GOEL INSTITUTE OF PHARMACY AND SCIENCES (0392)

ASSIGNMENT-I

BIOCHEMISTRY

BP203T

B. PHARMA Ist year

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CHAPTER-6

ENZYMES

Enzymes are biocatalysts — 'the catalysts of life'.
A catalyst is defined as a substance that increases the velocity or rate of a chemical reaction without itself undergoing any change in the overall process.
Enzymes may be defined as biocatalysts synthesized by living cells. They are protein in nature (exception- RNA acting as ribozyme), colloidal & thermolabile in character and specific in their action.

Properties —

- Enzymes are very specific in their action.
  Particular enzyme acts on a particular substrate only.
- Enzymes are very sensitive to heat & temperature. They are "thermolabile".
- Correct temperature for the maximum activity is called "optimum temperature".
- In the laboratory, hydrolysis of proteins by a strong acid at 100°c takes at least a couple of days. The same protein is fully digested by the enzymes in gastrointestinal tract at body temperature (37°c) with in a couple of hours.

HISTORICAL BACKGROUND

- Berzelius in 1836 coined the term catalysis (Greek: to dissolve).
- Isolation of enzyme system from cell-free extract of yeast was achieved in 1883 by Buchner.
- In 1926, James Sumner first achieved the isolation and crytallization of the enzyme "Urease" from jack bean and identified it as a protein.
- Buchner named the active principle as "Zymase" (later found to contain a mixture of enzymes), which could convert sugar to alcohol.
- In 1878, Kuhne used the word enzyme (Greek: in yeast) to indicate the catalysis taking place in the bio-logical systems.

NOMENCLATURE AND CLASSIFICATION

In early days, the enzymes were given names by their discoverers in an arbitarary manner. for example—the names pepsin, trypsin and chymotrypsin convey no information about the function of the enzyme or the nature of the substrate on which they act.

Sometimes, the suffix -ase was added to the substrate for naming the enzymes. for eg— lipase acts on lipids; nucleases on nucleic acids; lactase on lactose.

Enzymes are sometimes considered under two broad categories:

① Intracellular Enzymes
② Extracellular Enzymes

① Intracellular Enzymes—

Intracellular Enzymes are functional within cells where they are synthesised.

② Extracellular Enzymes—

Extracellular Enzymes are active outside the cell; all the digestive enzymes belongs to this group.

IUB SYSTEMS OF ENZYME CLASSIFICATION

The International Union of Biochemistry (IUB) appointed an Enzyme Commission in 1961. This commitee made a through study of the existing enzymes and devised some basic principles for the classification and nomenclature of enzymes.

Since 1964, the IUB system of enzyme classification has been in force. Enzymes are divided into six major classes —

① **Oxidoreductases** —

Enzymes involved in oxidation-reduction reaction known as Oxidoreductases.

Oxidation ⟶ Reduction

$AH_2 + B \longrightarrow A + BH_2$

② **Transferases** —

Enzymes that catalyse the transfer of functional groups known as transferases.

Group Transfer

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

③ **Hydrolases** —

Enzymes that bring about hydrolyses of various compounds, known as Hydrolases.

dralases —

Hydrolysis

$A-B + H_2O \longrightarrow AH + BOH$

) Lyases —

Enzymes specialised in the addition or removal of water, ammonia, CO_2 etc, known as lyases.

Addition ⟶ Elimination

$A-B + X-Y \longrightarrow AX - BY$

⑤ Isomerases —

Enzymes involved in all the isomerisation reactions known as Isomerases.

Interconversion of isomers

$A \longrightarrow A'$

⑥ Ligases —

Enzymes catalysing the synthetic reactions (Greek: ligate – to bind) where two molecules are joined together and ATP is used.

Condensation (usually dependent on ATP)

$A + B \longrightarrow A-B$

ATP ADP + Pi

CHEMICAL NATURE OF ENZYMES

All the enzymes are invariably proteins. The functional unit of the enzyme is known as "holoenzyme", which is often made up of Apoenzyme (the protein part) and a coenzyme (non-protein organic part).

Holoenzyme ⟶ Apoenzyme + Coenzyme

(active enzyme) (protein part) (non-protein part)

- **Monomeric enzyme** —

It is used if it is made up of a single polypeptide known as Monomeric enzyme.

for eg — Ribonuclease, trypsin.

- **Oligomeric enzyme** —

Some of the enzymes which posses more than one polypeptide (subunit) chain are known as Oligomeric enzymes.

for eg — lactate dehydrogenase, aspartate, transcarbomoylase etc.

- **Multienzyme complexes** —

There are certain multienzyme complexes possessing specific sites to catalyse different reactions in a sequence known as multienzyme complexes.

Enzyme Kinetics

Enzyme kinetics, deals with enzyme reactions which are time dependent and explains the mechanism of enzyme catalysis and its regulation.

Let's understand enzyme kinetics as a function for the concentration of the substrate available for the enzyme.

- Start the experiment with a series of tubes which contain substrate, [S].
- At the time (t) zero, addsome amount of the enzyme
- Wait for few minutes.
- Then, measure the newly formed product concentration We can also use spectrophotometer, if product absorbs light.
- At a time when the amount of substrate is greater than the amount of enzyme, then the rate is the initial velocity of V.

