociation of ES to E and S.

After the formation of ES, substrate (S) is converted into the products, thus making enzyme (E) available for further combination with more substrate. The rate of conversion of ES to the products of the reaction may be indicated by the constant K_3.

Each enzyme-catalyzed reaction has a characteristic K_m value, which is the Michalies-Menten constant, which is a measure of the tendency of the enzyme and the substrate to combine with each other.

$$K_m = \frac{K_2 + K_3}{K_1} = \frac{[E][S]}{[ES]}$$

In this way the K_m value is an index of the affinity of the enzyme for its particular substrate. Greater the affinity of an enzyme for its substrate, the lower the K_m value.

Enzymes Reduces the Energy of Activation:

Energy of activation is that minimal amount of energy which is required of a molecule to take part in a reaction. The effect of enzymes is to lower the activation energy requirements, thereby promoting appreciable reaction rates at lower temperatures than would be possible otherwise.

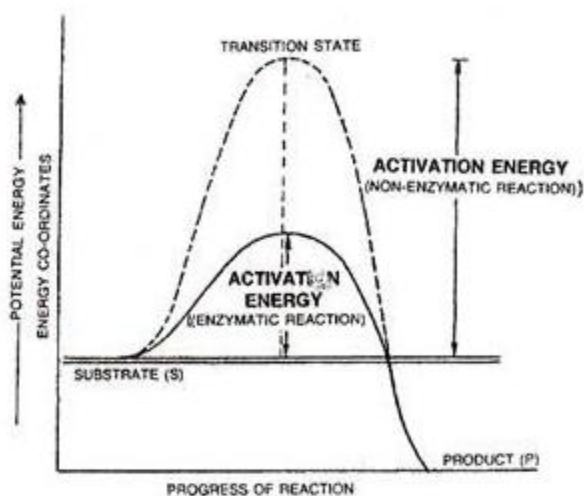
The Catalytic Sites:

Enzymes are much larger compared to the substrate molecules. In an enzyme-substrate therefore, the substrate is in contact with only a very small area of the enzymic surface. This part of the enzyme comprising amino acid residues and peptide bonds that are in physical contact with the substrate but essential for catalytic activity put together constitute an active site, presently referred as the catalytic site.

Excluding the catalytic site, the rest of the enzyme molecule may be necessary for maintaining the correct three-dimensional conformation of the catalytic site or it may just be there without any functional role.

The structure of a catalytic site has been studied in some enzymes. It is either a crevice on the enzyme as in papain and ribonuclease or a deep pit as in carbonic anhydrase. Whatever the shape of the catalytic site may be, it is believed that the correct substrate binds with the catalytic site producing a substrate-catalytic site complex.

The term productive binding is often applied to this complex. In productive binding, both the enzymes and substrates show conformational changes with a reduction in activation energy so that the substrate is converted into a product.



Lowering of activation energy by enzyme in the energy relations of a chemical reaction.

Theories of Enzyme Action:

1. Lock and Key Hypothesis:

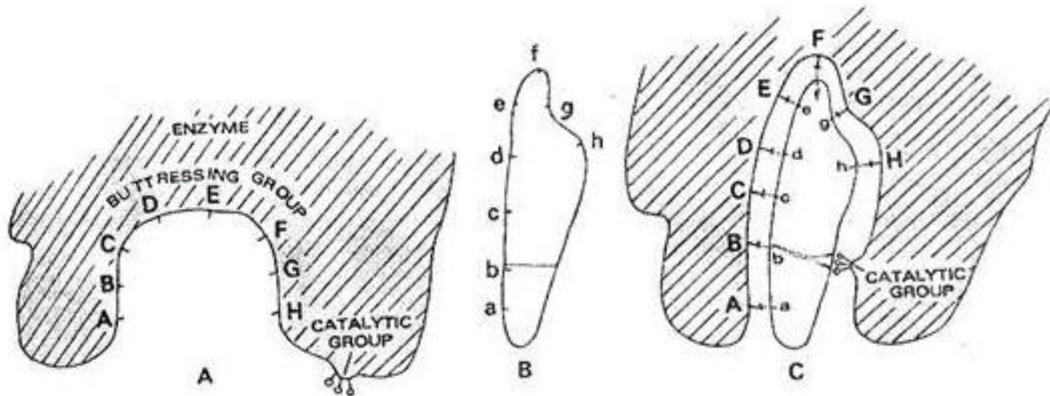
The enzyme-substrate complex first hypothesized by Emil Fischer in about 1884, assumed a rigid lock-and-key union between the two. The portion of the enzyme to which the substrate (or substrates) combines as it undergoes conversion to a product is called the active site.

If the active site were rigid and specific for a given substrate, reversibility of the reaction would not occur, because the structure of the product is different from that of the substrate and would not fit well.

2. Induced-Fit Theory:

As contrasted to a rigidly arranged active site of Fischer, Daniel E. Koshland (1973) found evidence that the active site of enzymes can be induced by close approach of the substrate (or product) to undergo a change in conformation that allows a better combination between the two.

This idea is now widely known as the induced-fit theory and is illustrated below. Apparently, the structure of the substrate is also changed during many cases of induced fit, thus allowing a more functional enzyme- substrate complex.



Induced-fit theory of enzyme action. (A) active site of enzyme. (B) substrate molecule. (C) enzyme-substrate complex with conformational changes so as to bring the catalytic group against the substrate bonds to be broken.

Properties of Enzymes:

1. The catalytic nature of the enzyme has been already discussed in details earlier.

2. Reversibility:

Theoretically, all enzyme controlled reactions are reversible. Reversibility is, however, dependent upon energy requirements, availability of reactant, concentration of end products and pH. If the chemical potential of reactants is very high compared to that of the products, the reaction might proceed only towards products formation, because of the chemical law of mass action. Most decarboxylation and hydrolytic reactions are irreversible.

The same enzyme facilitates forward and backward movement of a reaction if only it is possible thermodynamically. A convincing example is seen in the pathways of respiration and photosynthesis. The enzymes of glycolysis and pentose phosphate pathway dissimilate glucose. Some of these enzymes work in the reverse direction in photosynthesis and build glucose from carbon dioxide and water.

3. Heat sensitivity:

All enzymes are heat sensitive or thermolabile. Most enzymes operate optimally between 25°-35°C. They become inactive at freezing temperatures and denatured at 50°-55° C. However, thermal algae and bacteria are an exception. Their enzymes remain functional even at 80°C. Enzymes of seeds and spores are also not denatured at 60°-70°C.

4. pH-sensitive:

Each enzyme functions at a particular pH, e.g., pepsin (2 pH), sucrase (4-5 pH), trypsin (8.5 pH). A change of pH makes the enzymes ineffective.

5. Specificity of actions:

Enzymes show specificity towards the substrates on which they exercise their catalytic role. This unique property of the enzymes is decided by: (1) the structural configuration of the substrate molecule, (2) the conformation of the enzyme and (3) the active or catalytic sites on the enzyme. The substrate specificity of enzymes is of two kinds: group specificity and stereo-specificity.

Enzymes usually show group specificity i.e., they attack only a group of chemically related compounds. The group specificity may be a relative group specificity, in which case the enzyme functions on a number of homologous substrates.

Thus, hexokinase transfers phosphate group from ATP to at least 23 hexoses or their derivatives like glucose, mannose, fructose, and glucosamine. Some of the group specific enzymes exhibit an absolute group specificity, which means the enzyme acts only on a single compound and not its homologues. Mannose, glucokinase and fructokinase are involved in phosphorylations of the hexoses, mannose, glucose and fructose respectively.

Enzymes also show stereo-specificity towards the substrate and it is exhibited with both optical and geometric isomers.

(i) If the enzyme shows optical specificity, it acts on either dextro (D) or laevo (L) isomer of the compounds. Thus, D. aminoacid oxidase oxidises only D. amino-acids and L. aminoacid oxidases react only with L. aminoacids.

(ii) The geometrical specificity is exhibited towards the cis and trans isomers. Fumaric and malic acids are two geometrical isomers. Fumaric hydratase acts on only the trans-isomer fumaric acid but not on the cis-isomer malic acid.

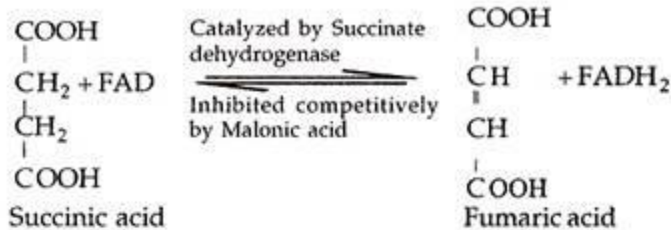
6. Enzyme inhibition:

Substances or compounds that decrease the rate of an enzyme-catalyzed reaction are known as inhibitors and the phenomenon is described as enzyme-inhibition. There are three types of inhibitions.

(i) Competitive inhibition:

When a compound competes with a substrate for the active site on the enzyme protein and thereby reduces the catalytic activity of that enzyme, the compound is considered to be a

competitive inhibitor. Inhibition by such structural analogues (called antimetabolites), which is reversed by simply adding more substrate to the reaction mixture, is known as competitive inhibition.



For example, succinate dehydrogenase readily oxidises succinic acid to fumaric acid. If increasing concentrations of malonic acid, which closely resembles succinic acid in structure, are added, succinic dehydrogenase activity falls greatly.

The inhibition can now be reversed by increasing in turn the concentration of the substrate succinic acid. The amount of inhibition in this type of inhibition is related to (i) inhibitor concentration, (ii) substrate concentration, and relative affinities of inhibitor and substrate. The inhibitory effect is reversible.

Whether an inhibitor is competitive or not can be found out by constructing the Lineiveaver-Burk Plot. Competitive inhibitors alter K_m of the enzyme because they occupy the active sites. They do not, however, alter the V_{max} or maximum velocity of the reaction.

(ii) Non-competitive inhibition:

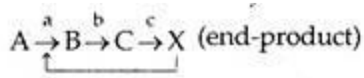
The type of inhibition that cannot be reversed by increasing the substrate concentration is called non-competitive inhibition. The inhibitor combines rather strongly with a site on the enzyme other than the active site and this effect is not overcome by simply raising the substrate concentration.

The amount of inhibition in this type of inhibition is related to (a) inhibitor concentration, and (b) inhibitors affinity for the enzyme. The substrate concentration has no effect on this system, and non-competitive inhibitors alter the V_{max} and not the K_m of the enzyme.

