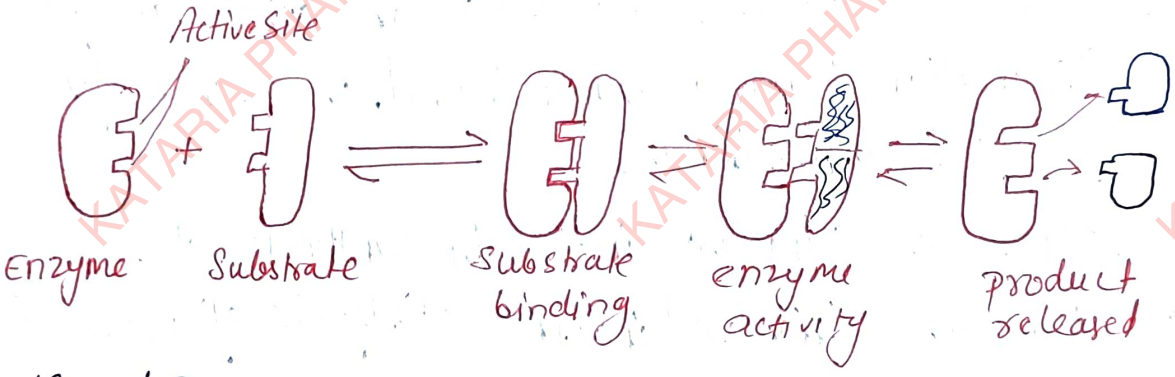
BIOCHEMISTRY UNIT-5

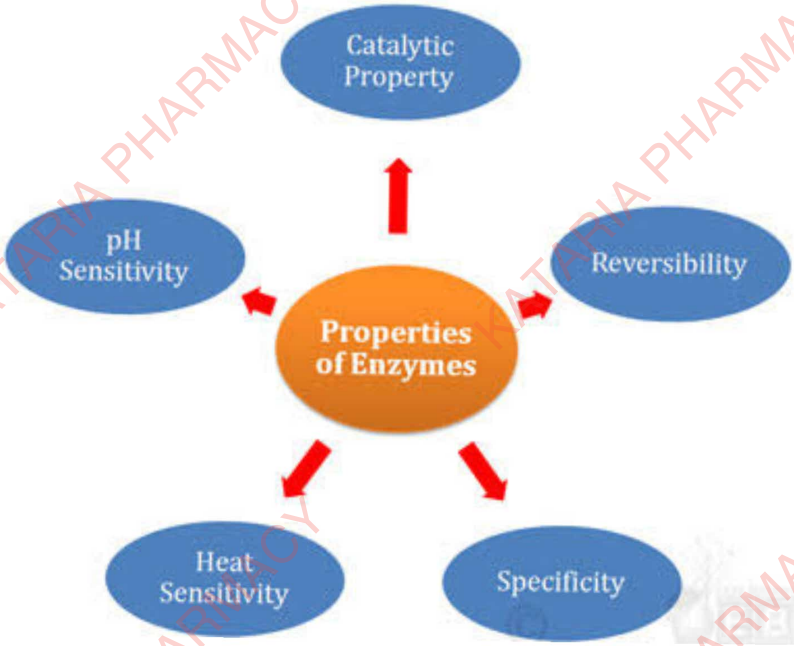
Biochemistry
UNIT-V

★ Enzymes:-

- Enzyme are biocatalysts present in cells that Speed up biochemical reactions without getting itself destroyed in the reaction.
- All types of biochemical reactions in the cell require enzyme.
- Enzymes are typically proteins.
- Certain types of RNA Can also Serve as Catalysts. These RNA molecule are Called Ribozymes and DNAzymes.



#properties of Enzyme:-



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Nomenclature of enzyme & IUB classification :-

- The Nomenclature Committe of the "International Union of Biochemistry and Molecular biology (IUBMB) adopted rules in 1992 for the Systemic classification and designation of enzymes based on reaction specificity.

- All enzymes are classified into Six major Classes according to the nature of the chemical reaction Catalyzed:

1. Oxidoreductase.
2. Transferase
3. Hydrolases
4. Lyases (cleave C-C, C-O, C-N, and other groups by elimination, leaving double bound, or Conversely adding groups to double bonds.