If we plot V_i as a function of [S]; following observations will be made:

- At low [S], the initial velocity Vi, rises linearly with increasing [S].
- When [S] increases, Vi settle down (rectangular hyperbolais formed).

e asymptote shows Vmax as the maximum velocity of the reaction.

- The substrate concentration which produces a V. equal to one-half of Vmax is called the Michaelis - Menten Constant (K_m).

EQUATION OF ENZYME KINETICS

① MICHAELIS MENTEN EQUATION —

In 1913, Michaelis (1875-1949) and Menten (1879-1960), proceeded the work which was previously done by frenchchemist V Henri (1872-1940), developed, a mechanism to explain how the initial rate of enzyme-catalysed reactions depends on the concentration.

Derivation of Michaelis-Menten equation:

Few considerable assumptions can be made for the Michaelis-Menten equation derivation:

- Assuming that reverse reaction ($P \rightarrow S$) is negligible.
- Assuming there exists only a single central complex (ES) i.e; E·S breaks down to E + P.
- Considering a situation when, [S] >> [E] Then, the immediate interaction of S and E to form ES does not significantly affect free [S].
- Usually, $([S]-[ES])/[S] \geq 99.9\%$.

nsidering the above assumptions, the reaction schme is follow as!

Following are two parts for this reaction:

① formation of ES complex (a second order process)

② The breakdown of ES complex to product P and free enzyme E (a first order process).

And the final Michaelis - Menten equation is given below —

$$v = \frac{V_{max}[S]}{[S] + K_M}$$

Where,

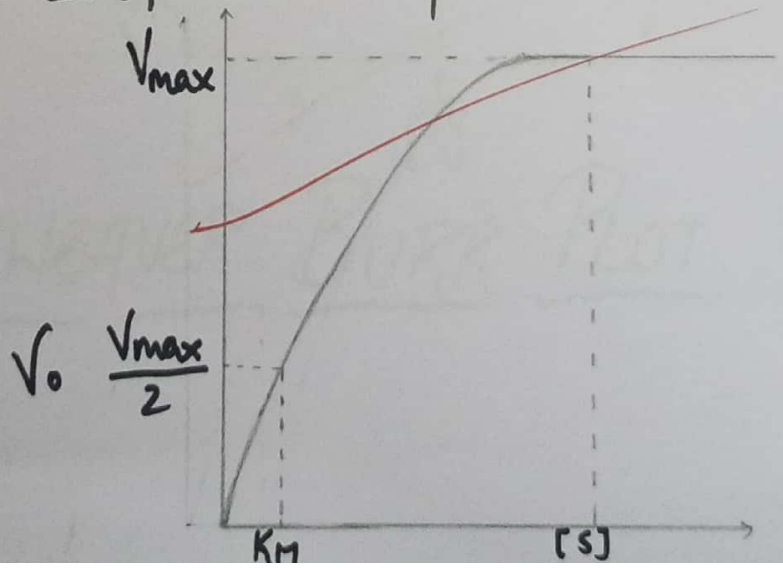
V_{max} = The maximum velocity achieved by the system at maximum (saturating) substrate concentration.

K_M = Michaelis constant

V_{max} = Velocity is 50% of V_{max}

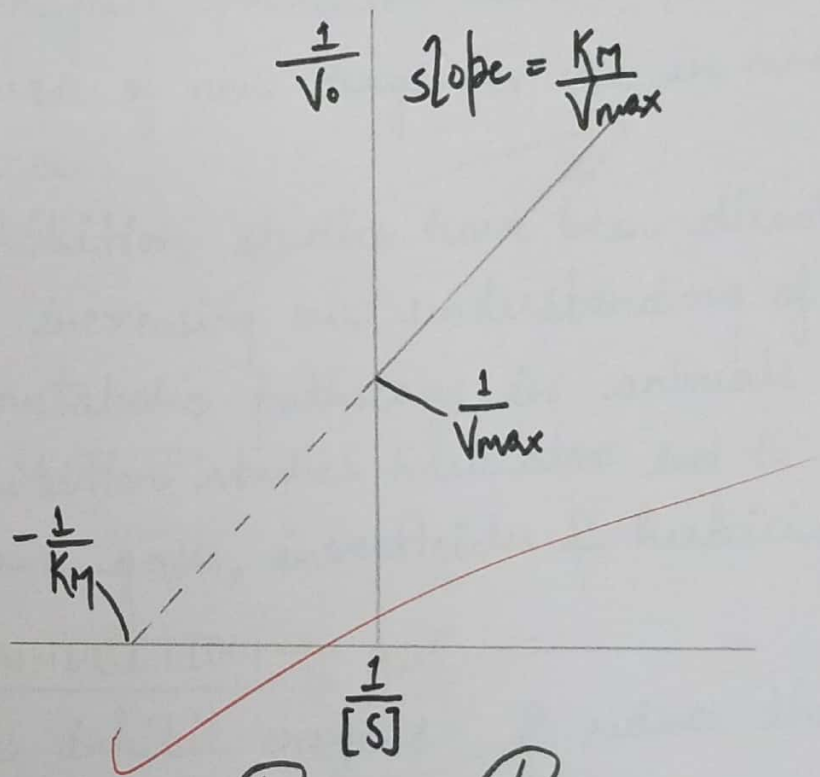
$[S]$ = Concentration of the substrate S

Graphical Representation/Determination for V_{max} & K_M



On the other hand, it is observed that in order to determine the value of Vmax, the plot of V_0 Vs. [S] is not useful since tracing the value of Vmax under very high. Substrate concentrations is difficult. So, American chemists H. Line Weaver & Burk employed the double - reciprocal plot. These are known as the "Lineweaver-Burk Plot". These are also called double-reciprocal plots.