Cyanide, azide and heavy metal like silver, mercury, lead, etc are some examples of non-competitive inhibitors that combine with or destroy essential sulfhydryl groups or the metal component of the enzymes.

(iii) Feedback (end-product) inhibition:

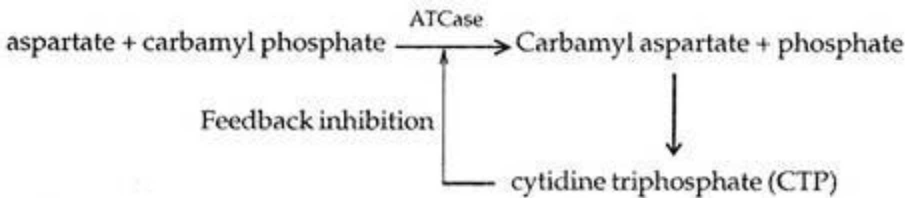
When end-product of a reaction serves to prevent the formation of one of its own precursors by inhibiting the action of the enzyme catalyzing the very- reaction, the inhibition is called feedback inhibition.



The inhibition of conversion of A to B by X would be such an inhibition. Here X, the ultimate product of the reaction, serves to prevent the formation of one of its own precursors (B) by inhibiting the action of enzyme a' which catalyzes the change from A to B.

In this instance, enzyme 'a' can be called the pacemaker since the entire sequence is effectively regulated by it. An actual example is the formation of cytidine triphosphate (CTP) from aspartic acid and carbamyl phosphate in E. coli.

As a critical concentration of CTP is built up, the triphosphate slows down its own formation by inhibiting the enzyme, aspartate transcarbamylase (ATCase), which catalyzes the pacemaker step of its own synthesis. When the concentration of triphosphate is sufficiently lowered by metabolic utilization, inhibition is released, and its synthesis is renewed.



Factors Affecting Enzyme Action and Enzyme Kinetics:

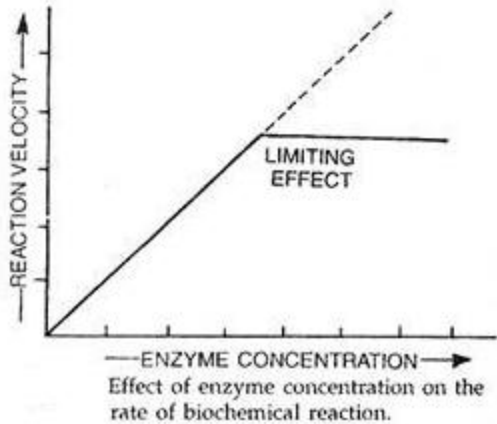
1. Enzyme Concentration:

The rate of a biochemical reaction rises with the increase in enzyme concentration upto a point called limiting or saturation point. Beyond this, increase in enzyme concentration has little effect.

2. Substrate concentration:

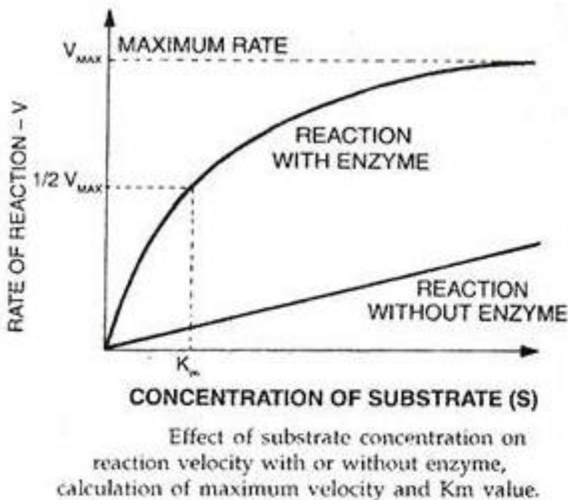
The first satisfactory mathematical analysis of the effect of substrate concentration on the reaction velocity of enzyme-catalysed reaction was made by Michaelis and Menten (1913). With fixed enzyme concentration, an increase of substrate will result at first in a very rapid rise in the velocity or reaction rate.

As the substrate concentration continues to increase, however, the increase in the rate of reaction begins to slow down until, with a large substrate concentration, no further change in the velocity is observed. The velocity of the reaction obtained at this high substrate concentration is defined as the maximum velocity (V_m) of the enzyme-catalyzed reaction under the specified conditions and the initial reaction velocity obtained with substrate concentrations below the saturation level is called V.



Effect of enzyme concentration on the rate of biochemical reaction.

The substrate concentration required to yield half the maximum velocity ($V_m/2$) can be readily determined from above figure and is an important constant in the enzyme kinetics. It defines the Michaelis constant or K_m. In other words, K is defined as the substrate concentration when V= ½ V_m-Under carefully defined conditions of temperature, pH, and ionic strength of the buffer, this constant K_m approximates the dissociation constant of an enzyme-substrate complex. The reciprocal of K_m or $1/K_m$, approximates the affinity of an enzyme for its substrate.



Effect of substrate concentration on reaction velocity with or without enzyme, calculation of maximum velocity and Km value.

Kinetics of Enzyme Action:

The Michaelis constant K is of considerable importance since it provides the mode of action of an enzyme catalyzing a reaction. It should be noted that at low substrate concentration the

relation of velocity to substrate is almost linear and obeys the first-order kinetics, i.e., the rate of the reaction A–>B is directly proportional to the substrate concentration [A].

$V = K' [A]$ low [substrate]

Where V is the observed velocity of the reaction at concentration [A] and K' is the specific rate constant. At high substrate concentration, however, the velocity of the reaction is maximum and is independent of the substrate [A]; hence it obeys the zero-order kinetics.

$V_m = K'$ Saturating [Substrate]

The Michaelis-Menten equation which describes this relationship and also satisfactorily explains curve, is as follows:

$V = Vm[S]/K_m + [S]$

Where V = initial reaction velocity at given substrate concentration [S]

K_m = Michaelis constant, moles/litre.

V_m = Maximum velocity at saturated substrate concentrations

[S] = Substrate concentration in moles / litre

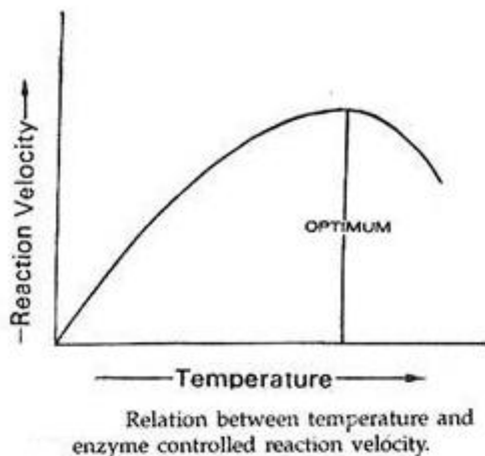
Determination of K_m of an enzyme reaction by Michaelis-Menten equation is in practice difficult. An outcome of this equation called Line-weaver-Burk plot is often used for such determination.

1. Temperature:

An enzyme is active within a narrow range of temperature. The temperature at which an enzyme shows its highest activity is called optimum temperature. Enzyme activity decreases above and below this temperature. As catalyst they show an increased reactivity with temperature but their proteinaceous nature makes them susceptible to thermal denaturation above the optimum temperature.

2. pH:

The pH at which the maximum enzyme activity occurs varies considerably from one enzyme to another. This is known as the pH optimum. Any minor shift to either direction tends to lower the enzyme activity considerably. Since enzymes are proteins, pH changes normally affect the ionic character of the amino and carboxylic acid groups on the protein surface and therefore markedly affect the catalytic nature of an enzyme.



Relation between temperature and enzyme controlled reaction velocity.

3. Hydration:

Enzyme functions maximally under the enhanced kinetic activity of the substrate as the continuous phase is higher. That is why the seeds which have low water content register a minimal enzymic activity though the substrates abound in them. On germination, however, the enzymic activity rises sharply and this is due to the absorption of water and consequent promotion of kinetic activity of substrate molecules.

Coenzymes:

In cellular physiology many enzymatic reactions are completed in the presence of coenzymes. These are compounds which function like the enzymes i.e., they speed up the biological reactions, but they are not proteins like the true enzymes.

Definition:

A coenzyme may be defined as a particular kind of cofactor, i.e., a non protein organic compound, or a carrier molecule functioning in conjunction with a particular enzyme.

If the cofactor is firmly bound to the apoenzyme, it is called a prosthetic group; and if, instead of being more or less permanently bound to the apoenzyme, the organic cofactor attaches itself to the enzyme protein only at the time of reaction, it is called a coenzyme.

In cellular processes sometimes hydrogen atoms or electrons are removed from one compound and transferred to another. In all such cases a specific enzyme catalyzes the removal, but a specific coenzyme must also be present to carry out the transfer. The coenzyme temporarily joins to, or accepts the removed group of atoms and may subsequently hand over them to another acceptor compound.

Chemical Nature of Coenzymes:

The majority of coenzymes are chemical derivatives of the nucleotides. More specifically, in most coenzymes the nitrogen base portion of the nucleotides is substituted by another chemical unit. This unit itself is usually a derivative of a particular vitamin. The following coenzymes are important in cellular physiology.

(i) Flavin derivatives or Flavin nucleotides (FMN and FAD)

(ii) Pyridine derivatives or Pyridine nucleotides (NAD and NADP).

(iii) Coenzyme A

(iv) Coenzyme Q

(iv) Cytochromes

(vi) Thiamine pyrophosphate

Here only two coenzymes are described.

1. Flavin Nucleotides or Flavoproteins:

A large group of respiratory enzymes use as their cofactor one of the two derivatives of riboflavin (vitamin B_2). They are flavin mononucleotide (FMN) and flavin adenine nucleotide (FAD).

Structure:

Riboflavin is a compound consisting of a ribose protein, and a flavin portion, the latter being a complex triple ring structure. In cells, a phosphate group is linked to riboflavin resulting in a nucleotide like complex known as flavin mononucleotide (FMN) or riboflavin monophosphate. If FMN joins to AMP, a dinucleotide known as flavin adenine dinucleotide (FAD) is formed.