5. Isomerases (involved in the Catalysis of Isomerizations within one molecule).
6. Ligases (involved in the biosynthesis of a Compound with the Simultaneous hydrolysis of a pyrophosphate bond in ATP or a Similar triphosphate.

BIOCHEMISTRY UNIT-5

☆ Enzyme kinetics :-

- Enzyme kinetics is the study of enzymatic reaction rate and how it change in response to different experimental parameters like substrate concentration and effect of inhibitors.
- It is an expression of chemical reaction in mathematical terms.
- It is an oldest approach to understand enzyme Mechnism.

Michaelis plot :-

- In 1913 Leonor Michaelis and Maud Menten postulated the ~~existence~~ existence of enzyme substrate Substrate Complex based on their observation on Sucrose.
- They proposed -

1. The enzyme Combines with Substrate to form enzyme Substrate Complex (ES Complex).
2. The Substrate gets Modified to form product and the product gets associated with enzyme (EP).
3. The product gets released from the enzyme.

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

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Line Weaver - Burk plot :-

- The Michaelis - Menten Curve was not useful in determining the exact value of V_{max}, so a more representating graph was suggest by Hans Lineweaver and Dean burk.
- They employed a double reciprocal plot of $1/V_0$ V/s $1/[S]$ from the Michaelis - Menten Equation.

Advantage:- More accurate determination of V_{max}.

Michaelis - Menten equation $\Rightarrow V_0 = \frac{V_{max}[S]}{K_m + [S]}$

Invert :- $\boxed{\frac{1}{V_0} = \frac{K_M + [S]}{V_{max}[S]}}$

Factor $\frac{1}{V_0} = \frac{K_m}{V_{max}[S]} + \frac{[S]}{V_{max}[S]}$

and Simplify

Double - Reciprocal or Lineweaves - Burk equation $\Rightarrow \boxed{\frac{1}{V_0} = \frac{K_m}{V_{max}(S)} + \frac{1}{V_{max}}}$

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☆ Enzyme inhibitors :-

There are numerous types of molecule that have the ability to interfere with the activity of an individual enzyme.

- Any molecule showing its direct effect on an enzyme in order to decrease rate is known as an inhibitor.
- Enzyme inhibitors are molecules that bind to enzyme and decrease their activity.
- Inhibitor are two type Reversible or irreversible.

① Irreversible inhibitors :-

- Usually react with the enzyme and change it chemically
- The enzyme become permanently inactive.
- These inhibitors modify key amino acid residues needed for enzymatic activity.

② Reversible inhibitors :-

- Bind non-covalently with the enzyme.
- The inhibition can be reversed on removal of the inhibitor from the enzyme.
- Most biological inhibitions are reversible and are involved in the regulation of metabolism.

