6th chapter: Enzyme properties and kinetics

ENZYMOGOLY: GDM 6106

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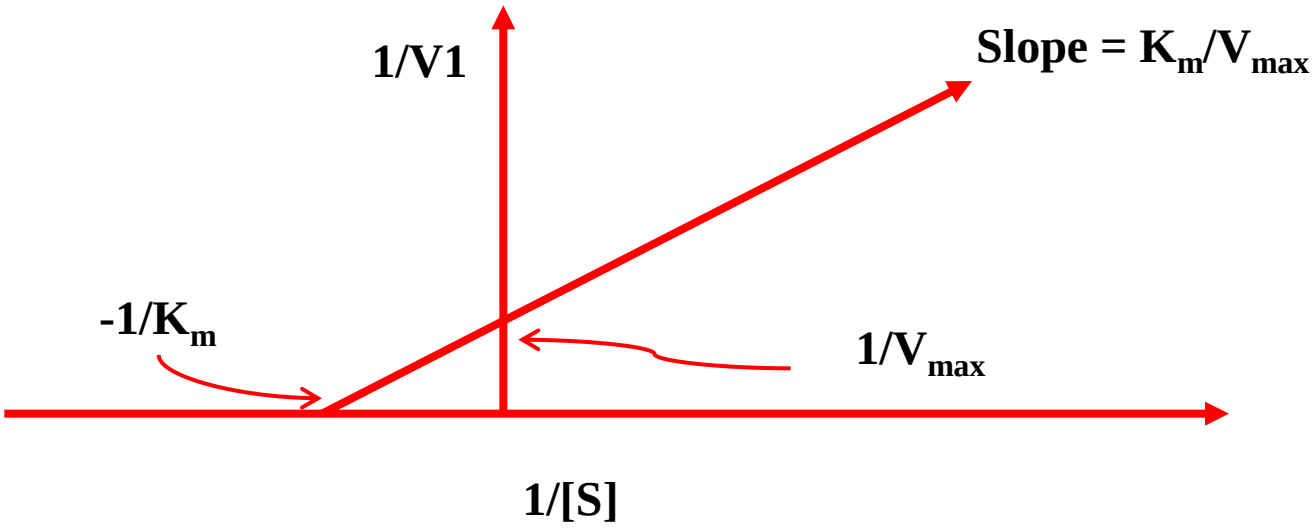
Second method of measuring velocity of an enzyme

B. Lineweaver-Burk Plot: (Double-reciprocal plot)

1. Here, the reciprocal of V, i.e. 1/V is plotted versus the reciprocal of S, i.e. 1/S.
2. The curve is a straight line.
3. The curve is more practical to estimate K_m because a few enzymes give the saturation curve.



A lineweaver-burke plot of the kinetic data

Factors effecting enzymes activities

3. Effects of temperature:

A. The optimal temperature for enzymatic activity in human body is that temperature similar of that of the living cell (37^{o}C).

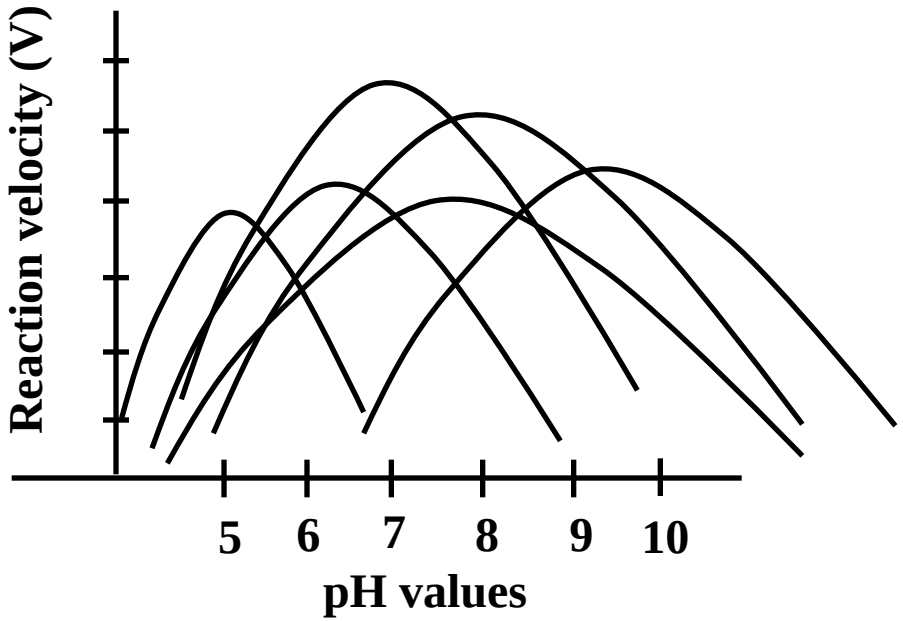
B. At zero temperature, the enzyme is inactive. The reaction velocity increases with increase of temperature until a maximum velocity is reached.

