CC5 - BIOCHEMISTRY AND BIOPHYSICS

Subject code :- 18KP2ZO5

UNIT - I.

Structure, properties, classification and function of carbohydrates, proteins and lipids.

STRUCTURE OF CARBOHYDRATES :-

Carbohydrates consists of carbon, hydrogen, and oxygen.

The general empirical structure for carbohydrate is $(CH_2O)_n$.

They are organic compounds organized in the form of aldehydes or ketones with multiple hydroxyl groups coming off the carbon chain.

The carbohydrate are a group of naturally occurring carbonyl compounds (aldehydes or ketones) that also contain serval hydroxyl groups.

It may also include their derivatives which produce such compounds on hydrolysis.

They are the most abundant organic molecules in nature and also referred to as "saccharides".

The carbohydrates which are soluble in water and sweet in taste are called sugars. The building block of all carbohydrates are simple sugars called monosaccharides.

A mono saccharide can be a poly hydroxy aldehyde (Aldose) or a poly hydroxy ketone (Ketose).

The Carbohydrates can be structurally represented in any of the three forms.

(1) Open chain structure
(2) Hemi - acetal Structure
(3) Haworth Structure.

Open chain Structure: It is the long straight chain form of carbohydrates.

Hemi acetal structure: Here the 1st carbon of the glucose condenses with the OH group of the 5th carbon to form a ring structure.

Haworth Structure: It is the presence of a the Pyranose ring structure.

PROPERTIES OF CARBOHYDRATES:

PHYSICAL PROPERTIES OF CARBOHYDRATES:

(1) Stereo isomerism.
(2) Optical activity
(3) Dia stereo isomers.
(4) Anno merism

CHEMICAL PROPERTIES OF CARBOHYDRATES:

(1) Osazone formation.
(2) Benedicts test
(3) Oxidation.
(4) Reduction to alcohols.

Stero isomerism: Compound having the same Structural formula but they differ in spatial configuration. example Glucose has two isomers with respect to the penultimate carbon atom.

They are D-glucose and L-glucose.

Optical Activity – It is the rotation of plane-polarized light forming (+) glucose and (-) glucose.

Diastereo isomers – It the configurational changes with regard to C_2, C_3, or C_4 in glucose.
Example :- mannose, galactose.

Anomerism :- It is the spatial configuration with respect to the first carbon atom in aldoses and second carbon atom in ketoses.

Chemical Properties of carbohydrates :-

(a) Osazone formation :
(b) Benedict's test.
(c) oxidation
(d) Reduction to alcohols.

(a) Osazone formation :-
Osazone are carbohydrate derivatives when sugars are reacted with an excess of phenylhydrazine. eg :- Glucosazone.

(b) Benedicts test :- Reducing sugars when heated in the presence of an alkali gets converted to powerful reducing species known as enediols.
When Benedicts reagent solution and reducing sugars are heated together, the solution changes its color to orange-red / brick red.

Oxidation: Monosaccharides are reducing sugars if their carbonyl groups oxidize to give Carboxylic acids.

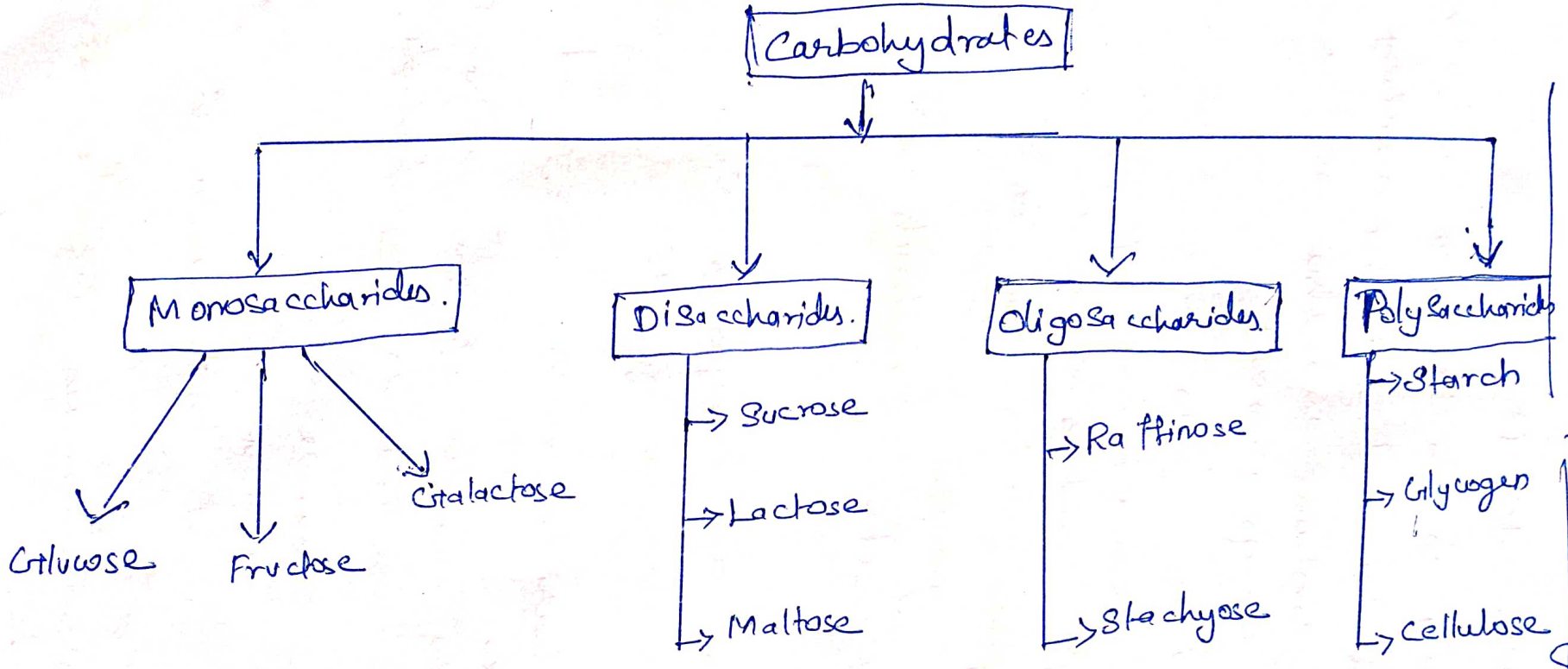
In Benedict's test, D-glucose is oxidized to D-gluconic acid thus, glucose is considered a reducing sugar.

Reduction to alcohols:

The C=O groups in open-chain forms of carbohydrates can be reduced to alcohols by sodium borohydride, $NaBH_4$, or catalytic hydrogenation (H_2, Ni, EtOH/H_2O). The products are known as "alditols".

Properties of Monosaccharides:

- Most Monosaccharides have a sweet taste (fructose is sweetest; 73% sweeter than sucrose).
- They are solids at room temperature.
- They are extremely soluble in water - Despite their high molecular weights, the presence of large numbers of OH groups make the monosaccharides much more water-soluble than most molecules of similar MW.
- Glucose can dissolve in minute amounts of water to make a syrup (1g/1ml H_2O)



Classification of Carbohydrates :- Types of Carbohydrates (5)

The simple carbohydrates include single sugar (monosaccharides) and polymers, oligosaccharides, and polysaccharides.

