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# FUNDAMENTALS OF BIOMOLECULE
## ASSIGNMENT

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Semester → II

Teacher → Mr. Manish Kumar Sachdeva Sir

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# Acknowledgement

* I would like to express my special thanks of gratitude to my Fundaments of Biomolecules professor Mr. Manish Kumar Sachdeva sir for giving me this golden oppurtunity to do this assignment on topic "Enzymology" which is also a part of our core syllabus. For completing this assignment a lot of references are taken which is also going to help in theory exam. I came to know about a lot of new things.

* Secondly, I would like to thank my parents for motivating me and supporting me in completing this assignment.

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Roll no: 23/22005
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Semester-II

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# History of Enzymes :-

* Biological catalysis was first recognised and described in late 1700s.
* Research continued in 1800s with examination of conversion of starch to sugar by saliva and various plant extracts.
* In the 1850s, Louis Pasteur concluded that fermentation of sugar into alcohol by yeast is catalysed by "Ferments".
* In 1897, Edward Buchner discovered that yeast extracts could ferment sugar to alcohol, proving that fermentation was promoted by molecules that continued to function when removed from cells.
→ First enzyme in Yeast extract [Ferment sugar].
* Frederick W. Kühne later on gave the name "ENZYMES" to the molecules detected by Buchner.
* In 1926, James Sumner did isolation and crystallization of first Enzyme = Urease.
→ He also postulated that all enzymes are proteins.
* John Northrop and Moses Kunitz crystallized Pepsin (2nd) and Trypsin (3rd) and found them also protein.
* J.B.S. Haldane wrote a treatise (book) titled "ENZYMES"

# Introduction to Enzymes :-

* Enzymes may be defined as biocatalysts synthesized by living cells. Life depends on powerful and specific catalysts: enzymes. Almost every biochemical reaction is catalyzed by an enzyme.
* With the exception of a few catalytic RNAs, all known enzymes are proteins. Many require non-protein coenzymes are protein. Many require coenzymes or co-factors for their catalytic function.

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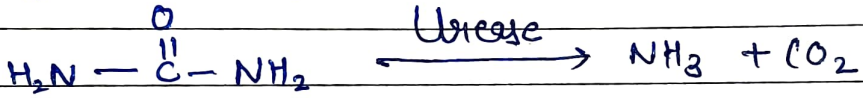
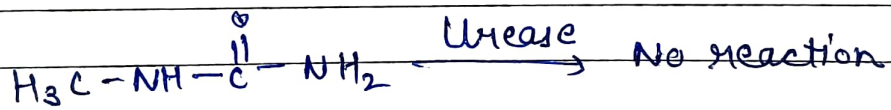
* Enzymes in minute quantities, catalyze the chemical reactions and are not broken down or changed in the process but are reusable.
* The species on which enzyme act is known as substrate.
Ex:) Salivary amylase acts on starch [substrate].

# General Characteristics :-

1) Enzymes have very high catalytic power which is much greater than that of synthetic or inorganic catalysts. They increase the rate of reactions by a factor of $10^6$ to $10^{12}$ time
2) They are required in small quantities.
3) Enzymes are specific in action.




Urea




N-methylurea

4) Enzymes are basically proteins in nature. They have molecular weights ranging from about 12,000 to 1 million.

5) They function in aqueous solutions under very mild conditions of temperature and pH.

6) They are synthesized by biological cells of all organisms; they are involved in chemical reactions related to metabolism. These reactions take place in a stepwise manner, involving different enzymes in organized sequences.

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7) Enzyme catalyzed biochemical reactions take place in a small part of the enzyme called the active site, while the rest of the protein acts as "scaffoldings".

8) Many enzymes need an additional chemical component called Cofactor or Coenzyme to work properly. These can be metal ions [such as $Fe^{2+}$, $Mg^{2+}$, $Cu^{2+}$] or organic molecule [such as haem, biotin, FAD, NAD or co-enzyme A]. A complete, catalytically active enzyme together with its bound co-enzyme or metal ion is called Holoenzyme. The protein part of such an enzyme is called Apoenzyme / Apoprotein.

# Nomenclature

* Earlier, the nomenclature of enzymes was based upon their discoverer which conveyed no information about their function or nature of the substrate on which they act. E.g. Ptyalin, Pepsin, Trypsin, Renin etc.

* Some enzymes have been given names on the basis of species of the origin e.g. Papain from papaya, Ficin from ficus; Subtilisin from Bacillus subtilis etc.

* Enzymes may be named on the basis of chemical reaction they catalyse such as dehydrogenases, Oxidases, Proteinases, Lipases Glycosidases etc.

* A customary practice now is to add the suffix -ase to the name of the substrate for naming the enzymes e.g. Enzyme that attacks starch (amylum) is amylase; Proteinase that attacks proteins, Urease catalyzes hydrolysis of urea and DNA polymerase catalyzes the polymerization of nucleotides.

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and the suffix -lytic is used to denote enzymes activity e.g. Proteolytic, Lipolytic, Amylolytic etc.

## (Classification)

* Enzymes were first divided into two classes as: organized ferments and Unorganized ferments.
* Later, "Internation Union of Biochem and Molecular Biology" (IUBMB) last amended in 1992 for systematic classification and and designation of Enzymes based on reaction specificity.
* All enzymes are classified into six classes :-

### (1) Oxidoreductases :-

→ These enzymes catalyze oxidation-reduction b/w two substances say A and B and are named as for examples oxidases, dehydrogenases, hydroperoxidases, hydroxylases etc.