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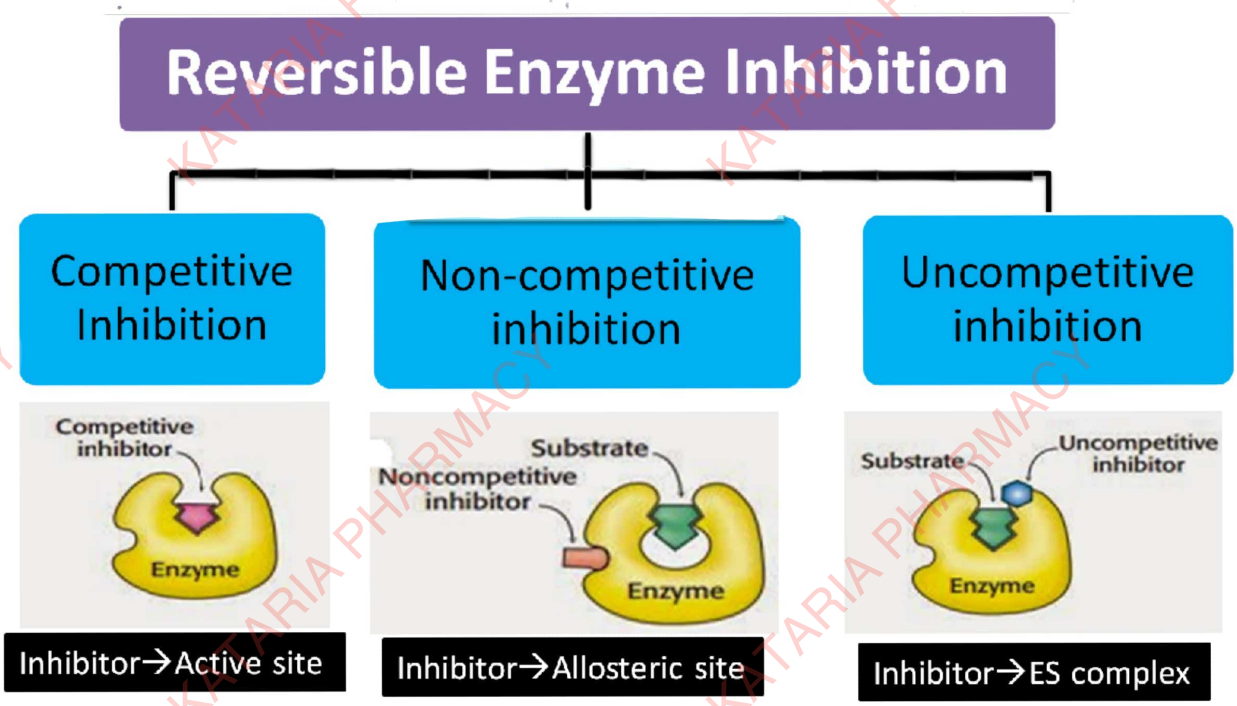
- There are three different reversible inhibition:
  - Competitive Inhibition
  - Non Competitive inhibition
  - Uncompetitive Inhibition:



☆ Regulation of enzymes :-

An enzyme's Catalytic activity Can be directly Controlled through structural alterations that influences the enzyme Substrate - binding affinity.

① Enzyme Induction
② Enzyme repression
③ Allosteric enzymes regulation.

① Enzyme 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.
- Enzyme that are Susceptible to induction are said to be "inducible".

② Enzyme repression :-

Enzyme repression is a regulatory Mechnism in cells that reduces or inhibits the synthesis of enzymes.

- Here are the key points.

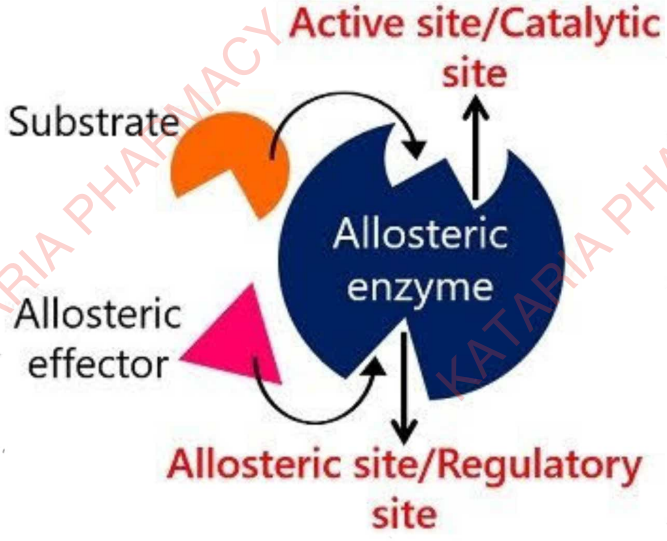
1. Mechnism :- Enzyme repression typically involves the binding of a repressor protein to the operator region of a gene preventing the transcription of mRNA and thus stopping the Synthesis of the enzymes.

Application :-

Understanding enzyme repression is crucial in fields like biotechnology and medicine, where controlling gene expression is important for developing treatments and producing bioengineered product.

③ Allosteric enzymes Regulation :-

- Allosteric enzyme regulation is a process by which the activity of an enzyme is modulated through the binding of molecules at specific sites other than the enzymes active site.
- These sites are known as allosteric sites.
- The binding of an allosteric effector can either enhance or inhibit the enzyme's activity.
- This form of regulation is crucial for controlling Metabolic pathway and maintaining homeostatasis with cells.



☆ Therapeutic And diagnostic applications of enzymes and isoenzymes.

