Name of Unit	Enzymes
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Class: B.Pharm. Semester	II
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Learning Outcome of Module 05

LO	Learning Outcome	Course Outcome Code
LO1	To understand the concept of Enzymes.	BP203.3
LO2	To understand the knowledge about Enzyme inhibition.	BP203.3
LO3	To give knowledge about diagnostic and therapeutic applications of Enzyme.	BP203.3

Content Table

Topic
• Introduction, properties, nomenclature and IUB classification of enzymes. • Enzyme kinetics (Michaelis plot, Line Weaver Burke plot), Enzyme inhibitors with examples. • Regulation of enzymes: enzyme induction and repression, allosteric enzymes regulation. • Therapeutic and diagnostic applications of enzymes and isoenzymes. • Coenzymes- Structure and biochemical functions.

ENZYMES

Enzymes are biological catalysts (also known as biocatalysts) that speed up biochemical reactions in living organisms. They can also be extracted from cells and then used to catalyse a wide range of commercially important processes. For example, they have important roles in the production of sweetening agents and the modification of antibiotics, they are used in washing powders and various cleaning products, and they play a key role in analytical devices and assays that have clinical, forensic and environmental applications.

The word 'enzyme' was first used by the German physiologist Wilhelm Kühne in 1878, when he was describing the ability of yeast to produce alcohol from sugars, and it is derived from the Greek words en (meaning 'within') and zume (meaning 'yeast').

Enzymes are catalysts, increase reaction rates without being used up. Most enzymes are globular proteins However, some RNA (ribozyme and ribosomal RNA) also catalyse reactions. In 1980s it was found that some ribonucleic acid (RNA) molecules are also able to exert catalytic effects. These RNAs, which arecalled ribozymes, play an important role in gene expression.

In the same decade, biochemists also developed the technology to generate antibodies that possess catalytic properties. These so-called 'abzymes' have significant potential both as novel industrial catalysts and in therapeutics. Notwithstanding these notable exceptions, much of classical enzymology, and the remainder of this essay, is focused on the proteins that possess catalytic activity. As catalysts, enzymes are only required in very low concentrations, and they speed up reactions without themselves being consumed during the reaction.

We usually describe enzymes as being capable of catalysing the conversion ofsubstrate molecules into product molecules as follows:

$$\text{Substrate} \underset{}{\overset{\text{Enzyme}}{\rightleftharpoons}} \text{Product}$$

Enzymes are potent catalysts. The enormous catalytic activity of enzymes can perhaps best be expressed by a constant, kcat that is variously referred to as the turnover rate, turnover frequency or turnover number. This constant represents the number of substrate

molecules that can be converted to product by a single enzyme molecule per unit time (usually per minute or per second). For example, a single molecule of carbonic anhydrase can catalyse the conversion of over half a million molecules of its substrates, carbon dioxide and water, into the product, bicarbonate, every second- a truly remarkable achievement.

NOMENCLATURE AND CLASSIFICATION

In the early days, the enzymes were given names by their discoverers in an arbitrary manner. For example, the names pepsin, trypsin and chymotrypsin convey no information about the function of the enzyme or the nature of the substrate on which they act. Sometimes, the suffix-ase was added to the substrate for naming the enzymes e.g. lipase acts on lipids; nuclease on nucleic acids; lactase on lactose. These are known as trivial names of the enzymes which, however, fail to give complete information of enzyme reaction (type of reaction, cofactor requirement etc.)

Enzymes are sometimes considered under two broad categories:

(a) Intracellular enzymes - They are functional within cells where they are synthesized.

(b) Extracellular enzymes - These enzymes are active outside the cell; all the digestive enzymes belong to this group.

The International Union of Biochemistry (IUB) appointed an Enzyme Commission in 1961. This committee made a thorough study of the existing enzymes and devised some basic principles for the classification and nomenclature of enzymes. Since 1964, the IUB system of enzyme classification has been in force. Enzymes are divided into six major classes (in that order). Each class on its own represents the general type of reaction brought about by the enzymes of that class. The word OTHLIL (first letter in each class) may be memorized to remember the six classes of enzymes in the correct orders.

CLASS	DESIGNATION	FUNCTION
EC1	Oxidoreductases	catalyze oxidation/reduction reactions
EC2	Transferases	transfer a functional group (e.g. a methyl or phosphate group)
EC3	Hydrolases	catalyze the hydrolysis of various bonds
EC4	Lyases	cleave various bonds by means other than hydrolysis and oxidation
EC5	Isomerases	catalyze isomerization changes within a single molecule
EC6	Ligases	join two molecules covalent bonds.

1. **Oxidoreductases:** Enzymes involved in oxidation-reduction reactions.
2. **Transferases:** Enzymes that catalyse the transfer of functional groups.
3. **Hydrolases:** Enzymes that bring about hydrolysis of various compounds.
4. **Lyases:** Enzymes specialized in the addition or removal of water ammonia, CO_2 etc.
5. **lsomerases:** Enzymes involved isomerization reactions.
6. **Ligases:** Enzymes catalysing the synthetic: reactions (greek : ligate-to bind) where two molecules are joined together and ATP is used.