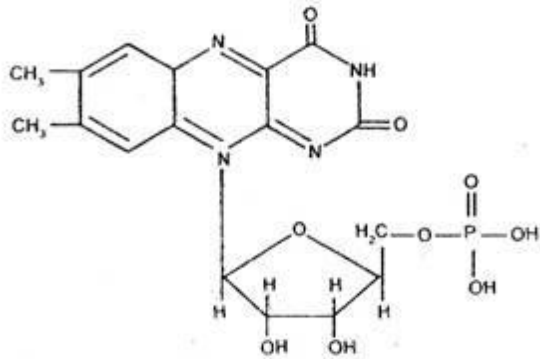
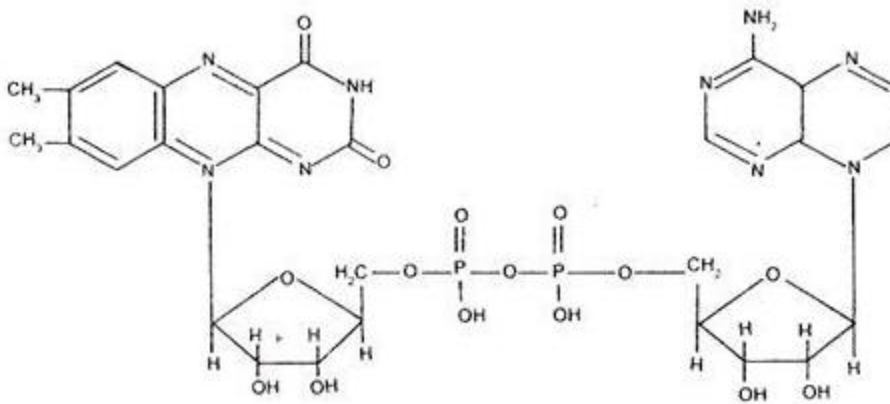


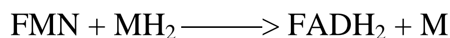
Fig. 1.37 Structure of Flavin mononucleotide (FMN).

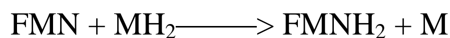


Structure of Flavin adenine nucleotide (FAD).

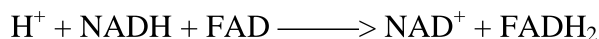
Functions:

The combination of either FMN or FAD with an apoenzyme is called flavoprotein (FP). Flavoproteins catalyzed the removal of hydride ion (H^-) and hydrozen ion (H^+) from a metabolite. In these coenzymes, it is the flavin portion of the molecule that provides the specific place for temporary attachment of hydrogen.


$FMN + MH_2 \longrightarrow FADH_2 + M$


$FMN + MH_2 \longrightarrow FMNH_2 + M$

In this reaction MH, represents a substrate, FADH, is the reduced form of FAD, and $FMNH_2$ is the reduced form of FMN. An important source of hydrogen for this reaction is the reduced pyridine nucleotide.


$H^+ + NADH + FAD \longrightarrow NAD^+ + FADH_2$

In all the cases reduced flavoproteins pass on their electrons to the cytochromes.

2. Coenzyme Q:

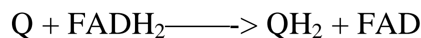
This enzyme is a quinone, known as ubiquinone, and is mainly found in the mitochondria but also in microsome and cell nuclei, etc.

Structure:

The coenzyme Q or ubiquinone consists of a quinone with a side chain whose length varies with the source of the mitochondria. In most animal tissues the quinone possesses 10 isoprenosid units in its side chain and is called coenzyme Q_{10}.

Function:

The coenzyme Q is a necessary component of the electron transport chain in the mitochondria. It serves as an additional hydrogen carrier between the flavin coenzymes (FAD and FMN) and the cytochromes.

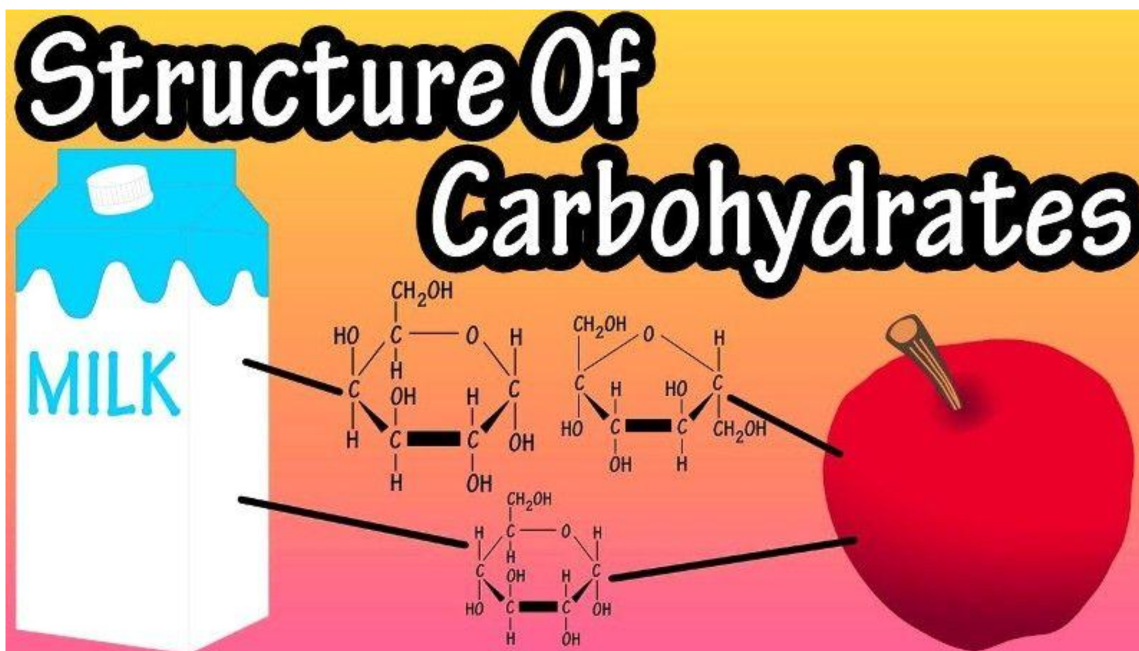


$Q + FADH_2$ ——> $QH_2 + FAD$

Reduced (QH_2) transfers its electrons to cytochrome b in the mitochondria.

Carbohydrates: Structure & Classification

Carbohydrates are one of the most important components of the biological world in addition to being one of the most abundant classes of biological molecules. The word 'carbohydrate' is derived from the Greek word '**sakcharon**' meaning '**sugar**'. Carbohydrates are nothing but aldehyde or ketone compounds with multiple hydroxyl groups. The literal meaning of carbohydrates is **'carbon hydrates'** which originates from their chemical composition. The chemical composition of carbohydrates or saccharides is $(CH_2O)n$ where n>3 or n=3.



Basic Functions Of The Carbohydrates

1. **Energy reserves**: Carbohydrates make up energy stores, fuels and metabolic intermediates.
2. **Structural framework of genetic material**: The sugars **ribose and deoxyribose** are a part of the structural framework of genetic material **RNA and DNA.**
3. **Structural element of cell wall**: Polysaccharides are the structural elements of the cell wall of bacteria and plants.
4. **Cellulose**, a polysaccharide and a principal component of the cell wall of pants, is one of the most abundant organic compounds in the biosphere.
5. **Conjugate with lipids and proteins**: Carbohydrates are extensively linked protein and lipid molecules. These glycoprotein and glycolipids are critical in choreographing interactions between cells and other biological elements.

Classification Of Carbohydrates

Carbohydrates can be classified into 2 categories-

1. mono-, oligo- and polysaccharides and
2. reducing and non- reducing sugars

Depending upon whether they undergo hydrolysis or not and if yes, then the number of products formed, the carbohydrates are classified into the following:

1. Monosaccharides: Monosaccharides are the **simplest** They cannot be hydrolyzed further into hydroxyl aldehyde and ketone unit.

2. Oligosaccharides: Oligosaccharides are polymers with two to ten monosaccharide units. The individual monosaccharide units are joined together by **glycosidic linkages**. They are often present in association with proteins (**glycoprotein**) and lipids (**glycolipids**). These two conjugates of carbohydrates with proteins and lipids are collectively called **glycoconjugates**. Depending upon the monosaccharide unit present, the oligosaccharides are further grouped into:

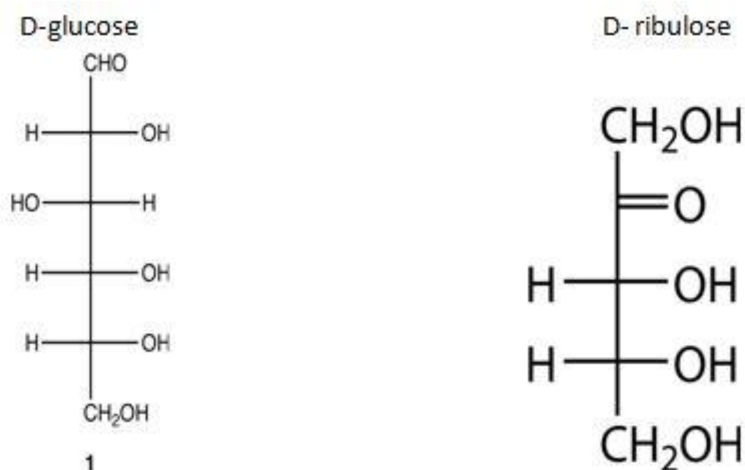
- Disaccharides- with two monosaccharide units.
- Trisaccharides- with three monosaccharide units.
- Tetrasaccharides- with four monosaccharide units.
- Pentasaccharides- with five monosaccharide units.

3. Polysaccharides: Polysaccharides have hundreds and even thousands of monosaccharide units **linked covalently**. The molecular mass of these polymers ranges into millions of Dalton.

They have a critical role in maintaining **the structural integrity** of the living organisms. **Cellulose** is a major structural polysaccharide in plants. **Starch** in plants and **glycogen** in case of animals are principal nutritional reserves.

Monosaccharides Or Simple Sugars

Monosaccharides are the simplest aldehyde or ketone derivatives which cannot be hydrolyzed further; for example **D-glucose and D- ribulose** cannot be hydrolyzed further.