① Terapeutic And diagnostic Application of Enzymes :-

Therapeutic Application :-

- Enzymes are used for aiding digestion e.g Amylases, proteases, lipase
- They are used as deworming agent E.g papain
- They act as anti-clotting agent like fibrinolytic and thrombolytic Eg. urokinase.
- They act to treat wound and swelling. e.g. Trypsin, chymotrypsin, Serratio-peptidase.
- They are used to assist metabolism like hyaluronidase.
- They are used as Surface disinfectant e.g. Trypsin.

Diagnostic application :-

- They are also used in the diagnosis purpose. E.g Glucose oxidase along with peroxidase to detect the level of glucose.
- Liver disease : SGPT (gamma-glutamyl transpeptidase)
- Heart attacks : Asparte aminotransferase (AST)
- myocardial infarcation : creatine phosphokinase
- Uric acid : Uricase.

(2) Therapeutic and diagnostic Application of Isoenzyme :-

- Isoenzyme - are enzyme that differ in amino acid Sequence but Catalyze the Same Chemical reaction.
- They have Similar Catalytic activity, but are different Biochemically or Immunologically.
- Different forms may be differentiated from each other based on certain physical properties.
  - Electrophoretic Mobility.
  - Differences in absorption properties
  - or By their reaction with a specific antibody

Diagnostic application :-

Enzymes involved in the diagnosis of different disease are as follows.

- In myocardia infarction
- In liver disease
- In Muscles Disease
- In Cancers.

BIOCHEMISTRY UNIT-5

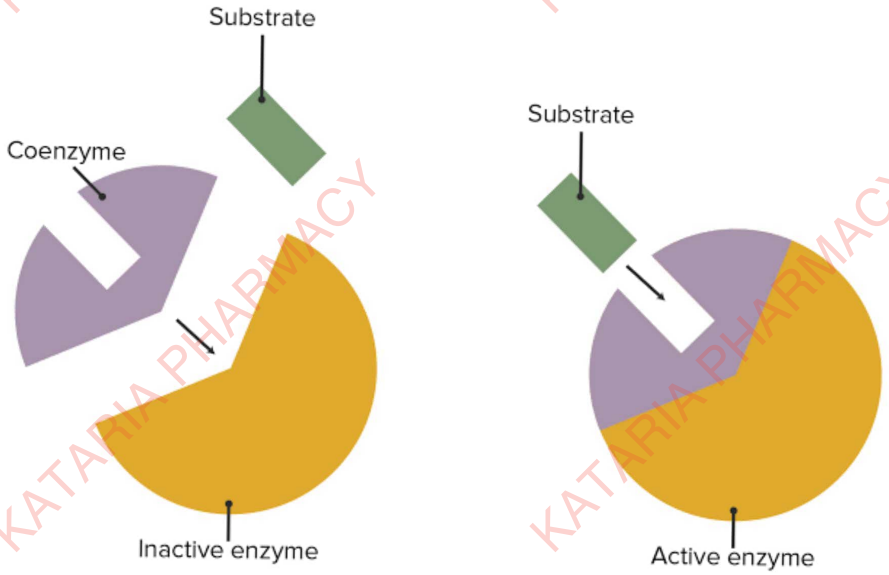
☆ Coenzymes:-

Small organic molecules, transporting the chemical groups from one enzyme to another, are known as Coenzymes.

Example- Thiamine, riboflavin, and folic acid

- The chemical groups that are carried from one enzyme to another, includes the acetyl group carried by coenzyme A,
- The hydride ion (H^-) carried by NAD or $NADP^+$, formyl, methenyl or methyl groups carried by folic acid, the phosphate group carried by adenosine triphosphate, and the Methyl group carried by S-adenosylmethionine.

- Coenzyme is a substance that enhance the action of an enzyme.



Biochemical function of Co-enzymes :-

- The Coenzyme is essential for the biological activity of the enzyme.
- A Coenzyme is a low Molecular weight organic Substance, without which the enzyme Cannot exhibit any reaction.
- Coenzyme prepares the active site for Catalytic activity.
- The function of Coenzyme is to transfer of group between enzymes.
- A Coenzyme is necessary helper for enzyme that assist in biochemical transformation.
- A Coenzyme transport a variety of chemical groups (such as Hydride, Acetyl, Formyl, methenyl, Methyl).