"Enzymes"

Introduction:-

* Enzymes are biocatalysts or Enzymes are catalytic proteins.
* They are colloidal, high molecular weight, non-dialysable, denaturable, structurally diverse groups of proteins. Biochemical reactions taking within the living cells are catalysed by enzymes (hence the name is biocatalysts). Enzymes increases the rate of reaction without undergoing any change themselves and without altering the equilibrium of the reaction.
* Enzymes help to speed up reactions of digestion and metabolism.
* They are soluble and colloidal.
* Cells can't existing without enzymes.
* The term enzyme was derived from Greek means "in yeast", because the yeast cells were the first to revel enzyme activity in living organism.
* It was first introduced by W. Kuhn in 1878. The study of enzymes is known as enzymology.
* Enzymes are located in the cells, cytoplasm, mitochondria, tissues and the body fluids.

Nomenclature of Enzymes:-

Enzymes are named based on substrates, the reaction, synthesis, chemical nature etc.

① Based on Substrate:-
ⓐ The enzymes acting on carbohydrates are named as carbohydrases.

(b) The enzymes acting on proteins = proteinases.
(c) The enzymes acting on Lipids = Lipases.
(d) The enzymes acting on nucleic acid = Nucleases.
(e) The enzymes acting on Maltose = Maltase.
(f) The enzymes acting on Lactose = Lactase.
(g) The enzymes acting on Sucrose = Sucrase.
(h) The enzymes acting on Urea = Urease.
Substrate is Substances on which an enzyme acts. Many enzymes are named by ading the Suffix -ase to the name of substrate.

(2) Based on Reaction :
The enzymes are highly specific as to the reaction they catalyze. Hence this had necessitated their naming by adding the suffix -ase in the name of the reaction.
(a) The enzyme catalysing hydrolysis = Hydrolase
(b) The enzyme catalysing oxidation = Oxidase.
(c) The enzyme catalysing reduction = Reductase.
(d) The enzyme catalysing Dehydrogenation = Dehydrogenase
(e) The enzyme catalysing Phosphorylation = phosphorylase.
(f) The enzyme catalysing transmination = Transminase.
(g) The enzyme catalysing isomerization = Isomerase.

(3) Based on Substrate reaction :

Some enzymes are named based on the substrate utilized and the type of reaction catalyzed.

(a) The enzyme removing CO_2 from pyruvic acid is named as pyruvic decarboxylase.
(b) The enzyme removing hydrogen from isocritic acid is named as isocritic dehydrogenase.

④ Based on synthesis.
⑤ Based on Discoverer.
⑥ Based on Enzyme-commission.
commission on enzymes named the enzymes into 6 groups based on the chemical reaction catalyzed. They are –
(a) ① Oxidoreductases (b) Transferases (c) Hydrolases.
(d) Lyases (e) Isomerases (f) Ligases.

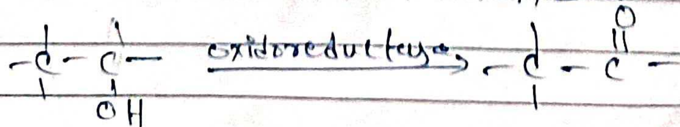
⑦ Based on E. Commission Number:
The commission on enzymes named the enzymes by a code number called Enzyme commission number. According each enzyme is named by 4 digit number.
Example: 1.1.1.1.
The first number represents the class of enzyme. The second number represents the sub-class. The third number represents the sub-sub class. And final number represents the Enzymes.

Classification of Enzyme:

Enzymes are classified into 6 ~~categories~~ classes based on reactions they catalyzed.

(a) ① Oxidoreductase: Oxidoreductases performs oxidations - reductions reaction and/or electron transfer.
Example: Alcohol dehydrogenase (E.C. 1.1.1.1)

(b) Alcohol dehydrogenase perform oxidation of ethanol to Acetaldehyde. Where NAD^+ is reduced into NADH.

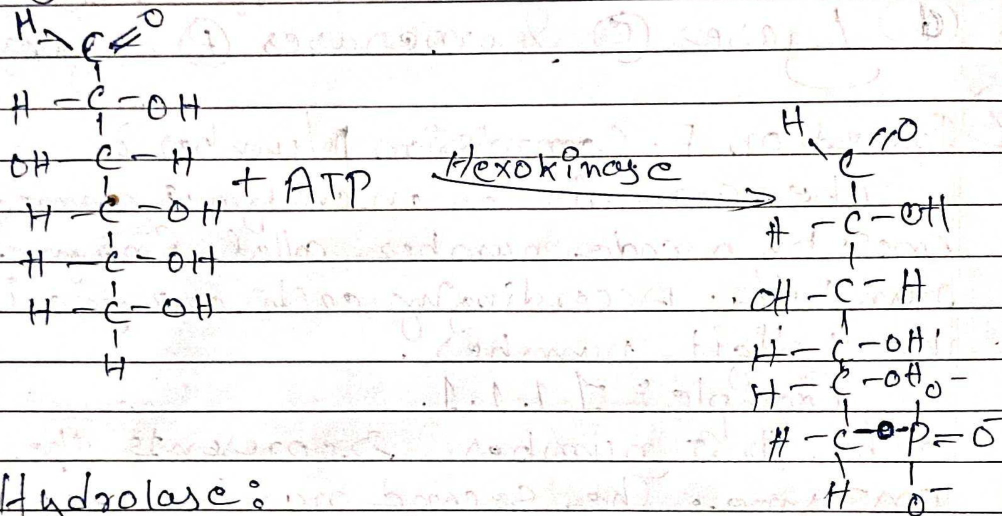


AMAS Page No. / Date / /

(b) Transferase:

They are transfer functional group from one molecule to another.

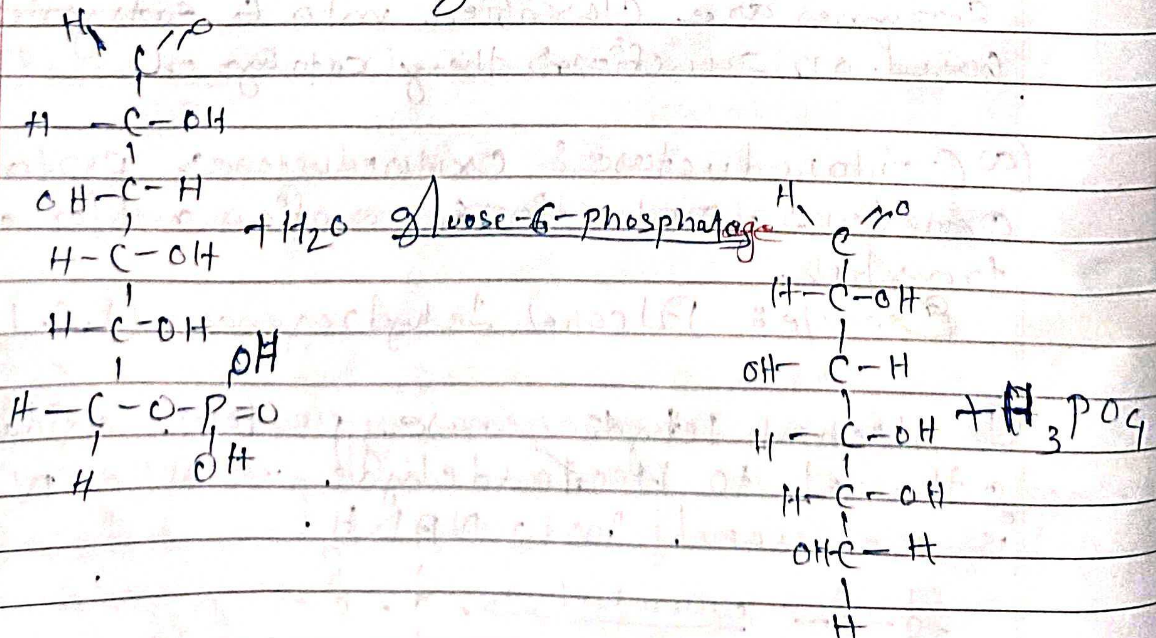
Example: Hexokinase (EC 2.7.1.1) perform phosphorylation of glucose to glucose-6-phosphate using the terminal phosphoryl group from ATP.