→ $A_{reduced} + B_{oxidized} \longrightarrow A_{oxidized} + B_{reduced}$

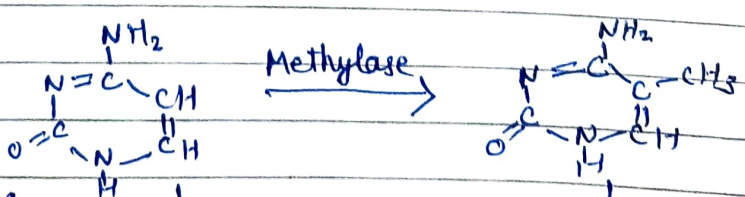
### (2) Tranferases :-

→ These enzymes catalyze the transfer of group or radical [acetyl, methyl, phosphate, amine etc].

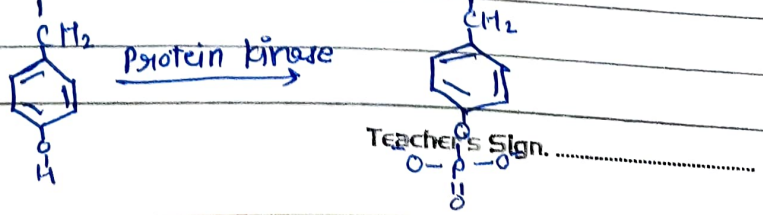
$$A-R + B \longrightarrow A+B-R.$$

(a) acetylation : $H-\underset{H}{N}-(CH_2)_4-$ acetyltransferase $\rightarrow CH_3-\overset{O}{\overset{\|}{C}}-\underset{H}{N}-(CH_2)_4$

(b) Methylation :




(c) Phosphorylation :



## (3) Hydrolases:-

→ These enzymes catalyze hydrolysis of ester, ether, peptide, glycosyl, or acid anhydride groups by the addition of water.
For example:- ~~gly~~ galactosidases, esterases, amidases etc.

→ β-Galactosidases + $H_2O$ $\xrightarrow{\beta\text{-galactosidase}}$ Alcohol + D-galactose.

→ Proteases splits protein molecules:-
Amides → Acid + Ammonia

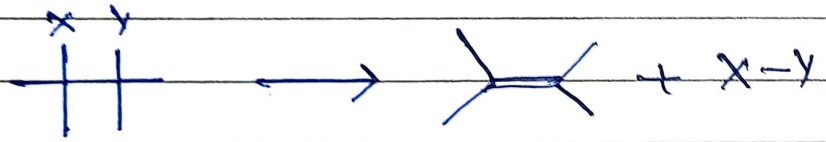
→ Nucleases. (splits nucleic acids).

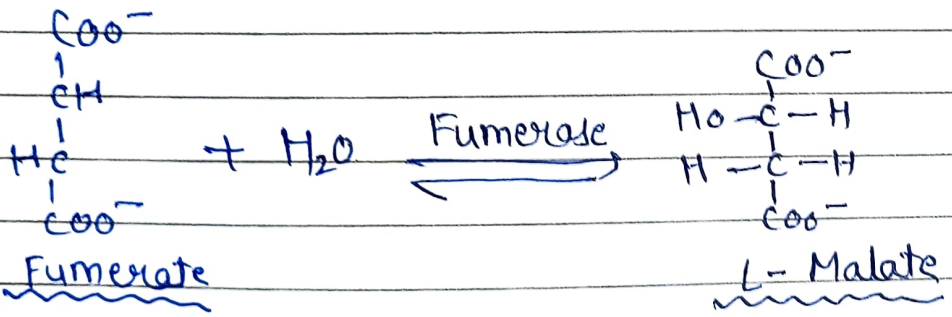
→ Phosphatase. [catalyzes dephosphorylation].

→ Glucosidases. [hydrolyzes fructose to glucose].

## (4) Lyases:-

→ Lyases are those groups which can cleave C-C, C-N, C-S and other bonds by removal of group from substrate other than hydrolysis leaving double bond or conversely adding groups to double bonds. For ex⇒ Aldolases, Fumerases etc.

→ 


→ 

Fumerate — L-Malate

## (5) Isomerases:-

→ These enzymes catalyze interconversion of optical, geometric or positional isomers, some examples are: Isomerases, Racemases and Epimerases.

→ L-Alanine $\xrightarrow{\text{Racemase}}$ D-Alanine.



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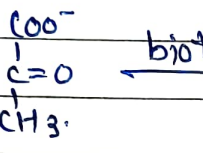
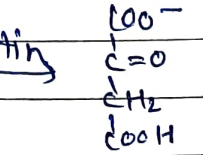
## (6) Ligases :-

→ These enzymes catalyze linking of two compounds by forming a new chemical bond. The reaction is coupled to the breaking of Pyrophosphate (diphosphate) bond in ATP or in a similar triphosphate compound. Ex: DNA ligases.