CHEMICAL NATURE AND PROPERTIES OF ENZYMES

All the enzymes are invariably proteins. In recent years, however, a few RNA molecules have been shown to function as enzymes. Each enzyme has its own tertiary structure and specific conformation which is very essential for its catalytic activity. The functional unit of the enzyme is known as holoenzyme which is often made up of apoenzyme (the protein part) and a coenzyme (non- protein organic part).

Holoenzyme ⟶ Apoenzyme + Coenzyme

(active enzyme) (protein part) (non-protein part)

The term prosthetic group is used when the non-protein moiety tightly (covalently) binds

with the apoenzyme. The coenzyme can be separated by dialysis from the enzyme while the prosthetic group cannot be.

Cofactors – either one or more inorganic ions

Coenzymes – complex organic or metalloorganic molecules, some enzymes require both

Prosthetic group – a coenzyme or cofactor that is very tightly (or covalently)bound to an enzyme

Holoenzyme– a complete active enzyme with its bound cofactor or coenzyme

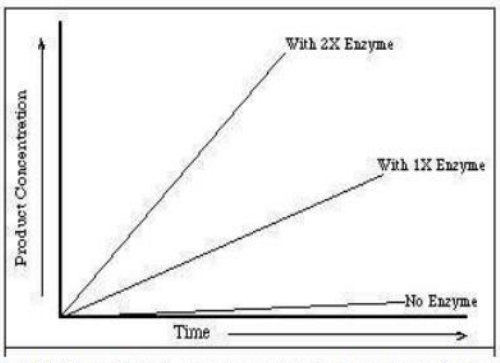
Apoprotein– the protein part of a holoenzyme

FACTORS AFFECTING ENZYME FUNCTION

- Enzyme concentration
- Substrate concentration
- Temperature
- pH
- Inhibitors
- Activators
- Effect of time
- Effect of light and radiation

1. Enzyme Concentration

In order to study the effect of increasing the enzyme concentration upon the reaction rate, the substrate must be present in an excess amount; i.e., the reaction must be independent of the substrate concentration. Any change in the amount of product formed over a



Fig# 01 'Zero Order' reaction rate is independent of substrate concentration

specified period of time will be dependent upon the level of enzyme present. Graphically this can be represented as:

These reactions are said to be "zero order" because the rates are independent of substrate concentration, and are equal to some constant k. The formation of product proceeds at a rate which is linear with time. The addition of more substrate does not serve to increase the rate. In zero order kinetics, allowing the assay to run for double time results in double the amount of product. The amount of enzyme present in reaction is measured by the activity it catalyzes. The relationship between activity and concentration is affected by many factors such as temperature, pH, etc

2. Concentration of substrate

Increase in the substrate concentration gradually increases the velocity of enzyme

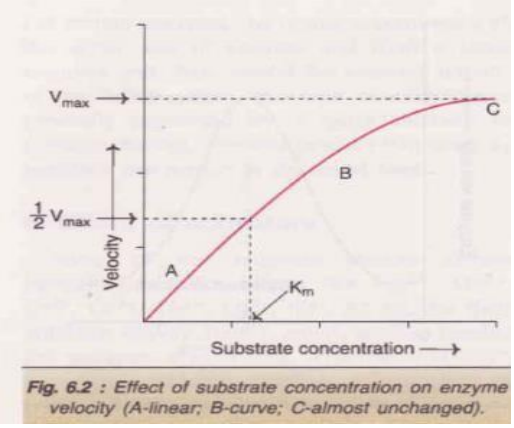


Fig. 6.2 : Effect of substrate concentration on enzyme velocity (A-linear; B-curve; C-almost unchanged).

reaction within the limited range of substrate levels. Three distinct phases of the reaction are observed in the graph (A-linear; B-curve; C-almost unchanged).

3. Effect of temperature: Velocity of an enzyme reaction increases with increase in temperature up to a maximum and then declines. A bell-shaped curve is usually observed Ternperature coefficient or Q_{1o} is defined as increase in enzyme velocity when the temperature is increased by 10"C. For a majority of enzymes, Q_{lo} is 2 between 0^0C and 40^oC. lncrease in temperature results in higher activation energy of the molecules and more molecular (enzyme and substrate) collision and interaction for the reaction to Proceed faster.

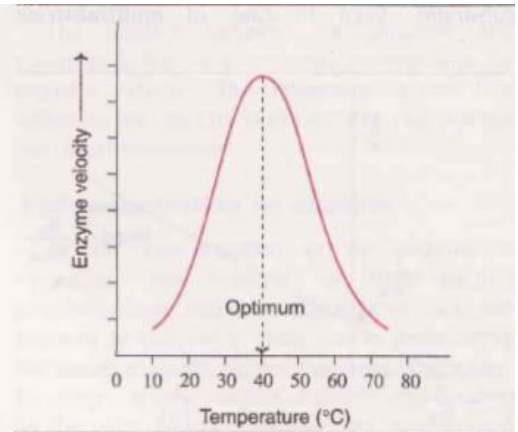


Fig. 6.4 : Effect of temperature on enzyme velocity.

4. **Effect of pH**: lncrease in the hydrogen ion concentration (pH) considerably influences the enzyme activity and a bell-shaped curve is normally obtained. Each enzyme has an optimum pH at which the velocity is maximum. Below and above this pH, the enzyme activity is much lower and at extreme pH, the enzyme becomes totally inactive.