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



LINEWEAVER BURK PLOT

ENZYME INHIBITION

The rate of an enzyme - catalyzed reactions can be decreased by specific inhibitors, i.e; compounds that combine with the enzyme and prevent normal enzyme substrate interactions in the active site, thus diminishing the rate of the reactions.

Certain enzymic inhibitors are poisonous for living organism, including cyanide, hydrogen, sulphide & carbon mono-oxide.

Application of enzyme inhibition

1). Studies on enzymes inhibition have led to the development of hundreds of new drugs for use in medicine and veterinary science.

2). Enzyme inhibition studies have been directed specifically toward increasing our understanding of specific reactions or metabolic pathways in animals and plants.

3). Enzyme inhibition studies have also led to the development of nerve gases, insecticides & herbicides (weed killers)

TYPES OF INHIBITION

Many substances inhibit enzymes & reduce the initial velocity (increase $1/v$).

The Lineweaver - Burk plots reveal the mechanism of the inhibition.

wing four types of inhibitions are
ion which are as follows —

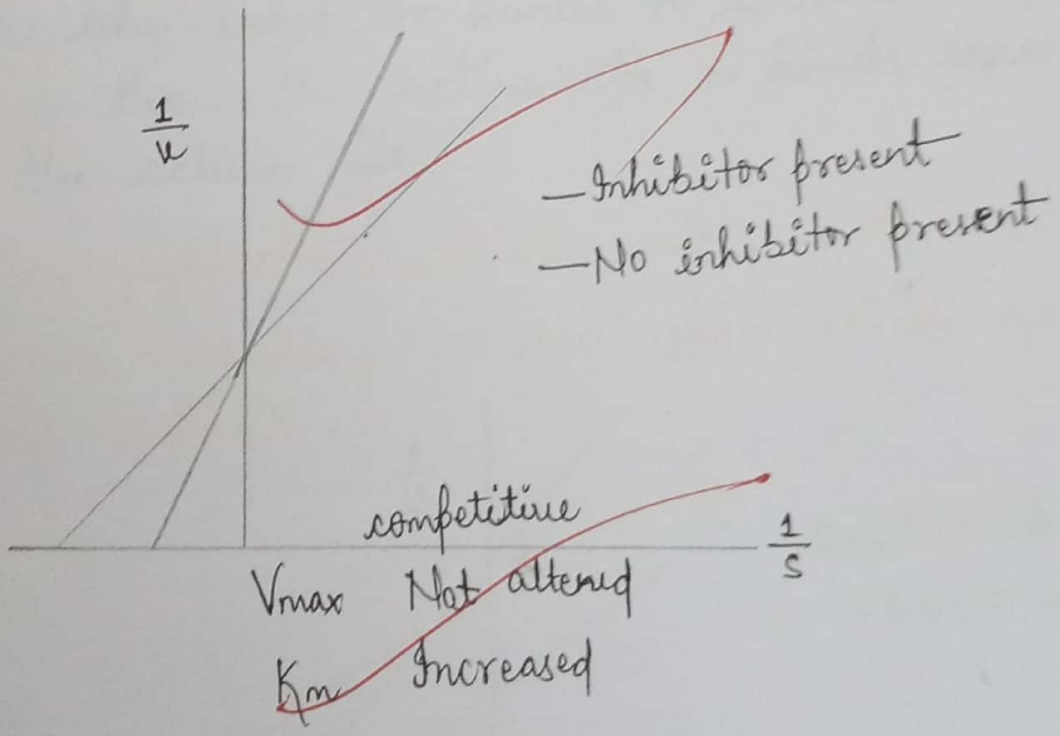
1. Competitive Inhibition
2. Non-competitive Inhibition
3. Partially competitive Inhibition
4. Uncompetitive Inhibition

