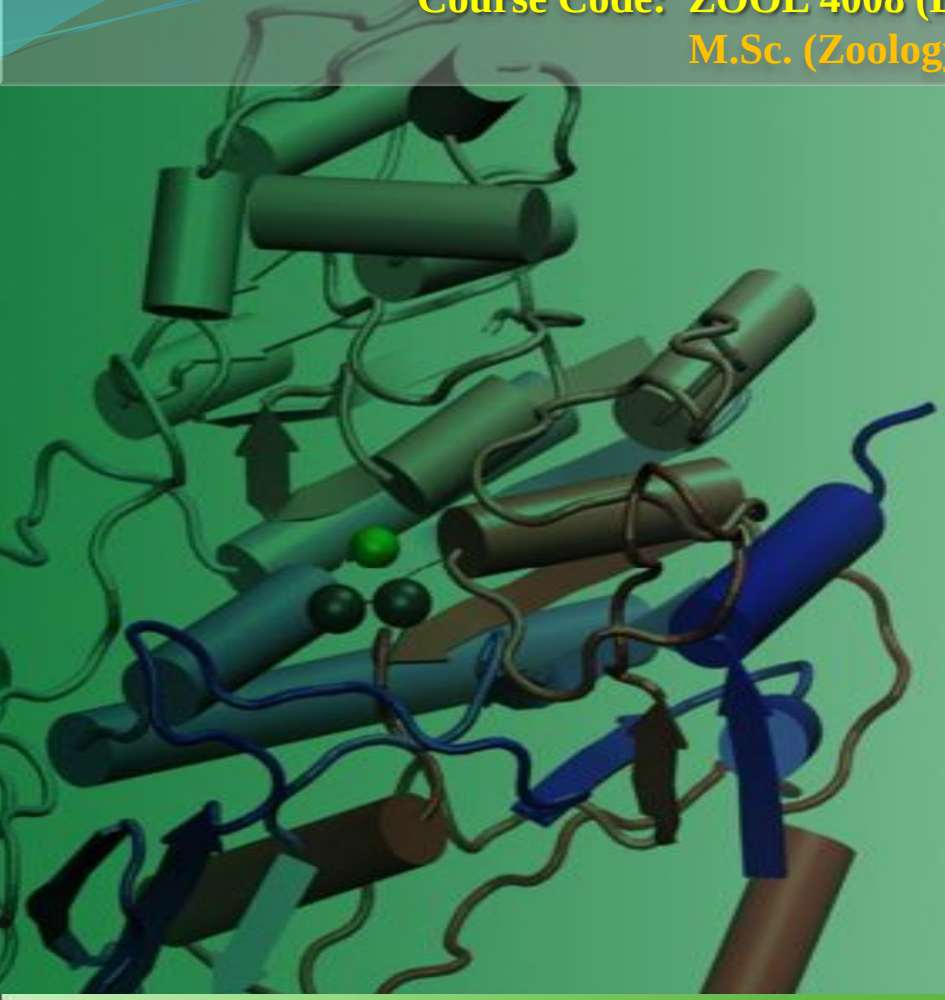
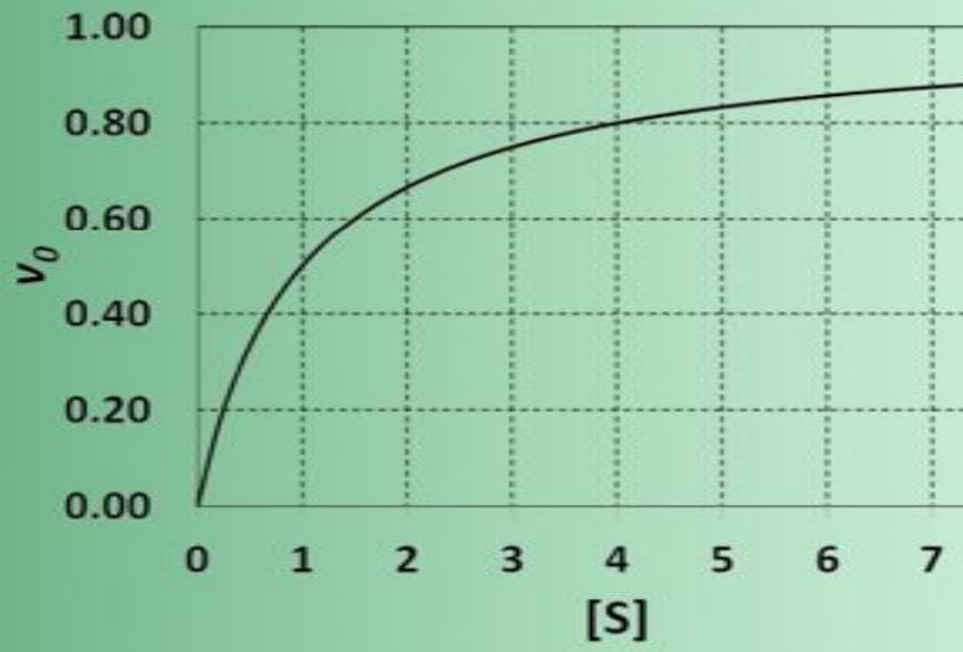
Regulation of Enzyme Activity

