

Chapter = 02

Enzymes

ENZYME:

In living systems biological reactions occur at very high rate which is due to biocatalyst. The biocatalysts is called enzyme. The word enzyme is derived from "EN" means inside and "ZYME" means yeast. The word enzyme was coined by Friedrich Wilhelm Kuhn in 1878. Enzyme may be defined as organic substance that can catalyse chemical reaction within the body without being consumed.



CHARACTERS OF ENZYME:

- Enzymes are produced in the cell.
- Enzymes are proteinacious in nature but few enzymes are non proteinacious in nature such as ribozyme.
 - Some proteinacious enzymes are made up of only protein such as amylase and pepsin or made up of protein and non-protein part such as Acetyl CoA.
 - Enzymes have high molecular weight ranging from 10000 to 1 million so each cell produce its own enzyme.
 - Enzymes may act within the cell or out of the cell. The enzyme which act within the cell is called endoenzyme or out of the cell called exoenzyme.
 - Enzymes are specific in their action.
 - Enzyme has particular site to react with substrate called active site.
 - Enzymes activity can be enhanced by activators such as Mn, Mg, Cl- etc.
 - Enzymes activity can be inhibited by inhibitors such as pH and temperature.
 - Enzymes are sensitive to temperature and pH.
 - Enzymes not consumed during and after the reaction.

STRUCTURE OF ENZYME:

ACTIVE SITE:

The specific charge bearing cavity of an enzyme in which substrate can fit is called active site.

The shape of active site is similar to substrate molecule. Active site is formed by 3-12 amino acids of polypeptide.

Active site has two regions;

BINDING SITE:

in binding site amino acid make bond with substrate

CATALYTIC SITE:

in catalytic site amino acids catalyse the reaction

STRUCTURAL PART:

Enzymes are made up of proteins but some enzymes have a non-protein part. The enzymes which have non protein part called conjugated enzyme. The protein part is Apo enzyme while the non-protein part of conjugated enzyme is co-factor. Co-factor may be organic or inorganic.

TYPES OF CO-FACTOR:

ACTIVATORS: if the cofactor is inorganic ion and detachable then it is called activator. Activators attach to enzyme at the time of enzyme-substrate complex formation.

CO-ENZYME:

if the cofactor is organic and detachable then it is called co-enzyme. Co-enzyme are the derivatives of vitamins. Co-enzyme also attach to enzyme at the time of enzyme-substrate complex formation. E.g. FAD, NAD

PROSTHETIC GROUP:

if the cofactor is organic and permanently attached then it is called prosthetic group. E.g. Mg Containing porphyrin of chlorophyll Fe* containing porphyrin of cytochrome oxidase. The activated form of conjugated enzyme is called holoenzyme. Some enzymes are secreted in inactive form. Such as pepsin secreted as pepsinogen which in the presence of HCl.

MECHANISM OF ENZYME ACTION:

Enzyme bind to substrate molecule and form enzyme-substrate complex which than transform into enzyme-product complex. Enzyme-product complex break into enzyme and product of the reaction.

MODE OF ACTION OF ENZYME:

Two models explain mode of action of enzyme.