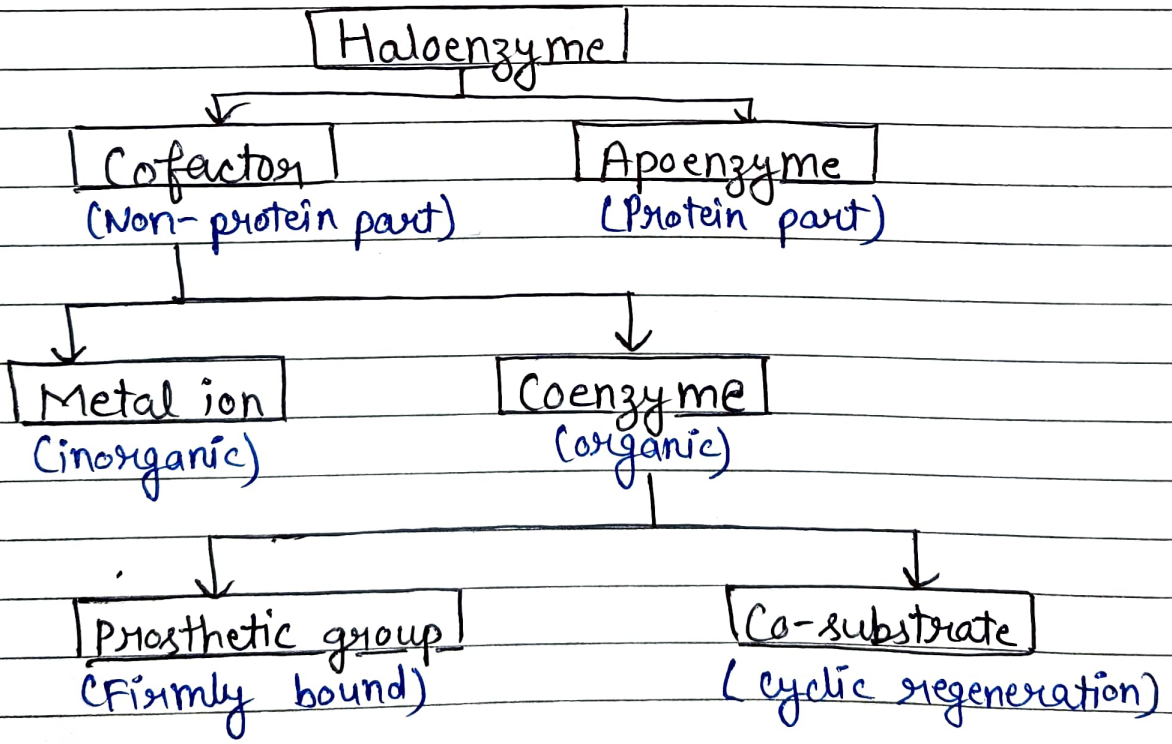
→ GTP + Succinate + CoA ⟶ GDP + iP + Succinyl CoA.

→ ATP + $HCO_3^-$ +  ⟶  + ADP + P

(Pyruvate) (Oxaloacetate)

## # Cofactor and Coenzyme :-

→ The functional enzyme is referred to as <u>holoenzyme</u>. It consists of protein part i/a <u>Apoenzyme</u> and a non-protein part i/a <u>Co-factor</u>.



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# Inorganic metal ions/metals as cofactors

| Cofactor | Enzymes |
|---|---|
| * $Fe^{2+}$ or $Fe^{3+}$ | Cytochrome oxidase, Catalase, Perodidase |
| * $Cu^{2+}$ | Cytochrome oxidase |
| * $Zn^{2+}$ | Carbonic anhydrase, Alcohol dehydrogenase |
| * $Mg^{2+}$ | Hexokinase, Glucose-6-phosphate, Pyruvate kinase |
| * $Mn^{2+}$ | Arginase, Ribonucleotide reductase |
| * $K^{+}$ | Pyruvate kinase |
| * $Ni^{2+}$ | Urease |
| * Mo | Dinitrogenase |
| * Se | Glutathione peroxidase |

# Vitamins as co-enzymes in some reactions

| Vitamin | Coenzyme | Typical reaction type |
|---|---|---|
| * Thiamine ($B_1$) | Thiamine pyrophosphate | Aldehyde transfer |
| * Riboflavin ($B_2$) | Flavin adenine dinucle-otide (FAD). | Oxidation – reduction |

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| | | |
|---|---|---|
| * Pyridoxine ($B_6$) | Pyridoxal phosphate | Group transfer to or from amino acids. |
| * Nicotinic acid (Niacin) | Nicotinamide adenine dinucleotide ($NAD^+$). | Oxidation - reduction |
| * Pantothenic acid | Coenzyme - A | Acyl-group transfer |
| * Biotin | Biotin-lysine adducts (biocytin). | ATP-dependent carboxylation and carboxyl-group transfer. |
| * Folic acid | Tetrahydrofolate | Transfer of one carbon component thymine synthesis. |
| * $B_{12}$ | 5'-Deoxyadenosyl Cobalamin. | Transfer of methyl groups; intramolecular rearrangements. |

# How enzyme work?

* Enzymes catalyze reactions by providing a specific environment within which a given reaction can occur more rapidly. Enzymatic reactions take place at the enzyme active sites.
* Active site is an area on an enzyme which is capable of attracting and holding particular substrate molecule by its specific charge and shape so as to allow the chemical reaction.
* Allosteric site: The site other than the active site where other molecules (activators, inhibitor or modulators) can bind is called as an allosteric site and the phenomenon of regulation of enzyme activity by binding with allosteric site is k/a Allosteric enzyme

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# Mechanism of Enzyme catalysis :-

1) Acid - Base Catalysis → General / Specific

2) Covalent Catalysis.