(3) Hydrolase:

These enzymes performs hydrolytic cleavage of one molecule into two molecules.

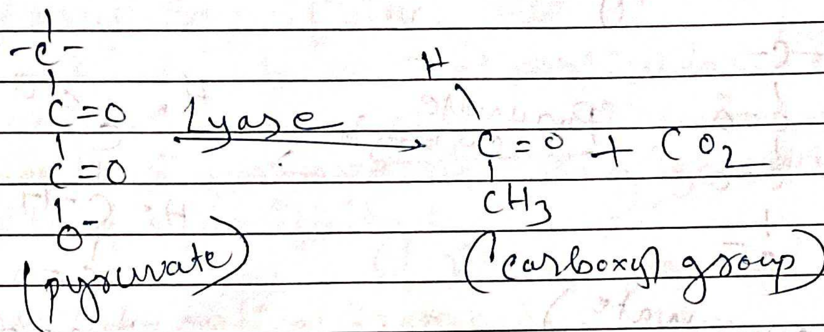
Example: Glucose-6-phosphatase (EC 3.1.3.9) breaks (glucose-6-phosphate) into glucose and organic phosphate using a H_2O molecule.



(4) Lyase:

These enzymes performs removal of a group from, or addition of a group to, a molecule.

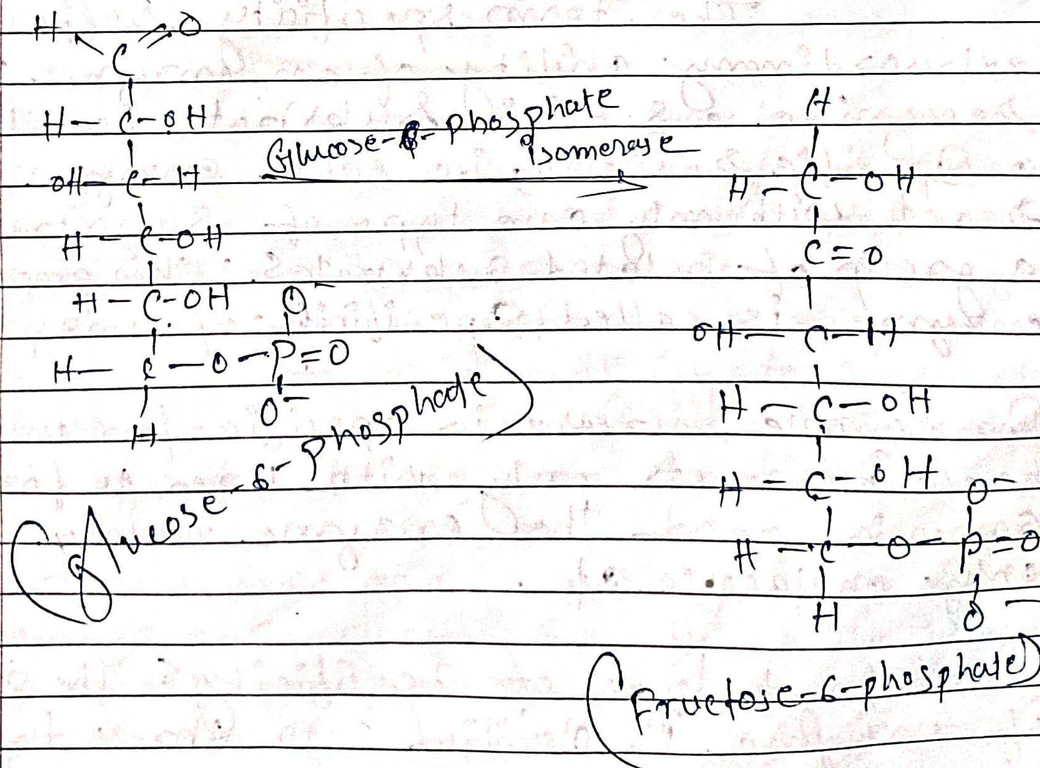
Example: Pyruvate decarboxylase (EC 4.1.1.1) Removes carboxyl group from pyruvate to produced acetaldehyde and carbon dioxied (CO_2)



(5) Isomerase:

Isomerase performs movement of functional group within a molecule.

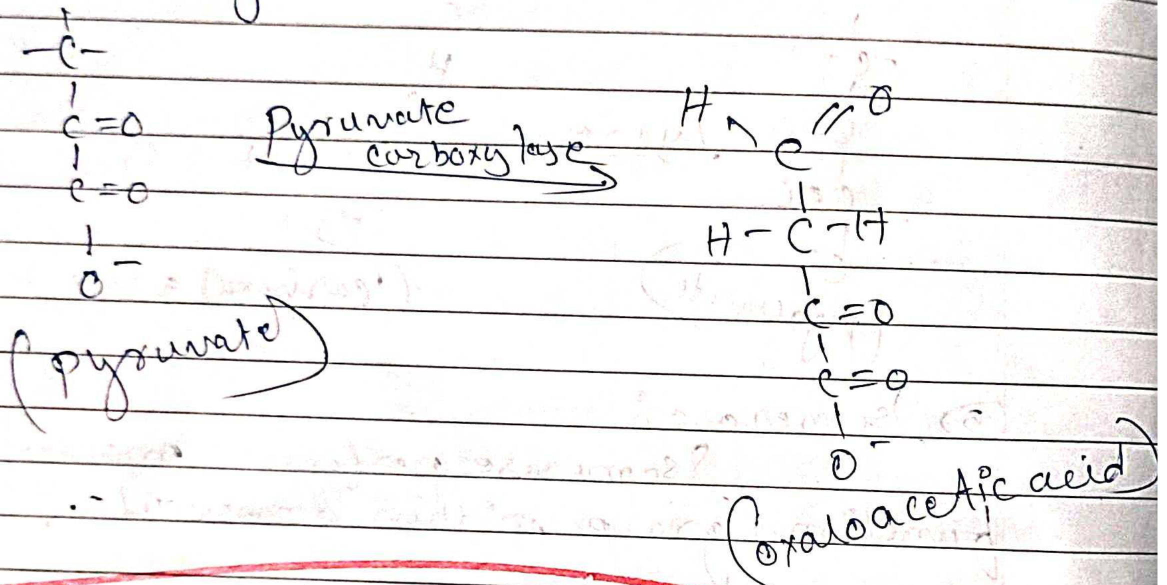
Example: Glucose-6-phosphate isomerase (EC.5.3.1.9) converts glucose-6-phosphate in fructose-6-phosphate by moving the carbonyl group from 1st carbon to 2nd carbon.