Course Code: ZOOL 4008 (Biochemistry and Metabolism)

M.Sc. (Zoology), Semester –II



$$v_0 = \frac{v_{max}[S]}{K_M + [S]}$$



Dr. Shyam Babu Prasad

Assistant Professor

Department of Zoology

Mahatma Gandhi Central University (MGCU),

Motihari-845401 (Bihar)

Email: shyambabuprasad@mgcub.ac.in

Basic concepts of Enzyme Regulations

- The cells of our body are capable of making many different enzymes.
- In living systems hundreds of different enzyme catalysed reactions occur simultaneously.
- Regulation of enzyme activity is important to coordinate the different metabolic processes.
- It is also important to maintained cellular homeostasis i.e. to maintain the internal environment of the organism constant.
- These reactions must be regulated for the proper functioning of a living system.
- Regulatory enzymes exhibit increased or decreased catalytic activity in response to certain signals.
- An enzyme's catalytic activity can be directly controlled through structural alterations that influence the enzyme's substrate-binding affinity.

Enzyme activity regulation

➢Regulation of enzyme activity can be achieved by two general mechanisms:

1. **Control of enzyme quantity:**

Enzyme quantity is affected by:

- A- Altering the rate of enzyme synthesis and degradation,
- B- Induction
- C- Repression
- D- Substrate, Metal ion and Coenzyme

2. Altering the catalytic efficiency of the enzyme by:

Catalytic efficiency of enzymes is affected by:

- A- Allosteric regulation
- B- Feedback inhibition
- C- Proenzyme (zymogen)
- D- Covalent modification
- E- Protein – Protein interaction

1. Control of Enzyme Quantity

As enzymes are protein in nature, it's synthesized from amino acids under genetic control and degraded after doing their work.

✓ **Quantity of the enzyme within cells depends on:**

- Rate of synthesis and degradation (Ex. Liver arginase enzyme increases after protein rich meal).
- Induction of Synthesis:

  Constitutive enzymes: Synthesis don't depends on inducers.

  Inducible enzymes: Synthesis depends on Inducers for example, induction of Lactase enzyme in bacteria grown on glucose media.
- Repression: Decrease in the rate of enzyme synthesis by substances called repressors (low mol. wt substance) and repression is sometimes called feedback regulation. For example dietary cholesterol decreases the rate of synthesis of HMG CoA reductase (β-hydroxy β-methyl glutaryl CoA reductase) involves in cholesterol biosynthesis.
- Concentration of substrates, coenzymes and metal ion activator: Presence of substrate, coenzyme or metal ion activator causes changes in the enzyme conformation decreasing its rate of degradation.

2. Control of catalytic efficiency of enzymes

A- Allosteric Regulation:

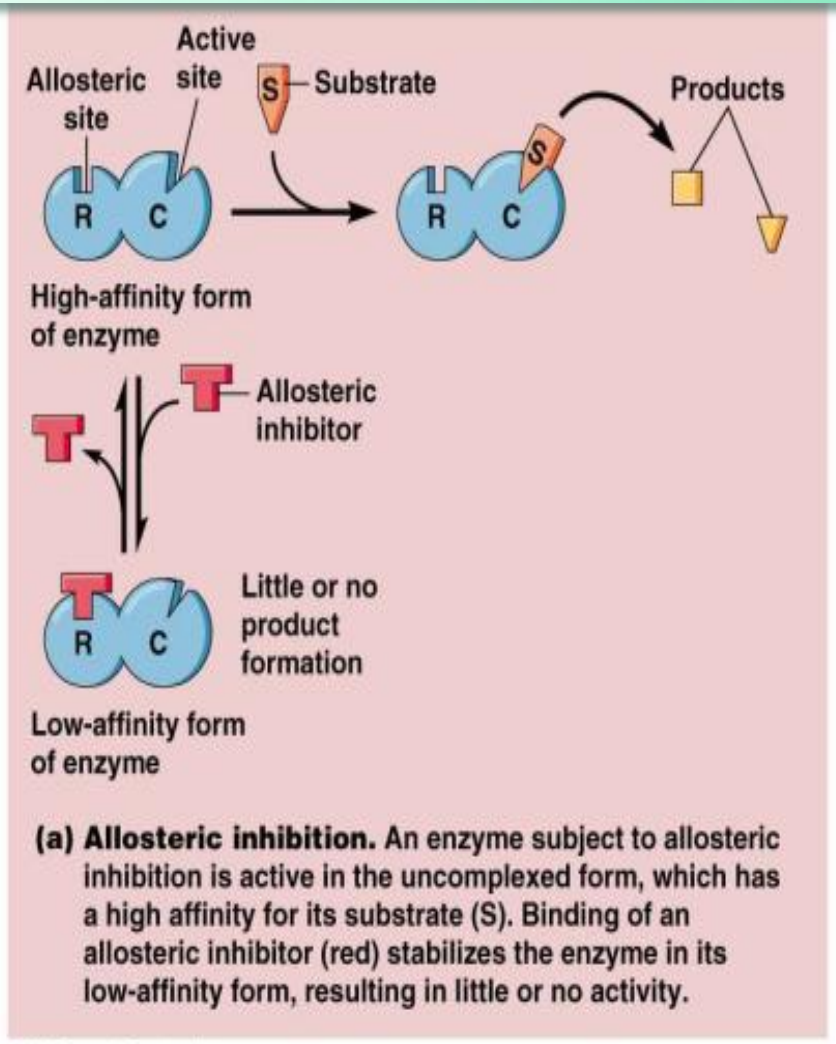
•Allosteric enzyme is formed of more than one protein sub unit. It has two sites; a catalytic site for substrate binding and another site called allosteric site , where effectors binds.
•Enzymatic activity can be controlled by positive and negative effectors/ metabolites through non-covalent interaction. This form of control is termed **Allosteric regulation**.

• **Positive effectors/allosteric activators** -Binding of the effectors to the enzyme increases its activity e.g. ADP is allosteric activator for phosphofructokinase enzyme.

•**Negative effector or allosteric inhibitor**- Binding of the effectors to the enzyme causes a decrease in its activity, e.g.

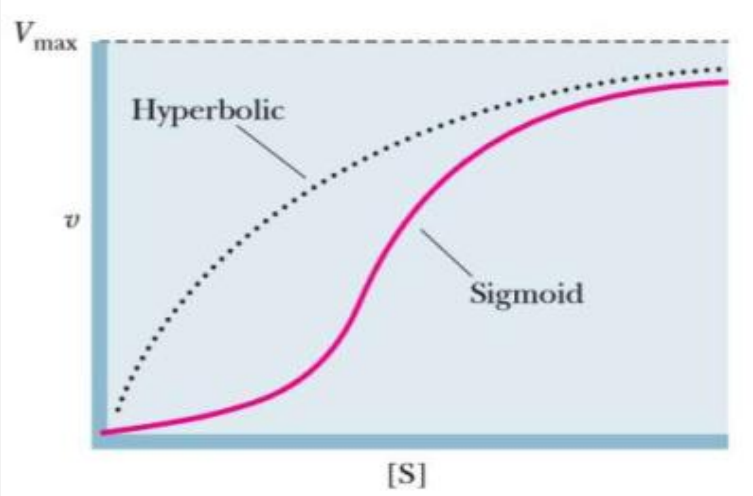
-ATP and citrate are allosteric inhibitors for phosphofructokinase enzyme.
-Glucose-6-phosphate is allosteric inhibitor for hexokinase enzyme

Mechanism of Allosteric regulation



Kinetics of Allosteric enzymes

- One of the common characteristics of an allosteric enzyme is that it shows a sigmoid plot when velocity is plotted against substrate concentration,



- Allosteric enzymes generally do not follow the Michaelis-Menten equation.
- The Lineweaver-Burk plot is concave upward.
- Allosteric enzyme show the property of **cooperativity** i.e., activity at one functional site affects the activity at others.