Monosaccharides:-

① Simplest group of carbohydrates and often called Simple sugar since they cannot be further hydrolyzed.

② Colorless, crystalline solid which are soluble in water and insoluble in a non-polar solvent.

(3) These are compound which possesses a free aldehyde or ketone group.

(4) the general formula is $C_n(CH_2O)_n$ or $C_nH_{2n}O_n$.

(5) They are classified according to the number of carbon atoms they contain and also on the basis of the functional group present.

(6) The monosaccharides thus with 3, 4, 5, 6, 7.... Carbons are called trioses, tetroses, pentoses, hexoses, heptoses, etc., and also as aldoses or Ketoses depending upon whether they contain aldehyde or ketone group.

Examples: Glucose, Fructose, Erythrulose, Ribulose

Oligosaccharides:-

(1) Oligosaccharides are compound sugars that yield 2 to 10 molecules of the same or different.

(2) The monosaccharide units are joined by glycosidic linkage.

(3) Based on the number of monosaccharides units, it is further classified as disaccharide, trisaccharide, tetrasaccharide etc.

(4) The general formula of disaccharides is $C_n(H_2O)_{n-1}$ and that of trisaccharides is $C_n(H_2O)_{n-2}$ and soon.

(5) Examples: Disaccharides include Sucrose, Lactose, maltose etc.

(6) Trisaccharides are Raffinose, Rabinose.

Polysaccharides:-

① They are also called as "glycans"

② Polysaccharides contain more than 10 monosaccharide units and can be hundreds of sugar units in length.

③ They yield more than 10 molecules of monosaccharides on hydrolysis.

④ They maybe homopolysaccharides containing monosaccharides of the same type or heteropoly-saccharides i.e., monosaccharides of different types.

⑤ Examples of Homopoly saccharides are Strach, glyogen, cellulose, peetin.

Hetero poly saccharides are Hyaluronic acid, Chondroitin.

FUNCTIONS OF CARBOHYDRATES:

① Living Organisms use carbohydrates as accessible energy to fuel cellular reaction. They are the most abundant dietary source of energy (4kcal/gram) for all living beings.

② carbohydrates along with being the chief- energy source, in many animals, are instant source of energy. Glucose is broken down by glycolysis / Kerb's cycle to yield ATP.

③ carbohydrates are intermediates in the biosynthesis of fats and proteins.

④ Formation of the structural framework est, RNA and DNA

(5) In animals, they are an important constituent of connective tissues.
(6) carbohydrates. Heat are rich in fiber content help to prevent constipation.
(7) Also, they help in the modulation of the Immune System.

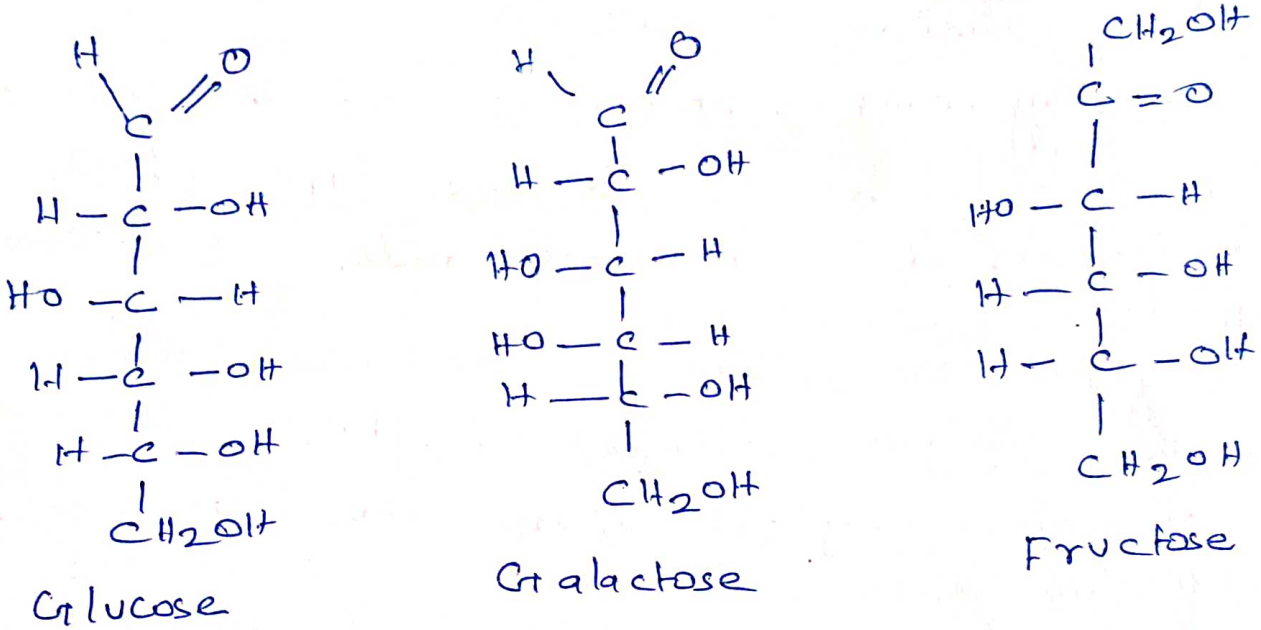


Fig: Carbohydrate isomers

STRUCTURE OF PROTEIN

Proteins are the important consitituents of protoplasm. Hence, Proteins are responsible for the structure and function of the cell.

Proteins are made up of one or more poly peptide chains. Each chain consists of many α amino acid residues covalently linked by peptide bonds.

Various order of protein structure:-

(a) Primary structure
(b) Secondary structure.
    (i). Ordered structure of polypeptides.
    (ii). Disordered or Random coil conformation of polypeptides.
    (iii) Super secondary structure.
(c) Tertiary structure.
(d) Quaternary structure.

Proteins are built from amino acids by liking them in linear fashion, it may be viewed as proteins having long chain like structure. However such an arrangement is unstable and polypeptide or protein folds to specific shape known as Conformation, which is more stable.

Various stages involved in the formation of final conformation from linear chain are divided into four levels or orders of protein structure. They are. Primary, Secondary, tertiary and Quaternary Structures

(a). Primary structure:-

The linear sequence of aminoacids residues in a polypeptide chain is called Primary structure. Amino acids are bonded with one another by a special type of bond called as peptide bond. But, th polypeptide chains of protein are linked with one another by disulphide bonds. Hence, bonds responsible for the maintenance of Primary structure are mainly peptide bonds and disulphide bonds. Both of them are covalent bonds.

B. Secondary Structure:

Folding of- polypeptide chain along its long axis is known an secondary structure of Protein. Folding of polypeptide chain can be ordered, disordered or random. The secondary structure is often referred to as conformation. So, protein has ordered Secondary structure or conformation and disordered or random Secondary structure or conformation.

① Ordered conformation of poly peptides.

The polypeptide chain of some proteins may exist in highly ordered conformation. The conformation is maintained by hydrogen bonds formed between peptide residues.

There are two types of ordered Secondary Structure observed in proteins.