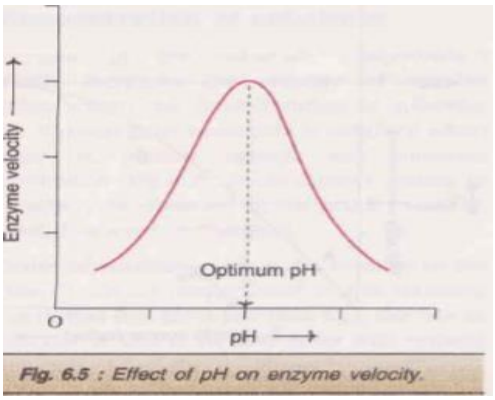


Fig. 6.5 : Effect of pH on enzyme velocity.

5. **Effect of product concentration:** The accumulation of reaction products generally decreases the enzyme velocity. For certain enzymes, the products combine with the active site of enzyme and form a loose complex and, thus, inhibit the enzyme activity. In the living system, this type of inhibition is generally prevented by a quick removal of products formed.

6. **Effect of activators:** Some of the enzymes require certain inorganic metallic cations like Mg^{2+}, Mn^{2+}, Zn^{2+}, Ca^{2+}, Na^{+}, K^{+} etc" for their optimum activity". Rarely, anions

are also needed for enzyme activity e.g. chloride ion for amylase. Metals function as activators of enzyme velocity through various mechanisms combining with the substrate, formation of ES- metal complex, direct participation in the reaction and bringing a conformational change in the enzyme.

Metal-activated enzymes: The metal is not tightly held by the enzyme and can be exchanged easily with other ions e.g. ATPase (Mg_2* and Ca_2*) Enolase (Mg_2*)"

Metalloenzymes: These enzymes hold the metals rather tightly which are not readily exchanged. e.g. alcohol dehydrogenase, carbonic anhydrase, alkaline phosphatase, carboxypeptidase and aldolase contain zinc. Phenol oxidase (copper); Pyruvate oxidase (manganese); Xanthine oxidase (molybdenum)

7. **Effect of time:** Under ideal and optimal conditions (like pH, temperature etc.), the time required for an enzyme reaction is less. Variations in the time of the reaction are generally related to the alterations in pH and temperature.

8. **Effect of light and radiation:** Exposure of enzymes to ultraviolet, beta, gamma and X-rays inactivates certain enzyrnes due to the formation of peroxides. e.g. UV rays inhibit salivary amylase activity.

MODE OF ENZYME ACTION

Two theories have been put forth to explain mechanism of enzyme-substrate complex formation

1. Lock and key model/ Fischer's template Theory
2. Induced fit theory/Koshland's mode

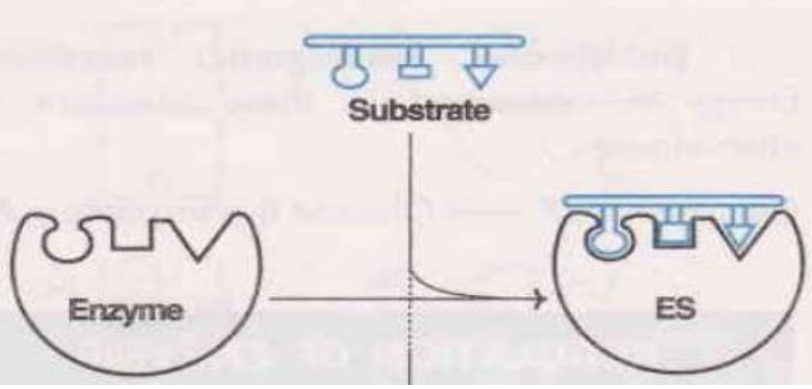
1. Lock and key model/ Fischer's template Theory: Proposed by a Emil Fischer. Very first model proposed to explain an enzyme catalyzed reaction. According to this model, the structure or conformation of the enzyme is rigid. The substrate fits to the binding site just as a key fits into the proper lock or a hand into the proper glove. Thus the active site of an enzyme is a rigid and pre- shaped template where only a Specific substrate can bind.

This model was not accepted because

A. Does not give any scope for the flexible nature of enzymes

B. Totally fails to explain many facts of enzymatic reactions

C. Does not explain the effect of allosteric modulator.



2. **Induced fit theory/Koshland's model:** Koshland proposed this model. The active site is not rigid and pre-shaped. The interaction of the substrate with the enzyme induces a fit or a conformation change in the enzyme, resulting in the formation of a strong substrate binding site. Furthermore the appropriate amino acids of the enzyme are repositioned to form the active site and bring about the catalysis.

This model was accepted because:

A. Has sufficient experimental evidence from the X-ray diffraction studies.

B. This model also explains the action of allosteric modulators and competitive inhibition on enzymes.

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. Studying an enzyme's kinetics in this way can reveal the

catalytic mechanism of enzyme.

Enzymes are usually protein molecules that manipulate other molecules the enzymes' substrates. These target molecules bind to an enzyme's active site and are transformed into products through a series of steps known as the enzymatic mechanism.

- These mechanisms can be divided into single substrate and multiple- substrate mechanisms.
- Kinetic studies on enzymes that only bind one substrate, such as triosephosphate isomerase, aim to measure the affinity with which the enzyme binds the substrate and the turnover rate.