  Positive cooperativity: Binding of one site facilities binding of others site.

  Negative cooperativity: Binding of one site inhibits binding of others site

- A slight change in substrate concentration can produce substantial changes in activity.

Enzyme regulatory Molecules

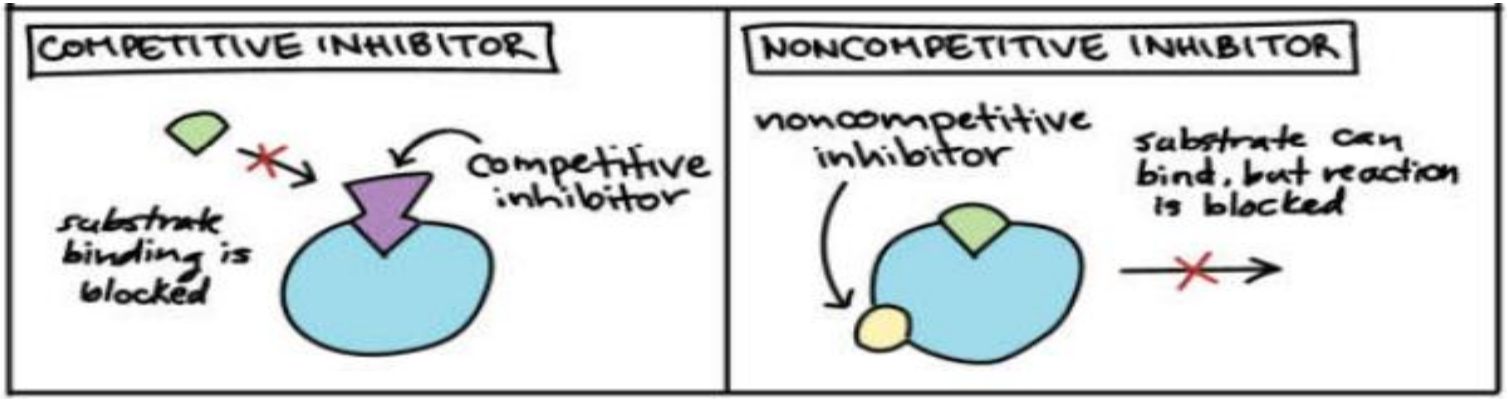
•Enzyme activity may be turned "up" or "down" by activator and inhibitor molecules that bind specifically to the enzyme active site.
• The binding of an activator or inhibitor's is reversible. It means doesn't permanently attach to the enzyme.

There to types of Inhibitors mostly affects enzyme activity:

•An inhibitor hat binds to the active site of the enzymes and blocks the binding of the substrate is called **competitive inhibition.** As a result, competitive inhibition increase only the K_m, leaving the V_{max} the same.
For example Inhibition of the enzyme **succinate dehydrogenase by malonate and** many other pharmaceutical drugs.

• The inhibitor doesn't block the substrate binding to the active site. Instead, it attaches at another site and blocks the enzyme activity. This inhibition is said to be "**noncompetitive Inhibition** " . **As a results Noncompetitive Inhibitors decreased *V*max but Do Not Affect *K*m**
For example , the action of **pepstatin on enzyme renin**.