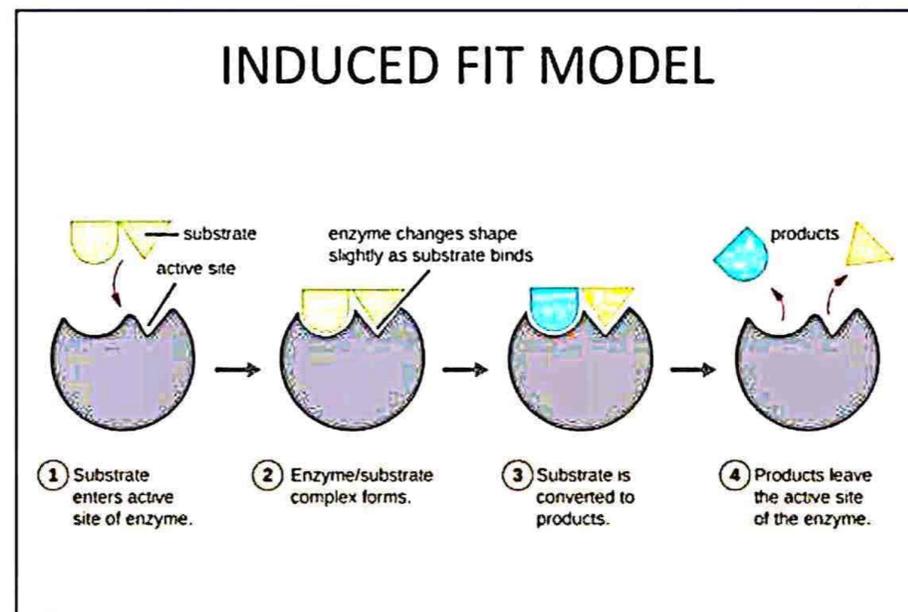
i- LOCK AND KEY MODEL:

Lock and key model was proposed by Emil Fischer in 1898. According to this a particular enzyme act on a particular substrate like a particular lock can be unlock by a particular key. This model explained that active site is fixed or rigid and can only act on a particular substrate. E.g. amylase.



ii-INDUCED FIT MODEL:

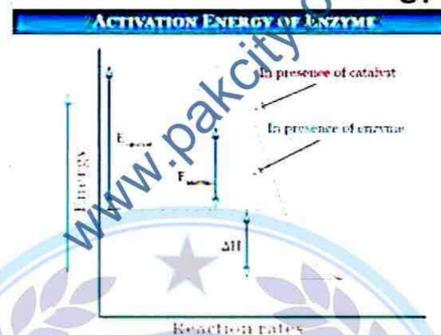
Induced fit model was proposed by Koshland in 1959. According to this model active site of enzyme is elastic and can change according to the shape of substrate molecule. Due to elasticity of active site enzyme can perform more catalytic function and can act on more substrate molecule. E.g. carbonic anhydrase



ENERGY OF ACTIVATION:

Minimum amount of energy required to break the covalent bond of substrate molecule is called energy of activation.

Energy of activation is required to transform substrate into product. In non-living system, heat energy is used to transform substrate into product. In living system biomolecules are sensitive to heat so, they use enzymes to decrease the amount of activation energy.

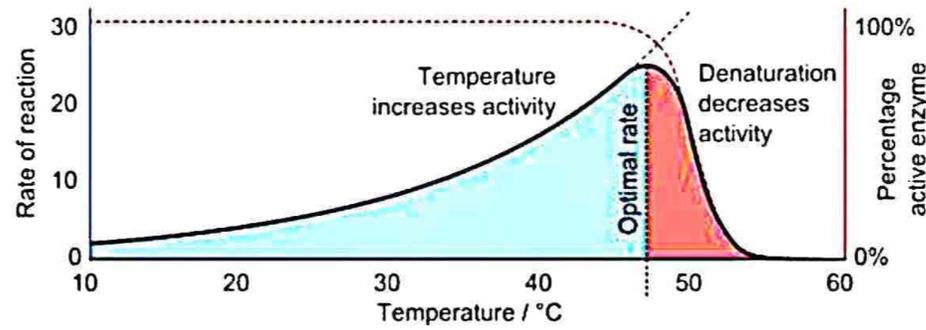


FACTORS AFFECTING ENZYME ACTIVITY:

Some factors affect the activity of enzymes such as temperature, pH, substrate concentration and enzyme concentration.