MICHAELIS-MENTEN HYPOTHESIS

Leonor Michaelis and Maud L. Menten (1913), while studying the hydrolysis of sucrose catalyzed by the enzyme invertase, proposed this theory. According to this theory

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

From the above equation theoretically one can explain the kinetics of theenzyme reaction, but practically not. For this reason Michealis and Menten proposed an equation. From that equation, these immeasurable quantities were replaced by those which could be easily measured experimentally. Following symbols may be used for deriving Michaelis Menten equation:

(Et) = total concentration of enzyme

(S) = total concentration of substrate

(ES) = concentration of enzyme-substrate complex (Et)

(ES) = concentration of free enzyme.

Derivation of the equation: The rate of appearance of products (i.e., velocity,V) is proportional to the concentration of the enzyme substrate complex.

$$V \alpha ES$$

$$V = k\,(ES) \quad \text{---------- (1)}$$

The maximum reaction rate, Vm will occur at a point where the total enzyme Etis bound to the substrate.

$$Vm \propto Et$$

$$Vm = k\,(Et)$$

(2)Dividing equation (1) by (2,) we get :

$$\frac{V}{Vm} = \frac{k\,(ES)}{k\,(Et)} \quad \text{---------- (3)}$$

Now coming back to the reversible reaction,

$$E + S \longrightarrow ES$$

one can write the equilibrium constant for dissociation of ES as Km which isequal to :

$$K_m = \frac{(E_t) - (ES) \times (S)}{(ES)}$$

$$(ES) \times K_m = (E_t) \times (S) - (ES) \times (S) \quad \text{---------- (4)}$$

$$(ES) \times K_m + (ES) \times (S) = (E_t) \times (S)$$

$$(ES) \times [K_m + (S)] = (E_t) \times (S)$$

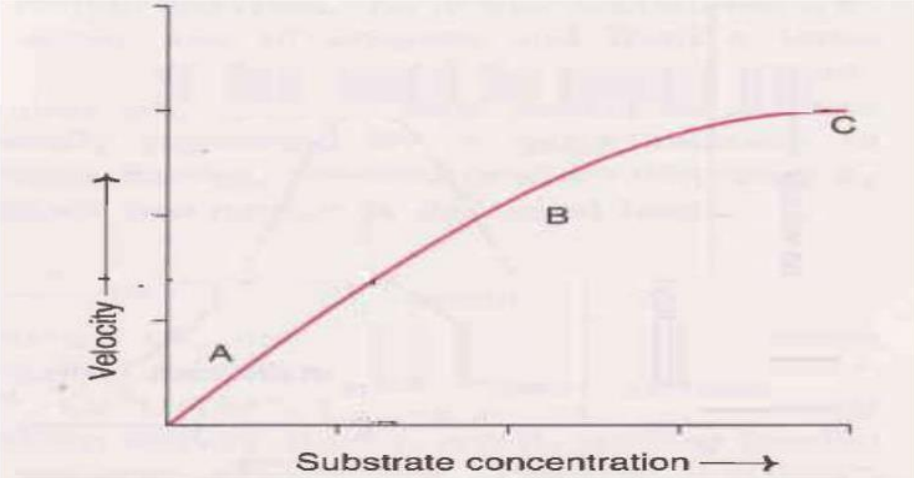
Substituting the value of $\frac{(ES)}{(E_t)}$ from equation (3) to equation (5), we get :

$$\frac{V}{V_m} = \frac{S}{K_m + (S)}$$

$$V = \frac{V_m \times (S)}{K_m + (S)}$$

Michaelis-Menten equation

Michaelis-Menten plot



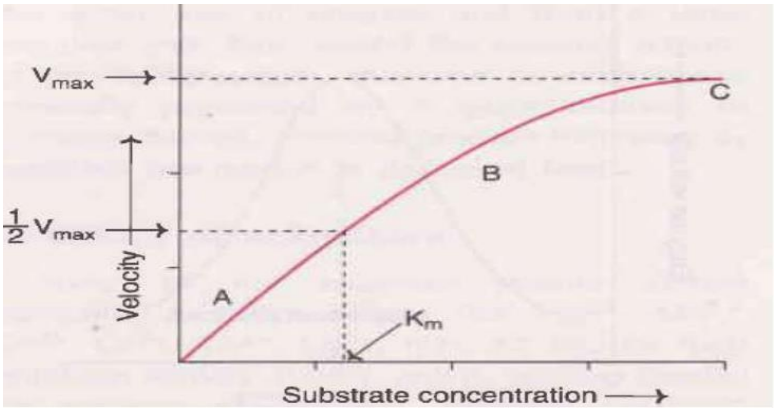
This plot is used to determine the Vm and Km value of the enzyme

Determination of Vm and Km value

When V =

½Vm

Km = S



Significance of Vm and Km value

- Km or Michaelis-Menten constant is defined as the substrate concentration (expressed in moles/l) to produce half-maximum velocity in an enzyme catalysed reaction.
- The Km values of the enzymes differ greatly from one to other, but it is a characteristic feature of a particular enzyme.

