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Enzymes

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Definition

Enzymes may be defined as biocatalysts that increases the rate of biochemical reaction without itself being changed in the overall process.

- Enzymes are the globular, large Proteins with molecular weight ranging from 13,000 to million dalton
- Mostly all the enzymes are protein in nature
- except ribozyme, in which RNA part is act as catalyst or enzyme.
- When an enzyme like any protein has secondary & tertiary structure, in that the backbone of the protein chain folds upon itself, the chain criss-crosses itself & due to this many crevices or pockets are made.
- one of the such pocket formed is acts as active site' of the enzyme.
- This active site is specific for the substrate & substrate get fit into enzyme

$$\underset{(S)}{\text{Substrate}} \xrightarrow{\text{Enzyme}} \underset{(P)}{\text{Product}}$$

- Enzymes are also called as Biocatalyst, organic catalyst, metabolism regulators & cell ferment.

- Term 'Enzyme' given by - Kohne
- First discovered enzyme is - Zymase
- First discoverer of enzyme - Bochnes
- First crystalised enzyme - Urease & it is crystalised by sumner.

On the basis of composition, enzymes are classified into two classes -

① Simple Enzymes

-consist entirely of amino acids or Proteins.

② Conjugated Enzymes

- consist of Protein as well as non-protein components. Non-protein component is called as co-factor which required for catalytic activity. Protein component is called as 'apo-enzyme' which is generally biologically inactive.

Holoenzyme = apoenzyme + cofactor

- A co-factor can be linked to the protein portion of the enzyme either covalently (ie) or non-covalently.
- Some cofactors are simple metal ions & some are complex organic compound. complex organic compounds are called as ~~apo~~ 'co-enzyme'.
- co-enzyme which are tightly associated with the protein covalently or non-covalently are called as Prosthetic group.

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Properties of Enzymes

1) Mostly all enzymes are protein (except ribozyn but all proteins are not enzymes.

2) Enzymes are highly specific. They are specialized proteins & have a high degree of specificity for their substrates.

3) Enzymes exibit enormous catalytic power. It increases the rate of a reaction by lowering the activation energy.

4) Enzymes do not change the equillibrium state of a biochemical reaction. It change only the rate at which equillibrium is achieved.

5) Every cell form their own enzymes. Enzymes act both inside & outside of the cell.

6) They are sensitive to temperature & p^H.

7) Life without enzyme is not possible.

Classification of Enzymes

Thousands of enzymes have been discovered, isolated & studied. Most of these enzymes have been classified into different groups based on the type of reaction they catalyse.

① Oxidoreductase

- Enzymes which catalyse oxidation & reduction between two substrates are called oxidoreductases.
- The substrate oxidised is regarded as hydrogen or electron donor.

$$A_{(red)} + B_{(oxi)} \longrightarrow A_{(oxi)} + B_{(red)}$$

eg.
i) Oxidases ii) Oxygerases iii) Dehydrogenases
iv) Peroxidases.

2) Transferases

The enzymes which transfer the groups from one substrate to another are included in the transferases class.

- eg. of such groups include amino, carboxyl, carbonyl, methyl, phosphoryl & acyl etc.
- common trivial names for the transferases often include the prefix 'trans.'

$$A + B-X \longrightarrow A-X + B$$

eg.
i) ~~Transferases~~ Transcarboxylases ii) Transaminases
iii) Kinases iv) Phosphorylases

3) Hydrolases

Hydrolases catalyze reaction in which cleavage of bond is accomplished by adding water or hydrolysis of H_2O.

$$A-B + H_2O \longrightarrow A-H + B-OH$$

eg. i) Phosphodiesterases
ii) Peptidases

4) Lyases

The enzymes that catalyzes breaking of C-C, C-O, C-N, C-S & other bonds by means other than hydrolysis are included in the lyases class.

These bonds are cleaved by Process of elimination & the result in the formation of a double bond or a new ring or conversely adding group to double bonds.

$$\begin{matrix} X & Y \\ | & | \\ C-C \end{matrix} \longrightarrow X-Y + C=C$$

eg.
i) aldolases
ii) Dehydratases
iii) Synthases
iv) Decarboxylases.

5) Isomerases

The enzymes which catalysing inter-conversion of optical, geometric or positional isomers, are included in this class.

$$A-B \longrightarrow B-A \quad \underline{or} \quad A \longrightarrow A'$$

eg.
i) Mutases ii) cis-trans isomerase
iii) Epimerases iv) Racemases.