① Competitive Inhibition —

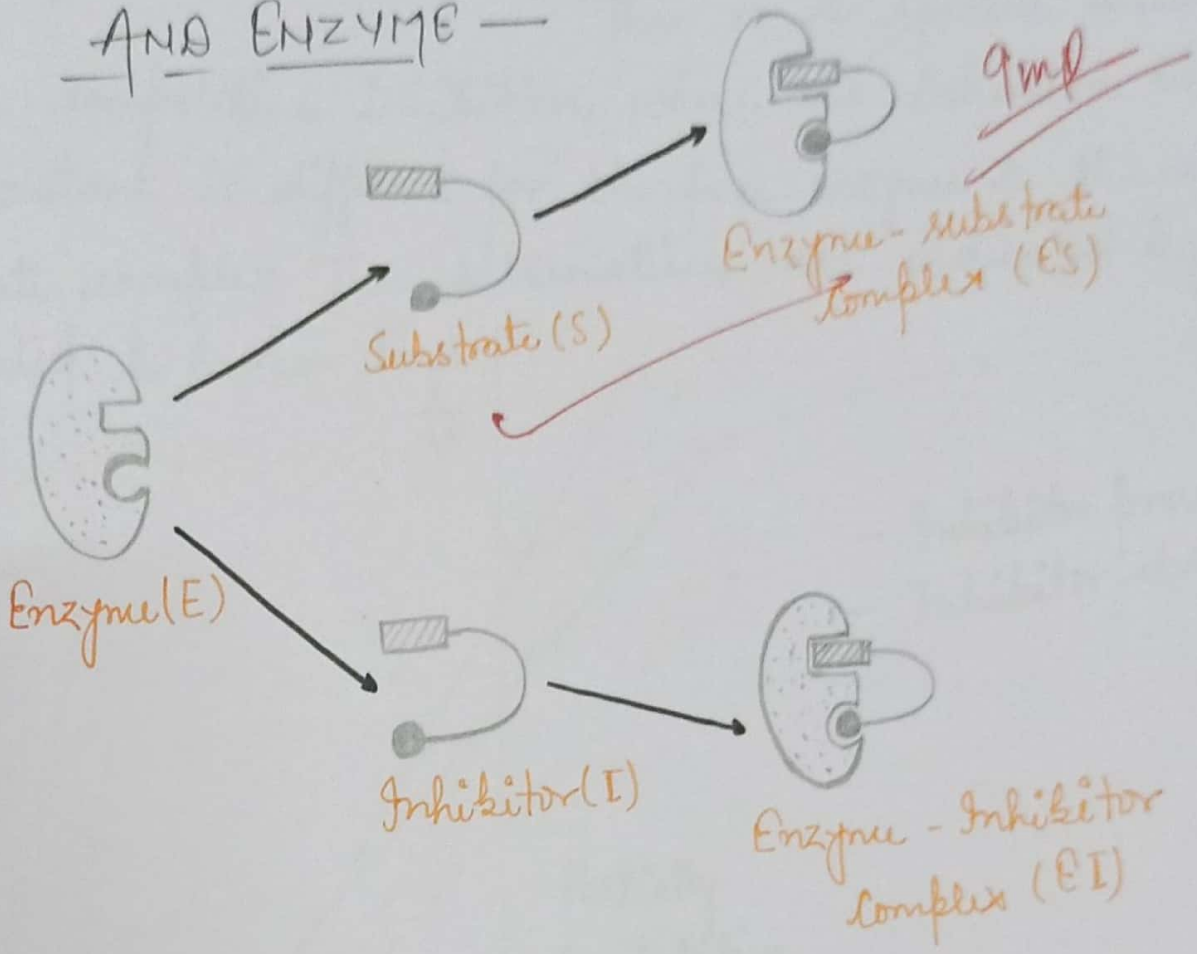
In this type of inhibition, compe-
-titive inhibitors can combine reversibly with the
active site of the enzymes & complete with the substrate
at the active site.