3) Metal - ion catalysis

## (1) Specific acid - base catalysis :-

- A charge intermediate formed by various biochemical reactions can often be stabilized by the transfer of protons to or from the substrate on intermediate. So, as to form a species or a compound that breaks down more reading to products.

$$[E] + [S] \rightleftharpoons [ES] \rightleftharpoons [EP] \rightleftharpoons [E] + [P]$$

- For non-enzymatic reaction the proton transfer can involve either the constituents of water alone or other weak proton donors or acceptors. Catalysis of this type that uses only proton or hydronium ($H_3O^+$) ion or Hydroxyl ion present in water is refer to as specific acid-base catalysis.
- If proton is transferred between intermediate and water faster than the intermediate breakdown to reactants. The intermediate is effectively stabilized everytime it is formed.
- No additional catalysis mediated by other proton acceptor or donors will occur.

$$A-B \xrightarrow{H_2O} A + B$$

## (2) General acid - base catalysis :-

- It refers to proton transfer mediated by other classes of molecule. For non-enzymatic aqueous solution, this occurs only when the unstable reaction intermediate breaks down to

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reactors faster than proton transfer to or from water.

- Many weak organic acids can supplement water as proton donors in this situation or weak organic bases can serve as proton acceptors.
- In the active site of the enzyme, a number of amino acid side chain can similarly act as proton donor or acceptor. These groups can be precisely positioned or located in an enzyme active site to allow proton transfer.
- Providing rate enhancement of order $10^2$ to $10^5$.

## (3) Covalent catalysis :-

- A transient covalent bond is formed between the enzyme and the substrate.

$$A-B + \underset{\text{Nucleophile}}{X} \longrightarrow A-X + B \xrightarrow{H_2O} A + X-B$$

- In the presence of covalent catalysis an enzyme with nucleophile group X in this particular reaction. So, the reaction will go in this way.
- This alters the pathway of reaction and it results in catalysis only when new pathway has a lower activation energy than the ~~new~~ uncatalysed reaction or pathway. Both of the new steps must be faster than the uncatalysed.
- The functional group of some enzymes co-factors can serve as neucliophiles in the formation of covalent bonds with substrate
- These covalent complexes (A-X, B-X) always undergoes further reaction to generate compounds. The covalent bond formed between the enzymes and substrate can accelerate a substrate for further reaction in a manner that is usually specific to the particular functional group or co-enzyme.

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(4) Metal-ion catalysis :-

- Ionic interactions between an enzyme bound metal and a substrate can help orient the substrate for reaction or stabilize charged reaction transition state. This use of weak bonding interaction between metal and substrate is similar to some of the uses of enzyme-substrate binding energy.
- Metals can also mediate oxidation-reduction reaction by reversible changes in the metal ions oxidation state.

## Enzyme Kinetics

→ The effect on initial rate $V_0$ of varying substrate concentration when the enzyme concentration is held constant.

→ At relatively low concentration of the substrate $V_0$ ↑ almost linearly with an ↑ in substrate concentration.

→ At higher substrate concentration $V_0$ increases by smaller and smaller amount in response to increase in substrate con$^n$.

→ Finally a point is reached beyond which increase in $V_0$ is vanishingly strong in substrate concentration increases and this plateau like $V_0$ region is close to the max. velocity ($V_{max}$).

# Postulates of Leonor Michaelis and Maud Menten (1913) :-

* Enzymes first combines reversibly with its substrates to form an enzyme-substrate complex. In a relatively fast reversible step

$$[E] + [S] \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} [ES]$$

Later, enzyme substrate complex breaks down in a slower

$$[ES] \rightleftharpoons [EP] \underset{K_{-2}}{\overset{K_2}{\rightleftharpoons}} [E] + [P]$$

* Second step to yield the free enzyme and the reaction products because the slower second reaction must limit the rate of

overall reaction. The overall rate must be proportional to conc. of the species that reacts in the second step at any given instance of time.

- An enzyme can be found in two states : [E], [ES]. (Enzyme uncombined, Enzyme-substrate complex).
- At low substrate concentration most of the enzyme is in uncombined state. Here, the rate is proportional to substrate concentration because the equilibrium of $[E] + [S] \rightleftharpoons [ES]$ is pushed towards formation of more [ES] as the substrate concentration increases. The max initial rate of catalyse reaction ($V_{max}$) is observed when virtually all the enzyme is present as [ES] complex and the concentration of enzyme is vanishingly small.
- Under these conditions the enzyme is saturated with its substrate so that further increase in substrate concentration have no effect on the rate.
- After the [ES] complex breakdown to yield the product [P] the enzyme is free to catalyse reaction of another molecule of substrate. The saturation effect is a distinguishing characteristic of the enzymatic catalysis and is responsible for Plateau formation.
- When the enzyme is first mixed with substrate. There is an initial period which is k/a pre-steady state. During which concentration of [ES] complex builds up. This period is usually too short to be easily observed the reaction quickly achieves a steady state in which [ES] complex remains approximately constant overtime. This concept of steady state was overtime. introduced by G.E Briggs and Haldane in 1925.
- The measured $v_o$ generally reflects the steady state even though the $V_o$ is limited to the early part of the reaction and the analysis of these initial rates is referred to as steady state kinetics.

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# Derivation of Michaelis Menten Equation

✱ Quantitative relation / expression of relationship between the substrate concentration and the rate of the reaction :-

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

$$\frac{1}{2} V_{max}[S] = (K_m)$$ → Michaelis-Menten constant.

→ ($V_0$ = Initial velocity) ($V_{max}$ = Maximum velocity)

→ $V_0$ is determined by breakdown of [ES].

→ $[E] + [S] \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} [ES] \rightleftharpoons [EP] \underset{K_{-2}}{\overset{K_2}{\rightleftharpoons}} [E] + [P]$

$[S] \gg [E]$.

$[E_t]$ = Total concentration of Enzyme.

$[E_t] - [ES]$ = Unbound enzyme concentration.