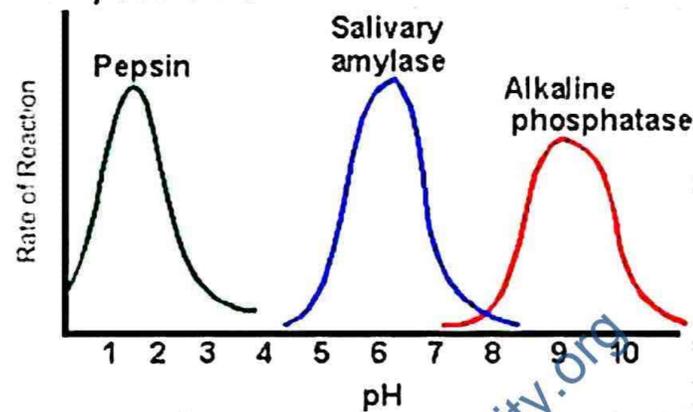
TEMPERATURE:

- Increase in temperature responsible for increase kinetic energy of molecules and increase chance of collision which causes increase rate of reaction.
- If we increase temperature by 10 C° then the amount of reaction will be double.
- High temperature for enzyme is not good because enzymes are protein molecules.
- The temperature at which the activity of enzyme is maximum called optimum temperature.
- The optimum temperature for most of the enzyme is $25\text{-}42\text{ C}^\circ$.
- The optimum temperature for human enzyme is 37 C° .
- Enzyme destroy at 100 C° .
- The optimum temperature of thermophilic bacteria is 70 C° .
- At 0 C° enzyme activity reduced but not destroyed.



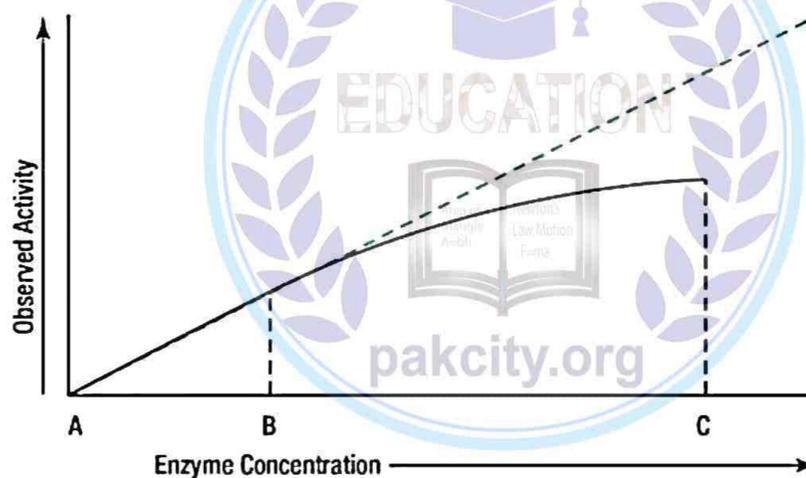
EFFECT OF PH:

- Enzyme activity change with change in pH.
- Each enzyme has specific pH.
- The pH at which Enzyme activity is at its maximum called optimum pH.
- Changes in pH effect charges of amino acid and enzyme becomes inactive.
- pH of pepsin is 1.4 and amylase is 8.5.



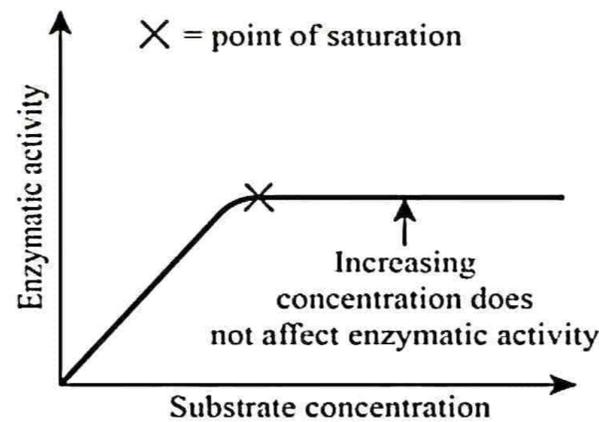
ENZYME CONCENTRATION:

- At optimum pH and temperature and maintained substrate concentration, rate of reaction becomes directly proportional to the enzyme concentration.
- Rate of reaction increase with increase in enzyme concentration.
- Less enzyme concentration reduces the rate of reaction.
- At very high concentration of enzyme the activity is reduced.



