ENZYMES

Enzyme

Enzyme may be defined as biocatalysts synthesized by living cells. They are protein in nature, colloidal and thermo liable in character and specific in their action.

History of Enzymology

* The term enzyme was coined in 1878 by friedrich wilhelm kuhne.
* 'biological catalyst' that had previously been called 'ferments'.
* manifestation of nature impatience
* The name 'enzyme' (en (Gr) = in ; zyme (Gr) = yeast) literally means 'in yeast'.
* Because of most recognizable reaction popularly known as alcohol fermentation by zymase enzyme in yeast.

Difference from catalysts

* Like catalysts the enzyme do not alter the chemical equilibrium point of a reversible reaction but only the speed of the reaction is changed.

* Differ from catalyst in being the biological products i.e. produced from the living cells.

* The enzyme are all protein and unlike catalyst, cannot last indefinitely in a reaction system since they, being colloidal in nature, often become damaged or inactivated by the reaction they catalyze. they must be replaced constantly by further synthesis in the body.

* unlike catalyze, most individual enzyme are very specific in that they act either on a single or at the most on some structurally related substrates.

Nomenculture

* Enzyme are generally named according to the reaction they

catalyze or by suffixing 'ase' after the name of substrate.

* The International union of Biochemistry and molecular biology developed a nomenculture for enzyme.

* Each enzyme is described by a sequence of four number proceded by "Ec". Ec denotes Enzyme commission and the number of enzyme is called Ec number.

General features of enzyme

CAUTION: peptidyltranferase is ribozyme.

$$(AK)_n\text{-tRNA}_1 + AK\text{-tRNA}_2 \xrightarrow{rRNA} tRNA_1 + (AK)_{n+1} - tRNA_2.$$

* biocatalysts.
* different distribution in cell and in the body, make isoforms.
* Specific, highly effective.
* work under mild conditions.
* in vivo - can be regulated in two ways.
* in vitro - sensitive to many factors.

classification of enzyme

ENZYME CLASS	GENERAL SCHMEI OF REACTION
oxidoreductases	$A_{red} + B_{ox} \leftrightarrows A_{ox} + B_{red}$
transferases	$A-B+C \rightarrow A+C-B$
Hydrolases	$A-B+H_2O \rightarrow A-H+B-OH$
Lyases	$A-B \leftrightarrows A+B$ (reverse reaction synthesis)
Isomerases	$A-B-C \leftrightarrows A-C-B$
Ligases	$A+B+ATP \rightarrow A-B+ADP+P_i$

oxidoreductases

catalyze the oxidation or reduction of substrate.

Subclasses:

* dehydrogenases catalyze transfers of two hydrogen atoms.
* oxygenases catalyze the incorporation of one/two O atoms into the substrate.
* oxidases catalyze transfers of electrons between substrates E.g. (cytochrome c oxidase, ferroxidase).

* peroxides catatyze the break down of peroxidases.

Example:

lactate + NAD^+ ⟷ pyruvate + NADH + H^+

Recommended name: lactate dehydrogenase.

Systematic name: (S)-lactate: NAD' oxidoreductase.

Transferases

* catalyze the transfer of a group from one to another substrate.

subclasses:

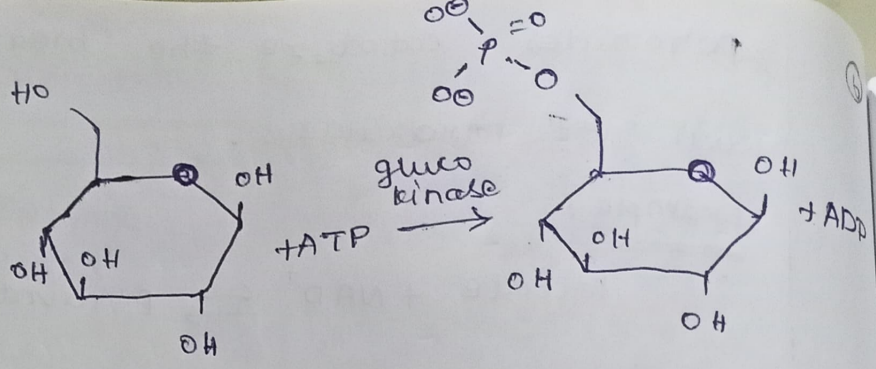
* Aminotranferases, methyltranferases, glucosyltranferases.
* phosphomutases - the transfer of group PO_3^{2-} within molecule.
* kinases phosphorylate substrate by the transfer of phosphoryl group PO_3^{2-} from ATP E.g. hexokinases.