Mechanism of Reversible Inhibitors



Double Reciprocal Plots use to evaluate Inhibitors effects on enzymes

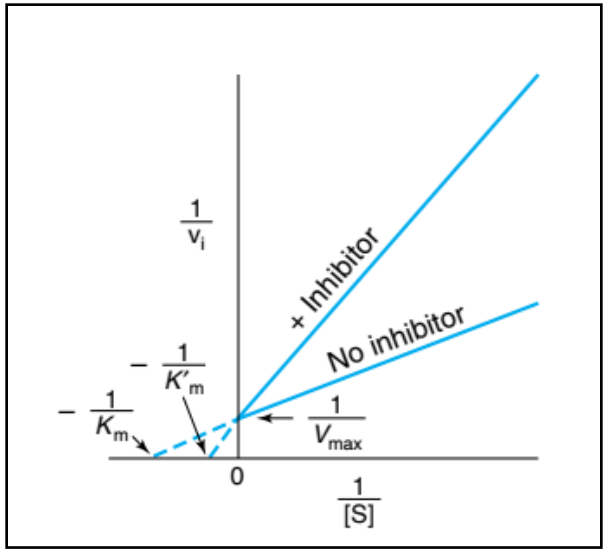


Fig. Lineweaver-Burk plot of competitive inhibition

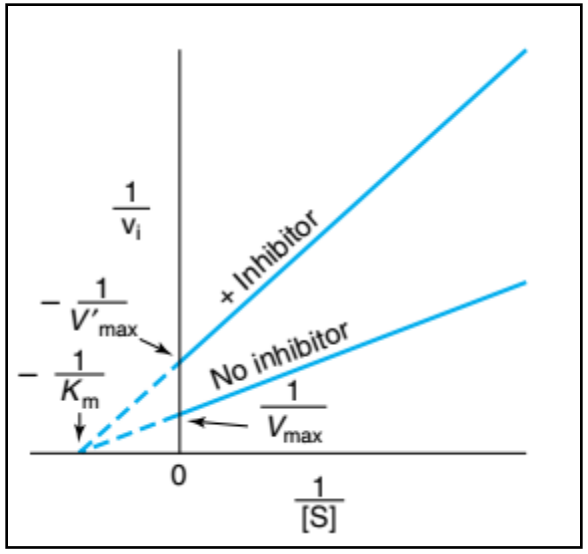
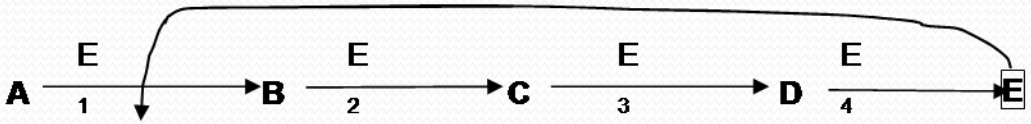


Fig. Lineweaver-Burk plot for simple noncompetitive inhibition

Note# There are some non-reversible Inhibitors that inhibits enzyme activity permanently, for example diisopropylphosphofluoridate (DIPF) blocks enzyme acetylecholinesterase and Penicillin bind to glycopeptide transpeptidase in bacterial cell wall formation.

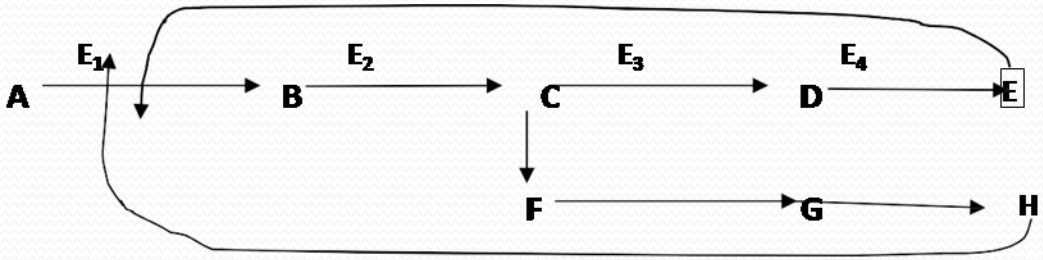
B. Feed Back Inhibition:

- An end product of biosynthetic pathways may directly inhibit an enzyme early in the pathway.



Simple feedback inhibition loop

Where A is the substrate, E is the end product, B, C, D are intermediate metabolites, E_1, E_2, E_3 and E_4 are enzymes in biosynthetic pathway.



Multiple feedback inhibition loop

Note# Feedback regulation does not affect the enzyme activity but decreased the enzyme quantity and it is slow process. Feedback inhibition does not affect enzyme quantity but decreases the enzyme activity and it is fast process.

C- Proenzymes (Zymogens)

•Zymogens are inactive precursors of enzymes, some enzymes secreted in this form, for example pepsinogen, trypsinogen, chymotrypsinogen, prothrombin,clotting factors and Insulin an important metabolic regulator.