for eg:- Malonic acid as an inhibitor of succinic dehydrogen

$$E + I \rightleftharpoons EI$$

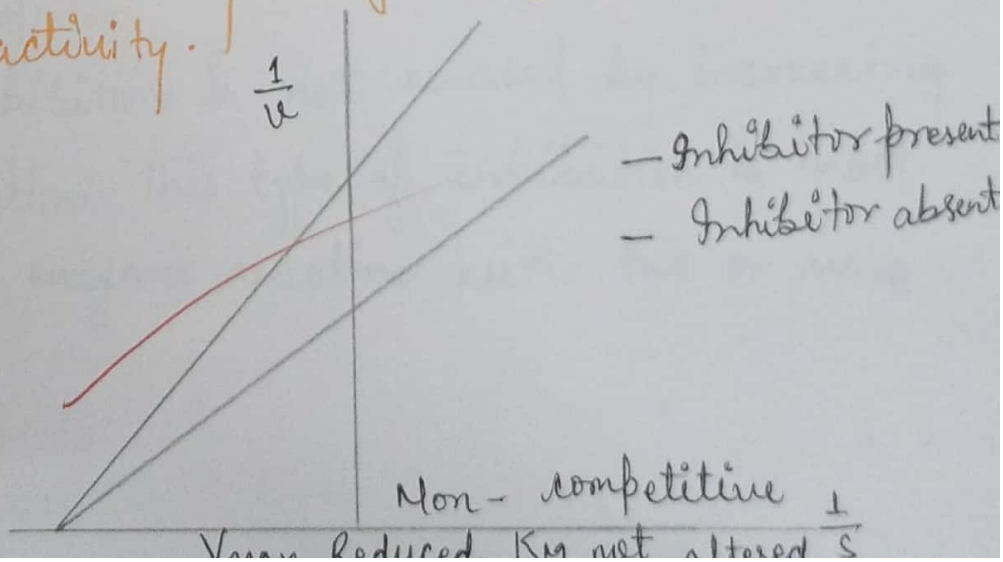


AND ENZYME —



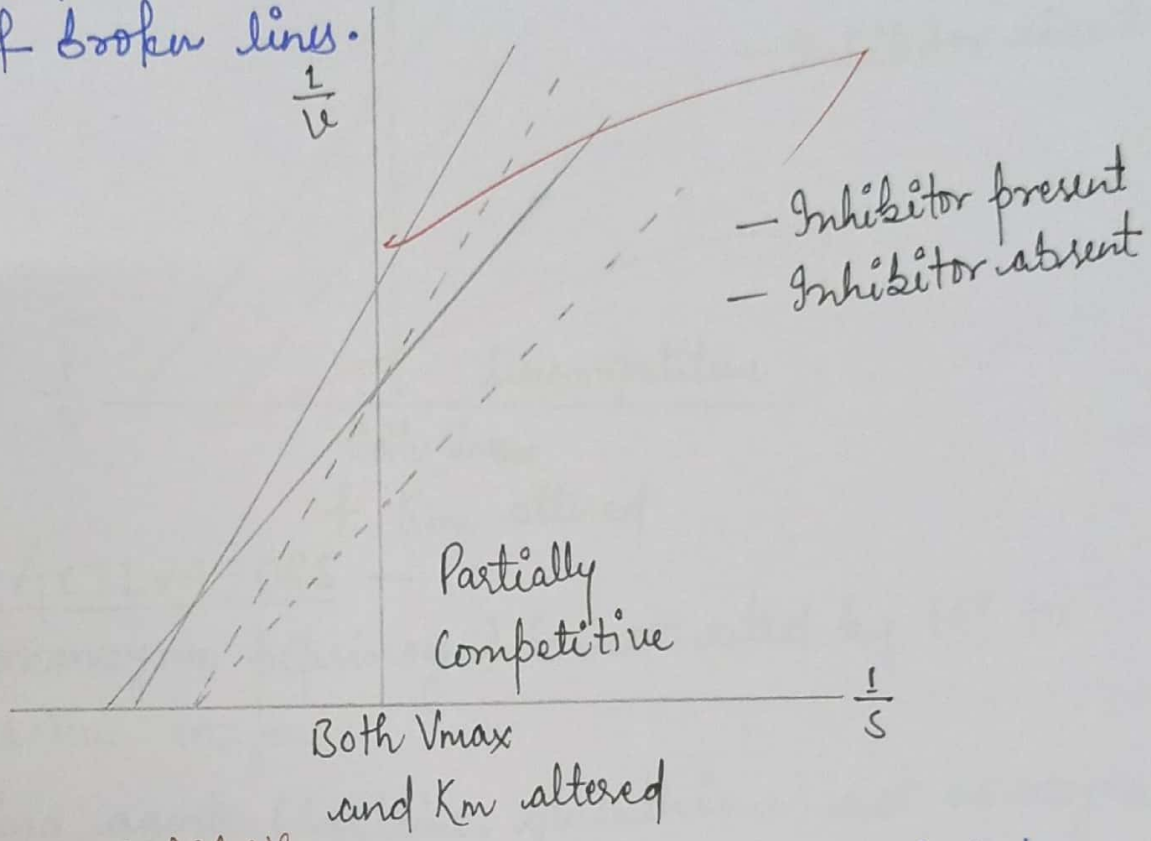
② Non-competitive Inhibition— In this type, inhibition occurs when the inhibitor binds to a site one the enzyme other than the active site or binds irreversibly to the active site.

Example— Diisopropylfluorophasphate reacts with various esterases including chymotrypsin which has some esterase activity.



tially Competitive Inhibition —

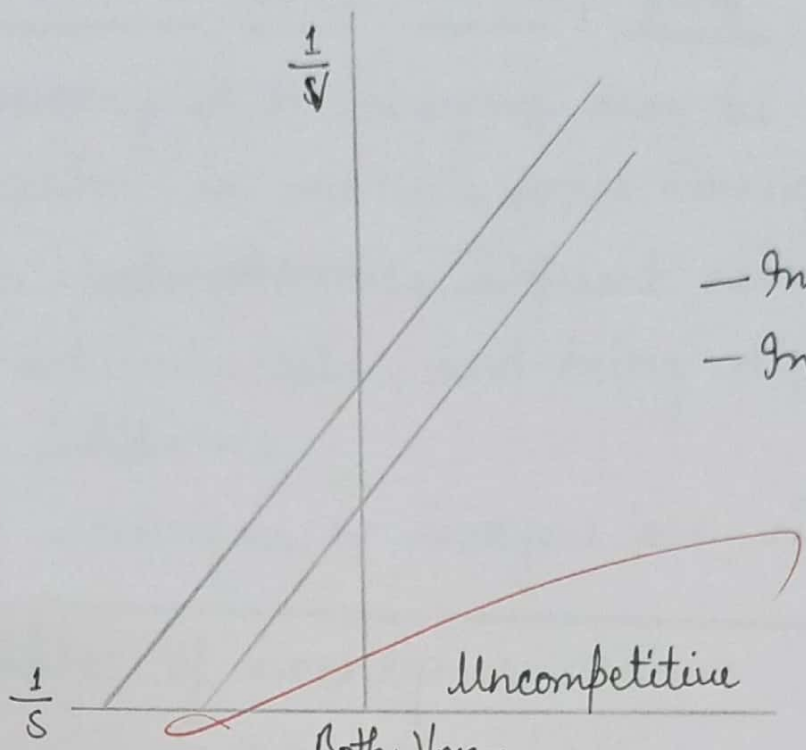
This is a special instance of non-competitive inhibition, where the inhibitor binding constant is different for the free enzyme & the enzyme substrate complex. Two alternatives are indicated by the solid & broken lines.



