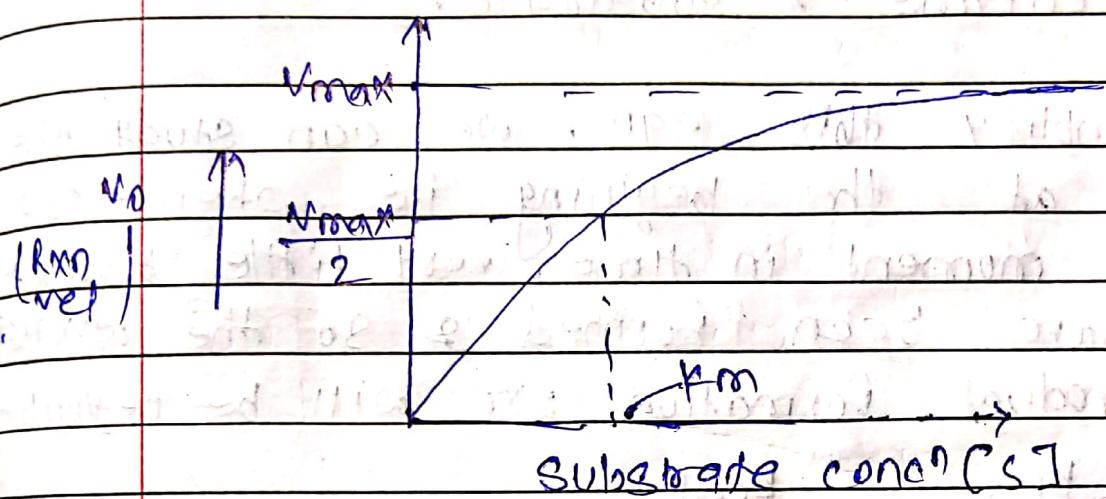


11th SEM

Topic - Enzyme kinetics

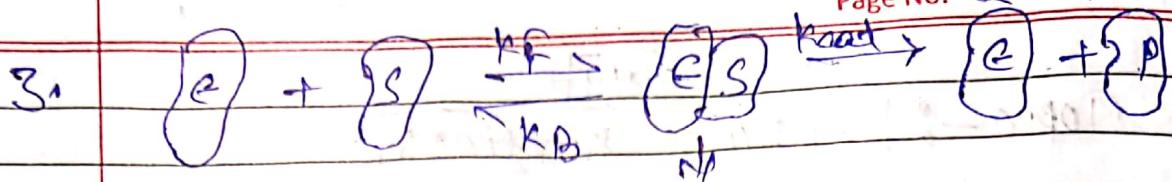
- it is the study of enzyme-catalyzed reactions.
- Michaelis-Menten model of enzyme is one of the best model for single substrate enzyme kinetics.



- V_0 is the rxn velocity at which the enzyme catalyzed rxn takes place.

Note - The curve shown above is known as Michaelis-Menten curve. It is a plot of rxn vel of enzyme versus the substrate concn.

- The curve shows that as we increase the concn of substrate, the velocity approached V_{max} asymptotically.

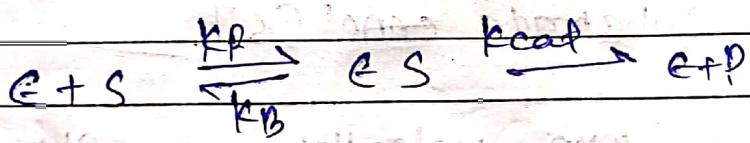


enzyme
substrate
complex

$k_F \rightarrow$ rate of formation of ES complex

$k_B \rightarrow$ rate of dissociation of ES complex
to enzyme & substrate.