- for most of the enzymes, the general range is between 10^{-1} and 10^{-6} M
- The Km value depends on the particular substrate and on the environmental conditions such as temperature and ionic concentration. But it is not dependent on the concentration of enzyme.
- Km is a measure of the strength of ES complex. The high Km value indicates weak binding whereas the low Km value signifies strong binding.
- The maximal rate (Vm) represents the turnover number of an enzyme, if the concentration of the active sites (Et) is known.

LINE WEAVER-BURKE PLOT

The Line weaver–Burke plot is a graphical representation of the Line weaver– Burke equation of enzyme kinetics, described by Hans Line weaver and Dean Burke in 1934. The plot provides a useful graphical method for analysis of the Michaelis-Menten

$$V = \frac{V_{max}[S]}{K_m + [S]}$$

equation:

Taking the reciprocal gives

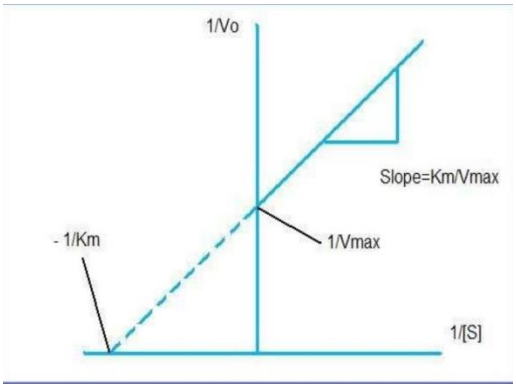
$$\frac{1}{V} = \frac{K_m + [S]}{V_{max}[S]} = \frac{K_m}{V_{max}}\frac{1}{[S]} + \frac{1}{V_{max}}$$

Km is the Michaelis—Menten constant

[S] is the substrate concentration

V is the reaction velocity (the reaction rate)

V_{max} the maximum reaction velocity



Apply this to equation for a straight line and we have:

$$y = \frac{1}{v}$$

$$x = \frac{1}{[S]}$$

$$m = slope = \frac{K_m}{V_{max}}$$

$$b = y - intercept = \frac{1}{V_{max}}$$

When we plot $y = \frac{1}{v}$ versus $x = \frac{1}{[S]}$, we obtain a straight line.

$$x - intercept = \frac{-1}{K_m}$$

$$y - intercept = \frac{1}{V_{max}}$$

The Line weaver–Burk plot was widely used to determine important terms in enzyme kinetics, such as Km and Vmax, 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 Vmax; the intercept of the graph represents −1/Km. It also gives a quick, visual

impression of the different forms of enzyme inhibition. The double reciprocal plot distorts the error structure of the data, and it is therefore unreliable for the determination of enzyme kinetic parameters.

ENZYME INHIBITION

Enzyme inhibitor is defined as a substance which binds with the enzyme and brings about a decrease in catalytic activity of that enzyme. Inhibitor may be organic or inorganic in nature.

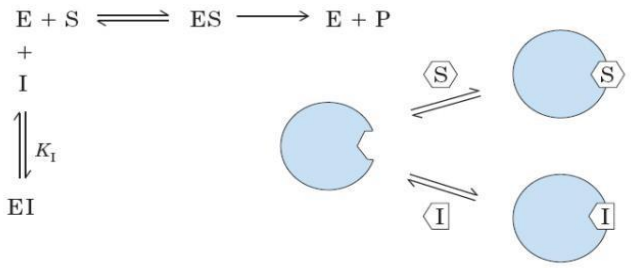
There are three broad categories of enzyme inhibition.

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

Reversible inhibition: The inhibitor binds non-covalently with enzyme**.** Enzyme inhibition can be reversed if the inhibitor is removed. The reversible inhibition is further sub-divided into

- Competitive inhibition
- Non-competitive inhibition

Competitive inhibition



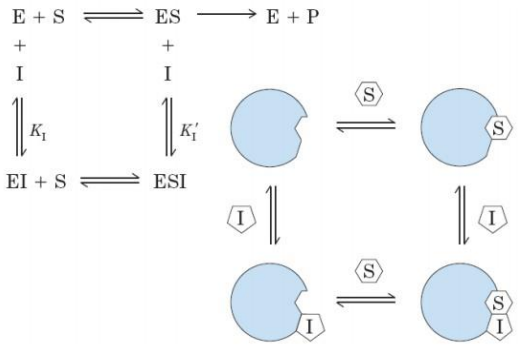
The rate of inhibition depends on:

- Concentration of substrate and inhibitor
- Affinity of inhibitor towards the enzyme
- The inhibition can be reversed by increasing the concentration ofsubstrate
- Km value increases whereas Vmax remains unchanged

- Eg., succinate dehydrogenase
- Original substrate - succinic acid
- Inhibitor – malonic acid, glutaric acid, oxalic acid

Non-competitive inhibition:

The rate of inhibition depends on the concentration of the inhibitor



- Km remains constant whereas Vmax value decreases
- Eg., Various heavy metals ions (Ag+, Hg2+, Pb2+) inhibitthe activity of avariety of enzymes.
- Urease, for example, is highly sensitive to any of theseions in traces.
- Heavy metals form mercaptides with sulfhydryl (-SH) groups ofenzymes.