④ Uncompetitive Inhibition —

It occurs when the inhibitor binds after the substrate has bound to the enzyme, and then stops the reaction from occuring; eventually no product is formed.

This type of inhibition is not reversed by increasing substrate concentration. This type of inhibition is most frequently found in enzymic reaction with two or more substrates.



* ENZYME ACTIVATORS —

- Pepsin (as proenzyme pepsinogen) is activated by H^+ to form the active enzyme.
- Many reducing agents (Cysteine, glutathione) act as enzyme activators of SH enzymes.
- Enterokinase activates trypsinogen to form active trypsin.

Enzyme Activators are —

1. Mg^{++}
2. Mn^{++}
3. Cobalt
4. Molybdenum
5. Calcium
6. Copper
7. Iron
8. Zn^{++}
9. Cysteine
10. Glutathione

Isoenzymes Or Isozymes

The term isoenzymes or isozymes may be defined as the enzymes existing in multiple forms having different mobilities on electrophoresis, different relative activities towards different substrates, and being differently depressed by inhibitors.

Examples of isoenzyme or isozyme are cited in "box".

Examples of isoenzymes are —

1) lactic dehydrogenase (LDH)
2) alkaline phosphatase (ALP)
3) Geatine phosphokinase (Geatinekinase), CPK or CK
4) Acid phosphatase (ACP)
5) Amylase
6) Malati dehydrogenase
7) Cholinesterase
8) Hexokinase
9) Phosphoglucomutase
10) Pyruvate kinase etc.

Regulation Of Enzyme Activity In The Living System

In biological system, regulation of enzyme activities occurs at different stages in one or more of the following ways to achieve cellular economy.

1) Allosteric regulation
2) Activation of latent enzyme
3) Compartmentation of metabolic pathways
4) Control of enzyme synthesis
5) Enzyme degradation
6) Isoenzymes

This control occurs in several ways —

- biosynthesis at the genetic level.
- covalent modification after biosynthesis.
- regulatory enzymes.
- feedback inhibition.

— A common covalent enzyme modification is the addition or removal of a phosphate group.
— under high-energy condition (high ATP & low ADP), phosphorylation is favored.
— under low-energy condition (low ATP & high ADP), dephosphorylation is favoured.
— this regulates the balance between biosynthesis and catabolism.