(a) The polypeptide chain of α-keratin.

(b) The polypeptide chain of β-keratin.

(a) The polypeptide chain of α-keratin.

It is present in hair, nails, epidermis of the skin. The poly peptide chain of α-keratin is arranged as α-Helix.

In α-helix, poly peptide back bone is tightly coiled the long axis of the molecule. The distance between two amino acid residues is 1.5 Å.

α-helix present in most fibrous proteins is right handed. The right handed α-helix is more stable than the left-handed helix.

α-helix is hydrophobic in nature because of intra chain hydrogen bonds.

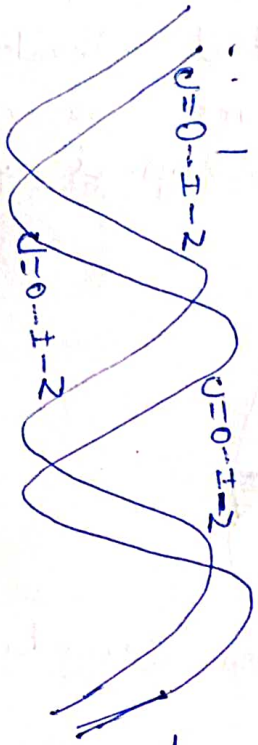


Fig:- Right handed α- helix.

b. The poly peptide chain of β-keratin.

It is present in silk fibroin and a spider web. It is arranged in β-pleated sheet, the poly peptide chain is fully extended.

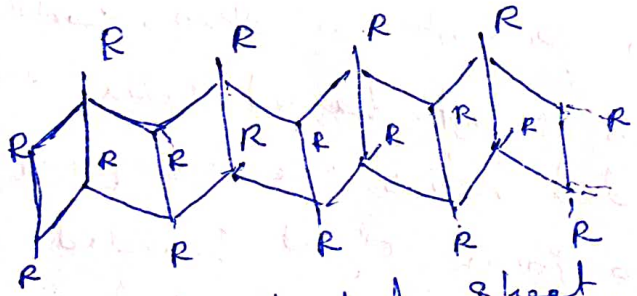


Fig:- β pleated sheet.

ii) Disordered or Random coil conformation:-

Regions of proteins that are not organized as helices and pleated sheet, are said to be present in a random coil conformation.

These are also equally important for the biological function of proteins as those of helices and β- pleated sheet.

iii) Super Secondary Structure:-

In some globular proteins, regions of α-helix and β-pleated sheet join to form Super Secondary structure or motifs. They are very important for biological function.

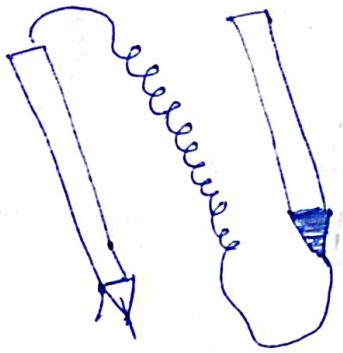


Fig:- Motif or Super Secondary Structure.

Super helix:-

α-Keratin consist of right-handed α-helix as the basic unit. Three such α-helices get cross linked by disulphide bonds and form super Secondary structure.

Triple helix:-

Collagen present in skin, cartilage, bone and tendons consists of left handed helix as basic unit. Three left handed helices are wrapped around each other to form right handed super Secondary structure triple helix.

c TERTIARY STRUCTURE:

The tertiary structure of protein is more complex than the secondary structure.

Tertiary structure is exhibited by proteins having only one polypeptide chain.

It is attained by globular proteins.

The folding is established by the appearance of more disulfide bond as well as hydrogen bonds, ionic bonds and hydrophobic bonds.

Myoglobin, ribonulease, chymotrypsin, cytochrome c etc. exist in tertiary structure. Ribonuclease is another globular protein with teritary structure.

It is made up of a single polypeptide chain containing 124 amino acids with 4 disulfide bonds.

D. QUATERNARY STRUCTURE

Two or more polypeptide chain associate together to produce a quaternary structure.

It is two types depending on the nature of the polypeptide chains.

① homogeneous quaternary structure.
② Hetero geneous " "

Homogeneous quaternary structure the polypeptide chains are identical. eg: Lactic acid

Heterogeneous quaternary structure the polypeptide chains are non-identical.
eg: keratin.

Properties of protein:

1. plysical State.

Most of the proteins are hydrophilic Colloids. A few proteins such as insulin, Tobacco mosaic virus, etc. are crystalline in nature.

(4) Colour:-
Proteins have no characteristic colour except chromoproteins.

(5) Taste and odour
A pure protein is tasteless and odourless.

(6) Viscosity proteins.
Proteins are highly viscous in nature. Generally fibrous proteins are more viscous (fibrinogen). than globular proteins (albumin).

(7) Molecular weight.
The molecular weight of proteins varies from 30,000 to a few million.

(8) Levo rotatory.
All the proteins are levorotatory. this property is due to the presence of α-amino acids, which are the building blocks of proteins.

FUNCTIONS OF PROTEINS:-

1. Enzyme catalysts. almost all chemical reactions in the biological system are catalyzed by enzymes.

2. Transport :- proteins transport ions and small molecules.
Haemoglobin, a conjugated protein of blood, transports oxygen.

3. Certain proteins function as a storage molecule.

(4) Nutrients – The egg contains ovalbumin.
The milk contains casein.

(5) Mechanical Support – Keratin, Fibroin.

(6) Immune protection – Antibodies are proteins, immunoglobulins.

(7) Blood clotting – fibrinogen and Thrombin.

(8) Transmission of Nerve impulse – acetylcholine.

(9) Gene Expression – repressor proteins.

(10) Hormonal Action – Insulin, growth hormone.

(11) Thermoregulation – The blood plasma of some Antarctic fish contains antifreeze protein, which protect the blood from freezing.

LIPIDS

Lipids are water insoluble oily or greasy organic compounds soluble in – non – polar organic solvents.

Chemically lipids are defined as the esters of alcohol and fatty acids.

STRUCTURE OF LIPIDS:

Lipids are esters of glycerol and fatty acids. They are formed by the combination of alcohol and fatty acids.

Usually a lipid is made up of a glycerol and three fatty acids. Such a lipid is called a triglyceride or a neutral fat.

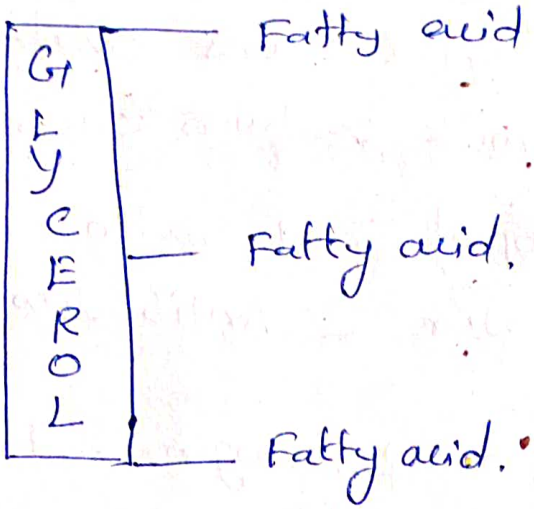


Fig:- A Simple Structure of a Simple Lipid.