Irreversible inhibition

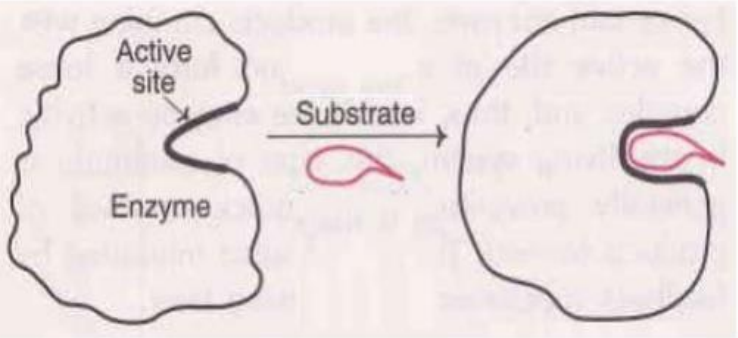
- The inhibitors bind covalently with the enzymes and inactivate them irreversibly
- These inhibitors are usually toxic poisonous substances
- Irreversible inhibitors combine with or destroy a functional group on the enzyme that is essential for its activity. Eg., lodoacetate – irreversible inhibitor of papain and glyceraldehydes-3-phosphate dehydrogenase . Iodoacetate combines with sulfhydryl (-SH) groups at the active site of these enzymes and makes them inactive.

Allosteric inhibition

The details of this type of inhibition are given under allosteric regulationas a part of the regulation of enzyme activity in the living system.

ACTIVE SITE

The active site (or active center) of an enzyme represents as the small region atwhich the substrate binds and participates in the catalysis.



Salient features

- The existence of active site is due to the tertiary structure of protein.
- Made up of amino acids which are far from each other in the linear sequence of amino acids.
- Active sites are regarded as clefts or crevices or pockets occupying a small region in a big enzyme molecule.
- The active site is not rigid; it is flexible to promote the specific substrate binding.
- Enzymes are specific in their function due to the existence of active sites.
- Active site possesses a substrate binding site and a catalytic site.
- The coenzymes or cofactors on which some enzymes depend are present as a part of the catalytic site.
- The substrate binds at the active site by weak non-covalent bonds.
- The commonly found amino acids at the active sites are serine (mostly found), aspartate, histidine, cysteine, lysine, arginine, glutamate and tyrosine.

The substrate binds the enzyme (E) at the active site to form enzyme- substrate complex (ES). The product (P) is released after the catalysis andthe enzyme is available for reuse.

Regulation of enzymes: Enzyme induction and repression, allosteric enzymes regulation

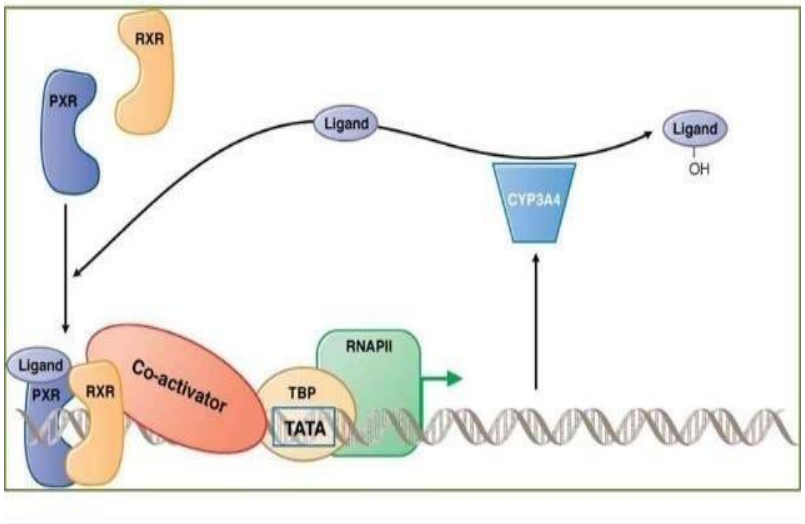
INDUCTION

A process in which a molecule (e.g. a drug) induces (i.e. initiates or enhances) the expression of an enzyme. An enzyme inducer is a type of drug which binds to an enzyme and increases its metabolic activity. Regulated by exposure to drugs and environmental chemicals leading to increased rates of metabolism. Example: lac operon Enzymes that are susceptible to induction are said to be "inducible" enzymes kick into production when needed, remain dormant otherwise. Enzyme induction can increase the metabolic clearance of a concomitantly administered drug, resulting in reduced efficacy, which may comprise the therapeutic effectiveness of a drug.

REPRESSION

Effectors can associate with the operator and alter the configuration so that the binding of the polymerase occurs less efficiently or not at all. This effect is known as repression. Example: trp operon, Feedback inhibition, Prevents wasting of energy.

Mechanism of enzyme induction:



Importance of enzyme induction

Essential to understand various reactions that occur inside the body. Important to analyse drug reactions Studies. Toxicity Causes Less or over production of hormones

Induction	Repression
It Turns the operon On	It turns the Operon OFF
It Starts transcription and translation	It stops transcription and translation.
It is caused by new metabolite, which needs new enzymes to getmetabolized.	It is caused by excess of existing metabolite.
It operates in a catabolic pathway	It operates in an anabolic pathway
Repressor is prevented by inducerfrom joining the operator gene.	Aporepressor is enabled by co repressor to join the operator gene.

Allosteric regulation

Allosteric regulation, broadly speaking, is just any form of regulation where the regulatory molecule (an activator or inhibitor) binds to an enzyme someplace other than the active site. The place where the regulator binds is called the **allosteric site**.