⑥ Ligase:

These enzyme join two molecule to formed a single molecule.

Example: Pyruvate carboxylase (EC 6.4.1.1) adds CO_2 to pyruvate oxaloacetic acid.



Specificity of Enzyme Action:

Specificity is one of the most important properties of enzymes.

The term specificity reffers to the extraordinary ability of an enzyme to recognize a specific substrate to catalyzed a specific reaction, i.e each enzyme will react with only one type of substrate or a group of related substrates. This property of enzyme is called specificity of enzymes.

For example, urease is specific for urea and hence it reacts only with urea to form amonia and the enzyme lactase acts only on lactose etc.

According to degree of specificity: The specificity of enzyme is classified into three types:

AMAS Page No.
Date / /

(a) Absolute Specificity:
When an enzyme acts ~~one~~ only one substrate, the specificity is called absolute specificity.

Example: Urease acts only urea.

(b) Group Specificity:
A particular enzyme act only on a particular chemical group.
Example: Glycosidase acts on glycosides.

(c) Optical Specificity:
An enzyme act only one of a pair of optical isomers.

Example: D-amino acid oxidase oxidises D-amino acids. L-amino acid oxidase oxidises L-amino-acids and not D-amino acid. D-and L amino acids refers to the position of NH_2 groups to the right or left of the carbonyl group respectively.

Isoenzymes:

Higher organism often elaborate several physically distinct versions of an given enzyme each of which catalyzed the same reaction which are called Isoenzymes. Isoenzymes may exibits subtal differences in properties such as ~~Sensti~~ Sensitivity to regulatory factors or substrate affinities that adapt them to specific tissues or conditions.

or

Isoenzymes are multiple forms of a given enzyme that occurs within the same animal species. They are also called isozymes.

They have different physical and chemical properties but perform similar catalytic activity. Isoenzymes are similar to the different forms of an one rupee coin. The one rupee coin may be small or big or may have different marking; but all coins have the same value.

They are coded by different genes and therefore differ in amino acid composition and in their isoelectric pH value.

They have different immunological behaviour.

Example:-

Lactic dehydrogenase (LDH) is an isoenzyme. This enzymes occurs in five different forms in various tissues.

Each enzyme molecule is formed of four polypeptide chains. There are two types of polypeptide chains, namely M chain and H chain. The amino acid contents of M chain is different from that of H chain.

The heart muscle contains only H type polypeptide chain. The skeletal muscle contains only M type polypeptide chains.

The x-ray structure of phosphorylase -a and phosphorylase -b are similar.

Mammals express 3 isoenzymes of glycogen phosphorylase in muscle, brain and liver.

Isoenzymes are produced by gene duplications processes

AMAS Page No.
Date / /

Mechanism of Enzyme action:

The breaking of substrate into end products by an enzyme is called enzyme action. The compound on which the enzymes acts is called the substrate.

Michalis and Menton proposed a hypothesis for enzyme action. The enzyme action involves the following steps:

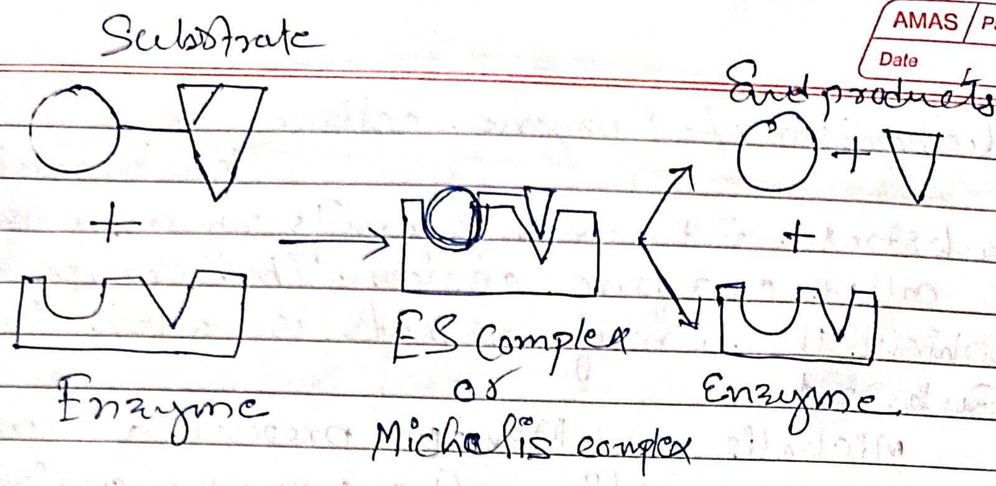
(1) The enzyme molecule (E) combines with a substrate molecule (S) to form an enzyme-substrate complex. It is called as Michalis complex.

Enzyme Substrate E-S complex

(2) The enzyme contains specific sites for the attachment of substrate. These sites are called active sites or catalytic centres. They are made up of amino acid residues.

(3) The active sites loosen the chemical bonds in the substrate and this leads to breaking of substrate into end product.

(4) Finally the enzyme dissociates from the end products.



Mechanism of enzyme Action.

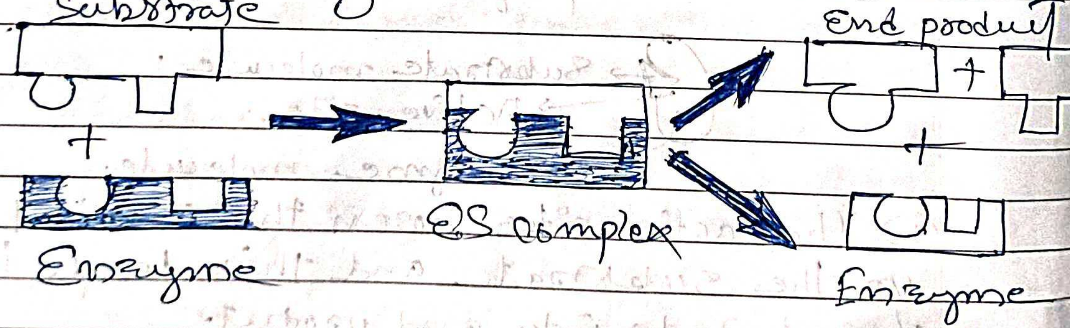
⑤ The enzyme is now free to Combine with another molecule of substrate.

There are two types of hypothesis to explain the mechanism of the formation of enzyme-Substrate complex.

ⓐ Lock and key hypothesis.
ⓑ Induced fit hypothesis.

(a) Lock and key mechanism:

This hypothesis was proposed by Emil fisher (1914)



This theory explain the mechanism of the formation of enzyme-Substrate complex. According to this hypothesis the enzyme molecule has one or more specific points this points are called active site or active centres.

The active site exist in the enzyme in a Rigid and proper conformation

~~on~~ even in the absence of substrate.
During enzyme action the substrate fits into the active site of the enzyme as a key fits into the lock.

(b) Induced fit theory:
This hypothesis was proposed by Koshland (1963). It explains the mechanism of the formation of enzyme-substrate complex.
This theory says that the active site does not possess a rigid and performed structure. The region of the active site is flexible.
When the enzyme reacts with the substrate the substrate induces a conformation change in the active site of the enzyme.
This change results in the development of attraction between enzyme and the substrate so that an enzyme-substrate complex is formed.
It leads to the ~~lost~~ loosening of the chemical bonds linking the components of the substrate.
As the reaction is completed the substrate is split into end products and enzyme is released.