When the fatty acids present in the Lipid are palmitic acid, the lipid is called tripalmitin.

Similarly, when the fatty acids present in the lipid are stearic acid the Lipid is called tristearin

When the fatty acids present in the Lipid are oleic acid, the lipid is called triolein

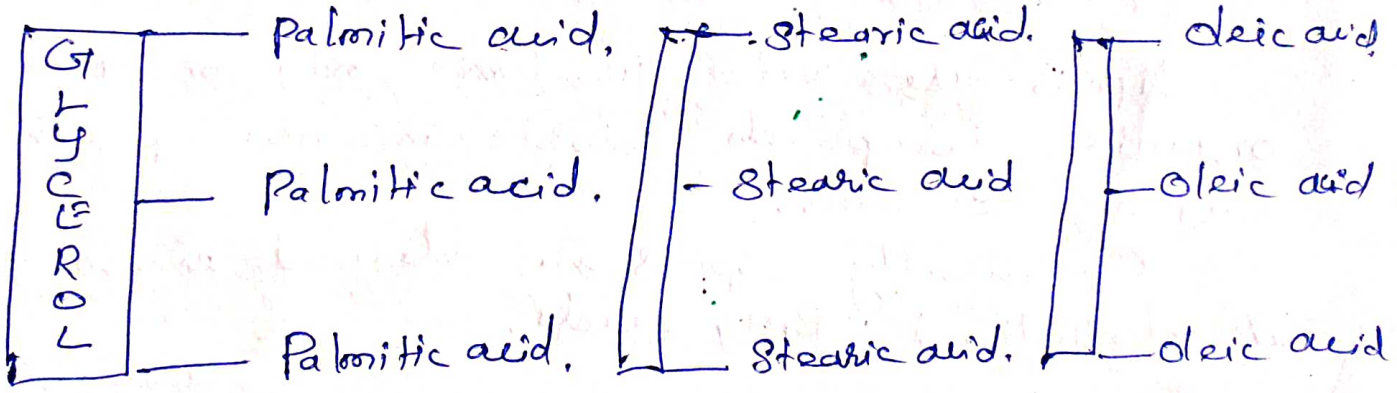


Fig:- Structure of Lipids.

CLASSIFICATION OF LIPIDS:-

Lipids are generally classified into three types. They are

1. Simple lipids or homolipids
2. Compound lipids or heterolipids.
3. Derived lipids.

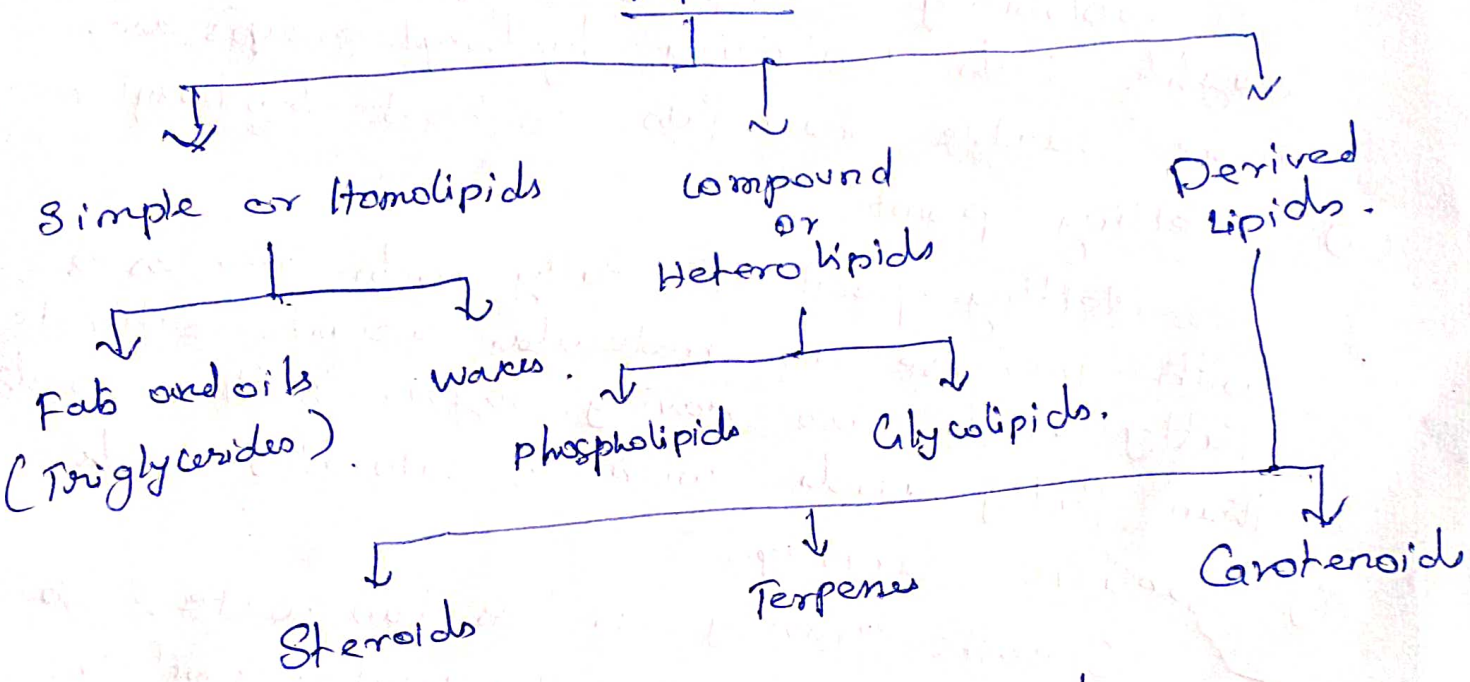


Fig:- Classification of lipids.

Properties of lipids:-

1. physical state:-
Fats containing saturated fatty acids and are solids. Animal fats are solids. Fats fat containing unsaturated fatty acids are liquids. plants fats are oil at room temperature.

2. Oily and Greasy.
Lipids are greasy to touch and they leave an oil impression on paper.

③ Colour:-
Pure fats are colourless and odourless.

④ Solubility.
Fats are sparingly soluble in water i.e. fats are hydrophobic.
They are highly soluble in organic solvents like alcohol, ether, etc.
Solubility decreases with increasing molecular weight. Fats containing hydroxyl groups are more soluble than fats without hydroxyl groups

⑤ Melting point.
Melting point of fatty acids increases with increase in molecular weight. Saturated fatty acids are having higher melting points than fatty acids with unsaturated bonds.

⑥ Specific Gravity:
Specific gravity is less than water. so they are floating on the water surface. Solid fats are lighter than liquid fats (oil)

⑦ Isomerism.
Due to the presence of double bonds in unsaturated fattyacids, geometrical isomerism (cis-trans) is possible.