✱ **Step: 1**

- It is assumed that the initial rate of reaction reflects a steady state. Rate of formation and breakdown of [ES] is determined by the steps governed by the Rate Constant $K_1$ and ($K_{-1}$), ($K_2$) respectively.

→ So, the Rate of formation: $K_1([E_t] - [ES])[S]$.

→ Rate of breakdown: $K_{-1}[ES] + K_2[ES]$.

✱ **Step: 2**

- It is assumed that the initial rate of reaction reflects a steady state in which [ES] is constant i.e. the rate of formation of [ES] is equal to rate of its breakdown.

→ Rate of Formation = Rate of Breakdown. Assumption.

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✱ Steps : 3.

- In other words, $K_1[[E_t]-[ES]][S] = K_1[ES] + K_2[ES]$.

$K_1([E_t]-[ES])[S] = (K_{-1}+K_2)[ES]$.

$K_1[E_t][S] - K_1[ES][S] = K_{-1}[ES] + K_2[ES]$.

$K_1[E_t][S] = K_{-1}[ES] + K_2[ES] + K_1[ES][S]$

$K_1[E_t][S] = [ES](K_{-1} + K_2 + K_1[S])$.

$$[ES] = \frac{K_1[E_t][S]}{(K_{-1}+K_2+K_1[S])}.$$

$$[ES] = \frac{K_1[E_t][S]/K_1}{\frac{K_{-1}+K}{K_1}+[S]}.$$

[Now the term, $\frac{K_{-1}+k_2}{k_1} = Km$ Michaelis-Menten equation]

$$[ES] = \frac{[E_t][S]}{Km+[S]}.$$

→ Express the $V_0$ (initial velocity) in terms of $[ES]$.

$$V_0 = \frac{K_2[E_t][S]}{Km+[S]}.$$

→ Because the Max. velocity occurs when enzyme is saturated

$[E_t] = [ES]$

Hence, we can say that $V_{max}$ can be defined as

$\boxed{V_{max} = K_2[E_t]}$ [as we know, $V_{max} = K_2[ES]$]

Now, our equation will become

$\boxed{V_0 = \frac{V_{max}[S]}{Km+[S]}}$ → Michaelis-Menten equation derived

When

$\boxed{V_0 = \frac{1}{2}V_{max}} \longrightarrow \boxed{\frac{V_{max}}{2} = \frac{V_{max}[S]}{Km+[S]}}$

$\frac{1}{2} = \frac{[S]}{(Km+[S])}$

$Km + [S] = 2[S]$

$\boxed{Km = [S]}$

# Units: $[S]$ = mM (milliMoles).

$[V_0]$ = MM/min (Micromole / minute).

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"Km value of every enzyme is different. Km value can vary greatly from enzyme to enzyme and even for different substrates of same enzyme. The actual meaning of Km depends on specific aspects of reaction mechanism such as the number and relative rates of individual steps."

→ When $K_2$ is rate limiting $\boxed{K_2 <<< K_{-1}}$ and Km ↓ $\boxed{Km = \frac{K_{-1}}{K_1}}$

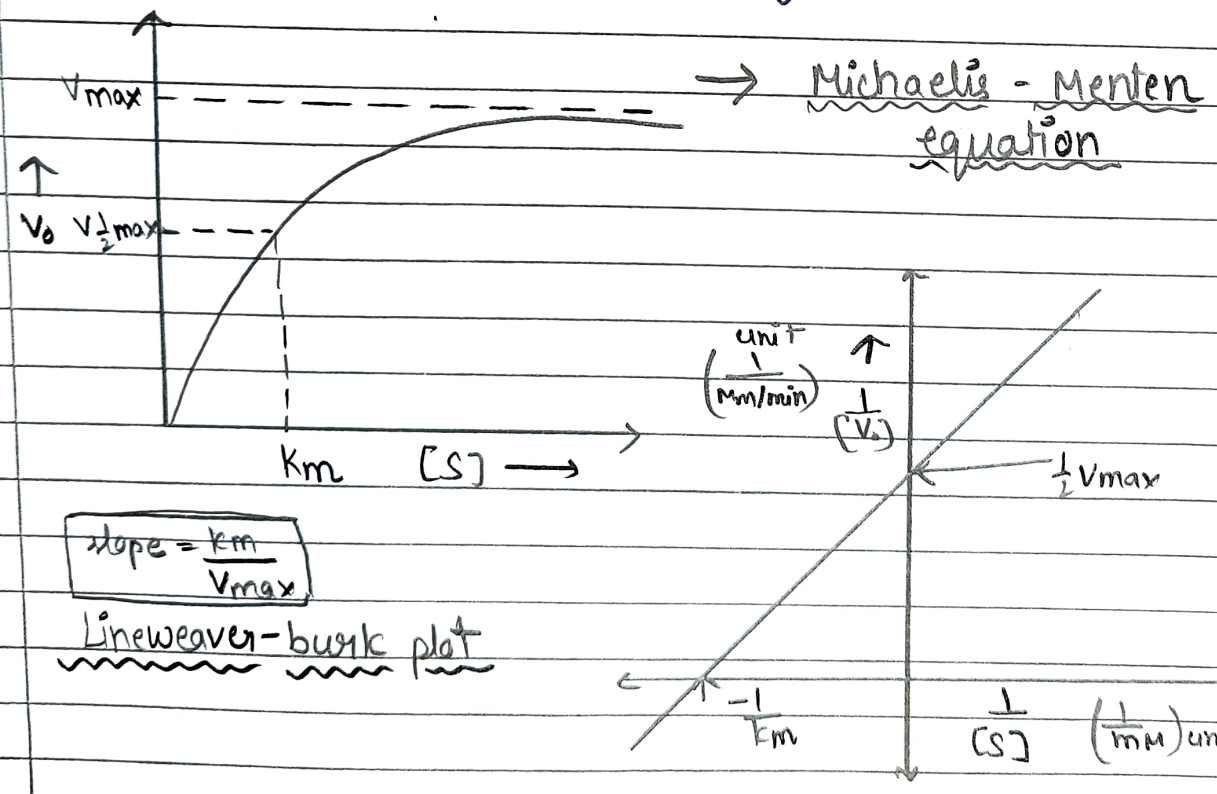
$K_d$ = dissociation constant

→ Km does not represent the measurement of affinity of the enzyme for its substrate.