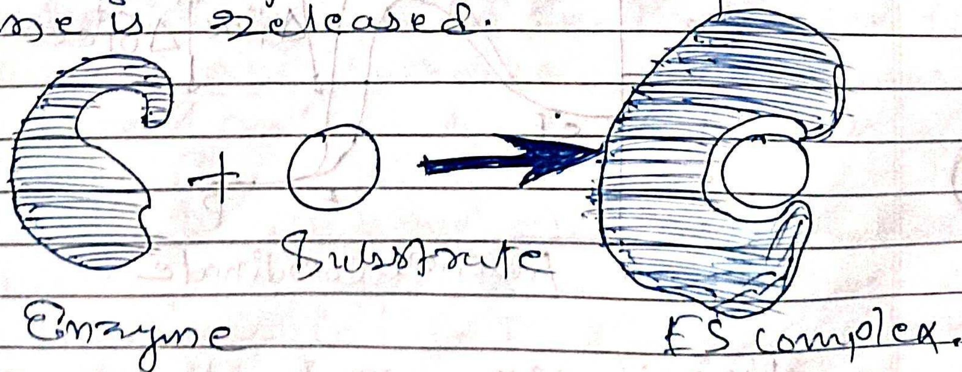
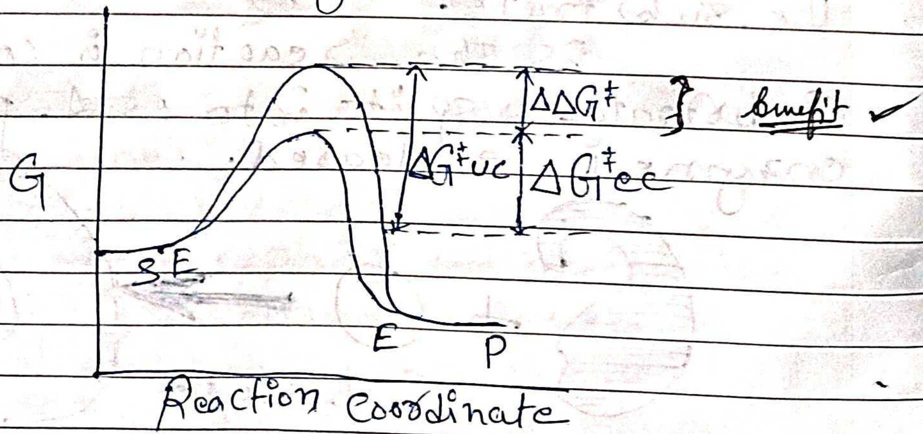


Fig: Induced-fit theory.

x_e $\boxed{\text{Catalytic efficiency}} = x_a - x_e$

Or

The energy required for formation of the transition state is called activation energy ($\Delta G^{\ddagger}$). Generally, an enzyme increases the reaction rate by lowering the activation energy. This can be explained as follows: After the substrate fits into the active site of the enzyme, ~~as per the model described in the preceding section~~, free energy is released; it is called intrinsic binding energy and is denoted as ΔG_b. When the ES complex is formed, the entropy of [S] is lost. The entropy loss is denoted as $-T\Delta S$. The amount by which the activation energy is lowered by the enzyme is called catalytic efficiency and is denoted as $\Delta\Delta G^{\ddagger}$. ΔG_b acts as a major source of free energy for the enzyme, ~~is called~~ to lower the activation energy. ΔG_b is equal to the amount by which $\Delta G^{\ddagger}$ is lowered, that is, $\Delta G_b = \Delta\Delta G^{\ddagger}$. This catalytic concept could be described by a transition state diagram, which is a plot of free energy (G) versus progress of the reaction



ES

Fig:- Transition state diagram showing mechanism of enzyme action.

ΔG_b : intrinsic Binding energy

$-T\Delta S$: Entropy loss.

AMAS Page No.
Date

where

G = Free energy.

$\Delta G^{\ddagger}_{uc}$ = Activation energy for uncatalyzed Reaction.

$\Delta G^{\ddagger}_{ec}$ = Activation energy for enzyme-catalyzed reaction.

$\Delta\Delta G^{\ddagger}$ = Amount of activation energy lowered by enzyme.

Enzyme Kinatics:

Enzyme kinatics is the central approse to study mechanism of an enzyme-catalyzed reaction and to determined the rate of reactions and how it changes in Response to changes in exprimental parameters.

(1) Substrate concentration [S] effects the rate of enzyme-catalyzed reaction. One simplificing approse in kinatics is to measure the initial rate or initial velocity (V_o) when [S] is much more greater than the concentration of the enzyme [E]. In a tipical reaction one enzyme may be present in nano-molar concentration. whereas [S] may be 5 or 6 orders of magnitude higher.

If only the beginning of the reaction is monitor changes in [S] can be limited to a few percent and [S] can be ~~greater~~ Regarded as constant and V_o can be explored as a function of [S].

At relatively low concentration of substrate, V_o increases almost linearly with increase in [S]. At higher substrate concentration, V_o increases by smaller and smaller amount in response to increase in [S]. Finally a point is reached beyond

which increase in V_0 is vanishingly small as [S] increases. The plateau like region is close to maximum velocity that is V_{max}

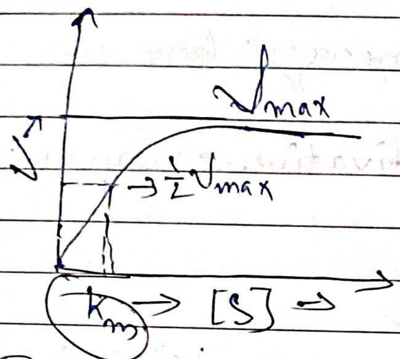
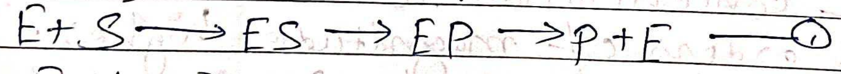


Fig: effect of [S] on the V of an enzyme catalyzed reaction.

V_{max} is extrapolated from this curve since the V never reaches the V_{max}. The [S] at which V is $\frac{1}{2}V_{maximal}$ is called K_m (Michalis constant).

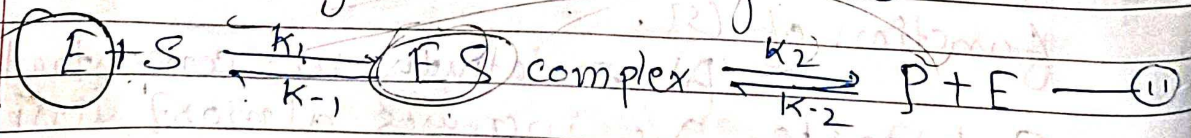
Michaelis-Menten equation:

The basic hypothesis for an enzyme catalyzed is the breakdown of enzyme substrates complex (ES) [ES] into product and free enzyme.



$$E + S \longrightarrow ES \longrightarrow EP \longrightarrow P + E \quad \text{—— (I)}$$

Early in the reaction, the concentration of the product (P) is negligible and we make assumption that the reverse reaction (P → S) denoted by k_{-2} can be ignored.