All the best

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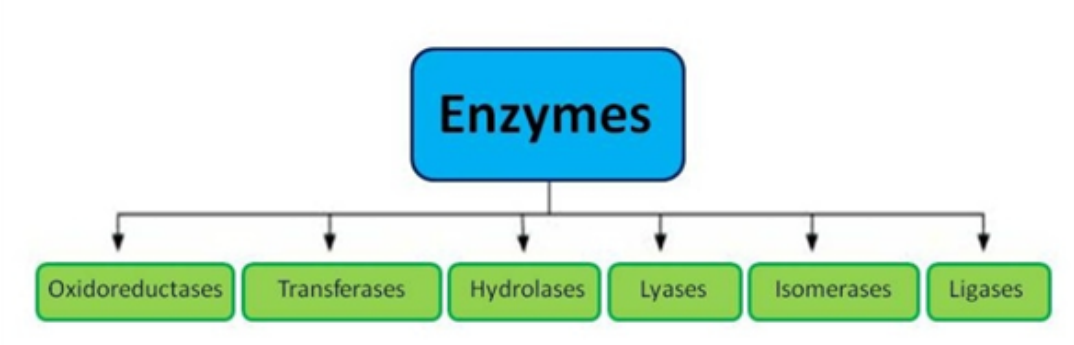
DEPARTMENT OF ZOOLOGY

UNIT II

Enzymes

Enzymes are proteins that act as biological catalysts. Catalysts accelerate chemical reactions. The molecules upon which enzymes may act are called substrates, and the enzyme converts the substrates into different molecules known as products.

Enzymes Classification



According to the International Union of Biochemists (I U B), enzymes are divided into six functional classes and are classified based on the type of reaction in which they are used to catalyze. The six kinds of enzymes are hydrolases, oxidoreductases, lyases, transferases, ligases and isomerases.

Listed below is the classification of enzymes discussed in detail:

Types	**Biochemical Property**
Oxidoreductases	The enzyme Oxidoreductase catalyzes the oxidation reaction where the electrons tend to travel from one form of a molecule to the other.

Transferases	The Transferases enzymes help in the transportation of the functional group among acceptors and donor molecules.
Hydrolases	Hydrolases are hydrolytic enzymes, which catalyze the hydrolysis reaction by adding water to cleave the bond and hydrolyze it.
Lyases	Adds water, carbon dioxide or ammonia across double bonds or eliminate these to create double bonds.
Isomerases	The Isomerases enzymes catalyze the structural shifts present in a molecule, thus causing the change in the shape of the molecule.
Ligases	The Ligases enzymes are known to charge the catalysis of a ligation process.

Oxidoreductases

These catalyze oxidation and reduction reactions, e.g. pyruvate dehydrogenase, catalysing the oxidation of pyruvate to acetyl coenzyme A.

Transferases

These catalyze transferring of the chemical group from one to another compound. An example is a transaminase, which transfers an amino group from one molecule to another.

Hydrolases

They catalyze the hydrolysis of a bond. For example, the enzyme pepsin hydrolyzes

peptide bonds in proteins.

Lyases

These catalyze the breakage of bonds without catalysis, e.g. aldolase (an enzyme in glycolysis) catalyzes the splitting of fructose-1, 6-bisphosphate to glyceraldehyde-3-phosphate and dihydroxyacetone phosphate.

Isomerases

They catalyze the formation of an isomer of a compound. Example: phosphoglucomutase catalyzes the conversion of glucose-1-phosphate to glucose-6-phosphate (phosphate group is transferred from one to another position in the same compound) in glycogenolysis (glycogen is converted to glucose for energy to be released quickly).

Ligases

Ligases catalyze the association of two molecules. For example, DNA ligase catalyzes the joining of two fragments of DNA by forming a phosphodiester bond.

Properties of enzymes:

(1) Enzymes are complex macromolecules with high molecular weight.

(2) They catalyze biochemical reactions in a cell. They help in the breakdown of large molecules into smaller molecules or bring together two smaller molecules to form a larger molecule.

(3) Enzymes do not start a reaction. However, they help in accelerating it.

(4) Enzymes affect the rate of biochemical reaction and not the direction of the reaction.

(5) Most of the enzymes have a high turnover number. Turnover number of an enzyme is the number of molecules of a substance that is acted upon by an enzyme per minute under saturated substrate concentration. High turnover number of enzymes increases the efficiency of the reaction.

(6) Enzymes are specific in action.

(7) Enzymatic activity decreases with increase in temperature and all enzymes show maximum activity at an optimum of 30-40oC.

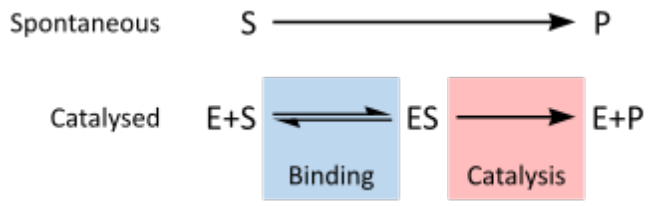
(8) They show maximum activity at an optimum pH of 6 – 8.

(9) The velocity of enzyme increases with increase in substrate concentration and then, ultimately reaches maximum velocity.

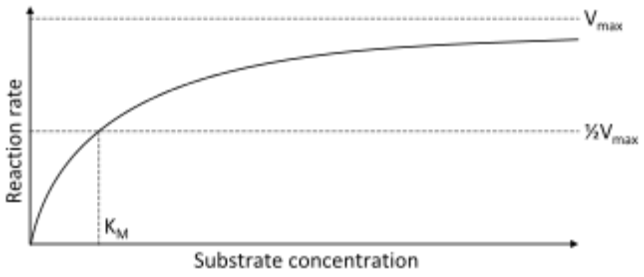
Enzyme kinetics

Enzyme kinetics is the study of the chemical reactions that are catalysed by enzymes. In enzyme kinetics, the reaction rate is measured and the effects of varying the conditions of the reaction are investigated.

Michaelis–Menten kinetics



A chemical reaction mechanism with or without enzyme catalysis. The enzyme (E) binds substrate (S) to produce product (P).

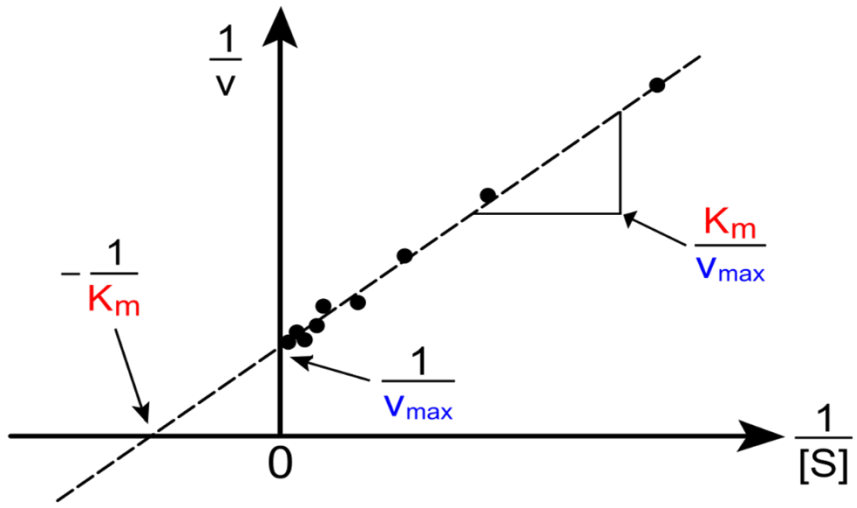


Saturation curve for an enzyme reaction showing the relation between the substrate concentration and reaction rate.