SUBSTRATE CONCENTRATION:

- Substrate concentration is the amount of substrate present that can be turned into product.
- Active sites of enzyme waiting for substrate to bind.
- Rate of reaction increase with increase in substrate concentration.
- At very high concentration of substrate the activity reduces because of complete Saturation of enzyme's active sites.



ENZYME INHIBITION:

- The phenomena in which an enzyme fails to catalyze a reaction is called enzyme inhibition.
 - The molecules which react with enzyme but are not converted into desired products are called enzyme inhibitors.
 - Molecules which act as inhibitors include: poisons, metabolites, penicillin, sulpha drugs etc.
- Inhibition may be competitive, non-competitive and uncompetitive



COMPETITIVE INHIBITION:

- A type of enzyme inhibition in which enzyme activity is blocked by the presence of a chemical that compete with the substrate for binding to the active site due to structural similarity with substrate is called competitive inhibition.
- Competitive inhibition is usually temporary and the inhibitor eventually leaves the enzyme hence it is also called reversible inhibition.
- This inhibition depends on the relative concentration of substrate and inhibitors.
- If the substrate concentration increase relative to concentration of inhibitors the active site will usually be occupied by the substrate.
- Example: penicillin is a competitive inhibitor that block the active site of responsible for cell wall formation. Enzyme.



NON-COMPETITIVE INHIBITION:

- In noncompetitive inhibition the inhibitor molecule binds to an enzyme other than active site.
- The other binding site is called allosteric site.
- The noncompetitive inhibitors inactivate the enzyme temporarily (reversible inhibition) or they denature the enzyme permanently (irreversible inhibition).



UNCOMPETITIVE INHIBITION:

Uncompetitive inhibitor binds to Enzyme Substrate complex and form enzyme substrate inhibitor complex. This complex stops enzyme action and prevent product formation.



uncompetitive inhibitors

SIGNIFICANCE OF ENZYME INHIBITION:

- For understanding the regulation of enzyme activity within the cell.
- Provide information about substrate specificity of the enzyme.
- Some drugs inhibiting certain enzyme so enzyme inhibitors have therapeutic applications.
- Useful to study the mechanism of catalytic activity of enzyme.

FEEDBACK INHIBITION:

- In feedback inhibition the activity of enzyme is inhibited by its own product. It is reversible inhibition.
- Feedback inhibition occurs when product is abundant.
- Product bind to allosteric site.
- When product is consumed then it separates from allosteric site.
- Enzyme become active again.

CLASSIFICATION (6 GROUP) OF ENZYMES BASED ON REACTION

TYPES AND REACTION MECHANISM:

ON THE BASIS OF TYPE OF REACTION CATALYSED:

A systematic nomenclature and classification of enzymes based on reaction types and reaction mechanism was given by International Union of Biochemistry in 1961.

On that basis all the enzymes have been classified into 6 groups.

1. OXIDOREDUCTASES (OXIDATION-REDUCTION ENZYMES):

These enzymes catalyse oxidation/reduction of their substrate and act by removing or adding electron or H⁺ ions from or to the substrate.

FOR EXAMPLE: Cytochrome oxidase oxidizes cytochrome.

2. TRANSFERASES:

These enzymes catalyse the transfer of specific functional group other than hydrogen from one substrate to another. The chemical group transferred in the process is not in a free state.

FOR EXAMPLE:

Hexokinase transfer a phosphate group from ATP to glucose.

3. HYDROLASES:

These enzymes bring about the breakdown of large complex organic molecules into smaller ones by adding water (hydrolysis) and breaking the specific covalent bonds.

FOR EXAMPLE:

Proteolytic enzymes (proteinases) which breakdown proteins into peptones and peptides, eg, pepsin, rennin (chymosin), trypsin and trypsin.