→ Similarly. Vmax also varies from one enzyme to the next.

# Lineweaver – burk plot :-

Transform the graph (Michaelis-Menten equation) into a double reciprocal plot (straight line) plot




slope = $\frac{Km}{Vmax}$

Lineweaver-burk plot

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→ This plot is to determine the value of $V_o$ and [S] at $V_{max}$.
→ All type of inhibitor/inhibition can be understood by this graph.
→ Important for Bisubstrate reaction.

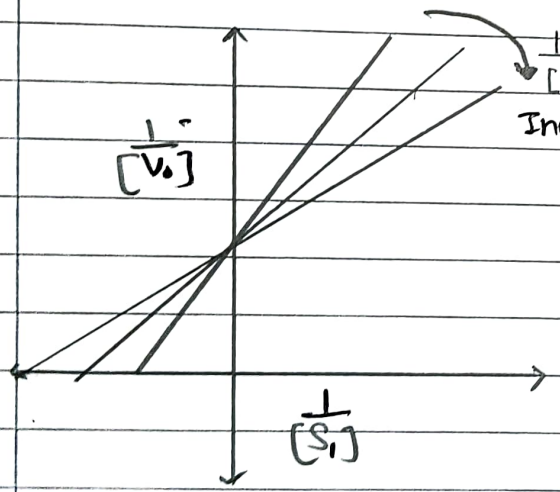
⇒ $$V_o = \frac{V_{max}[S]}{K_m + S}$$

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

$$\boxed{\frac{1}{V_o} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}}$$ → Lineweaver-burk equation

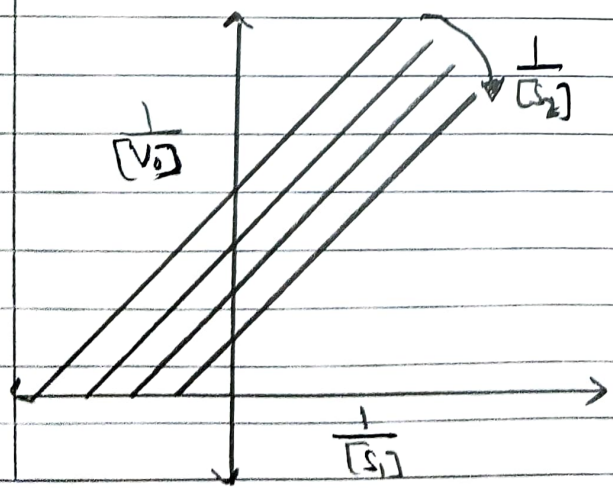
# Steady state kinetics for a substrate and Multi-substrate reaction :-

(i)




⇒ Intersecting lines indicate that a ternary complex is formed.
⇒ $[E_1] + [S_1] \rightleftharpoons [ES] \rightleftharpoons [EP] \rightleftharpoons [E] + [P]$
$[S_2] \rightleftharpoons [ES]$

(ii)




⇒ Parallel lines indicates a Ping-Pong [Double-displacement] Pathway.
⇒ [ES] is not formed for longer time immediately forms the [E].



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## # Significance of steady state kinetics:-

1) Steady state kinetics yields two main parameter.
(i) Kcat (ii) Kcat/Km.
→ The variation in Kcat and Kcat/Km with changes in pH or temperature can provide additional information about the steps in the reaction pathway.
2) In the Bisubstrate reaction the steady state turns as ternary complex or ping-pong pathway (Double-displacement).

## Factors affecting enzyme activity

### (1) Concentration of Substrate:-

- For an enzyme catalysed reaction, a curved graph is obtained measuring reaction velocity at different concentrations.
- We studied the effect of concentration of substrate on activity of enzyme in Michaelis-Menten equation.
- Keeping all other things constant, an increase in substrate concentration ↑ in enzyme activity till maximum is reached, but further ↑ in substrate concentration does not ↑ the rate of reaction. A rectangular hyperbola is observed when rate of reaction is plotted.

### (2) Concentration of Enzyme:-

- Rate of enzyme catalyzed reaction ↑ proportionally as enzyme concentration is increased. The relationship is linear. At low enzyme concentration there are more substrate molecules than the available active sites. Increasing the number of active sites by ↑ the enzyme concentration effectively ↑ the rate of reaction.

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(3) Concentration of Products :-

- An ↑ in product concentration ↓ the enzyme activity. If the products are allowed to remain in the area of reaction, the rate of forward reaction will fall and reverse reaction may start.

(4) pH :-

- Enzymes are affected by changes in pH of the medium. The most favorable pH i.e. the pH value at which the enzyme has max. activity is known as optimum pH. Each enzyme has optimum pH.
- At a pH one unit below or above this value the enzymes are only partially active. At pH values far removed from optimum, the enzymes can be denatured and lose activity.
- While overall change on enzyme is important, denaturation of the active site structure results in loss of activity.
- Optimum value :-

Lipase pancrease (8.0), Lipase stomach (4-5), Lipase castor oil (4.7), Amylase pancrease (6.7-7.0), Amylase malt (4.5-5.2) etc.