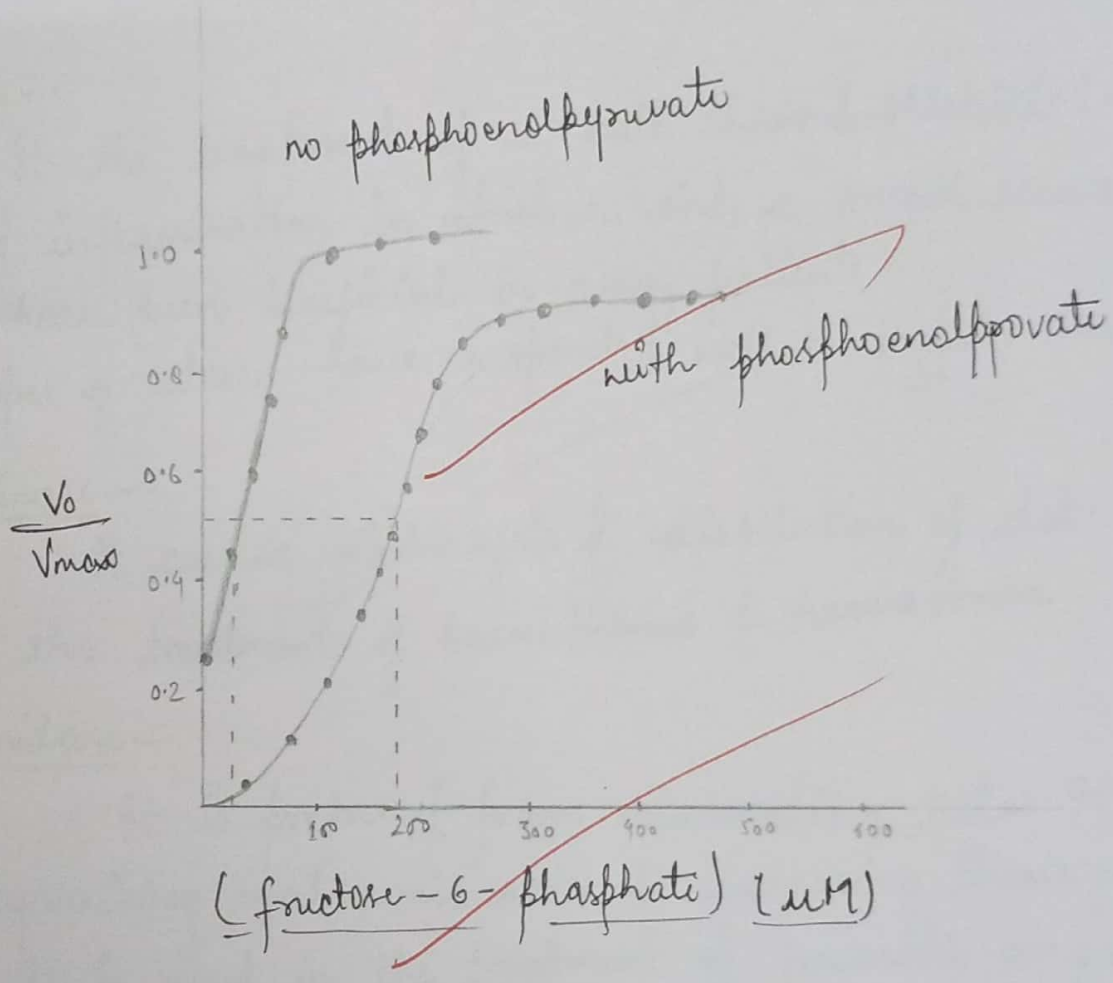
INDUCTION AND REPRESSION

S.No.	Induction	Repression
1.	It turns the operon on.	It turns the operon off.
2.	It starts transcription and protein synthesis.	It stops transcription and protein synthesis.
3.	It is caused by a new metabolite which needs enzymes to get metabalised.	It is caused by an excess of existing metabolite.
4.	It operates in a catabolic pathway.	It operates in a anabolic pathway.
5.	Repress-or is prevented by the inducs from joining the operator gene.	Apporepressor is enabled by co-repressor to join the operator gene

ALLOSTERIC ENZYMES REGULATION

Phosphofructokinase.

- Binding of F6P hyperbolic in absence of PEP.
- Obeys Michaelis - Menten Kinetics.
- Becomes sigmoidal in presence of PEP.
- Effector changes pattern of binding.



THERAPEUTIC USES OF ENZYMES

Several enzymes are known today which have got beneficial important role in several disorders / diseases. So, as to make the sick person better one.

Since, the enzymes are inactivated in gastrointestinal tract, hence these are administered parenterally.

Some of the important examples of such enzymes are as follows—

Trypsin—

It the treatment of a cute thrombophlebitis (a blood clot and inflammation in which a vein), a small recurrent injections have been beneficial in many patients.

Some types of ulcers have responded well to trypsin therapy.

Streptokinase—

It causes fibrinolysis & dissolution of clot. It is used in the treatment of haemothorax & haematoma.

Hyaluronidase—

It is prepared from mammalian testes. It acts by depolymerizing hyaluronic acid & increasing tissue permeability. It is used in the treatment of traumatic or postoperative edema.

Pepsin—

It is obtained from hog stomach. It is used in the treatment of gastric achlia (congentially undeveloped gastric glands.)

mboplastin —

This is prepared by lysis of blood platelets & is ed for blood coagulation.

rokinase —

It is prepared from human urine & is used in the treatment of pulmonary embolism & in myocardial infarction to dissolve the clot.

Fibrinolysin —

It is prepared by activating fibrinolysinogen by streptokinase & is used to in the treatment of venous thrombosis, pulmonary & arterial embolism.

Renin —

It is obtained from the glandular layer of calf stomach & is used in the therapy of gastric achylia.

DIAGNOSTIC IMPORTANCE OF ENZYMES

The assay of serum enzymes is used as an important aid to diagnosis. Some enzymes of diagonostic importance with principal conditions involved are as follows—

Enzymes of diagnostic importance with principal conditions involved—

ENZYMES	PRINCIPAL CONDITIONS IN WHICH LEVEL OF ACTIVITY IN SERUM GETS ELEVATED
Amylase	Acute pancreatitis
Acid phosphate (optimum pH 5)	Prostatic Carcinoma
Alkaline phosphatase (optimum pH 10)	Diagnosis of liver diseases, especially billary obstruction & detection of osteoblastic bone diseases eg. rickets.
Aspartate transaminase (AST - previously GOT)	Myocardial infarction, liver diseases especially with liver cell damage
Alanine transaminase (ALT - previously GPT)	Liver diseases especially with liver cell damage.

COENZYMES

They are organic molecules, often derived from the B. Vitamin. that participate directly in enzymatic reactions. Some co-enzymes are attached to their companion enzymes are tightly bound prosthetic groups where as others can be easily removed by dialysis. The complete functional complex of enzyme & co-factors is called a "holoenzyme" the protein part, free of the co factors, is called a apoenzyme.

Characteristics of Co-enzymes —

1. They are stable towards heat.
2. Generally derived from vitamins.
3. Function as co-substrates.
4. They participate in:
5. Electron transfer reactions for eg:- NAD^+, NADH, FMN, FAD etc
6. CoA, TPP, pyridoxal phosphate tetrahydro folic acid etc.