Example:

Glucose + ATP → glucose 6-P + ADP

Recommended name: glucokinase

systematic name: ATP: D-glucose phosphotranferase



Glucose Glucose 6- Phosphate

phosphorylation of glucose.

Hydrolases

Catalyze the hydrolytic splitting of esters, glycosides, amides, peptides etc.

Subclasses:

* Esters (phosphatases, lipases).
* Glycosidases (E.g. Sucrase, maltase)
* proteinases and peptidases (pepsin)
* Amidases (glutaminase)
* ATPases

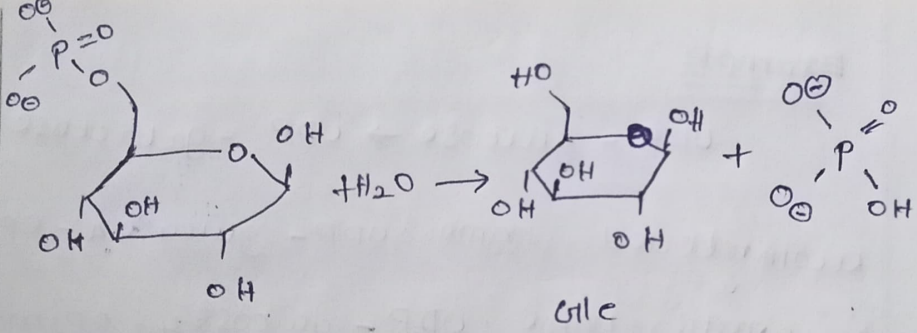
Example

Glucose 6-P + H_2O → glucose + P_i

Recommended name of the enzyme:

glucose 6- phosphatase

Systematic name: glucose 6-phosphate phosphohydrolase.



Glc 6-P

Glucose 6- phosphatase

Glc

lyases

Catalyze non-hydrolytic splitting or forming bond C-C, C-O, C-N, C-S through removing or adding, respectively, a small molecule (H_2O, CO_2, NH_3).

Frequent recommended names:

- Ammonia lyases
- decarboxylases
- aldolases
- (de)hydratases

Example

Fumarate $+ H_2O \rightleftharpoons$ L-malate

Recommended name: Fumarate hydratase,

Systematic name: (S)- malate hydro-lyase

Isomerases

catalyze intra molecular rearrangement of atoms

Example:

Epimerases, racemases, mutases

Example

UDP - glucose → UDP - galactose

Recommended name : UDP - glucose 4 - epimerase

Systematic name : UDP - glucose 4 - epimerase.

Ligases

catalyze formation of high energy bonds C-C, C-O, C-N. in the reactions coupled with hydrolysis of ATP.

Frequent recommended name :

* carboxylases.
* Synthetases.

(E.g. glutamine synthetase : glutamate + ATP + NH_3 → glutamine + ADP + P_i).

Example

pyruvate + CO_2 + ATP + H_2O → oxaloacetate + ADP + P_i

Recommended name : pyruvate carboxylase

Systematic name : pyruvate : carbon - dioxide ligase (ADP - forming).

Cofactors of enzymes

* low molecular non-protein compounds
* many of them are derived from B - complex vitamins.

many of them are nucleotides.

* transfer 2H or e^- (cooperate with oxidoreductase)

* transfer group (cooperate with transferase)

* tightly attached - prosthetic groups

* loosely attached - coenzyme (co-substrates).

Three different components in enzyme reaction

$$\text{Substrate} + \text{cofactor} \underset{\longrightarrow}{\overset{\text{enzyme}}{\longleftarrow}} \text{product} + \text{cofactor}_{\text{altered}}$$

substrate (s) } react to each other.
cofactor

enzyme catalyze the whole process.

* one or two substrate may be involved (dehydrogenation x transamination).

* substrate can be low / high molecular (hexokinase x protein kinase).

* some reaction proceed without cofactor (hydrolysis, isomerization)

* reaction can be reversible or irreversible (dehydrogenation x decarboxylation).

Mechanism of enzyme action :

Active sites

* The active site of an enzyme is the region that binds substrate co-factors and prosthetic groups and contains residues that help to hold the substrate.

* Active site generally occupy less than of the total surface area of enzyme.

* Active site has a specific shape due to tertiary structure of protein.