$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \text{ complex} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} P + E \quad \text{—— (II)}$$

V_0 is determined by the breakdown of ES to form product.

$$V_0 = k_2[ES] \quad \text{—— (III)}$$

we introduced the term $[E_t]$ representing total enzyme concentration (free enzyme + substrate bound enzyme)
free enzyme can then be represented by $[E_t] - [ES]$. Also because $[S]$ is far greater by the enzyme at any given time is negligible compared to $[S]$ with this conditions, we derived Michaelis-Menten equation.

Step I:

The rate of formation and breakdown of $[ES]$ are determined by the steps govern by the rate of constant k_1 (formation) and $k_{-1} + k_2$ (breakdown) as follows.

Rate of $[ES]$ formation equal to
$$k_1([E_t] - [ES])[S] \quad \text{--- (iv)}$$
Rate of $[ES]$ breakdown equal to
$$k_{-1}[ES] + k_2[ES] \quad \text{--- (v)}$$

Step II:

we now make an important assumption : that the initial rate of reaction reflects a steady state in which $[ES]$ is constant. That means rate of formation of ES is equal to rate of its breakdown. This is called steady state assumption. Now the above equation (iv) and (v) for the steady state as follows –
$$k_1([E_t] - [ES])[S] = k_{-1}[ES] + k_2[ES] \quad \text{--- (vi)}$$

Step III:

Through algebraic steps the left side is multiplied out and right side is simplified.

From equation (vi) :

$$k_1([E_t]-[ES])[S] = k_{-1}[ES] + k_2[ES]$$

$$\Rightarrow k_1[E_t][S] - k_1[ES][S] = [ES](k_{-1}+k_2)$$

Adding $k_1[ES][S]$ on both side-

$$k_1[E_t][S] - k_1[ES][S] + k_1[ES][S] = k_1[ES][S] + [ES](k_{-1}+k_2)$$

$$\Rightarrow k_1[E_t][S] = [ES](k_1[S] + (k_{-1}+k_2))$$

$$\Rightarrow \frac{k_1[E_t][S]}{k_1[S]+(k_2+k_{-1})} = [ES]$$

$$\Rightarrow [ES] = \frac{[E_t][S]}{[S] + (k_2+k_{-1})/k_1}$$

$\left(\frac{k_2+k_{-1}}{k_1}\right)$ is called Michaelis ~~Menten~~ constant (K_m)

$$\Rightarrow ES = \frac{[E_t][S]}{[S]+K_m} \quad \text{--- (VII)}$$

Step IV: Expressing V_0 in terms for [ES]

we get

$$V_0 = k_2 \times \frac{[E_t][S]}{[S]+K_m} \quad \text{--- (VIII)}$$

Because the maximum velocity occurs, when the enzyme is saturated, that is $[ES]=[E_t]$ V_{max} can be defined as

$$k_2 \times [E_t]$$

So,

$$V_0 = \frac{V_{max}[S]}{[S]+K_m} \quad \text{--- (IX)}$$

This equation is Michaelis - Menten equation the rate ~~rate~~ equation for any one substrate enzyme - catalyzed reaction. It is the quantitative relationship betn. V_0, V_{max} and initial [S] all related through Michaelis constant (K_m).

AMAS Page No.
Date / /

Line weaver-Burk plot:

The Michaelis-Menten equation can be algebraically transformed into equation that are more useful in ploting exprimantel data. One common transformation is by taking the reciprocal of both the sides of the Michaelis-Menten equation.

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

on simplification:

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

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

This form of Michaelis-Menten equation is called Line weaver-Burk equation.

For enzyme obeying Michaelis-Menten equation, a plot of $\frac{1}{V_0}$ vs $\frac{1}{[S]}$ (double reciprocal of V_0 and [S]) gives a straight line.

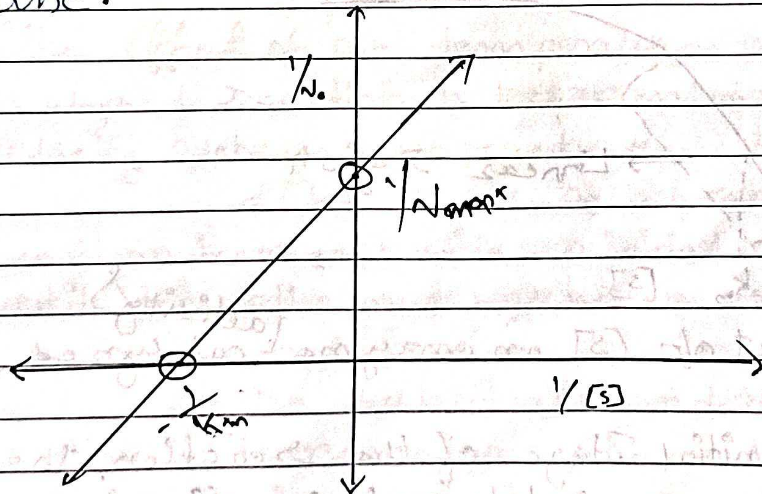


Fig: Line-weaver-Burk plot.

Advantage:

The double reciprocal Michaelis-Menten equation is called Line weaver-Burk plot equation.

This equation can be represented graphically and is called Lineweaver-Burk plot. This plot has the advantage of allowing a more accurate determination of Vmax.

Factor affecting rate of enzyme-catalyzed reaction:

Various factors influences the activity of enzymes. This factors include [S], Reaction temperature, and pH of the reaction medium.

Effect of Substrate Concentration:

The effect of Substrate concentration on enzyme activity can be well described by plotting the velocity of the reaction against Substrate concentration.

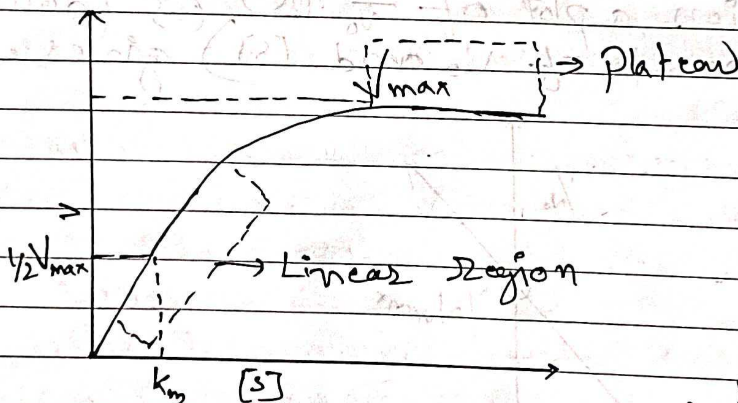


Fig: Effect of [S] on enzyme activity catalyzed reaction.

At the initial Stage of the reaction, the velocity of the enzyme-catalyzed reaction increases linearly with an increase in Substrate-concentration. This is also termed as initial velocity of the reaction. Generally, it is assumped that the velocity of the enzyme catalyzed reaction is directly propotional to the enzyme

concentration in a reaction mixture.
As the substrate concentration is raised gradually, the velocity reaches a maximum (V_{max}). Once V_{max} is attained, the velocity is constant even with increase in substrate concentration. This leads to a plateau region. Thus, a hyperbola is obtained.