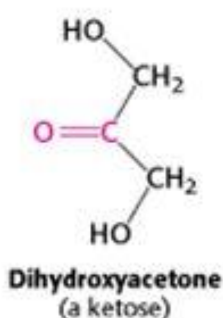


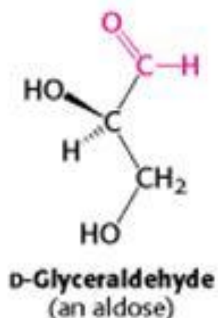
Monosaccharides are further classified into two subgroups depending upon the

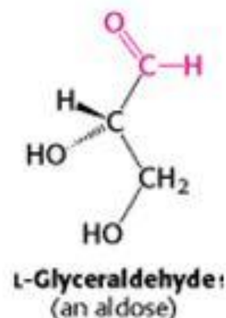
- **Number of carbon atoms present*:*** the smallest monosaccharide is the one with three carbon atoms, known as **trioses**. Therefore, the monosaccharide with four, five, six or seven carbon atoms are called **tetroses, pentoses, hexoses and heptoses**
- **Chemical nature of their carbonyl group or presence of aldehyde or ketone unit**: if the carbonyl group is **aldehyde** in nature, the monosaccharide is called **aldose**. If the carbonyl group is **ketone**, then the monosaccharide is called **ketose**.

The monosaccharide **glucose** can hence be referred to as '**aldohexose'**. This implies that it is a six-carbon monosaccharide with carbonyl group which is aldehyde in nature. Similarly, **fructose** is a **'ketohexose'** containing a six-carbon monosaccharide and a ketone group.

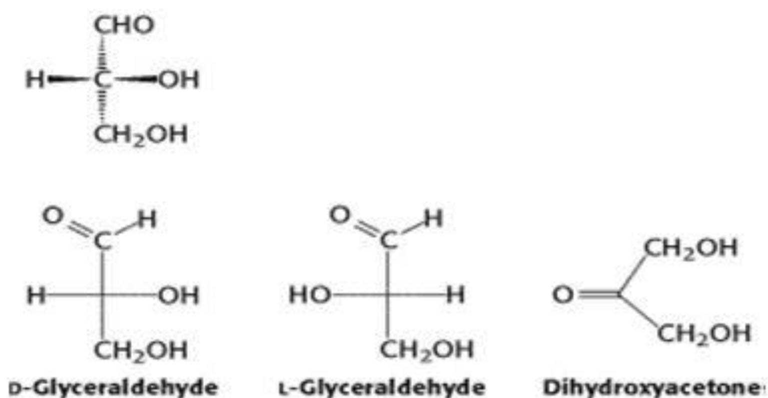
The smallest monosaccharides or **trioses** (n=3) are **dihydroxyacetone**, **D- and L-glyceraldehyde**.



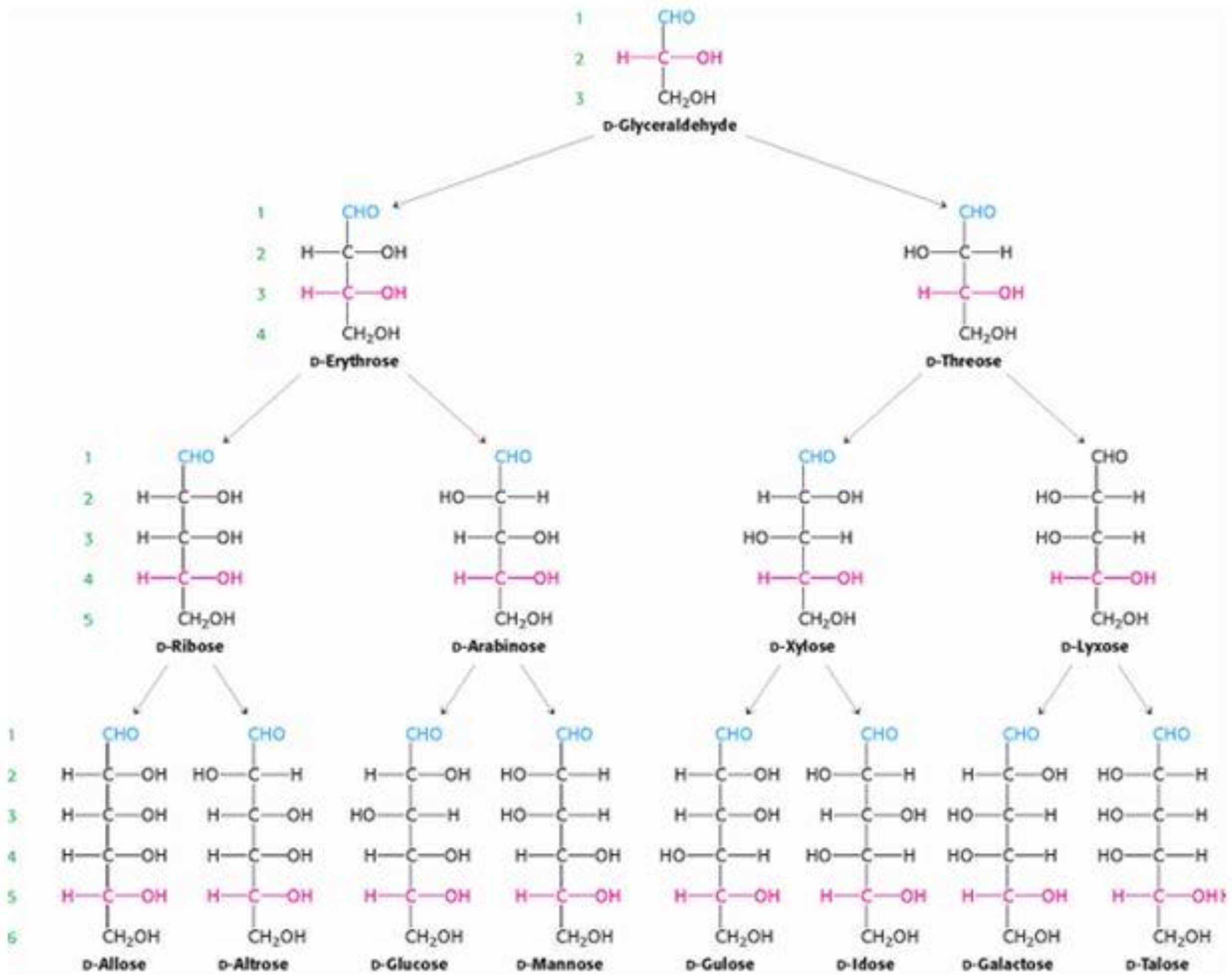




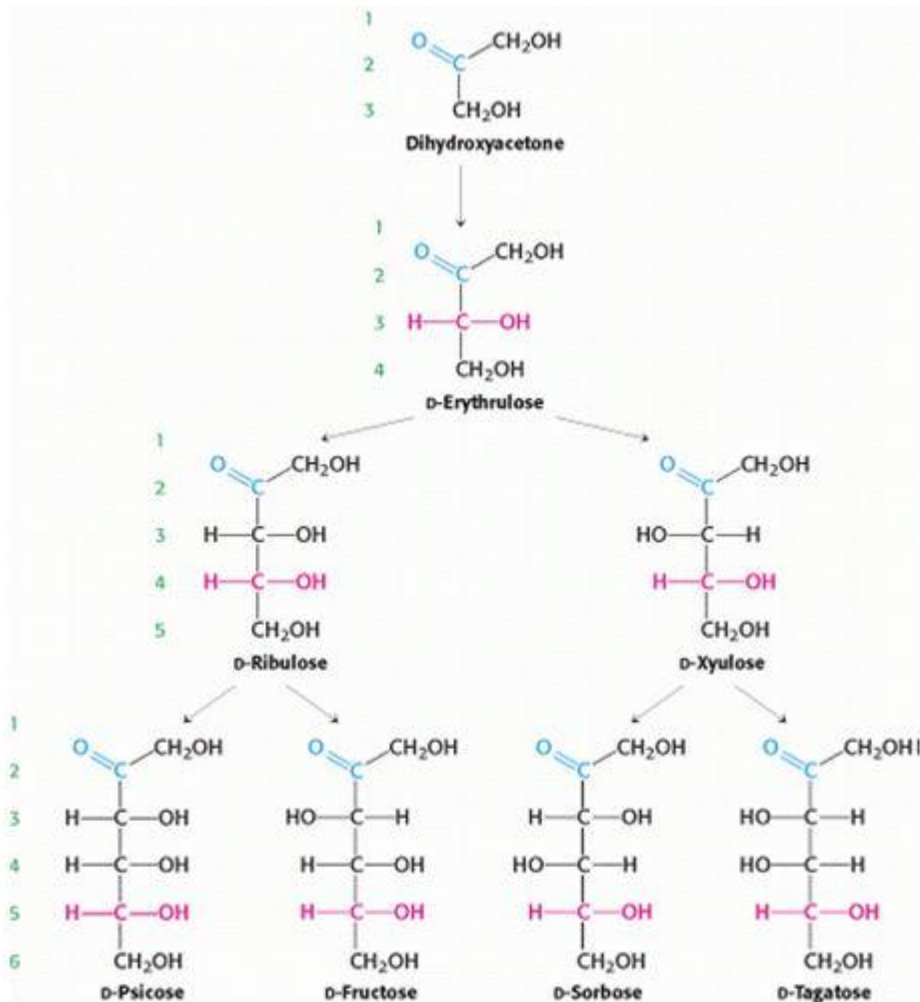
Glyceraldehyde with C-2 atom is **chiral or asymmetric** in nature, henceforth, there are two **stereoisomers** of this sugar. Chiral compounds like glyceraldehyde usually exist in two forms which are **non- superimposable mirror images** of each other. These non-superimposable mirror images are known as **enantiomers**. They are often represented as **Fischer projections**. In Fischer projections, atoms which are linked to an asymmetric carbon atom by horizontal bonds are present in front of the plane of the page whereas those linked to asymmetric carbon by vertical bonds are present behind. In case of glyceraldehyde, when the hydroxyl group which is attached to the asymmetric carbon is present on the left of the Fischer projection, the configuration is referred to as **'L'** and when the hydroxyl group is present on the right, the configuration is '**D'**.



Since other polymers have more than one chiral or asymmetric carbon, they generally exist as **diastereoisomers**. Diastereoisomers are not mirror images of each other. A compound with 'n' chiral carbon atoms will have a maximum of $\mathbf{2^n}$ **stereoisomers**. Taking into consideration **glucose** here, we observe that 4 out of its 6 carbons are chiral. Going by the general formula for calculating the number of stereoisomers, 2^n, **16 possible stereoisomers** can arise comprising all possible aldohexoses. The absolute configuration of monosaccharides which contain multiple chiral carbons is, however, determined by comparison of the configuration at the highest- numbered chiral carbon to the configuration of the single chiral carbon of glyceraldehyde. Except for dihydroxyacetone, all monosaccharides occur in optically active isomeric forms.