•Zymogens or proenzymes acquire full activity only upon specific proteolytic cleavage of one or several of their peptide bond and it's activation is irreversible process .

•Zymogen is inactive because it contains an additional polypeptide chain that masks (blocks) the active site of the enzyme.

Note # Biological importance of zymogens

1.Some enzymes are secreted in zymogen form to protect the tissues of origin from auto digestion.
2.Zymogens is to insure rapid mobilization of enzyme activity at the time of needs in response to physiological demands.

Activation of zymogens can occur by one of the following methods:

- **Activation by HCl**

$$\text{Pepsinogen} \xrightarrow{\text{HCl}} \text{Pepsin}$$

- **Activation by other enzymes:**

$$\text{Trypsinogen} \xrightarrow{\text{Enterokinase}} \text{Trypsin}$$

$$\text{Prothrombin} \xrightarrow{\text{Thrombokinase} + Ca^{++}} \text{Thrombin}$$

- **Auto activation** i.e. the enzyme activates itself.

$$\text{Pepsinogen} \xrightarrow{\text{Pepsin}} \text{Pepsin}$$

Table. Examples of Pancreatic and Gastric Zymogens

Origin	Zymogen	Active Protease
Pancreas	Trypsinogen	Trypsin
Pancreas	Chymotrypsinogen	Chymotrypsin
Pancreas	Procarboxypeptidase	Carboxypeptidase
Pancreas	Proelastase	Elastase
Stomach	Pepsinogen	Pepsin

D- Covalent modification

➢It means modification of enzyme activity through formation of covalent bonds with some specific group:

•Phosphorylation (addition of phosphate group at the hydroxyl group of serine, threonine or tyrosine)

•Methylation (addition of methyl group).

•Hydroxylation (addition of hydroxyl group).

Adenylation (addition of adenylic acid) etc.

Examples of enzymes inactivated by phosphorylation:

•Glycogen Synthetase, which catalyzes biosynthesis of glycogen.

•Acetyl CoA carboxylase, an enzyme in fatty acid biosynthesis.

•HMG CoA reductase, an enzyme in cholesterol biosynthesis.

Examples of enzymes activated by phosphorylation:

•Glycogen phosphorylase that breaks down glycogen into glucose.

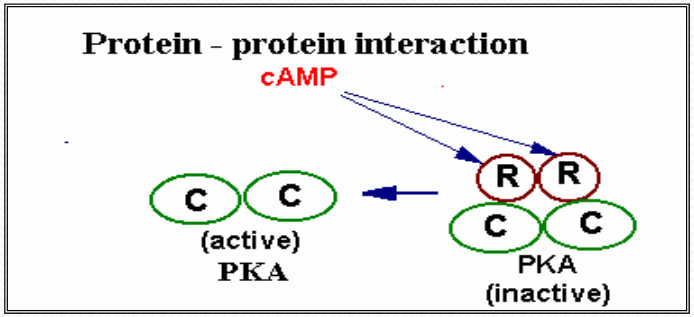
•Citrate lyase, which breaks down citrate.

•Lipase that hydrolyzes triglyceride into glycerol and 3 fatty acids.

Note# Phosphorylation is the most common kind of covalent modification in the enzymes. After covalent modification enzyme get either active or inactive depends on modification. **Phosphatases**: Removal of phosphate group from the hydroxyl group of serine, threonine or tyrosine.

E- Protein-protein interaction

- An Enzymes that are formed that have many protein subunits, the enzyme may be present in an inactive form through interaction between its protein subunits.
- The whole enzyme, formed of regulatory and catalytic subunits, is inactive.
- Activation of the enzyme occurs by separation of the catalytic subunits from the regulatory subunits, for example Protein kinase A enzyme is an example for regulation of enzyme activity through protein interaction.
- The PKA having 2R2C domain and it is an inactive form. cAMP (cyclic adenosine monophosphate) activates the enzyme by binding to the 2 regulatory (2R) subunits releasing the 2 catalytic (2C) subunits and hence activating the enzyme.



References:

•Stryer, Lubert. *Biochemistry (Third Edition)*.New York, NY: W.H. Freeman and Company, 1988. Page 187-191 .

• Lehninger principles of biochemistry (4th ed.): Nelson, D., and Cox, M, W.H. Freeman and Company, New York, 2005 .

Thank You