Effect of temperature:

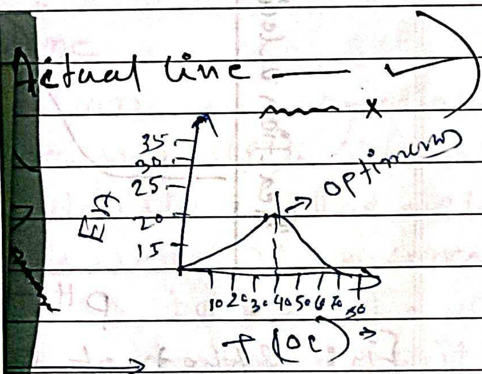


Fig: Effect of temperature on the rate of Enzyme-catalyzed Reaction.

The effect of the temperature on the rate of enzyme-catalyzed reaction is represented as a plot of velocity versus temperature.

The rate of an enzyme-catalyzed reaction increases with an initial rise in temperature untill the optimum temperature is reached. This rise is due to effective collision between reactive groups of the substrate and the enzymes. At optimum temperature, the velocity is maximum. A further increase in temperature leads to a fall in velocity due to disturbances in conformation of active site of the enzyme. This leads to be a bell-shaped curve.

Effect of pH:

The effect of pH on the velocity of an enzyme-catalyzed reaction is represent in the fig. in below. The bell-shaped curve of pH versus v is simillar to that of T versus v.

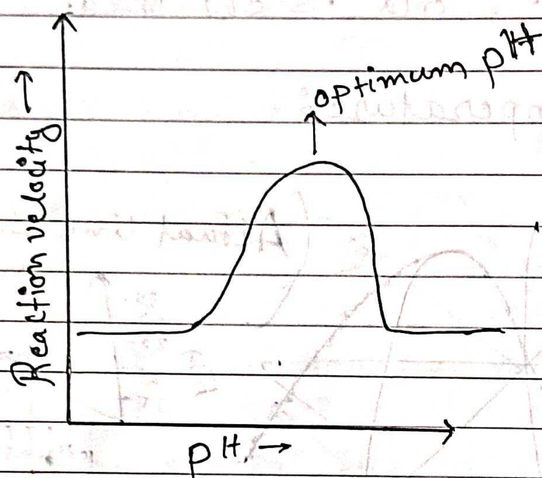
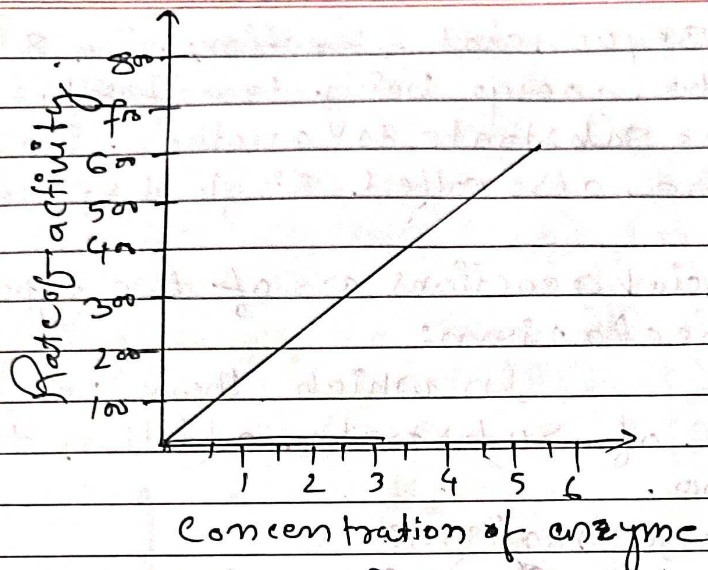


Fig: Effect of pH on velocity of an enzyme-catalyzed reaction.

In the initial stage of the reaction, the velocity increses with increases in pH due to increase in enzyme-substrate binding. At a particular pH call the optimum pH (usually 6-8), the velocity reaches a maximum. Beyond the optimum pH, the 3-D structure of the enzyme is altered leading to a dissociation of enzyme-substrate complex and a fall in velocity.

Effect of enzyme Concentration:

An enzyme works even when it is present in low quantity. The velocity of the reaction increase with the increase in the concentration of enzymes.

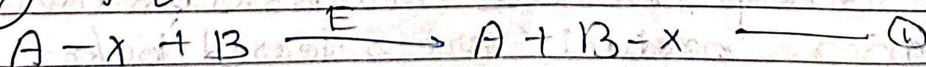


Concentration of enzyme

The velocity (V) of a reaction is propotional to the concentration of enzyme (E). When the enzyme concentration is doubled, the velocity is also doubled. This because when concentration is doubled as much as twice the active sites become available to combine with the substrate.

Multi-substrate Reaction:

Enzymetic reaction requiring more than one substrate and yeilding multiple products are vary common. Those yeilding two products from two substrate account for approximatly 60% of biochemical reaction and these are either transfer reaction where the enzyme catalyzes transfer of a functional group from one substrate to another.



$$A - x + B \xrightarrow{E} A + B - x \quad \text{--- (1)}$$

or oxidation-reductions reactions in which reducing equivalants are transfer between two substrates.

(I) Single displacement Reaction:

Reactions in which all substrates must combined with the enzyme before a reaction can occur and products are released.

are called sequencial reactions. In such reactions the group being transferred is directly pass from one substrate to another. Such reactions are also called single displacement reaction.

Sequancial reactions are of two types:

(a) Order mechanism:

In which there is a compulsory order of substrate addition to the enzyme.

(b) Random mechanism:

In which there is no preferance for the order of substrate addition.

In ordered mechanism, binding of the 1st substrate to the enzyme creates the active side for binding of the second substrate.

In random mechanism, binding side for all the substrates are available in the enzyme.

Example of orderd mechanism enzyme:
NADH dehydrogenase (mitochondrial complex I)

Example of random mechanism enzyme:
Kinase.

(2) Double displacement (ping-pong) reaction:

Group transfer reactions in which one or more products are released before all substrates have been added are called ping-pong reaction.

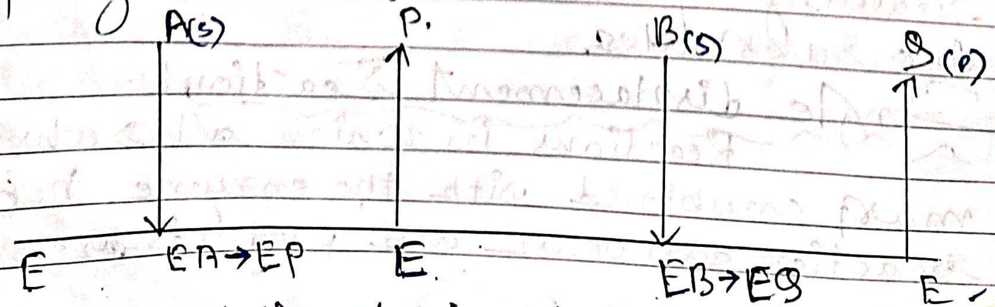
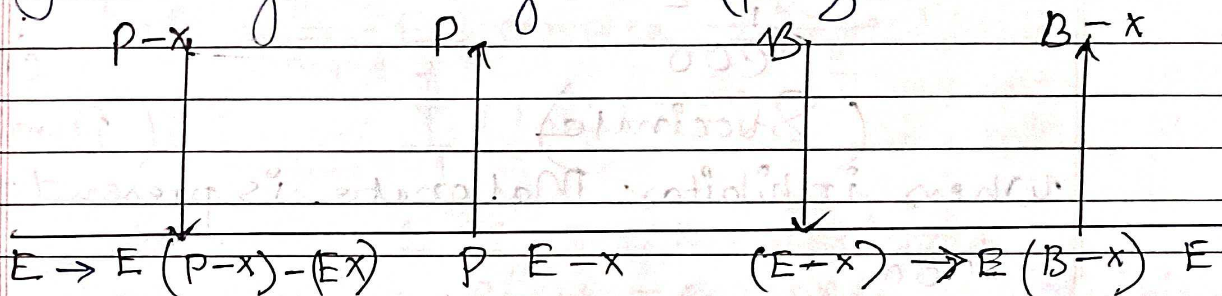


Fig. Representation of bi-substrate ping-pong reaction

Here, a functional group X of the first substrate A (P-X) is displaced by the enzyme E to produced the first product P and a stable enzyme form F (E-X), in which X is tightly bound to the enzyme (ping). In the second stage of the reaction X is displaced from the enzyme by the second substrate B to yeild the product Q (B-X) thereby regenerating the original enzyme E (pong).