6) Ligases

The enzymes which catalyses joining of C-O, C-S, C-N, P-O, etc. bonds with simultaneous hydrolsis of ATP are included in ligases class.

$$A + B + ATP \longrightarrow A-B + ADP$$

eg.
i) carboxylases - use CO_2 as substrate.

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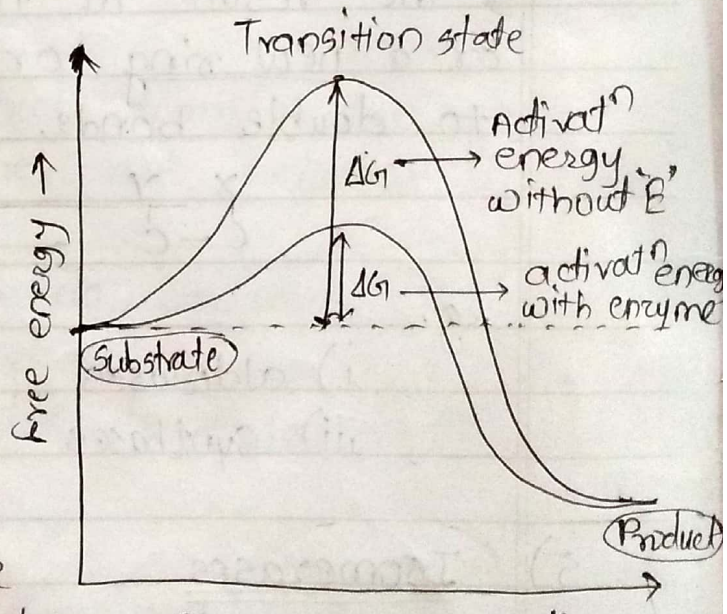
Mechanism of Enzyme Action

Enzymes are highly specific in nature. They increases the rate of reaction by lowering the activation energy. The energy difference between transition state & substrate level is called as activation energy. Due to the lowering of activation energy by the enzyme, the reaction proceed at a lower temperature.



Enzyme do not alter the equilibrium constants, they

only ~~en~~ enhance the velocity of the reaction. The role of enzyme or catalyst is comparable with a tunnel made in a mountain to reduce the barrier. ~~es~~

Steps in the enzyme action -

1) Binding of substrate at the active site of enzyme.
2) Formation of Enzyme - substrate complex.
3) ultimately results in product formation.

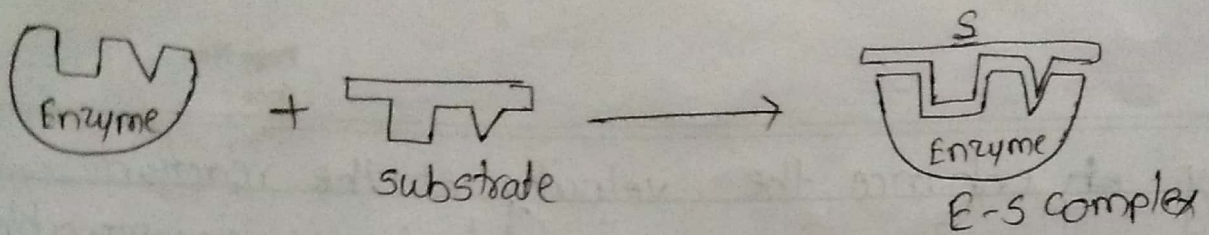
$$E + S \rightleftharpoons E\text{-}S \longrightarrow E\text{-}P \longrightarrow E + P$$

A few theories have been put forth to explain mechanism of enzyme - substrate complex formation.

1) Lock & key model or Fisher's template theory

This theory was proposed by Emil Fischer. This is in fact the very first model proposed to explain an enzyme catalysed reaction.

According to this model, the structure or conformation of the enzyme is rigid. according to this model, substrate is work like the key & Enzyme is like lock. The substrate fits to the binding site just as a key fits into the proper lock. Because of the active site of enzyme is fixed or rigid & pre-shaped template where only a specific substrate can bind.

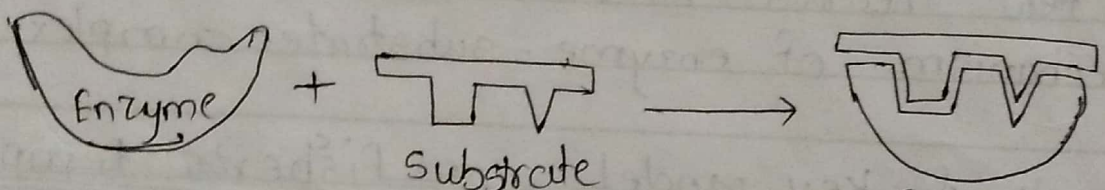


E-S complex

② Induced fit Theory (or) Koshland's model

This theory was proposed by Koshland in 1958. & it was more acceptable & realistic model for enzyme - substrate complex formation.