(5) Effect of temperature :-

- Temp. is one of the most important factors affecting the enzyme activity because enzymes are mostly proteins and denature at high temperature. [above 37°C]
- The temp. at which, the rate of reaction is maximum is known as optimal temperature.
- Like non-enzymatic reactions, enzyme activity ↑ with ↑ in temperature usually doubling the rate at every 10°C rise in temperature.

6) Radiations :- UV rays, γ-rays, β-rays produce peroxides which oxidize the enzyme and inactivate them.

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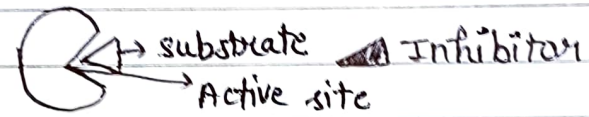
## (8) Enzyme Inhibition :-

* Enzyme inhibitors are substances that alter the catalytic action of enzyme and consequently slow down or in some cases stop catalysis. There are three types of common enzyme inhibition.

### (i) Competitive Enzyme Inhibition :-

- This type of inhibition occurs when inhibitor resemble in structure to that of substrate are present in the active site of enzyme. It prevents the binding of the substrate to the enzyme active site.

$$[E] + [S] \rightleftharpoons [ES] \rightleftharpoons [EP] \rightleftharpoons [E] + [P]$$
$$+I \uparrow\downarrow$$
$$[EI]$$




→ Many competitive inhibitors are the compounds that resemble the substrate and enzyme combine to form [EI] complex.

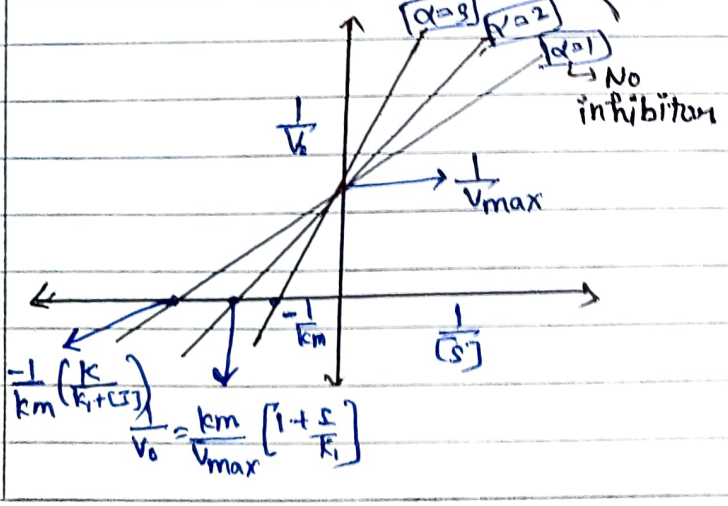
→ So, the presence of competitive inhibitor makes Michaelis–Menten equation :–

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

$$\left[\alpha = 1 + \frac{[I]}{K_I}\right]$$

$$\left[K_I = \frac{[E][I]}{[EI]}\right]$$

→ Lineweaver - burk plot :– (Double - reciprocal plot)




→ The competition can be biased to favor the substrate simply by prevailing the conditions for product formation from substrate.

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→ When concentration of [S] ⋙ [I] the probability that an inhibitor molecule will bind to the enzyme is minimised and the reaction exhibits a normal Vmax. However, the substrate concentration at which $V_o = \frac{1}{2} V_{max}$. The apparent Km ↑ in the presence of inhibitor by factor ($\alpha$). The effect on apparent km combined with the absence of an effect on Vmax is diagnostic of C.I.

## (2) Uncompetitive Inhibition :-

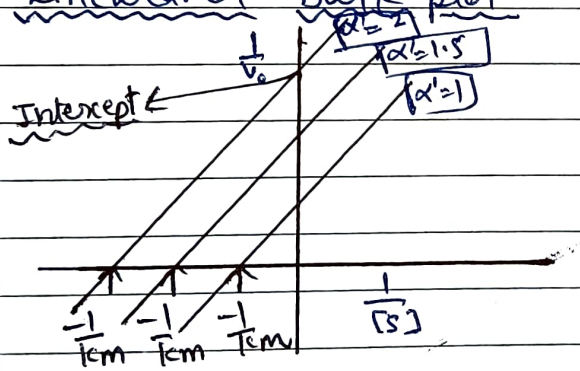
→ The uncompetitive inhibitor bind at the site distinct from the substrate active site. Unlike competitive inhibition, bind only to active site [ES] complex. In the presence of an uncompetitive inhibition Michaelis-Menten equation is altered to :-

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

$$\left[\alpha' = 1 + \frac{[I]}{K_i}\right]$$

$$\left[K'_i = \frac{[ES][I]}{[ESI]}\right]$$

* Lineweaver - burk plot :-



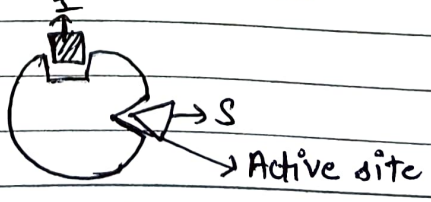

$$\text{Intercept} = \frac{1}{V_{max}}\left[1 + \frac{[I]}{K_i}\right]$$

$$\frac{1}{V_o} = \frac{K_m}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}}\left[1 + \frac{[I]}{K_i}\right]$$