As enzyme-catalysed reactions are saturable, their rate of catalysis does not show a linear response to increasing substrate. If the initial rate of the reaction is measured over a range of substrate concentrations (denoted as [S]), the initial reaction rate increases as [S] increases, as shown on the right. However, as [S] gets higher, the enzyme becomes saturated with substrate and the initial rate reaches V_{max}, the enzyme's maximum rate.

Lineweaver–Burk plot

Lineweaver–Burk plot (or double reciprocal plot) is a graphical representation of the Lineweaver–Burk equation of enzyme kinetics, described by Hans Lineweaver and Dean Burk in 1934.



The plot provides a useful graphical method for analysis of the Michaelis–Menten equation, as it is difficult to determine precisely the V_{max} of an enzyme-catalysed reaction:

Taking the reciprocal gives:

$$\frac{1}{V_O} = \frac{K_m + (S)}{V_{max}(S)}$$

$$\frac{1}{V_O} = \frac{K_m}{V_{max}(S)} + \frac{(S)}{V_{max}(S)}$$

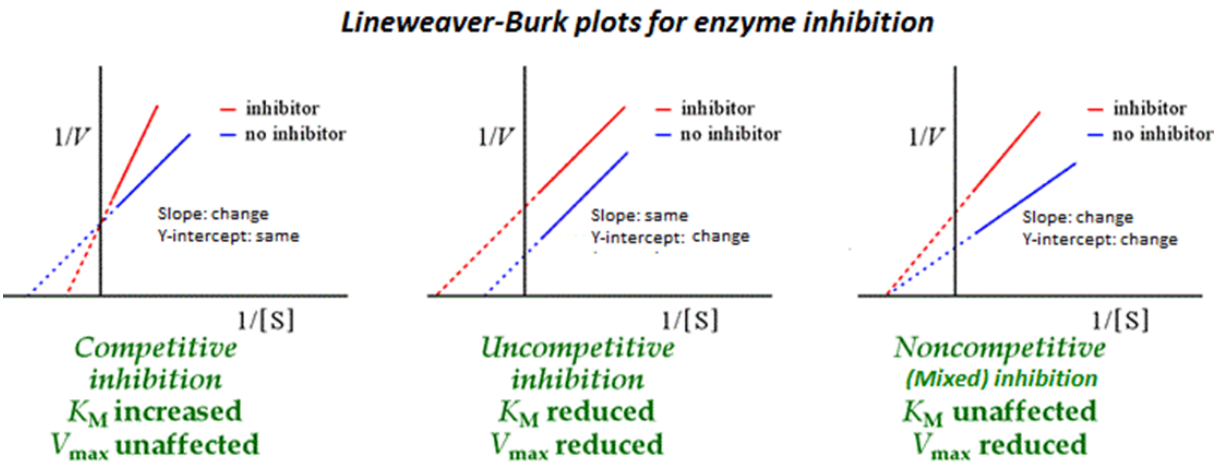
$$\frac{1}{V_O} = \frac{K_m}{V_{max}(S)} + \frac{1}{V_{max}}$$

where V is the reaction velocity (the reaction rate), K_m is the Michaelis–Menten constant, V_{max} is the maximum reaction velocity, and [S] is the substrate concentration.

The Lineweaver-Burk plot puts 1/[S] on the x-axis and 1/V on the y-axis

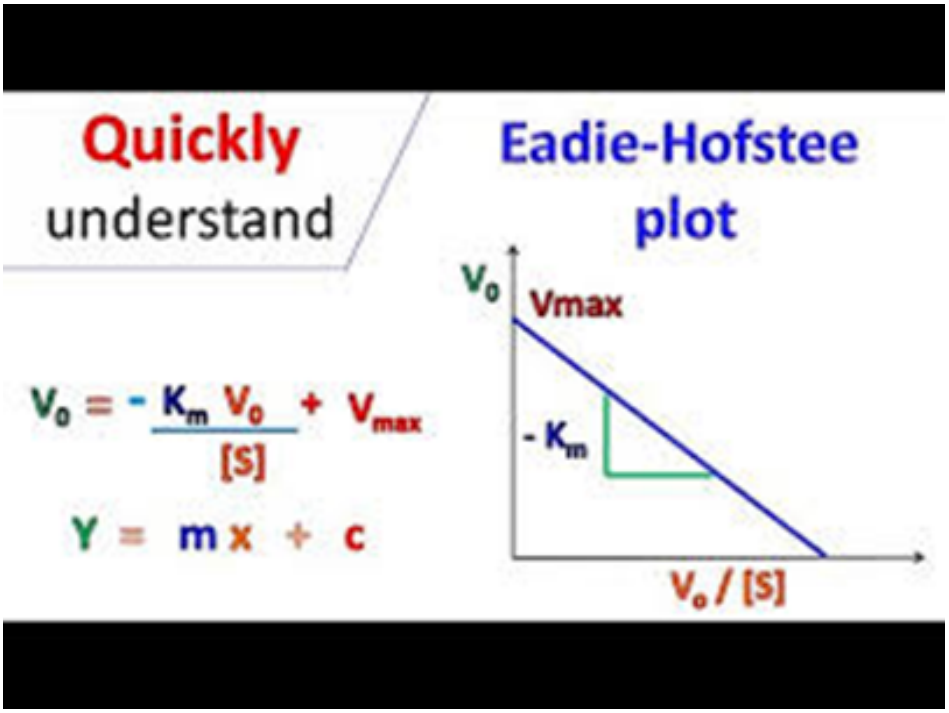
The Lineweaver–Burk plot was widely used to determine important terms in enzyme kinetics, such as K_m and V_{max}, before the wide availability of powerful computers and non-linear regression software. The *y*-intercept of such a graph is equivalent to the inverse of V_{max}; the *x*-intercept of the graph represents $-1/K_m$. It also gives a quick, visual impression of the different forms of enzyme inhibition.

When used for determining the type of enzyme inhibition, the Lineweaver–Burk plot can distinguish competitive, non-competitive and uncompetitive inhibitors. Competitive inhibitors have the same *y*-intercept as uninhibited enzyme (since V_{max} is unaffected by competitive inhibitors the inverse of V_{max} also doesn't change) but there are different slopes and *x*-intercepts between the two data sets. Non-competitive inhibition produces plots with the same *x*-intercept as uninhibited enzyme (K_m is unaffected) but different slopes and *y*-intercepts. Uncompetitive inhibition causes different intercepts on both the *y*- and *x*-axes.