This figure represents d- aldoses containing three, four, five and six carbon atoms.

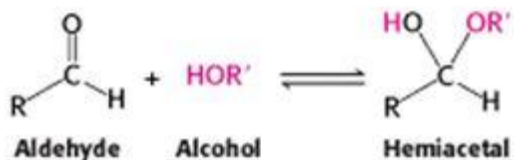


The figure illustrates d- ketoses containing three, four, five and six carbon atoms.

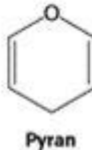
Epimers– sugars which **differ** at only a **single asymmetric** or chiral carbon are called epimers. For example, D- glucose, and D- mannose differ only at C-2. In addition to this, even D- glucose and D- galactose differ at C-4.

Cyclic Forms- Pentoses And Hexoses Cyclize To Form Pyranose And Furanose Ring Structure

Monosaccharide polymers like glucose, fructose, and others do **not exist as open chains in solution**. The open chains of these simple sugars cyclize to form **rings**. Aldehyde and ketone groups react readily with alcohols to form **hemiacetals** and **hemiketals** respectively. In aldohexoses like glucose, the aldehyde at C-1 in the open chain of glucose reacts with the hydroxyl group at C-5 in order to give rise to hemiacetal. The result of this process is a cyclic structure of six carbons known as **pyranose**.

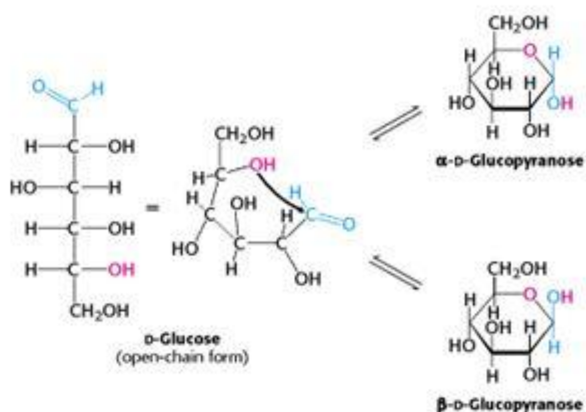


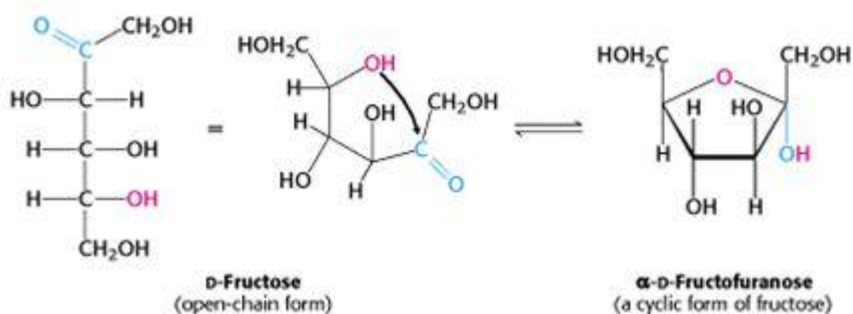
In a similar way, a ketone reacts with alcohol in order to yield hemiketal. In the open chain form of ketohexose, say fructose, the keto group at C-2 reacts with either the hydroxyl group at C-6 to form a six-membered cyclic hemiketal or the hydroxyl group at C-5 to form a five-membered cyclic hemiketal. The five-membered cyclic ring thus formed is called **furan**.



The whole process of pyranose and furanose formation is described as follows. The depictions of **glucopyranose** and **fructofuranose** are referred to as **Howarth** projections. During the process of formation of cyclic hemiacetal, an additional asymmetric center is created. The C-1 in case o fan open-chain glucose becomes the asymmetric center. The end product is the formation of two ring structures, **α– D- glucopyranose and β– D- glucopyranose.** In case of the D- sugars represented as Howarth projections, the symbol **α** represents that the **C-1 hydroxyl group is below** the plane of the ring; **β** represents that the same **hydroxyl group is above** the plane of the ring. These two **diastereoisomers** are called **anomers**. A similar process occurs during the formation of the furanose ring of fructose. The only difference is that the hydroxyl group is attached to the C-2 carbon atom.

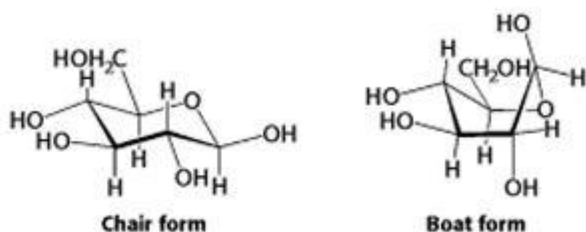
The α and β forms interconvert via the open-chain form to give an equilibrium mixture. This **process of interconversion** is commonly referred to as **mutarotation**. A mixture of glucose at equilibrium contains approximately one-third α anomer, two-thirds β anomer and less than 1% of the open-chain.





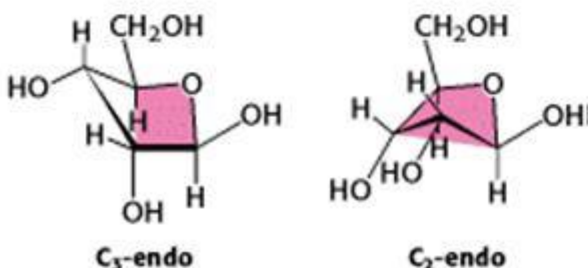
Conformations Of Pyranose And Furanose Forms

The pyranose form can easily adopt two conformations called **chair and boat**. The substituents in case of the chair form have two orientations, the **axial and the equatorial orientation**. The **axial** groups which are **close- fitting** usually extend parallel to the threefold rotational axis of the ring. If they manage to extend out from the same side of the ring, they hinder each other sterically. **Equatorial** orientation is usually **less crowded** compared to the axial substituents. In case of glucose, the chair form of β– **D- glucopyranose predominates** and is more **stable** only because all the axial positions are occupied by hydrogen atoms.



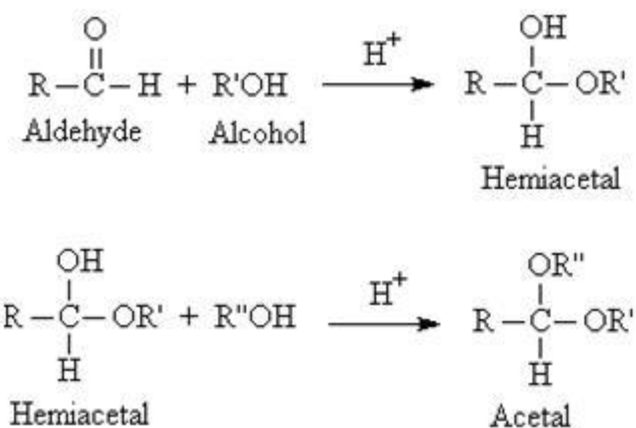
Furanose rings are **not planar**. The four atoms are nearly **coplanar** and so the conformation can be **puckered**. Just because this particular conformation resembles an opened envelope, it is called **envelope form**. Say, for example, the ribose moiety has either C-2 or C-3 is out of the

plane and on the exact same side as C-5. These conformations are particularly called **C2- endo and C3- endo,** respectively.

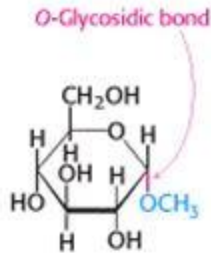


Monosaccharides And Their Derivatives

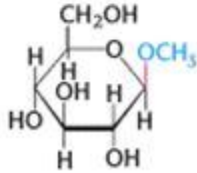
Monosaccharides react readily with alcohols and amines to form modified products called **adducts**. **Alcohols** react with hemiacetals to yield **acetals** and when they react with hemiacetal of sugars to form the acetal, it is commonly called **glycoside**. When glucose is the hemiacetal, the result is the formation of **glucoside**, if galactose, then **galactoside**. **Ouabain** is the most common glycoside. It particularly inhibits the action of enzymes, which pump Na^+ and K^+ across the biological cell membranes. Antibiotics such as **streptomycin** are also glycosides.



Say, for example, **methanol** reacts via an acid- catalyzed process with D- glucose. Two products are formed by the reaction between the anomeric carbon and the hydroxyl group of methanol, **methyl α -D-glucopyranoside and methyl β -D-glucopyranoside.**

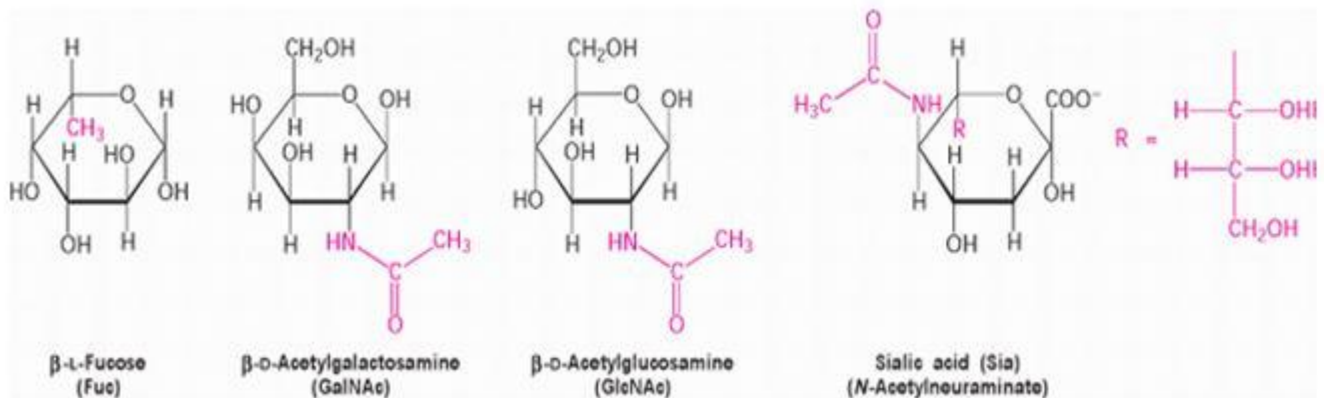


Methyl α-D-glucopyranoside



Methyl β-D-glucopyranoside

Some other modified sugars are as follows:



Complex Sugars Are Formed By Glycosidic Linkage Between Monosaccharides

Monosaccharides readily form glycosidic bonds because of the presence of multiple hydroxyl groups. Disaccharide sugars are a result of 2 monosaccharides linked by **O- glycosidic bond** and oligosaccharides are formed by joining of 2 or more monosaccharide by *O*- glycosidic bond.