* A change in the shape of protein affects the shape of active site and function of the enzyme.

Substrate molecule :

Substrate molecule are the chemicals that an enzyme acts on. They are drawn into the cleft of the enzyme.

Enzyme molecule :

* The complexity of the active site is what mixture makes each enzyme so specific. (i.e. piece of in terms of the substrate it acts on).

Active site further divided on

* Binding site : It chooses the Substrate and binds it to active site.

* catalytic site : It performs the catalytic action of enzyme

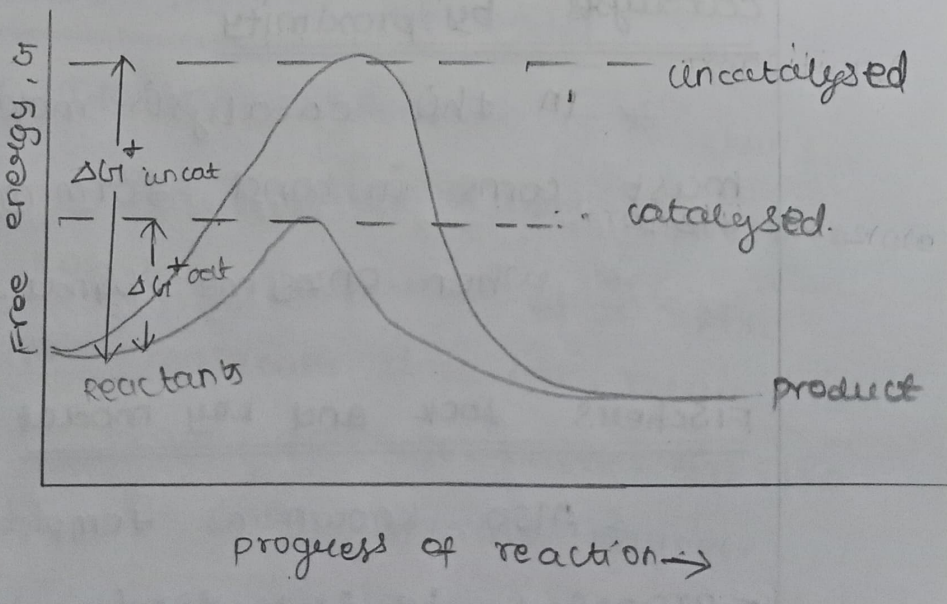
Mechanism of enzyme action

* The catalytic efficiency of enzyme is explained by two perspectives.

Thermodynamic change :

* All chemical change reaction have energy barriers between reactants and products.

* The difference is transitional State and Substrate is called activational barrier.



processes at the active site

covalent catalysis :

* Enzyme form covalent linkage with substrate forming transient enzyme -substrate complex with very low activation energy.

* Enzyme is reacted unaltered after completion of reaction.

Acid base catalysis :

* mostly undertaken by oxido-reductase enzyme.

* mostly at the active site, histidine is present which act as both proton donor and proton acceptor.

Catalysis by proximity

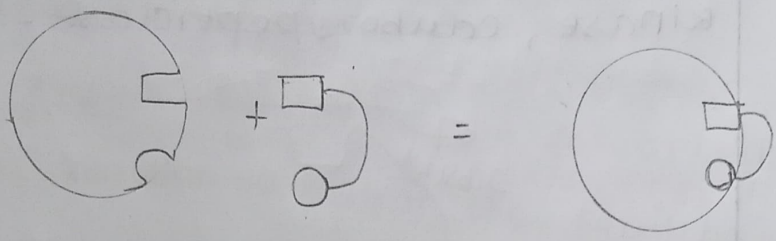
* In this catalysis molecule must come in bond forming distance.

* when enzyme binds.

Fischer's lock and key model :

* Also known as template model - proposed by Emil Fischer in 1898.

* The union between the substrate and the enzyme takes place at the active site more or less in a manner in which a key fit a lock and result in formation of an enzyme substrate complex.



* And as the two molecule are involved, this hypothesis is also known as the concept of intermolecular fit.

* In this activated state, certain bond, of the substrate molecule become more susceptible to cleavage.

Koshland's Induced fit model

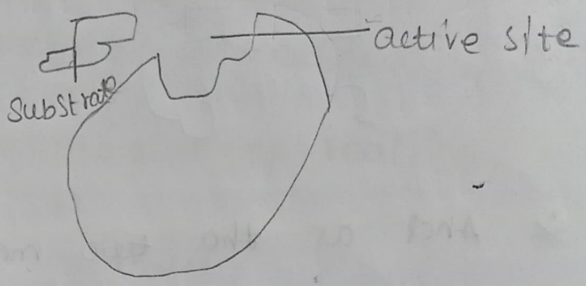
* unfortunate feature of fischer's model is the rigidity of the active site.