Example of double displacement reaction: Tripsin.

Enzyme inhibition:

Many substances alter the activity of an enzyme by combining with it in a way that influences binding of substrate and/or its turn over. Substances that reduced an enzymes activity are known as inhibitors.

(1) Competitive inhibition:

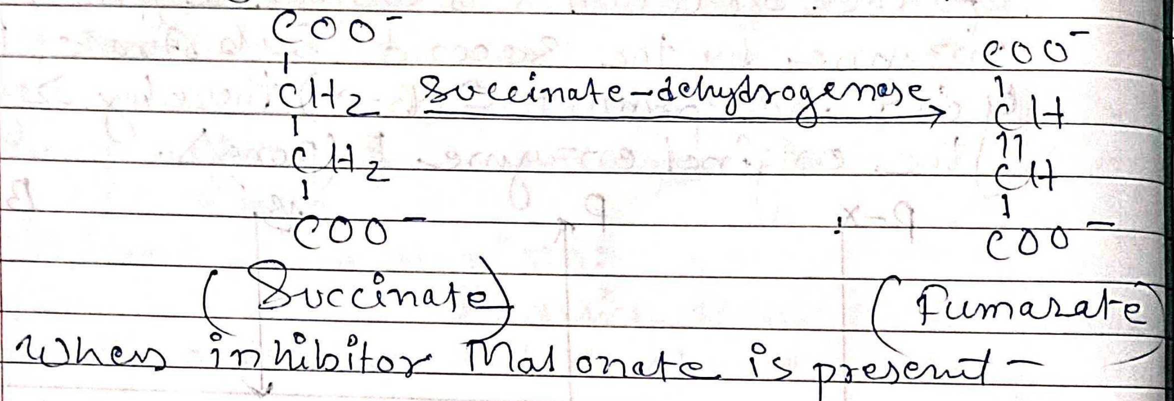
A substances that compete directly with a normal substrate for an enzyme substrate binding site is known as competitive inhibitor. Such inhibitors usually ressamble the substrate so that specifically binds to the active site, but differs from the substrate, so that it can't react as the substrate does.

Example:

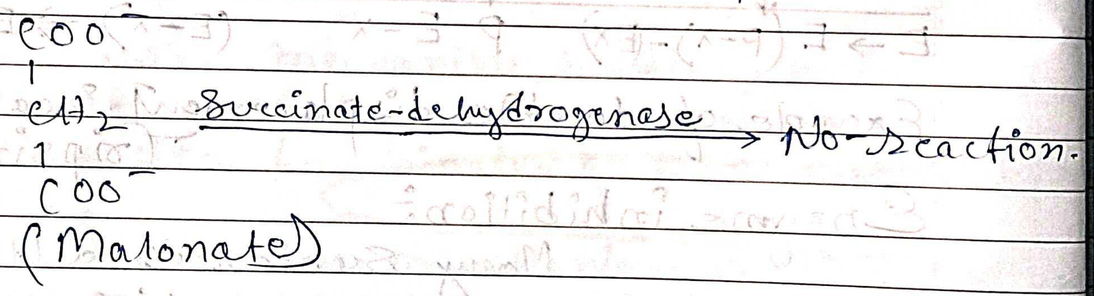
Succinate - dehydrogenase (mitochondrial complex II). It is a citric acid cycle

AMAS Page No.
Date

enzyme that converts succinates to fumarate and is competetively inhibited by Malonate. Malonate structurally ressamble succinates but can't be dehydrogenatesed.



When inhibitor Malonate is present -

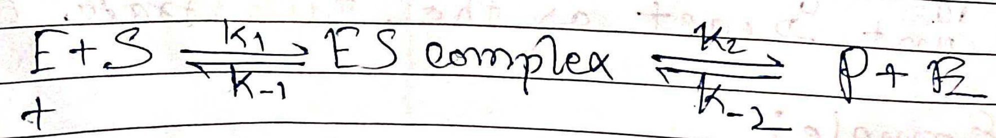


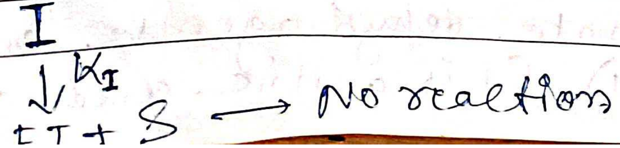
Product inhibition:

A product of the reaction, which neressarily is able to bind to the active side of the enzyme may accumulated and compete with substrate for binding to the enzyme is sabsiquant catalytic cycle.

A competitive inhibition reduces the concentration of free enzyme available for substrate binding.

$$E_t = [ES] + [E_f] + [EI]$$



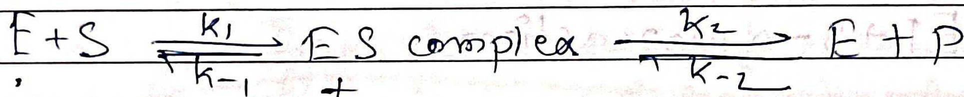


AMAS Page No.
Date / /

In order to increase activity of enzyme we will have to increase substrate concentration.

(2) Un-Competitive inhibitions:

In un-competitive inhibition, the inhibitor binds to the [ES] but not to the free enzyme.



$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \text{ complex} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

+ I (inhibitor)

ESI → No reaction.

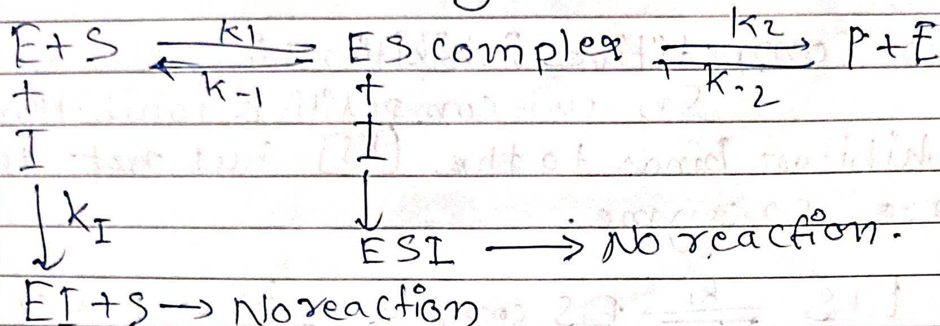
The binding of un-competitive inhibitors which need not reassemble substrate distorte; the active side thereby rendering the enzyme catalytically inactive. In Contrast competitive inhibition, adding substrate doesn't reverse. The effect of an un-competitive inhibitor because binding of the ~~inhibitor~~ substrate doesn't affects binding of the inhibitor and also binding of the substrate is essantial for binding of the inhibition, such inhibition request that the inhibitor effect The catalytic function of the enzyme but not substrate binding. It is common in multisubstrate enzyme.