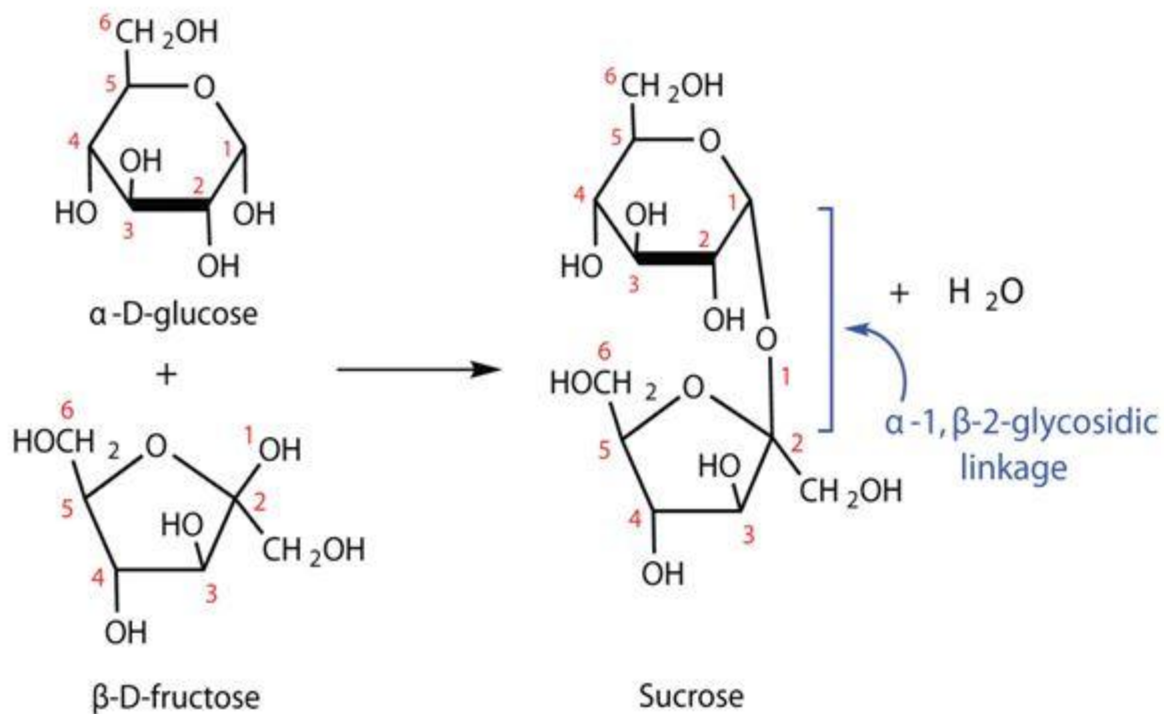
In this example, two molecules are linked by an *O*- glycosidic bond to yield the disaccharide, maltose.

Disaccharides And Glycosidic Linkage

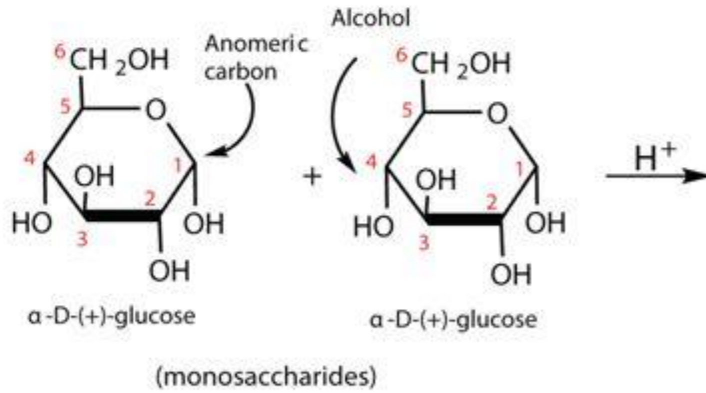
When a disaccharide is formed, two monosaccharides are joined to each other by **glycoside or acetal formation**. Loss of water molecule occurs when the hemiacetal -OH of one monosaccharide and the -OH of the second monosaccharide react in order to establish a glycosidic bond. Therefore, it can be said that the glycosidic bond results because of the reaction between the **anomeric carbon** and the **alkoxy oxygen**. Going by the convention, the glycosidic linkages are read from left to right.

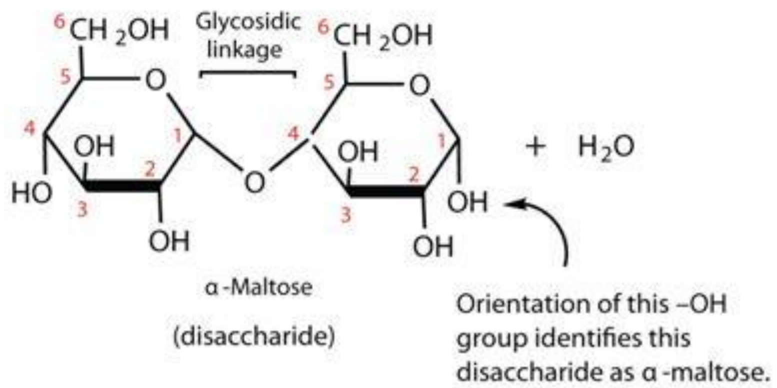
The most abundant disaccharides are **lactose, maltose, and sucrose** (common table sugar).

Sucrose, which is commercially available, is obtained from cane or beet and is the result of a reaction between **α- anomeric carbon of glucose** residue (C1) and **β- anomeric carbon of fructose** residue (C2). Therefore, the glucose and the fructose residues are joined via **α1-2β glycosidic linkage**. The configuration is always α for glucose and β for fructose. Sucrose can in turn be cleaved into its constituents monosaccharides by the action of **sucrase**. The hydrolysis of sucrose is often accompanied by the change in optical rotation from dextro to levo. Therefore, sucrose is also known as **invert sugar or invertose**. This process is catalysed by an enzyme called **invertase or β-D- fructofuranosidase.**

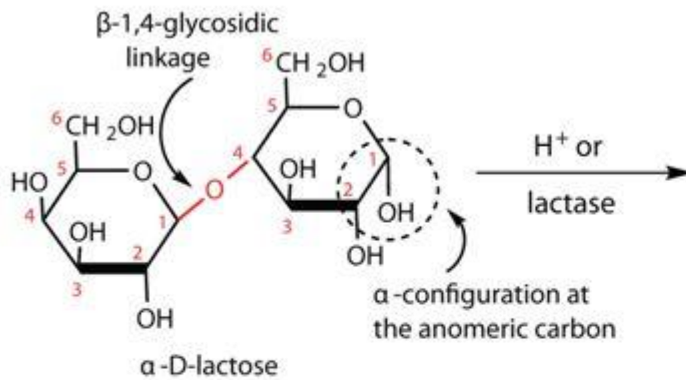


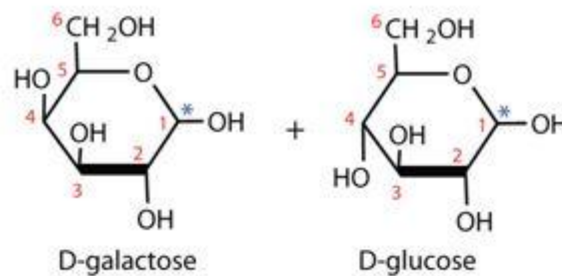
Maltose is a disaccharide of glucose. The glycosidic linkage is formed between **α- anomeric C-1 of one glucose** and **C-4 hydroxyl atom** of the adjacent **glucose** residue. Hence, such a linkage is known as **α-1, 4- glycosidic bond**.





The dissacharide of milk, **lactose**, is a linkage of **galactose** with **glucose** through **β- 1,4-glycosidic** linkage. Lactose is cleaved by **lactase** in humans and **β- galactosidase** in bacteria.

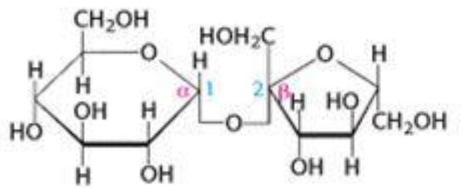


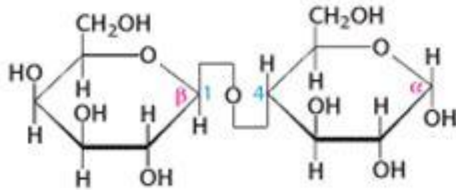


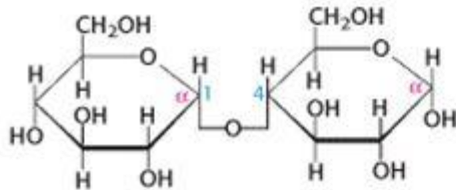
*We use this convention for writing the hydroxyl group on the anomeric carbon when we do not wish to specify either the α or the β anomer.