Coenzymes	Functions performed
1) NAD^+	Hydrogen transfer.
2) $NADP^+$	Hydrogen transfer.
3) FAD	Hydrogen transfer
4) FMN	Hydrogen transfer
5) TPP (Thiamine pyrophosphate)	Acetyl group transfer
6) PP (Pyridoxal Phosphate)	Amino group transfer

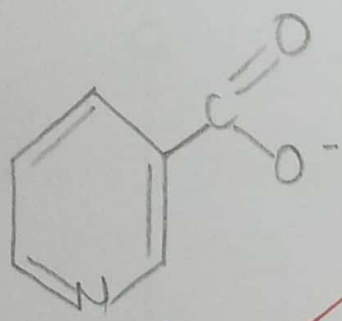
7) Biotin — Carboxy group transfer

8) Coenzyme A — Acyl group transferase.

Biochemical functions —

Nicotinamide nucleotides —

NAD^+, $NADP^+$ & their reduced forms are involved in a great variety of dehydrogenase reactions in the mitochondria, cytosol & endoplasmic reticulum of the cell. They are water soluble & are usually free to diffuse away from the enzyme after conversion to the oxidized or reduced form, to take part in another dehydrogenase reaction catalysed by another enzyme.



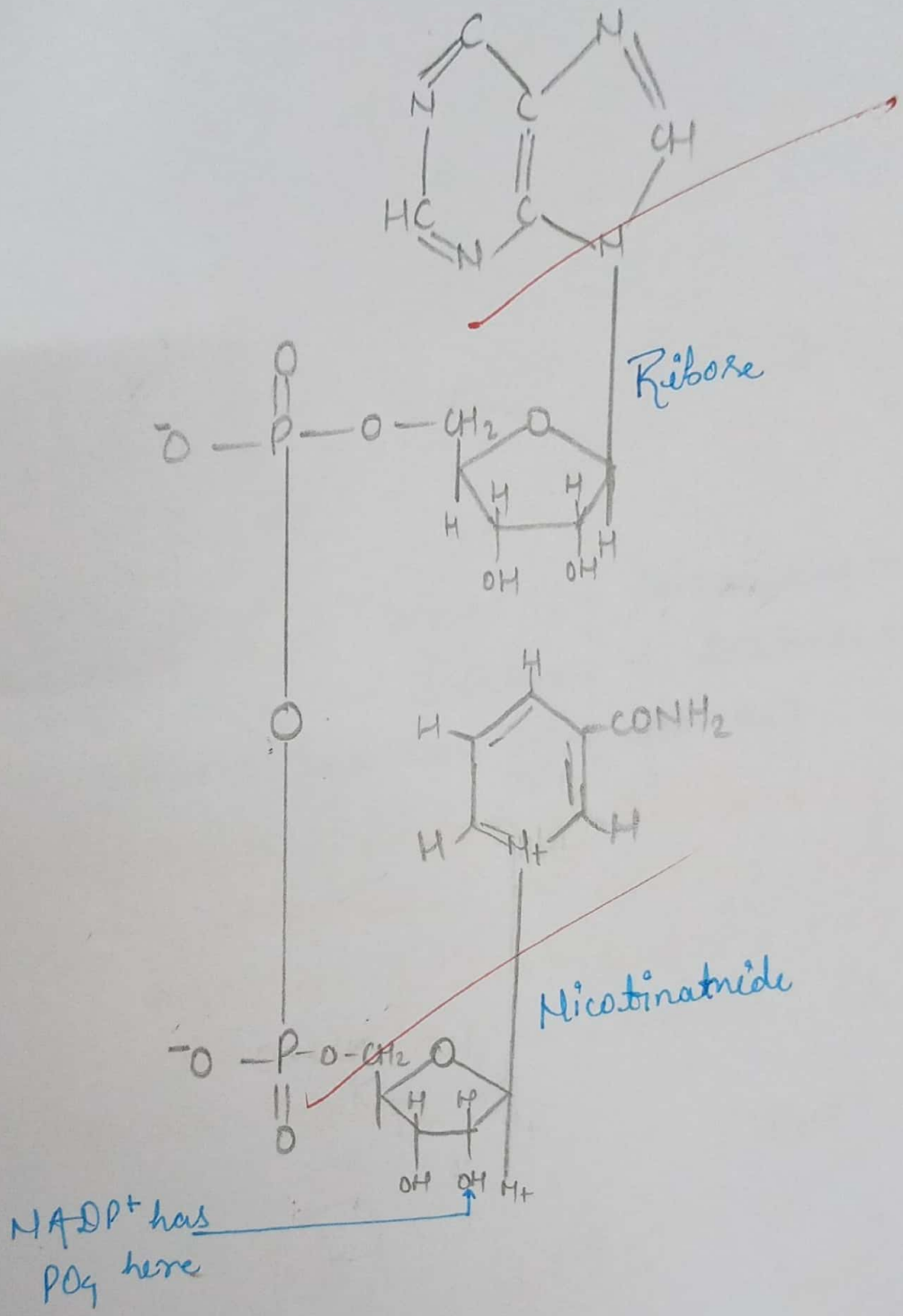
Nicotinic Acid

Nicotinic acid (niacin) is a vitamin used as a percursor of the nicotinamide moiety of the nicotinamide nucleotides.

Niacin difficiency causes pellagra, a disease categorised by dermatitis, diarrhoea and dementia.

tinamide adenine dinucleotide (NAD); DP is nicotinamide adenine dinucleotide phosphate

* $NADP^+$ has got PO_4 in place of OH



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05/05

Good

Keep it up

13/5/22