→ At increasing concentration of substrate $V_o$ approaches $\frac{V_{max}}{\alpha'}$ thus an uncompetitive inhibitor lowers the measure of $V_{max}$ apparent $K_m$ also decreases because the substrate concentration required to reach $\frac{1}{2}(V_{max})$ decreases by the factor $\alpha'$

$$[E] + [S] \rightleftharpoons [ES] \rightleftharpoons [EP] \rightleftharpoons [E] + [P]$$

$$+[I] \quad K_i \downarrow\uparrow \quad [ESI]$$

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## (iii) Non-Competitive Inhibition : (Mixed inhibition)

* Mixed inhibitor also binds to the site distinct from the substrate binding site. But it binds to either [E] or [ES] complex so the rate equation becomes.

$$V_0 = \frac{V_{max}[S]}{\alpha K_m + \alpha'[S]}$$

* Mostly occur in bisubstrate reaction.

$$[E] + [S] \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [ES] \rightleftharpoons [EP] \overset{k_2}{\rightleftharpoons} [E] + [P]$$

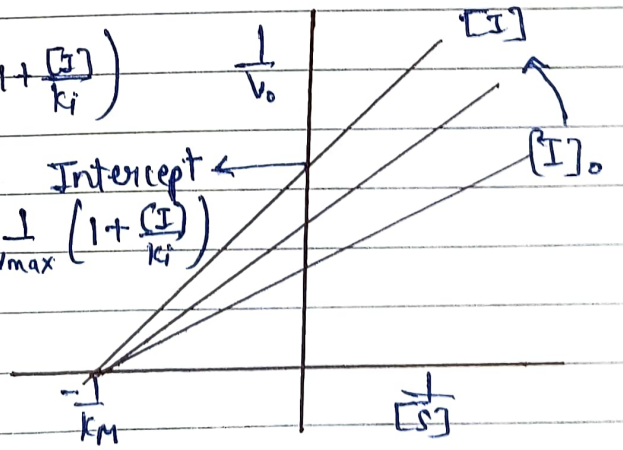
$$\begin{array}{ccc} + & & + \\ [I] & & [I] \\ k_i \downarrow\uparrow & & k_i \downarrow\uparrow \\ [EI] + [S] & & [ESI] \end{array}$$

* Mixed inhibitor affects both $K_M$ & $V_{max}$ but it is rarely occured. For all reversible inhibitors apparent

$$V_{max} = \frac{V_{max}}{\alpha'}$$

* Lineweaver - burk plot :

* $\frac{1}{V_0} = \frac{K_M}{V_{max}}\left(1 + \frac{[I]}{K_i}\right)\frac{1}{[S]} + \frac{1}{V_{max}}\left(1 + \frac{[I]}{K_i}\right)$




* $V_{max}$ reduces by factor $\left(1 + \frac{[I]}{K_i}\right)$.

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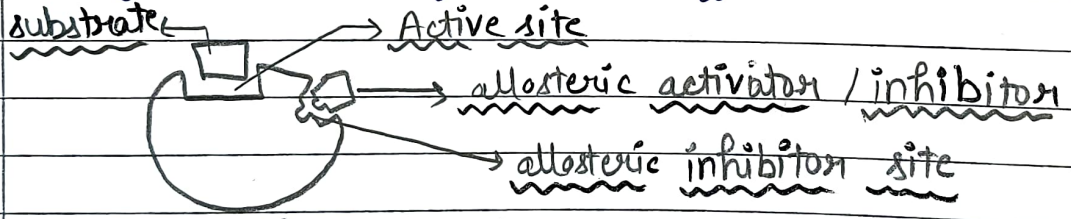
9) **Activators :- (Allostery)**

- It is regulation of an enzyme by an effect or molecule by binding at the enzyme's allosteric site [site other than the active site]. Effectors that enhance the enzyme activity are called as allosteric activator whereas those that decrease the activity are refferred to as allosteric inhibitors.
- Allosteric inhibition is a natural example of feedback control and is a non-competitive inhibition. Metals are also known as enzyme activators. They change the charge on active site, enabling the enzyme substrate complex to form e.g:- $Fe^{2+}$ in catalase.

# Allosteric inhibitors and activators :-

* Allosteric activators and inhibitors are compounds that alter the activity of multimeric allosteric enzymes.
* Their binding sites on the enzyme are different from the substrate binding site and their effect on the enzyme activity is through a distal conformational effect on substrate binding site.




Schematic model of Enzyme

* **Properties of Allosteric enzymes :-**

1) Catalyze irreversible reactions; are rate limiting.
2) Generally contain more than one polypeptide chain.
3) Do not follow Michaelis-Menten kinetics.
4) Can be upregulated by allosteric activators at constant [S].
5) Can be down regulated by allosteric inhibitors at constant [S].
6) Activators & Inhibitors need not have any structural resemblance to substrate structure.



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# Application of Enzymes:-

1) Alcoholic fermentation and other important industrial processes. The enzyme synthesized by the yeasts and bacteria are used for this purpose.

2) Enzyme rennin is used for the conversion milk to curd. It encourages casein, one of the proteins in milk, to solidify and clump together or coagulate.

3) Enzymes are capable of producing one of the enantiomers of the product (asymmetric synthesis).

4) A number of enzymes are used for medical purposes such as for the treatment of local inflammation, thrombosis etc.

5) Enzymes are used for the treatment of industrial, agricultural or municipal wastes. Here, the function of the enzyme is to convert a toxic substance into the non-toxic one.

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Aradhana