Eadie-Hofstee plot

The Eadie-Hofstee plot is a more accurate linear plotting method with v is plotted against v/[S]. A plot of v against v/[S] will hence yield Vmax as the y-intercept, Vmax/Km as the x-intercept, and Km as the negative slope



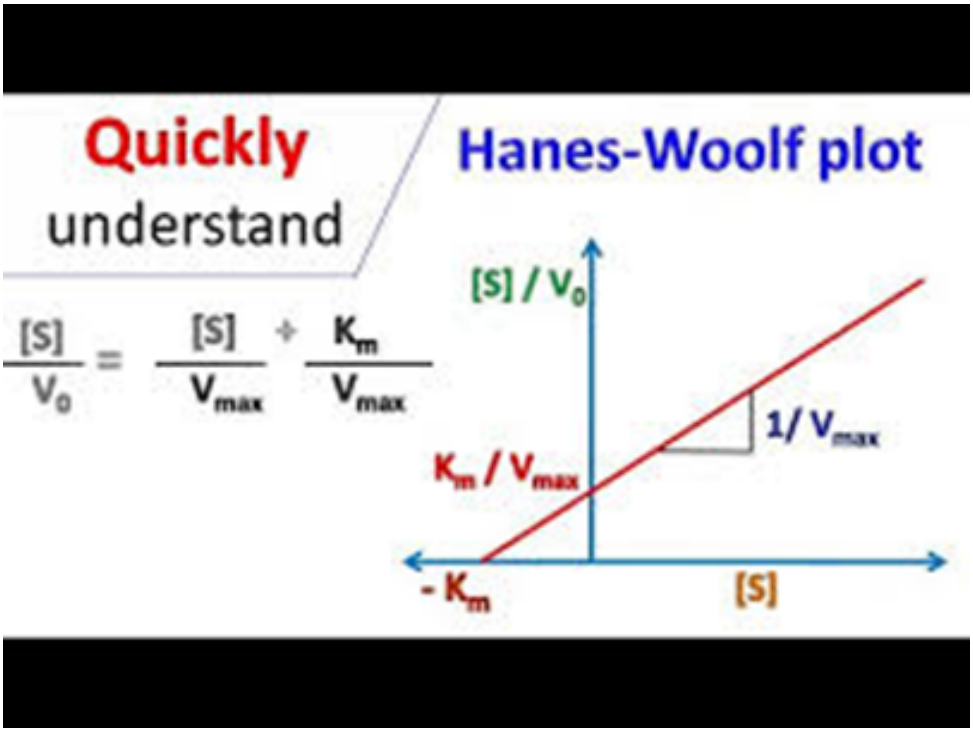
A plot of v against $v/[S]$ will hence yield V_{max} as the y-intercept, V_{max}/K_M as the x-intercept, and K_M as the negative slope.

Usage

Like other techniques that linearize the Michaelis–Menten equation, the Eadie–Hofstee plot was used historically for rapid identification of important kinetic terms like K_M and V_{max}, but has been superseded by nonlinear regression methods that are significantly more accurate and no longer computationally inaccessible. It is also more robust against error-prone data than the Lineweaver–Burk plot, particularly because it gives equal weight to data points in any range of substrate concentration or reaction rate. (The Lineweaver–Burk plot unevenly weights such points.) Both plots remain useful as a means to present data graphically.

Hanes–Woolf plot

Hanes–Woolf plot is a graphical representation of enzyme kinetics in which the ratio of the initial substrate concentration [S] to the reaction velocity v is plotted against [S]. It is based on the rearrangement of the Michaelis–Menten equation shown below:



As is clear from the equation, perfect data will yield a straight line of slope $1/V_{max}$, a *y*-intercept of K_m/V_{max} and an *x*-intercept of $-K_m$.

Like other techniques that linearize the Michaelis–Menten equation, the Hanes–Woolf plot was used historically for rapid determination of the important kinetic parameters K_m, V_{max} and V_{max}/K_m, but it has been superseded by nonlinear regression methods that are significantly more accurate and no longer computationally inaccessible. It remains useful, however, as a means to present data graphically.

Mechanism of enzyme action

An enzyme attracts substrates to its active site, catalyzes the chemical reaction by which products are formed, and then allows the products to dissociate (separate from the enzyme surface). The combination formed by an enzyme and its substrates is called

the enzyme–substrate complex.

"Lock and key" model

To explain the observed specificity of enzymes, in 1894 Emil Fischer proposed that both the enzyme and the substrate possess specific complementary geometric shapes that fit exactly into one another. This is often referred to as "the lock and key" model. This early model explains enzyme specificity, but fails to explain the stabilization of the transition state that enzymes achieve.

Induced fit model

In 1958, Daniel Koshland suggested a modification to the lock and key model: since enzymes are rather flexible structures, the active site is continuously reshaped by interactions with the substrate as the substrate interacts with the enzyme. As a result, the substrate does not simply bind to a rigid active site; the amino acid side-chains that make up the active site are molded into the precise positions that enable the enzyme to perform its catalytic function. In some cases, such as glycosidases, the substrate molecule also changes shape slightly as it enters the active site. The active site continues to change until the substrate is completely bound, at which point the final shape and charge distribution is determined. Induced fit may enhance the fidelity of molecular recognition in the presence of competition and noise via the conformational proofreading mechanism.

Coenzymes

Coenzymes are small organic molecules that can be loosely or tightly bound to an enzyme. Coenzymes transport chemical groups from one enzyme to another. Examples

include NADH, NADPH and adenosine triphosphate (ATP). Some coenzymes, such as flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD), thiamine pyrophosphate (TPP), and tetrahydrofolate (THF), are derived from vitamins. These coenzymes cannot be synthesized by the body *de novo* and closely related compounds (vitamins) must be acquired from the diet. The chemical groups carried include:

- the hydride ion (H^-), carried by NAD or $NADP^+$
- the phosphate group, carried by adenosine triphosphate
- the acetyl group, carried by coenzyme A
- formyl, methenyl or methyl groups, carried by folic acid and
- the methyl group, carried by S-adenosylmethionine

Since coenzymes are chemically changed as a consequence of enzyme action, it is useful to consider coenzymes to be a special class of substrates, or second substrates, which are common to many different enzymes. For example, about 1000 enzymes are known to use the coenzyme NADH.

Coenzymes are usually continuously regenerated and their concentrations maintained at a steady level inside the cell. For example, NADPH is regenerated through the pentose phosphate pathway and *S*-adenosylmethionine by methionine adenosyltransferase. This continuous regeneration means that small amounts of coenzymes can be used very intensively. For example, the human body turns over its own weight in ATP each day.

Allosteric enzymes

Allosteric sites are pockets on the enzyme, distinct from the active site, that bind to molecules in the cellular environment. These molecules then cause a change in the conformation or dynamics of the enzyme that is transduced to the active site and thus affects the reaction rate of the enzyme. In this way, allosteric interactions can either inhibit or activate enzymes. Allosteric interactions with metabolites upstream or downstream in an enzyme's metabolic pathway cause feedback regulation, altering the activity of the enzyme according to the flux through the rest of the pathway.

Enzyme inhibition

Enzyme reaction rates can be decreased by various types of enzyme inhibitors

Types of inhibition

Competitive

A competitive inhibitor and substrate cannot bind to the enzyme at the same time. Often competitive inhibitors strongly resemble the real substrate of the enzyme. For example, the drug methotrexate is a competitive inhibitor of the enzyme dihydrofolate reductase, which catalyzes the reduction of dihydrofolate to tetrahydrofolate. The similarity between the structures of dihydrofolate and this drug are shown in the accompanying figure. This type of inhibition can be overcome with high substrate concentration. In some cases, the inhibitor can bind to a site other than the binding-site of the usual substrate and exert an allosteric effect to change the shape

of the usual binding-site.