- To simplify this eqn. we can study the rxn at the beginning i.e. when $t \approx 0$. At this moment in time, very little product will have been formed & so the reverse of product formation rxn will be negligible.



$$① \quad v_0 = k_{cat}[ES]$$

$$② \quad \text{Rate of formation of } [ES] = k_F[E][S]$$

$$③ \quad \text{Rate of dissociation of } [ES] = k_B[ES] + k_{cat}[ES]$$

- Assuming the steady-state cond'n i.e. the concn of enzyme substrate complex remains constant. This means the rate of formation of $[E[S]$ is the same as rate of dissociation of $[E[S]$.

$$k_f [E] [S] = k_d [E[S] + k_{cat} [E[S]$$

$$\frac{[E] [S]}{[E[S]} = \frac{k_d + k_{cat}}{k_f}$$

- The term $\frac{k_d + k_{cat}}{k_f}$ is defined as k_m (Michaelis constant).

$$k_m = \frac{k_d + k_{cat}}{k_f}$$

- Now, at the beginning of rxn, i.e. when $t = 0$

$$[S]_{\text{total}} = [S] + [E[S] \approx [S]$$

This is so because, initially, a lot less substrate would have bound to the enzyme.

$$[E]_{\text{total}} = [E] + [ES]$$

$$\Rightarrow [E] = [E]_{\text{total}} - [ES]$$

So, $k_m =$

$$\frac{[E][S]}{[ES]}$$

$$k_m = \frac{[E] - [ES]}{[ES]} [S]$$

$$So, V_0 = k_{\text{cat}} [ES]$$

$$V_0 = \frac{k_{\text{cat}} [E][S]}{k_m + [S]} \rightarrow ①$$

$$[ES] = \frac{[E][S]}{k_m + [S]}$$

When all the substrates have binded to the active site of enzyme, then our reaction velocity would be maxim.

$$\therefore V_{max} = k_{cat} [E_S]_{max}$$

$$= k_{cat} [E_T]$$

$$[E_T] = \frac{V_{max}}{k_{cat}} \quad \text{--- (2)}$$

put (2) in (1)

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

↳ Michaelis-Menten eqn.

note:- Michaelis-Menten eqn. is a mathematical expression describing the relationship b/w reaction velocity & substrate concn.

- info that we can extract from Michaelis-Menten eqn.

① if we set $k_m = [S]$, then $V_0 = \frac{V_{max}}{2}$

∴ k_m is the substrate concn at which the rate of enzyme is exactly equal to half of the maximal velocity.

(iii) This eqn can be used to describe the enzyme activity at beginning ($t \approx 0$)

when $t \approx 0 \Rightarrow km \gg [S]$

$$\Rightarrow km + [S] \approx km$$

$$\therefore v_0 = \frac{V_{max}[S]}{km + [S]} = \frac{V_m [S]}{km}$$

Hence, $v_0 \propto [S]$

So, this implies that in the beginning (at lower conc' levels) the rxn follows a 1st order rxn i.e. rate of rxn is directly prop. to substrate concn.

We can also use the eqn to describe enzymatic activity at the end of rxn

$$[S] \gg km$$

$$\therefore v_0 = \frac{V_m [S]}{km + [S]} = \frac{V_{max} [S]}{km}$$

$$\underline{v_0 = V_{max}}$$

This implies that at the end of rxn (higher concn levels) the rxn follows a zeroth order rxn. This means that increasing the rate of rxn will not affect rate.

NOTE:-

① Assumption while deriving the Michaelis-Menten eqn.

(a) This eqn is derived for initial time ($t \approx 0$), so k_{cat} is not possible i.e. the rxn will proceed in both dirn.

(b) concn of substrate is much larger than concn of enzyme.

(c) steady state assumption i.e. E_S dissociation is insignificant & it remains constant throughout measured portion of rxn.

2) $[S] \ll \ll k_m \Rightarrow V_0 \propto [S]$ (1st order rxn)

$[S] \gg \gg k_m \Rightarrow V_0 = V_{max}$ (0th order rxn)

$$\text{At } [S] = k_m = \boxed{V_0 = \frac{V_{max}}{2}}$$

So, Michaelis menten const (km) is defined as substrate conc for which rxn velocity becomes half of maximum velocity.