(3) Mixed-inhibition:

Many reversible inhibitors interact with an enzyme in a way that effects both substrate binding and catalytic activity. In other words both enzyme as well as ES complex bind to the inhibitors.

AMAS Page No.
Date

In mixed inhibition the inhibitors binds to such as side that participate in substrate binding and catalysis.



Regulation of enzyme activity:

An organism must be able to control catalytic activity of its components enzymes, so that it can co-ordinate metabolic processes respond to changes in the environment grow and differentiated. There are two ways that this may occur:

(a) Control of enzyme availability:
The amount of a given enzyme in a cell depends on the both its rate of synthesis and rate of degradation. Each of these rates is directly control by the cell and is subject to dramatic changes over time spans of minute (in bacteria) to hours (in higher organism).

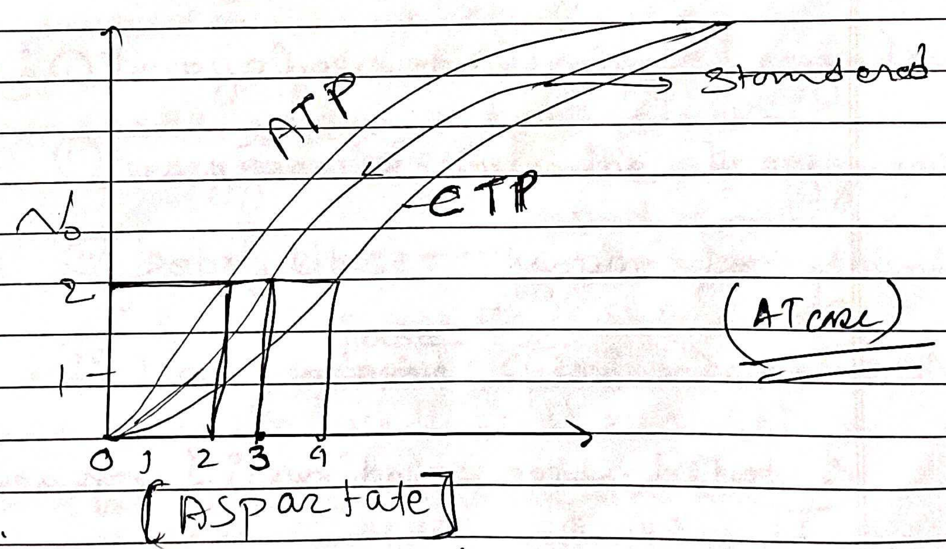
(b) Control of enzyme activity:
An enzyme can be inhibited by accumulation of products or by the presence of other types of inhibitors. In fact an enzymes catalytic activity may be modulated either negatively or positively through structural alterations that influence the ES binding affinity or turn over number. Just as

hemoglobins, oxygen effinities is allosterically regulated by binding of legands such as O_2 ~~an~~ CO_2 and H^+ on enzymes substrate binding effinity may like-roise vary with the binding of small molecule called allosteric effectors.

ⓐ Allosteric regulation:

Aspartate trans carbamoylase (ATcase)

ATcase catalyzes formation of carbamoyl aspartate from carbamoylphosphate and aspartate. This reaction is the first step in the biosynthesis of pyrimidine. ATcase is allosterically inhibited by cytidine tri-phosphate (a pyrimidine nucleotides) and allosterically activated by Adenosinetriphosphate (ATP)

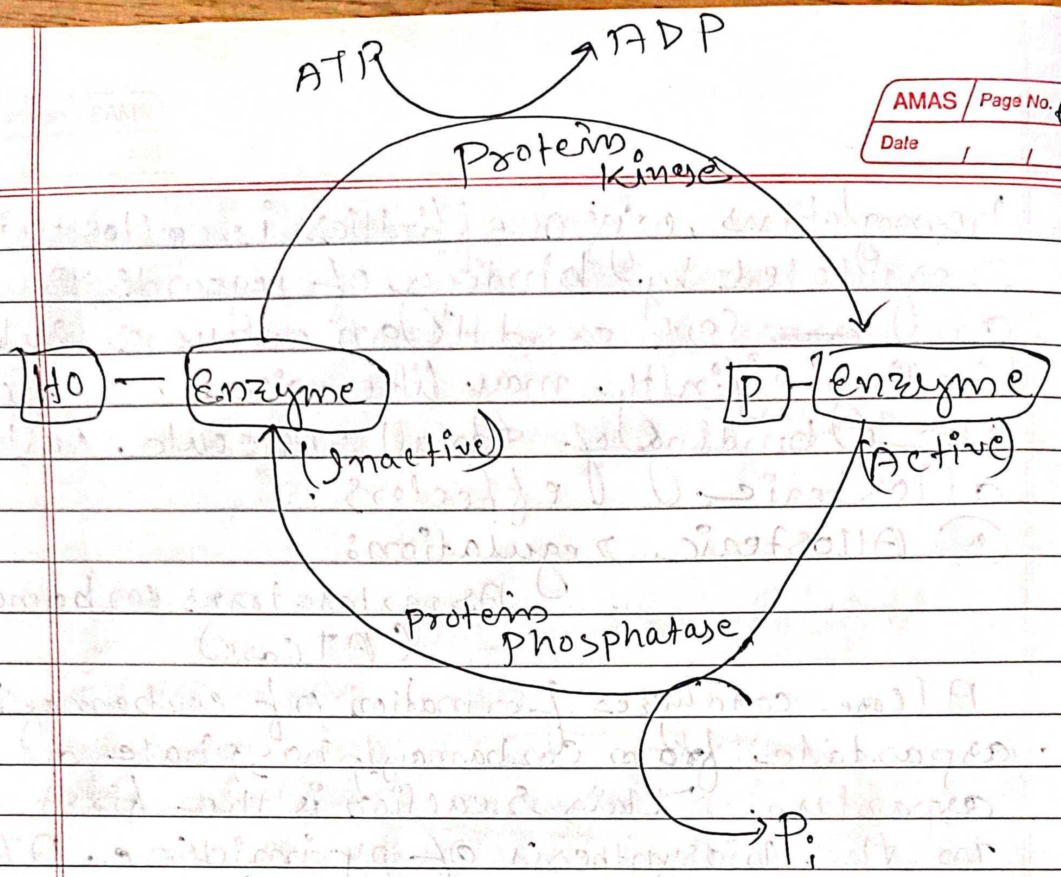


CTP is a feedback inhibitor because it inhibites an ~~enzyme~~ erlier step of its own biosynthesis.

ⓑ Covalent modification:

Many enzymes are controled by covalent modification. The most common modification are phosphorylation and dephosphorylation.

Carbamoyl-P + Aspartate ATcase Carbamoyl-aspartate


ATP
ADP
Protein kinase
HO
Enzyme
(Inactive)
P
Enzyme
(Active)
Protein Phosphatase
P_i