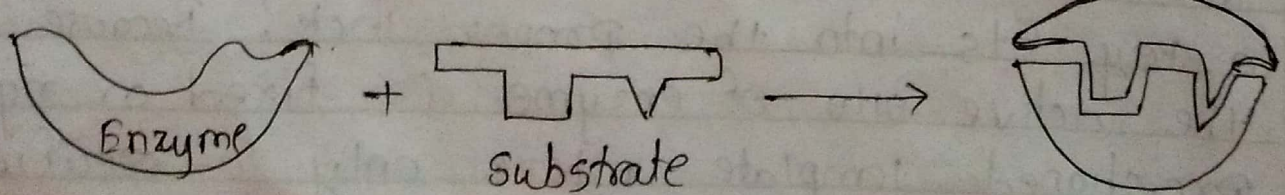
According to this model, the active site is not rigid & preshaped. It is flexible in nature. The interaction of substrate with the enzyme induces conformational change in the enzyme, resulting in the formation of a strong substrate binding site.



E-S complex.

3) Substrate strain Theory

According to this model also, enzyme's active site is not rigid & Pre-shaped. When substrate binds to active site, the conformational change in enzyme occur. Due to this, the substrate is strained. The enzyme induces a strain to the substrate. The strained substrate leads to the Product formatio



E-S complex.

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Factors Affecting Enzyme Activity

The contact between the enzyme & substrate is the most essential pre-requisite for enzyme activity. The important factors that influence the velocity of the enzyme reaction are as follows -

① concentration of Enzyme.

As the concentration of the enzyme is increased, the velocity of the reaction proportionately increases.

[Enzyme] ∝ velocity of reaction

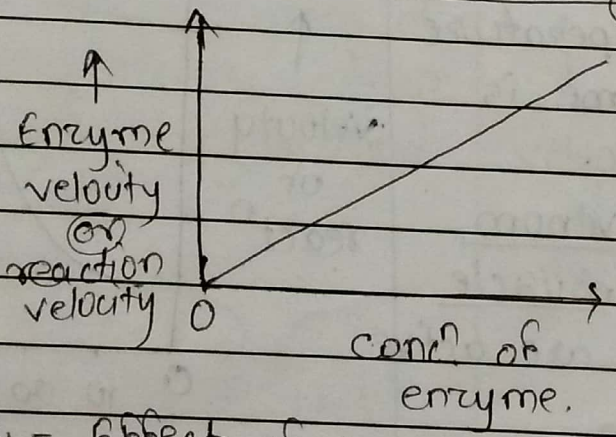

Fig. - Effect of enzyme conc^n on reaction velocity.

2) concentration of substrate

As the substrate concentration increases, the velocity of enzyme reaction also increases initially or within the limited range of substrate level. After that velocity of reaction remain same or constant.

A rectangular hyperbola is obtained when velocity is plotted against the substrate concentrat?

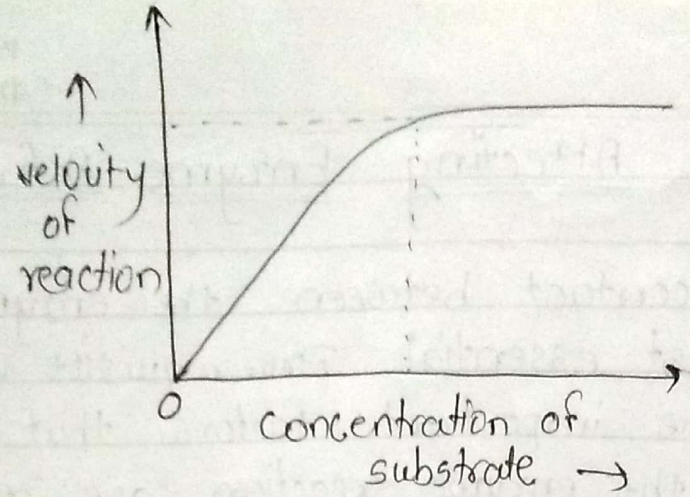

fig. - Effect of substrate concentration on velocity of reaction

3) Effect of temperature

velocity of an enzyme reaction increases with increase in temperature up to a maximum & then declines. A bell-shaped curve is usually obtained.

- The optimum temperature of most of the enzyme is between 40°C - 45°C.
- few enzymes like venom phosphokinases & muscle adenylate kinase are active even at 100°C.
- some plant enzymes like urease have optimum activity around 60°C.