The left part of this diagram shows allosteric inhibition. The allosteric inhibitor binds to an enzyme at a site other than the active site. The shape of the activesite is altered so that the enzyme can no longer bind to its substrate. The right part of this diagram shows allosteric activation. The allosteric activator binds to an enzyme at a site other than the active site. The shape of the active site is changed, allowing substrate to bind at a higher affinity. Pretty much all cases of noncompetitive inhibition (along with some unique cases of competitive inhibition) are forms of allosteric regulation.

However, some enzymes that are allosterically regulated have a set of unique properties that set them apart. These enzymes, which include some of our key metabolic regulators, are often given the name of **allosteric enzymes**^22squared. Allosteric enzymes typically have multiple active

sites located on different protein subunits. When an allosteric inhibitor binds to an enzyme, all active sites on the protein subunits are changed slightly so that they work less well

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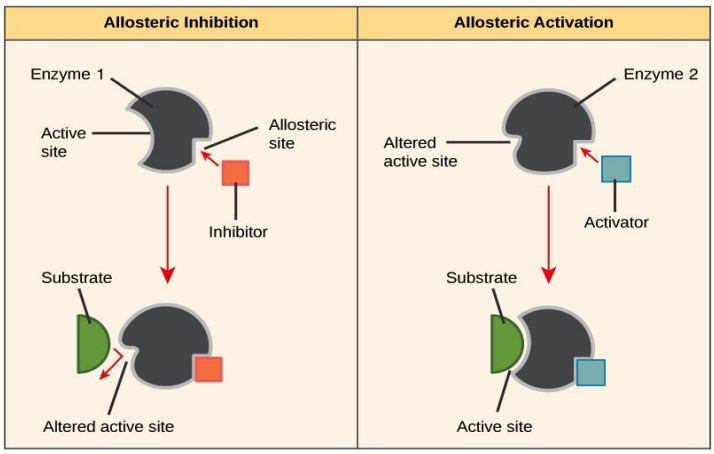


There are also allosteric activators. Some allosteric activators bind to locations on an enzyme other than the active site, causing an increase in the function of the active site. Also, in a process called **cooperativity**, the substrate itself can serve as an allosteric activator: when it binds to one active site, the activity of the other active sites goes up.^{3}3cubed. This is considered allosteric regulation because the substrate affects active sites far from its binding site.

ISO-ENZYMES: They are physically distinct forms of the same enzyme activity. Multiple molecular forms of an enzyme are described as iso-enzymes or isozymes. Different molecular forms of the same enzyme synthesized from various tissues are called iso-enzymes. Hence, study of iso-enzymes is very useful to understand diseases of different organs. If the subunits are all the same, the protein is a homomultimer represented by a single gene. If the subunits are different, protein is said to be a heteromultimer, produced bydifferent gene.

Iso-enzymes may be Formed in Different Ways

They may be products of different genes (more than one locus) in which case they are known as true iso-enzymes. The genes may be located on different chromosomes, e.g. salivary and pancreatic amylase.

In certain cases, all the different forms are present in the same individual, e.g. Lactate dehydrogenase (LDH) has 5 iso-enzymes.

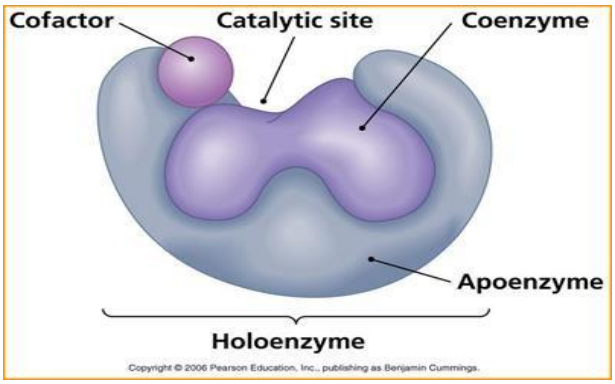
ENZYMES AS THERAPEUTIC AGENTS

1. Streptokinase prepared from streptococcus is useful for clearing the blood clots. Streptokinase activates plasma plasminogen to plasmin which, in turn, attacks fibrin to convert into soluble products.
2. The enzyme asparaginase is used in the treatment of leukemias. Tumor cells are dependent on asparagine of the host's plasma for their multiplication. By administering asparaginase, the host's plasma levels of asparagine are drastically reduced. This leads to depression in the viability of tumor cells.
3. Some enzymes are useful in the clinical laboratory for the measurement of substrates, drugs, and even the activities of other enzymes. The biochemical compounds (e.g. glucose, urea, uric acid, cholesterol) can be more accurately and specifically estimated by enzymatic procedures compared to the conventional chemical methods.

4. Enzymes can be used as catalytic agents in industrial and medical applications. Some of these enzymes are immobilized by binding-.them to a solid, insoluble matrix which will not affect the enzyme stability or its catalytic activity. Beaded gels and cyanogen bromide activated sepharose are commonly used for immobilization of enzymes.

5. Various enzyme estimations in serum and body fluids viz. CS fluid, peritoneal/pleural fluids have been used for diagnosis and prognosis of diseases.