* Koshland presumed that the enzyme molecule does not retain it original shape and structure. But the contact of the substrate induces.

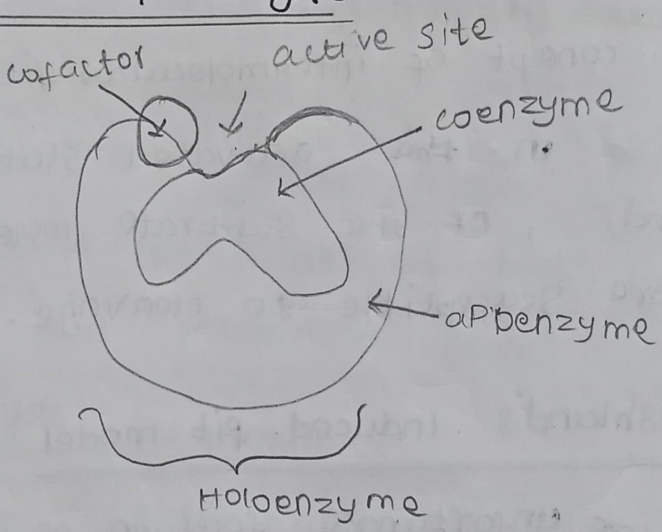
* some configurational or geometrical change in the active site

of the enzyme molecule.

* Koshland's model has now gained much experimental support, conformation changes during substrate binding and catalyst have been demonstrate for various enzyme such as phosphoglycomutase, creatine kinase, carboxypeptidase.



Structure of enzyme



Enzyme → protein part + Non protein part

Holoenzyme (Apoenzyme) (coenzyme)

catalytic properties of enzymes

* They accelerate the rate of catalysis of lowering the activation energy.

* They are highly specific in nature, because they only catalyse as specific biochemical reaction.

* Enzyme accelerate the forward and reverse reaction to attain the equilibrium.

* temperature : optimum temperature of enzyme is 20 - 35°c. They become inactivated at very low temperature and denatured at very high temperature (greater than 45°).

* pH : most enzyme exhibit optimal activity at pH value between 5 and 9.

Enzyme kinetics

Michaelis menten equation (mm plot)

Many enzyme, the rate of catalysis V varies with the substrate concentration (S), at a fixed concentration of enzyme. velocity (V) is almost linearly proportional to the [S].

M. M. Equation:

1. $E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \longrightarrow E + P$

Define the rate by

2. $V = k_2 [ES]$

The change in $[ES]$ is given by

3. $d[ES]/dt = k_1 [E][S] - k_2 [ES] - k_2 [ES]$.

$\therefore d[ES]/dt = 0$ gives

The steady state assumption.

4. $k_1 [E][S] = (k_1 + k_2)[E.S]$

Nothing that $[E_2] = [E] + [E \cdot S]$. $[E]$

$= [E_2] + [E.S]$ and

Substituating in 4 gives.

5. $k_1 ([E_2] - [ES])[S] = (k_1 + k_2)[ES]$

Solving for $[ES]$ gives

6. $$[ES] = \frac{k_1 [E_1][S]}{k_1 [S] + k_1 + k_2}$$

Dividing top and bottom by k_1 and define $K_m = \frac{(k_3 + k_2)}{k_1}$ give

7. $$[ES] = \frac{[E_2][S]}{K_m + [S]}$$

Remembering that $V = k_2 [ES]$ we get

8. $$V = \frac{k_3 [E_2][S]}{k_m + [S]}$$

Defining $V_m = k_3 [E_2]$, we arrive at the final equation

9. $$V = \frac{V_m [S]}{k_m + [S]}.$$

$k_m = [S]$ at $\frac{1}{2} V_{max}$

Relation between V_{max} and k_m

$$V_m = \frac{V_{max} [S]}{k_m + [S]}$$

$$\frac{1}{2} V_{max} = \frac{V_{max} [S]}{k_m + [S]}$$

$$\frac{1}{2} = \frac{[S]}{k_m + [S]}$$

$$k_m + [S] = 2[S]$$

$$2[S] = k_m + [S]$$

$$k_m = 2[S] - [S]$$

$$k_m = [S]$$

k_m is micromoles /sec.