Disaccharides	***Structure***	***Physiological Role***
Sucrose	Glucose (α1-2β) fructose	A product of photosynthesis
Lactose	Galactose (β1-4) glucose	A Major animal energy source
Trehalose	Glucose (α1-1α) glucose	Energy reserve; a major ciculatory sugar in insects
Maltose	Glucose (α1-4) glucose	The dimer derived from starch and glycogen
Cellobiose	Glucose (β1-4) glucose	The dimer of cellulose polymer
Gentiobiose	Glucose (β1-6) glucose	Constitutes plant glycosides and some polysaccharides

Occurrence and biochemical roles of disaccharides

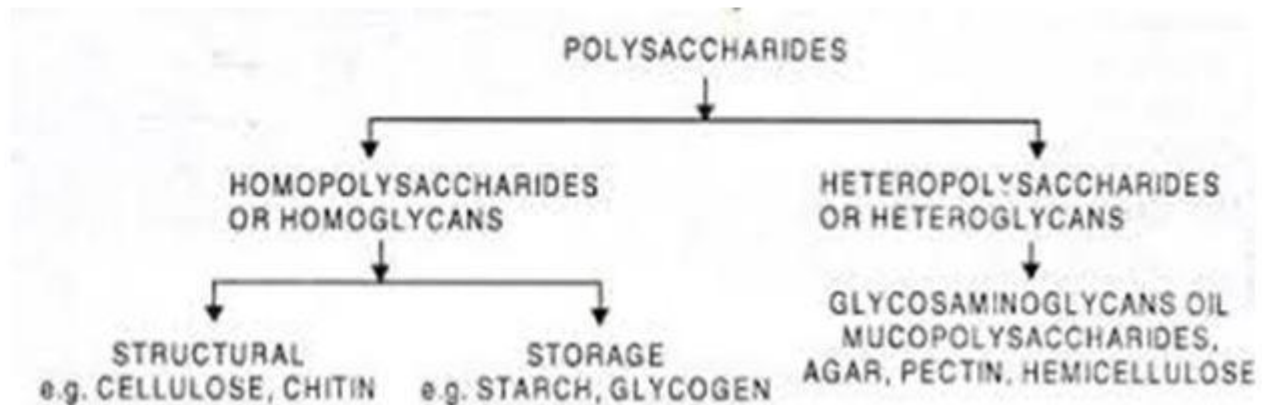

Sucrose
(α-D-Glucopyranosyl-(1→2)-β-D-fructofuranose


Lactose
(β-D-Galactopyranosyl-(1→4)-α-D-glucopyranose


Maltose
(α-D-Glucopyranosyl-(1→4)-α-D-glucopyranose

Polysaccharides

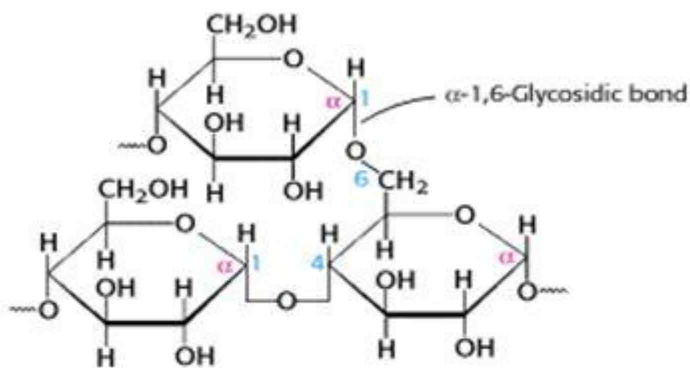
Multiple monosaccharides link to form large polymeric oligosaccharides called **polysaccharides,** which are also known as **glycans**. Polysaccharides are versatile in their functions. They are classified into two groups, **homopolysaccharides** (which contain only one type of monomeric unit) and **heteropolysaccharide** (which contain more than one or different types of monomeric units).



Homopolysaccharide

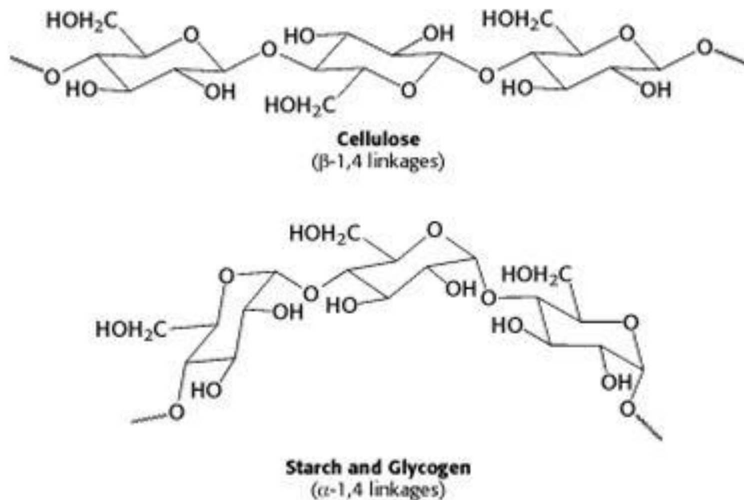
A branch of **D- glucose** units give rise to **starch**. Starch is a major storage form of glucose in plants. It contains **amylose and amylopectin**. **Amylopectin** is a branched structure consisting of **α- D- glucose** with **α1-4 glycosidic** linkages and **α1-6 branching points.** These branching points occur at approximate intervals of 25 to 30 α-D- glucose residues. **Amylose** is an unbranched linear polymer of **α-D- glucose** units with a repeat sequence of **α1-4 glycosidic** linkages. **Iodine test** is widely used to detect the **presence of starch**. The deep blue colour, which is formed in the presence of iodine is because of the presence of amylose in starch.

A major storage form of carbohydrate in animals is **glycogen**. It is found in liver and muscle. This large, branched polymer of glucose has **glucose** residues linked by **α-1,4- glycosidic** bonds. The **branches** present about **once in 10 units** are formed by **α-1,6- glycosidic** bonds.



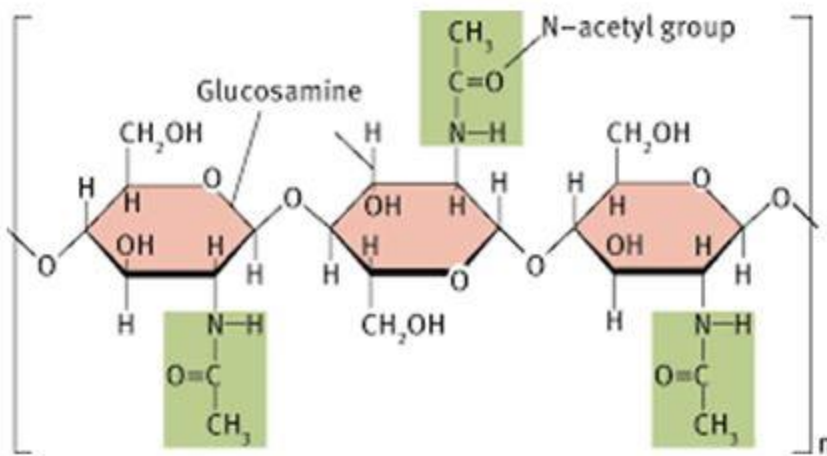
Branch point in glycogen

Another linear, unbranched homopolysaccharide of **D-glucose** is **cellulose**. The individual glucose residues in cellulose are joined by **β-1,4- glycosidic** linkages. It is important for maintaining **the structural integrity** of plant cells. Human enzyme systems are unable to hydrolyse cellulose. Cellulose is famous for being one of the most abundant organic compounds in the biosphere.



It is to be noted that straight chains are more favoured by β-1,4- linkages. They ae optimal for structural purposes whereas α-1, 4- linkages favor bent structure. The bent structures are much favourable for storage purposes.

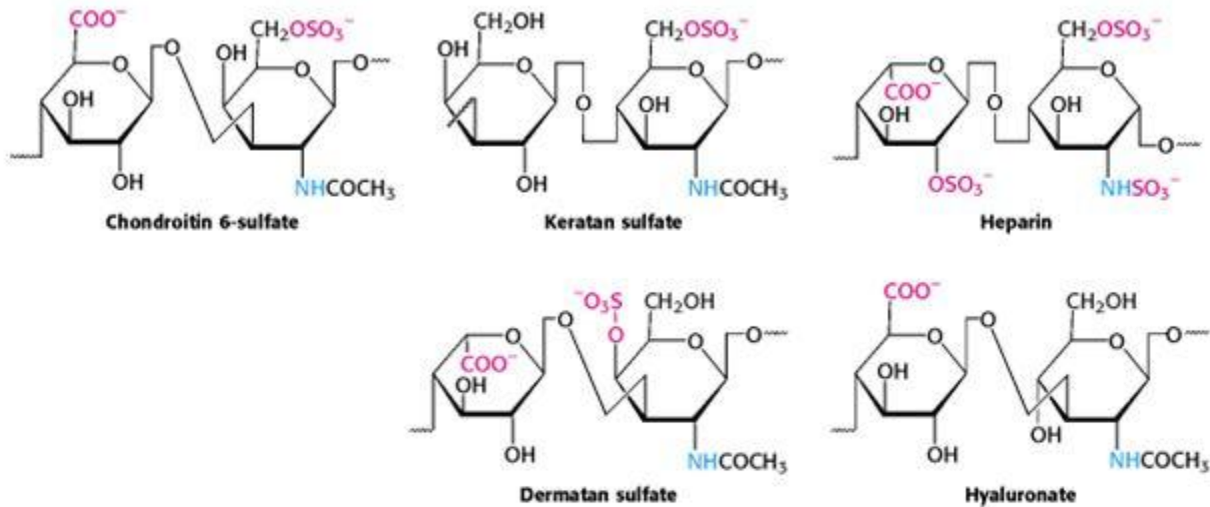
Chitin is yet another homopolysaccharide which is composed of **N- acetyl- D-glucosamine** residues. These residues are joined together by **β-1,4- glycosidic** linkage. It is critical for maintaing structural integrity in the exoskeleton of insects and crustaceans.



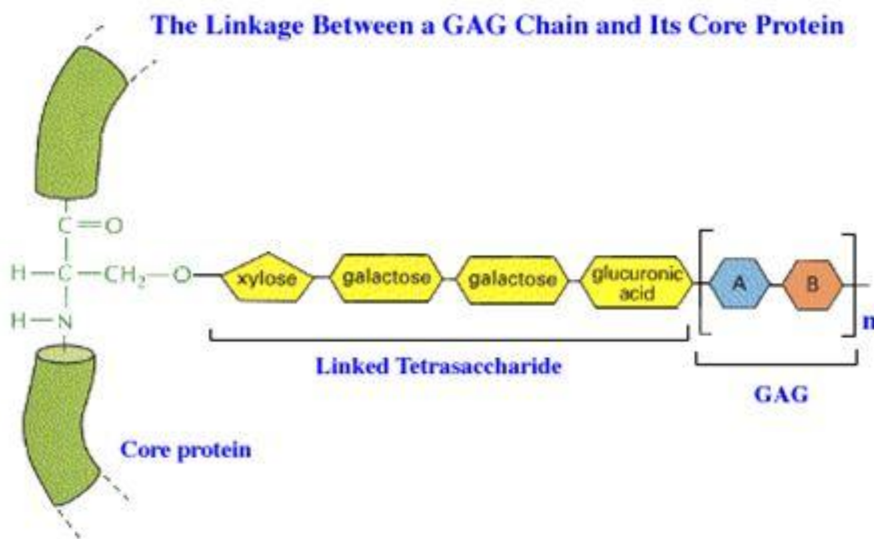
Heteropolysaccharides

Gylcosaminoglycans are **polysaccharides,** which are unbranched as well as negatively charged heteropolysaccharides. These heteropolysaccharides are composed of repeating dissacharide units, **[Acidic sugar- Amino sugar]$_n$.** **Amino sugars** in most of the cases is **either N-acetylglucosamine or N- acetylgalactosamine** and the **acidic sugar is uronic acid derivative**, mostly glucuronic acid.