COENZYME: The non-protein, organic, Iow molecular weight and dialysable substance associated with enzyme function is known as coenzyme. The functional enzyme is referred to as holoenzyme which is made up of a protein part (apoenzyme) and a non-protein part (coenzyme); The term prosthetic group is used when a non-protein moiety is tightly bound to the enzyme which is not easily separable by dialysis. The term activator is referred to the inorganic cofactor (like Ca2+, Mg2+, Mn2+ etc.; necessary to enhance enzyme activity. lt may, however, be noted that some authors make no distinction between the terms cofactor, coenzyme and prosthetic group and use them interchangeably.

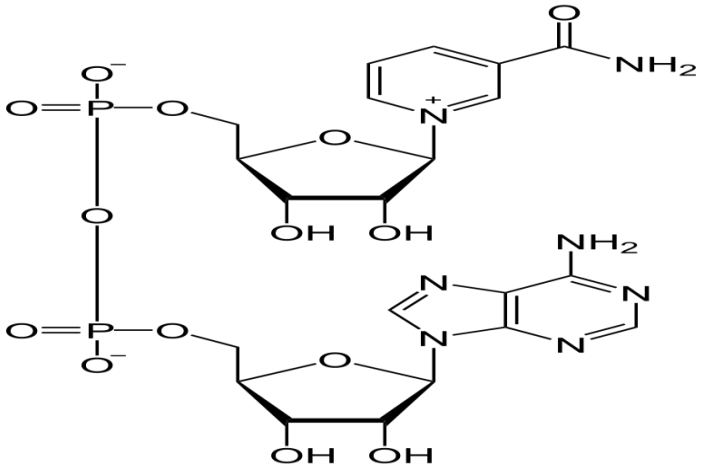


Functions of Coenzymes

An enzyme without a coenzyme is called an *apoenzyme*. Without coenzymes or cofactors, enzymes cannot catalyze reactions effectively. In fact, the enzyme may not function at all. If reactions cannot occur at the normal catalyzed rate, then an organism will have difficulty sustaining life.

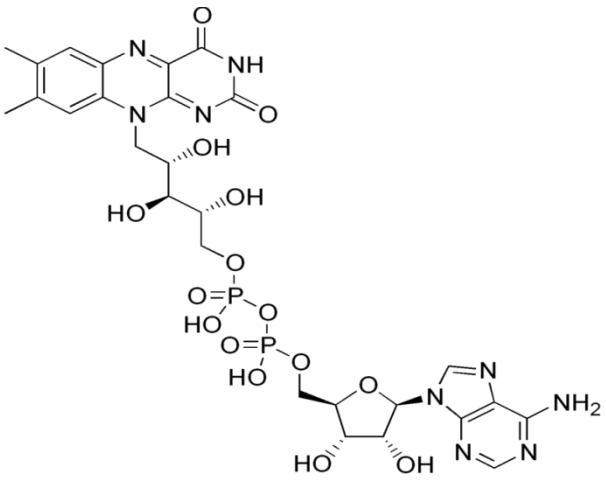
NADH

Nicotinamide adenine dinucleotide is a coenzyme derived from vitamin B3. In NAD+ the functional group of the molecule is only the nicotinamide part. NAD+ is capable of carrying and transferring electrons and functions as oxidizing agent in redox reactions. It also works as a substrate for DNA ligases in posttranslational



modification, where the reaction removes acetyl groups use every day. In addition to catabolic reactions, NADH is also involved in anabolic reactions such as gluconeogenesis, and it also aids in the production of neurotransmitters in the brain.

FADH



Flavin adenine dinucleotide is a prosthetic group that, like NADH, functions as areducing

agent in cellular respiration and donates electrons to the electrontransport chain.

Vitamin H

Also named *Biotin*, Vitamin H is a carboxyl carrier; it binds CO_2 and carries it until the CO_2 is donated in carboxylase reactions. It is water soluble and important in the metabolism of fatty acids and the amino acid Leucine. Deficiency leads to dermatitis and hair loss, thus making it a popular ingredient in cosmetics.

Vitamin K

Vitamin K is needed for the process of clotting of blood and Ca2+ binding. Vitamin K can be synthesized by bacteria in the intestines. Vitamin K is needed for catalyzing the carboxylation of the γ-carbon of the glutamate side chain in proteins.

Vitamin B3

Vitamin B_3 is Niacin or nicotinic acid with the formula C5H4NCO2H. Vitamin B3 is a precursor to NADH, NAD^+, $NADP^+$ and NADPH which are coenzymes found in all living cells. NAD^+ and $NADP^+$ are oxidizing agents. NADH and NADPH are reducing agents.

Short Answers Question 2marks

1) Define enzymes. Explain nomenclature and IUBMB classification with suitable examples.
2) Define enzyme inhibition.
3) Define isoenzymes.
4) Define Thimine Pyrophosphate.(TPP)

Short Answers Question 5marks

1) Write short note on Specificity of enzyme.
2) Explain factors affecting enzyme activity.
3) How will you classify Enzymes?
4) What are the properties of Enzymes?

Long Answer Questions 10marks

1. Explain in detail the different types of inhibitions with suitable examples.
2. Explain the structure, organ distribution and diagnostic importance of isoenzyme.
3. Explain the different theories proposed for mechanism of enzyme substrate complex formation.
4. Explain in detail Michaelis Plot.