③ $km = \frac{k_p + k_{p^{-}}}{k_p}$ ← Sum of rate constants for E_S dissociation
 , rate constant for E_S formation.

④ km is different for
 (i) different enzymes
 (ii) different substrate for same enzyme
 (iii) diff temp, pH, ionic strength.

⑤ km does not depend on concn of enzyme.

⑥ km is also a measure of affinity of an enzyme for its substrate.

Affinity of an enzyme \propto
 for its substrate

$\frac{1}{km}$

$km \uparrow \Rightarrow$ less formation \Rightarrow affinity \downarrow
 of E_S complex

$km \downarrow \Rightarrow$ more formation \Rightarrow affinity \uparrow
 of E_S complex

→ LineWeaver-Burk's Double reciprocal plot.

1.) Using Michaelis-Menten hyperbolic curve, it is diff to precisely locate V_{max} & K_m on graph. A double reciprocal plot of same eqn when plotted gives a value of K_m & V_{max} accurately.

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

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

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

For finding y intercept, x co-ord = 0

$$\text{i.e. } \frac{1}{[S]} = 0$$

$$\therefore \frac{1}{V_0} = \frac{1}{V_{max}}$$

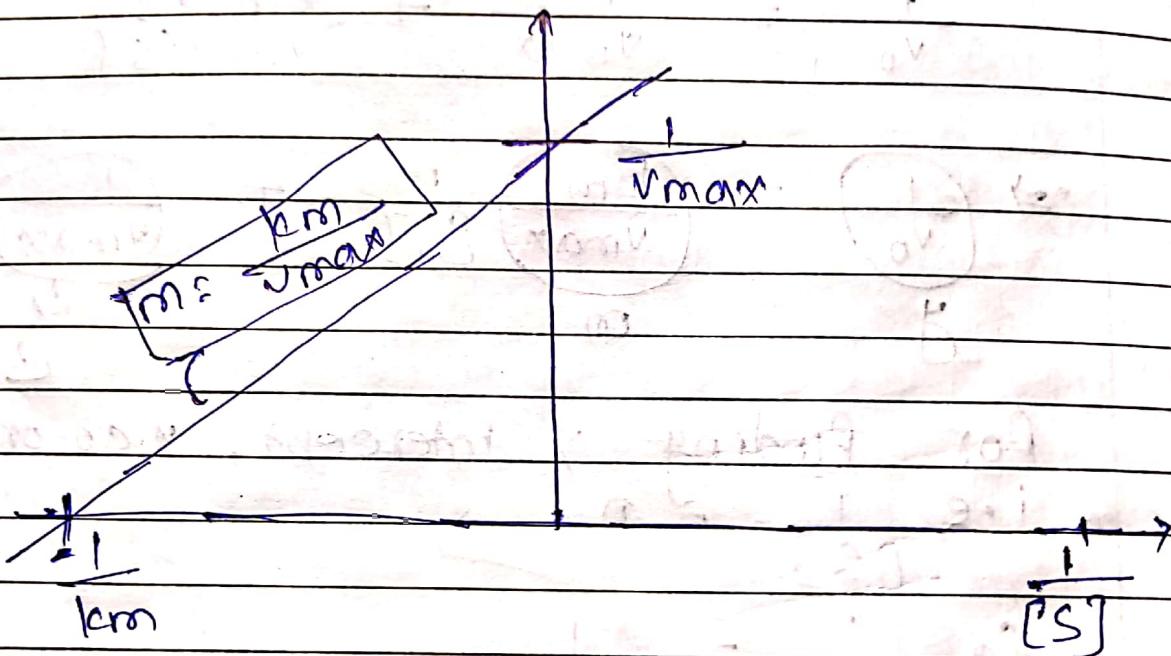
$$\text{So, y intercepts} = \frac{1}{V_{max}}$$

For x Intercept, y cord = 0 $\Rightarrow \frac{1}{v_0} - \frac{1}{v_m} = 0$

$$\frac{1}{v_{max}} - \frac{1}{[CS]} = -\frac{1}{v_m}$$

$$\Rightarrow \frac{1}{[CS]} = 1 - \frac{1}{v_m}$$

Plot $\frac{1}{v_0}$ and $\frac{1}{[CS]}$



To obtain v_{max} : Take reciprocal of y intercept

To obtain km : Take reciprocal of x intercept with -ve sign.