One of the simplest heteropolysaccharide is **hyaluron or hyaluronic acid**. It contains alternating residues of **D- glucuronic acid and N- acetylglucosamine**. Other major glycosaminoglycans are chondroitin sulfate, keratin sulfate, heparin, heparan sulfate, dermatan sulfate and hyaluronate. These polysaccharides are unique in the sense that their presence is only limited to bacteria and animals.



Glycosaminoglycans usually link to proteins in order to yield proteoglycans except for hyaluronic acid. The site for **assembly of polysaccharides** is the **core protein in the golgi bodies**. A specific link tetrasaccharide is initially assembled on a serine residue. It is only after the assembly on serine residue that the GAG chain is synthesised with a single sugar residue being added at one time. The O-glycosidic bond formation takes place between Ser residue of protein and xylose sugar residue of the link tetrasaccharide.



Characteristics of GAGs

GAG	Localization	Comments
Hyaluronate	synovial fluid, vitreous humor, ECM of loose connective tissue	large polymers, shock absorbing
Chondroitin sulfate	cartilage, bone, heart valves	most abundant GAG
Heparan sulfate	basement membranes, components of cell surfaces	contains higher acetylated glucosamine than heparin
Heparin	component of intracellular granules of mast cells lining the arteries of the lungs, liver and skin	more sulfated than heparan sulfates
Dermatan sulfate	skin, blood vessels, heart valves	
Keratan sulfate	cornea, bone, cartilage aggregated with chondroitin sulfates	

Peptidoglycan or **murein** is present widely in the bacterial cell wall. It is a heteropolymer consisting of alternating **(β1-4)** linked **N- acetyl- glucosamine** (NAG) and **N- acetyl- muramic acid** (NAM) units. **Lysosyme** hydrolyses this linkage and henceforth degrades cell wall.

Class	Name	Source	Composition	Linkages
Structural polysaccharides	Cellulose	Plant cell walls	Glucose (beta linkage)	Unbranched 1→4
	Mannan	Yeast cell walls	Mannose (beta linkage)	Branched 1→2, 1→3, and 1→6
	Chitin	Arthropod shells, fungal cell walls	Acetylglucosamine and glucuronic acid (beta linkage)	Unbranched 1→4
	Hyaluronic acid	Synovial fluid (joints), subcutaneous tissue	Acetylglucosamine and glucuronic acid (beta linkage)	Unbranched 1→3 and 1→4
	Peptidoglycans	Bacterial cell walls	Acetylglucosamine and acetylmuramic acid	Unbranched 1→4
Nutrient polysaccharides	Inulin	Artichokes, dandelions	Fructose (beta linkage)	Unbranched 2→1
	Paramylum	Certain protozoa (e.g., *Euglena*)	Glucose (beta linkage)	Unbranched 1→3
	Glycogen	Certain protozoa (e.g., *Tetrahymena*) and most animals	Glucose (alpha linkage)	Branched 1→4 and 1→6
	Starch: Amylopectin	Plant cells and some protozoa (e.g., *Polytomella*)	Glucose (alpha linkage)	Branched 1→4 and 1→6
	Amylose		Glucose (alpha linkage)	Unbranched 1→4

Reducing And Non- Reducing Sugars

Reducing sugars are the ones which are capable of reducing **ferric or cupric ions**. Reducing sugars always have a **free aldehyde group** which enables them to act as the reducing agents. It is interesting to note that all the **monosaccharides** (aldoses or ketoses) in their hemiketal or hemiacetal form are **reducing sugars**. The **free anomeric carbon** of the dissacharide or polysaccharide chain, which is not involved in the glycosidic linkage, is usually referred to as **reducing end** of the chain.

Also, all **dissachrides** with the exceptions of sucrose and trehalose are **reducing** sugars. All the sugars which act as reducing agents undergo **mutarotation** in aqueous solution. Since **sucrose** and **trehalose** are not capable of reducing ferric or cupric ions, they are commonly referred to as **non- reducing sugars.** The two non- reducing sugars have anomeric carbon engaged in glycosidic bond and so, have no free reducing end.

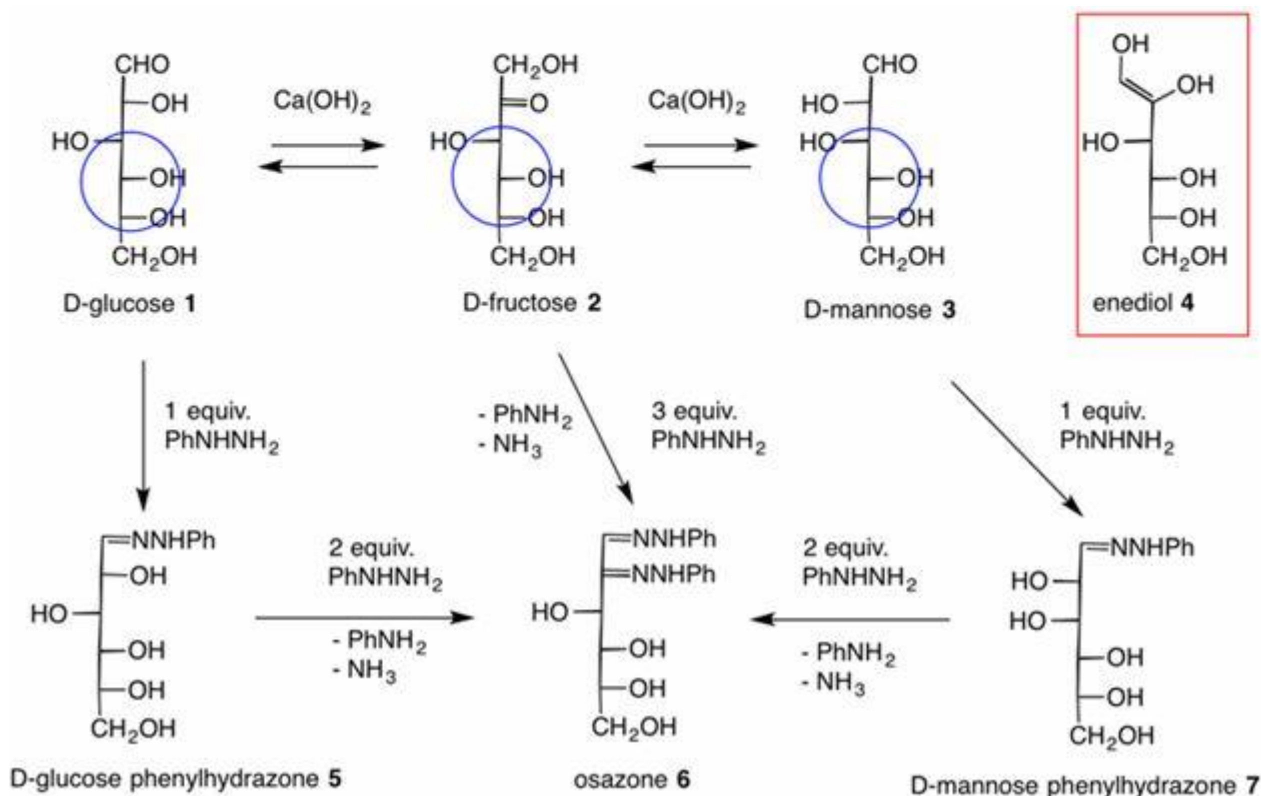
Osazone Formation

Famous German chemist Emil Fischer in 1875 prepared phenylhydrazine ($PhNHNH_2$) by the reduction of phenyldiazonium salt. This compound **phenylhydrazine** has been extensively popular for the study of **the stereochemistry of glucose**.

The **aldohexoses** namely, D-glucose **1** and D-mannose **3**, and the **D-ketohexose**, D-fructose **2 in the presence of calcium hydroxide are inter-convertible into one another. This reaction, which involves free carbonyl group (reducing end), takes place in the presence of excess phenylhydrazine when kept at boiling temperature,** does not alter the stereochemistry at C_3,

C_4, and C_5. Therefore, it can easily be said that **osazones** are nothing but **carbohydrate derivates** which are formed only when **sugars react with phenylhydrazine** (present in excess). Osazones are formed from all **reducing sugars.** Sucrose fails to form osazone crystals since it is a non-reducing sugar.

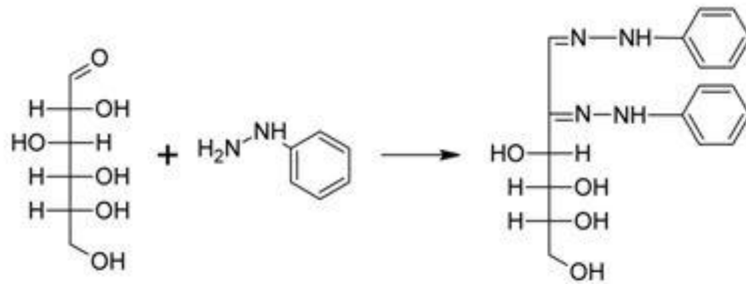
Accompanying the oxidation of hydroxymethyl group of alpha carbon (carbon atom next to the chiral carbon), a pair of phenylhydrazone group is also formed. Enolization occurs and leads to the formation of the intermediate in this process, enediol 4. The process is known as the Lobry de Bruyn-Alberda van Eckstein rearrangement.



Osazone formation is important because it helps in the **identification of monosaccharides**. This process occurs in two steps. Firstly, phenylhydrazine and glucose react with each other to yield **glucosephenylhydrazone** concomitant to the elimination of water molecule from the functional group. In the second step, one equivalent of glucosephenylhydrazone reacts with two equivalents of phenylhydrazine (present in excess). The first phenylhydrazine initially oxidises the **alpha carbon** to a carbonyl group and the second phenylhydrazine removes one water molecule with the newly- formed carbonyl group of previously oxidised carbon. This gives rise to a **carbon- nitrogen bond**. The alpha carbon involved in this reaction is much more reactive than the other carbon atoms.

Osazones can be easily detected because they are vibrant in colour and crystalline in nature. Each sugar forms a distinctive crystalline osazone form.

- Maltose forms petal-shaped crystals.
- Lactose forms powder puff-shaped crystals.
- Galactose forms rhombic-plate shaped crystals.
- Glucose, fructose and mannose form broomstick or needle-shaped crystals.



General reaction illustrating formation of glucose osazones.