Other digestive enzymes that work in digestive tract are also the examples of hydrolases.

4. LASES:

These enzymes catalyst the breakdown of specific covalent bonds and removal of groups without hydrolysis.

FOR EXAMPLE:

Histidine decarboxylase breaks the covalent bonds between carbon atoms in histidine forming carbon dioxide and histamine.



5. ISOMERASE:

These enzymes bring about intra-molecular rearrangement of atoms in the molecules and thus forming one isomer from another.

FOR EXAMPLE:

Phosphohexose isomerase changes glucose 6-phosphate to fructose 6- phosphate.

6. LIGASES (SYNTHETASES):

These enzymes bring about joining together of two different molecules. The energy is derived by hydrolysis of ATP.

FOR EXAMPLE:

Polymerase are responsible for linking monomers into a polymer such as DNA or RNA.

DIFFERENC BETWEEN BINDING SITE ENZYME AND CATALYTIC SITE

BINDING SITE OF ENZYME	CATALYTIC SITE OF ENZYME
It recognizes the specific substrate and attaches enzyme substrate complex (binds) with it to form i.e.; ES complex	The activated catalytic site changes the substrate into products.
This reaction or complex then activates the catalytic site.	

DIFFERENCE BETWEEN APOENZYME AND HOLOENZYME

APOENZYME	HOLOENZYME
Enzyme without is proper cofactor is called apoenzyme.	Enzyme with its proper cofactor is called holoenzyme.
It cannot perform a chemical reaction, so it is inactive form of enzyme.	It can perform a chemical reaction so it active form of enzyme.
Examples: Hexokinase enzyme without Mg ions etc.	Examples: Hexokinase enzyme with Mg ions etc.

CO-ENZYMES	PROSTHETIC GROUP
These are also organic compounds which are loosely bounded to apoenzyme.	These are organic co-facter that are tightly bound to apoenzyme.

The essential component of many co-enzymes is vitamins.	They usually become a permanent part of enzyme.
Examples: NAD and NADP contain niacin.	Examples: n peroxidase and catalase, haem is prosthetic group.

ORGANIC COFACTOR	INORGANIC COFACTOR
Cofactor composed of C and H together in their molecules.	Cofactor that are not composed of C and H instead contain metallic ions in their atoms/molecules.
Such cofactors are Coenzymes and prosthetic group.	Such cofactors are metallic ions activators.
Examples: Home group, Vitamins C/A, NADH, NADPH, FADH ₂ , ATP, Coenzyme A etc. NADH, NADPH, FADH ₂	Examples: Mg ⁺² , Fe ⁺² , Cu ⁺² , Zn ⁺² Ca ⁺² , Co ⁺² , K ⁺ etc.

LOCK AND KEY MODEL	INDUCED FIT MODEL
Emil Fischer, in 1890, proposed a lock and key model of enzyme and substrate interaction.	Koshland in 1959, proposed this model on the basis of new evidences.
According to this model. "as one specific key can open a specific lock in this manner a enzyme can transform only one substrate into products.	According to this model. "when a substrate combines with an enzyme, it induces changes in the enzyme structure, This change allows the enzyme to do its catalytic activity more effectively.
Active sites of enzymes are rigid structures.	Active sites of enzymes are not rigid structures.
Active sites of an enzyme cannot be modified (flexible).	Active sites of an enzyme can be modified (flexible)
Active sites act as a template.	Active sites do not act as a template.
Later studies have not support this model in all reaction.	This model is thoroughly accepted.

COMPETITIVE INHIBITORS	NON-COMPETITIVE INHIBITORS
Their shape and structure resemble with substrate.	Their shape structure does not resemble with substrate.
They complete with substrate to attach with enzyme.	They do not complete with substrate.
They do not change globular shape of enzyme.	They can change the giobular shape of enzyme.
Maionic acid inhibits succinic dehydrogenase enzyme.	Urea, penicillin etc.