UNIT - 1

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Enzymes & their classification

Enzymes are biocatalyst (it ^{helps in} catalyse ~~happens~~ in living organism).

Increase the rate of chemical reaction.

Most are protein except Ribozyme (RNA).

High degree of specificity.

Lower the activation energy.

Classification

Simple enzymes: only protein.

Conjugated enzymes: protein + co-factor (Non protein).

Part of protein is called Apoenzyme and when co-factor adds with apoenzyme called Holoenzyme.

Enzyme Nomenclature

- Common names.
- SUFFIX - ale is added.
- International Commission of Enzymes (EC)
 - A rule through which one enzyme have only one name.

• EC 2.7.1.1.2 (Hexokinase)

• EC 1.1.1.1 (Alcohol dehydrogenase).

SIX CLASSES

- EC 1: Oxidoreductases
- EC 2: Transferases
- EC 3: Hydrolases
- EC 4: Lyases
- EC 5: Isomerases
- EC 6: Ligases

EC 1. ~~and~~ oxidoreductases

- Oxidation reduction reactions.



Example: Oxidases, Dehydrogenases, Oxygenases, peroxidases.

involved in transfer of e^- from one substrate to another.

Transfereases EC 2.

Transfer of groups from one molecule to another.

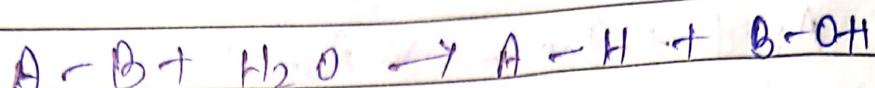


Examples: Transaminases, kinase, phosphorylase.

Transfer of some functional group from one substrate to another.

EC 3 Hydrolases:

Cleavage ^{covalent} of bonds by adding water.

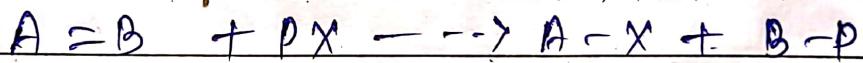


- Examples: phosphatases, peptidases, phosphodiesterases.

They are responsible for addition & elimination rxn (break / form bonds) without involvement of energy.

EC 4
Lyases

- Break the C-C, C-O, C-N, C-S bonds.



- Examples: Aldolases, Synthases, Decarboxylases.

Aldolases \rightarrow remove aldehyde.

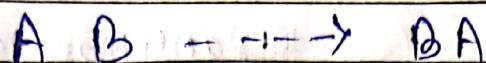
Synthases \rightarrow help in adding two molecule but ATP require to add.

Decarboxylases \rightarrow remove CO_2 help in Decarboxylation.

Rearrangement of functional grp
within the molecule, Date / /
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EC 5: Isomerases

intramolecular rearrangement and yield
isomeric form.



examples: mutases, cis-trans isomerases,
epimerases.

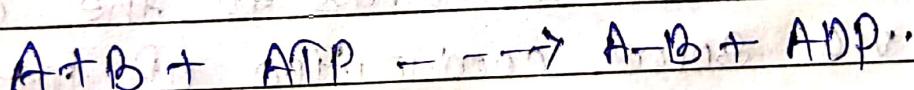
mutases \rightarrow intramolecular transfer of
functional grp.

Cis-trans isomerases \rightarrow made geometry
isomeric.

epimerases \rightarrow invert asymmetric carbon.

EC 6: Ligases

Formation of C-C, C-S, C-O and C-N bonds



example: carboxylases.