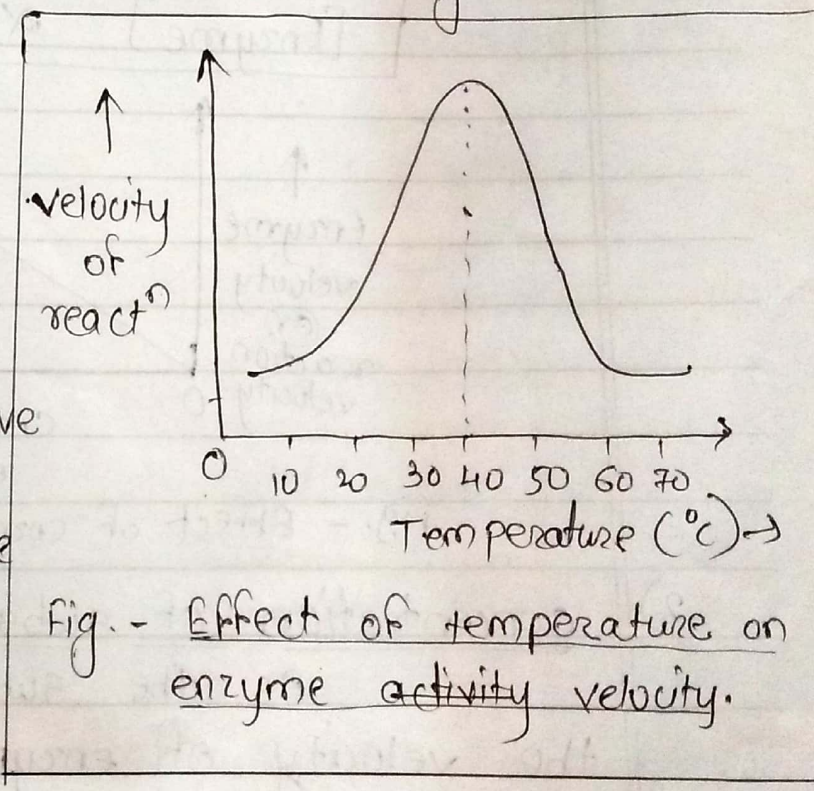

fig. - Effect of temperature on enzyme ~~activity~~ velocity.

In general, when enzymes are exposed to a temperature above 50°C, denaturation occur & if exposed above 70°C, the enzymes get inactivate

optimum range of temperature of enzyme is different for different enzymes & organisms.

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4) Effect of p^H

Increase in the hydrogen ion concentration (p^H) considerably influences the enzyme activity & a bell-shaped curve is normally obtained.

- Each enzyme has an optimum p^H at which the velocity is maximum. Below & above this p^H the enzyme activity is much lower.
- And at extreme high p^H, the enzyme becomes totally inactive.
- Most of the enzymes of higher organisms show optimum activity around neutral (6-8) p^H.

exceptions - p^H

- pepsin (1-2)
- phosphatase (p^H = 4-5)
- alkaline phosphatase (p^H = 10-11)

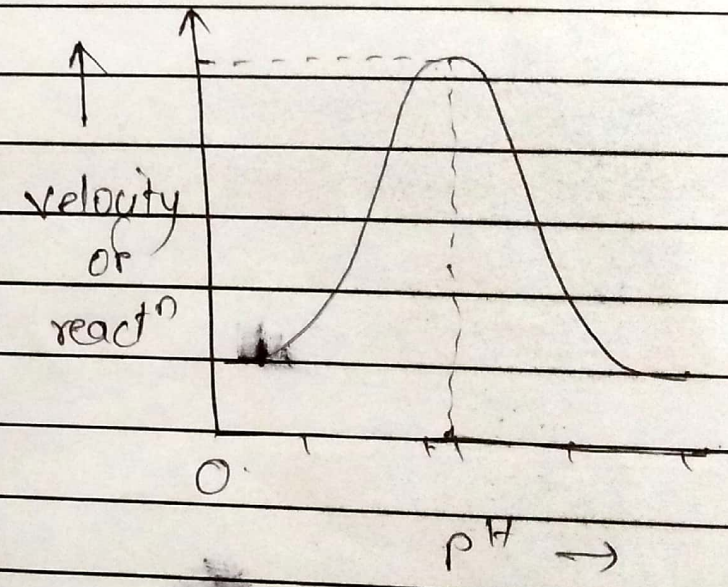

Fig. Effect of p^H on velocity of react^n

- eg. Taq polymerase enzyme from the bacteria Thermus aquaticus work at even 72°C.

5) Effect of Product concentration

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