Line weaver burk plot

MM plot we can attain an approximate value of km but attaining V_{max} of an enzyme from MM plot a little bit difficult.

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

Taking the reciprocal of both side

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

Separating the components of the numerator on the right side of the equation

$$\frac{1}{V_o} = \frac{K_m[S]}{V_{max}[S]} \quad \frac{V_{max}[S]}{V_{max}[S]}$$

which Simplifies to

$$\frac{1}{V_o} = \frac{K_m}{V_{max}[S]} \quad \frac{1}{V_{max}}$$

significance

LB plot has the great advantage of allowing the more accurate determination of V_{max} and K_m value than in mm equation

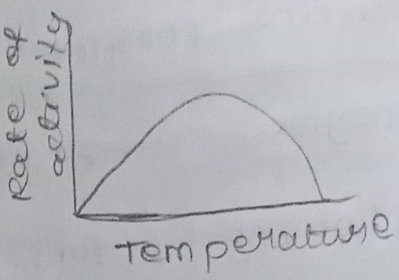
Factors affecting enzyme activity

The enzyme activity is unfluenced by the following factors.

* Temperature
* pH
* Enzyme concentration
* Substrate concentration.

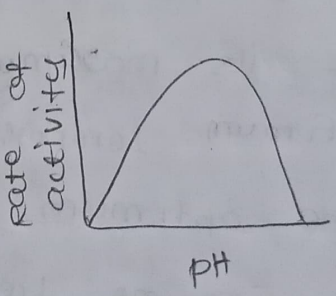
Temperature

* The temperature affects the rate of enzyme activity.
* At very low temperature the rate of enzyme activity is very low.
* If the temperature increased, the rate of activity also increases.
* Instead, the rate of activity decrease.
* The temperature at which the enzyme of its maximum activity is called optimum temperature.
* The optimum temperature for many enzyme lies around 37°c.
* At high temperature for example at 65°c the enzyme becomes inactive.



pH

* The pH also affects the rate of activity.
* At a particularly pH alone the rate of enzyme activity is maximum.
* This pH is known as the optimum pH.
* The other pHs the rate of activity is less.
* The optimum pH varies from enzyme to enzyme.
* However the optimum pH for many reactions 5 to 7.5.



Enzyme concentration

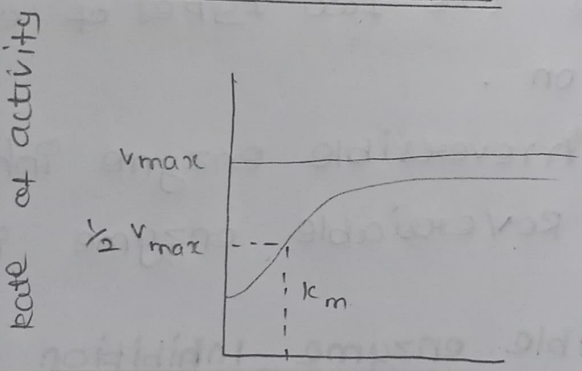
* An enzyme works even when it is present in low quantity.

* when the enzyme concentration is increased, the enzyme activity also increase.

* This is due to an increase in the number of active sites in the enzyme

* Thus the rate of activity is more. However beyond a particular concentration of enzyme, there will not be any increase in the rate of activity.

Substrate concentration



* The substrate concentration has significant effect on the rate of enzyme action.

* At very low concentration of the substrate, the initial rate of reaction is very low.

* But the increase in the rate of activity will be only to a particular concentration of the substrate.

* The rate of reaction reaches the maximum (V_{max}).

* This saturation effect is exhibited by all enzyme.

Enzyme inhibition

* Enzyme activity is inhibited by specific small molecule and ion and these are called inhibitors.

* There are two types of enzyme inhibition.

* Irreversible enzyme inhibition
* Reversiable enzyme inhibition

Irreversible enzyme inhibition

At the active sites of the enzyme the inhibition bind themselves permanently and so the enzyme cannot bind to its substrate and thus the action of enzyme is inhibited.

Reversible enzyme inhibition

* The inhibitory molecule bind the enzyme temporarily can cause inhibition in the rate of enzyme action.
* The inhibitory molecules can be removed and the enzyme can become normal.
* They are two types of reversible enzyme inhibition.
  * competitive inhibition
  * NON-competitive inhibition.