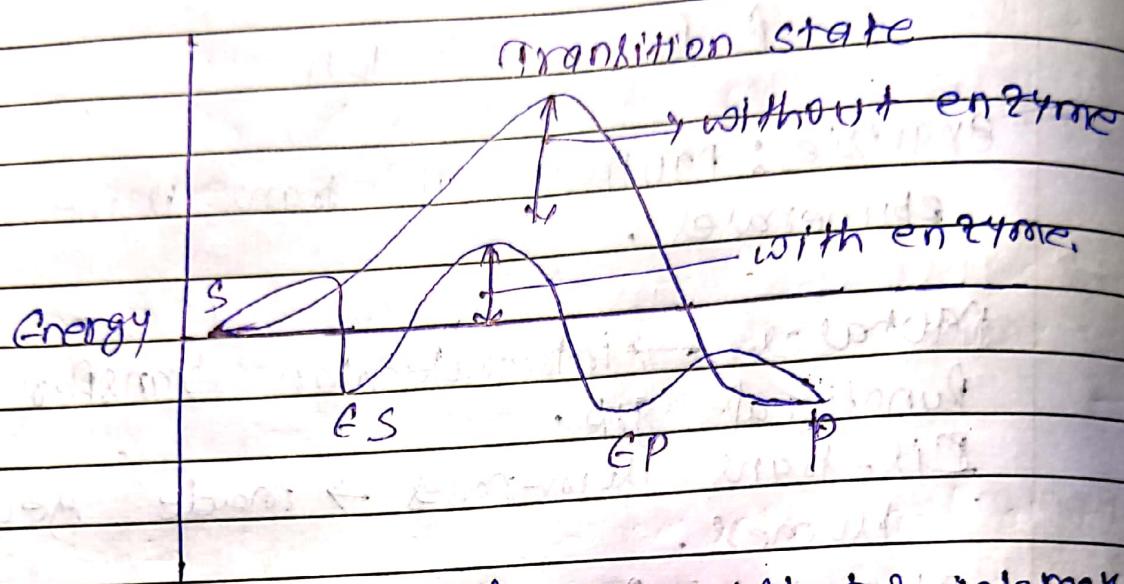
carboxylases - use CO_2 as a

substrate.

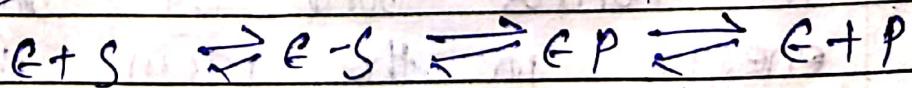
it forms bond by utilization of
energy in the form of ATP/CTP.

Mechanism of enzyme action.

What enzyme does?



- The enzyme and its substrate interact only over a small region of the surface of the enzyme, called the active site.
- When a substrate binds to the active site via some combination of intermolecular forces, an enzyme-substrate (ES) complex is formed.
- Once the complex forms, the conversion of the substrate (S) to product (P) takes place.



E-S complex

- Models
 - 1. Lock and key model
 - 2. induced fit model

Lock and key model :-

- First postulated in 1894 by Emil Fischer.
- The lock is the enzyme and the key is the substrate.
- High degree of similarity between the shape of the substrate and the binding site of the enzyme.
- A limitation of this theory is that it requires enzymes conformations to be rigid.

Induced fit model

- Koshland suggested that induced fit model in 1963.
- Proteins have 3D flexibility.
- The substrate plays a role in determining the final shape of an enzyme and that the enzyme is partially flexible.

- Catalysts → Catalysts are defined as substances which enhance the rate of rxn without undergoing any permanent change during them & therefore are reqd in small quantities.
- Non-enzymatic Catalyst are non-specific i.e same catalyst can participate in multiple rxn while biocatalyst are substrate specific as well as stereospecific.
- Simple enzyme → enzyme which is only made up of protein.
- Inactive protein molecule of the enzyme is known as apoenzyme.
- Apoenzyme with co-factor called Holoenzyme.