Non-competitive

A non-competitive inhibitor binds to a site other than where the substrate binds. The substrate still binds with its usual affinity and hence K_m remains the same. However the inhibitor reduces the catalytic efficiency of the enzyme so that V_{max} is reduced. In contrast to competitive inhibition, non-competitive inhibition cannot be overcome with high substrate concentration.

Uncompetitive

An uncompetitive inhibitor cannot bind to the free enzyme, only to the enzyme-substrate complex; hence, these types of inhibitors are most effective at high substrate concentration. In the presence of the inhibitor, the enzyme-substrate complex is inactive. This type of inhibition is rare.

Mixed

A mixed inhibitor binds to an allosteric site and the binding of the substrate and the inhibitor affect each other. The enzyme's function is reduced but not eliminated when bound to the inhibitor. This type of inhibitor does not follow the Michaelis–Menten equation.

Irreversible

An irreversible inhibitor permanently inactivates the enzyme, usually by forming a covalent bond to the protein. Penicillin and aspirin are common drugs that act in this manner.

Enzyme activators

Enzyme activators are molecules that bind to enzymes and increase their activity. They are the opposite of enzyme inhibitors. These molecules are often involved in the allosteric regulation of enzymes in the control of metabolism. An example of an enzyme activator working in this way is fructose 2,6-bisphosphate, which activates phosphofructokinase 1 and increases the rate of glycolysis in response to the hormone glucagon. In some cases, when a substrate binds to one catalytic subunit of an enzyme, this can trigger an increase in the substrate affinity as well as catalytic activity in the enzyme's other subunits, and thus the substrate acts as an activator.

Hexokinase-I

Hexokinase-I (HK-I) is an enzyme activator because it draws glucose into the glycolysis pathway. Its function is to phosphorylate glucose releasing glucose-6-phosphate (G6P) as the product. HK-I not only signals the activation of glucose into glycolysis but also maintains a low glucose concentration to facilitate glucose diffusion into the cell. It has two catalytic domains (N-terminal domain and C-terminal domain) which are connected through an α-helix. The N-terminal acts as an allosteric regulator of C-terminal; the C-terminal is the only one involved in the catalytic activity. HK-I is regulated by the concentration of G6P, where G6P acts as a feedback inhibitor. At low G6P concentration, HK-I is activated; at high G6P concentration, the HK-I is inhibited.

Glucokinase

Glucokinase (GK) is an enzyme that helps in the glycolytic pathway by phosphorylating glucose into glucose-6-phosphate (G6P). It is an isozyme of hexokinase and is found mainly in pancreatic β cells, but also liver, gut, and brain cells where glycolysis cause glucose-induced insulin secretion. Glucokinase activator lowers blood glucose concentrations by enhancing glucose uptake in the liver and increasing insulin production by the pancreatic β cells. Due to this, Glucokinase and glucokinase activators are the focus of treatment for those with type 2 diabetes mellitus. Glucokinase have a single allosteric site where the glucose-regulating protein (GKRP) binds in the nucleus of the cell in its inactive form when there is a low concentration of glucose present in the cell. However, when the glucose concentration of the cell increases the glucokinase-GKRP complex breaks apart and GK proceeds to the cytoplasm where it then phosphorylates glucose. Glucose when abundant in cells acts as an enzyme activator for glucokinase. Glucokinase activation in the β cells and liver cells results in the uptake of glucose and production of glycogen. This activation in the β cells leads to insulin secretion, promoting glucose uptake storing it as glycogen in the muscles.

Abzyme

An **abzyme** (from antibody and enzyme), also called *catmab* (from *catalytic monoclonal antibody*), and most often called *catalytic antibody*, is a monoclonal antibody with catalytic activity. Abzymes are usually raised in lab animals immunized against synthetic haptens, but some natural abzymes can be found in normal humans (anti-vasoactive intestinal peptide autoantibodies) and in patients with autoimmune diseases such as systemic lupus erythematosus, where they can bind to and

hydrolyze DNA. To date abzymes display only weak, modest catalytic activity and have not proved to be of any practical use.[1] They are, however, subjects of considerable academic interest. Studying them has yielded important insights into reaction mechanisms, enzyme structure and function, catalysis, and the immune system itself.

Enzymes function by lowering the activation energy of the transition state of a chemical reaction, thereby enabling the formation of an otherwise less-favorable molecular intermediate between the reactant(s) and the product(s). If an antibody is developed to bind to a molecule that is structurally and electronically similar to the transition state of a given chemical reaction, the developed antibody will bind to, and stabilize, the transition state, just like a natural enzyme, lowering the activation energy of the reaction, and thus catalyzing the reaction. By raising an antibody to bind to a stable transition-state analog, a new and unique type of enzyme is produced.

So far, all catalytic antibodies produced have displayed only modest, weak catalytic activity. The reasons for low catalytic activity for these molecules have been widely discussed. Possibilities indicate that factors beyond the binding site may play an important role, in particular through protein dynamics. Some abzymes have been engineered to use metal ions and other cofactors to improve their catalytic activity.

Isozymes

Isozymes (also known as **isoenzymes** or more generally as **multiple forms of enzymes**) are enzymes that differ in amino acid sequence but catalyze the same chemical reaction. These enzymes usually display different kinetic parameters (e.g. different K_M values), or different regulatory properties. The existence of isozymes

permits the fine-tuning of metabolism to meet the particular needs of a given tissue or developmental stage. In biochemistry, isozymes (or isoenzymes) are isoforms (closely related variants) of enzymes. In many cases, they are coded for by homologous genes that have diverged over time. Although, strictly speaking, allozymes represent enzymes from different alleles of the same gene, and isozymes represent enzymes from different genes that process or catalyse the same reaction, the two words are usually used interchangeably.

Allosteric enzymes

This type of enzymes presents two binding sites: the substrate of the enzyme and the effectors. Effectors are small molecules which modulate the enzyme activity; they function through reversible, non-covalent binding of a regulatory metabolite in the allosteric site (which is not the active site). When bound, these metabolites do not participate in catalysis directly, but they are still essential: they lead to conformational changes in a concrete part of the enzyme. These changes affect the overall conformation of the active site, causing modifications on the activity of the reaction.

Properties

Allosteric enzymes are generally larger in mass than other enzymes. Different from having a single subunit enzyme, in this case they are composed of multiple subunits, which contain active sites and regulatory molecule binding sites.

They present a special kinetics: the cooperation. In here, configuration changes in each chain of the protein strengthen changes in the other chains. These changes occur at the tertiary and quaternary levels of organisation.

Based on modulation, they can be classified in two different groups:

- *Homotropic allosteric enzymes*: substrate and effector play a part in the modulation of the enzyme, which affects the enzyme catalytic activity.

- *Heterotropic allosteric enzymes*: only the effector performs the role of modulation.

Questions

5 marks

1. Write the properties of enzymes
2. Give a note on active sites
3. Briefly describe the Lineweaver–Burk plot
4. Comment on abzymes

10 marks

1. Describe the classification of enzymes with examples
2. Explain the mechanism of enzyme action