PS. The velocity is **almost doubled every 10^{o}C**. This increase in reaction velocity is due to the increase number of molecules having sufficient energy to pass over the energy barrier and from the products of the reaction.

C. Further elevation of the temperature results in a decrease in reaction velocity. At 55- 60^{o}C, most of enzymes are denaturated and become permanently inactive.

4. Effects of pH

A. The optimal pH for the enzyme activity is that pH as which the enzyme acts maximally.
B. Above or below this pH, the ionic state of both enzyme and substrate will be changed, and the rate of reaction will therefore decline.
C. Each enzyme has its own optimal pH e.g. pancreatic lipase 7.5.
D. Extreme of pH can also lead to denaturation of the enzyme.



Effect of pH on an enzyme-catalyzed reaction.

$[H_3O^+]$	pH	$[OH^-]$	Example
1×10^{0}	0	1×10^{-14}	HCl (4%)
1×10^{-1}	1	1×10^{-13}	Stomach acid
1×10^{-2}	2	1×10^{-12}	Lemon juice
1×10^{-3}	3	1×10^{-11}	Vinegar
1×10^{-4}	4	1×10^{-10}	Soda
1×10^{-5}	5	1×10^{-9}	Rainwater (unpolluted)
1×10^{-6}	6	1×10^{-8}	Milk
1×10^{-7}	7	1×10^{-7}	Pure water
1×10^{-8}	8	1×10^{-6}	Egg whites
1×10^{-9}	9	1×10^{-5}	Baking Soda
1×10^{-10}	10	1×10^{-4}	Ammonia
1×10^{-11}	11	1×10^{-3}	
1×10^{-12}	12	1×10^{-2}	Drano®
1×10^{-13}	13	1×10^{-1}	NaOH (4%)
1×10^{-14}	14	1×10^{0}	

5. Enzyme activators:

Certain substrates may be needed to activate the enzyme. They include:

A. **Metal ions:** e.g. chloride ions which activates salivary amylase and calcium ions which activates blood clotting enzymes.

B. **Enzymes**: some inactive enzymes (zymogens) may need other enzymes for activation. This includes:

1. Auto-activation e.g. pepsenogen (pepsin) >>>> Pepsin
2. Other enzymes e.g. trypsinogen (Enterokinase) >>>>Trypsin

C. HCL: it starts activation of pepsinogen into pepsin.

D . Bile salts: activate pancreatic lipase enzyme.

6. Enzyme inhibitors:

NOTE: To understand the mechanisms of enzyme inhibitors: let us talk about the enzyme inhibitors as a subtopic.

6. Enzyme inhibitors:

Any substance that can diminish the velocity of an enzymatic reaction is called inhibitor. The inhibition of enzymes can be classified either as reversible and irreversible or competitive and non competitive.

A. Reversible and irreversible inhibitors:

1. Reversible inhibitors: these inhibitors bind to an enzyme through non covalent bonds.

a. Dilution of the enzyme-inhibitor complex results in dissociation of the reversibly bound inhibitor and recovery of enzyme activity.

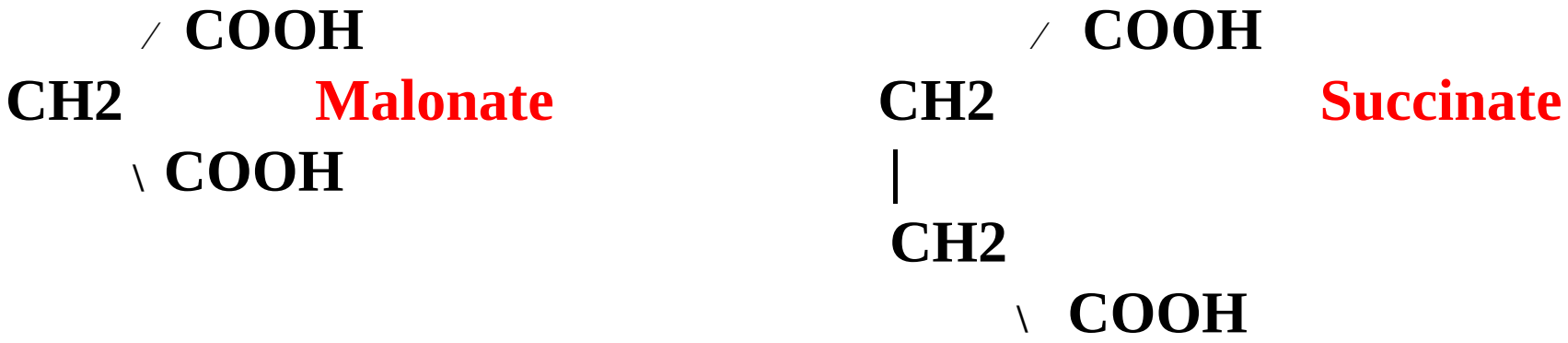
b. Reversible inhibition may be competitive or non competitive.

2. Irreversible inhibitors: this type of inhibition takes a place when an inhibited enzyme does not regain activity upon dilution of the enzyme-inhibitor complex. Irreversible inhibitors bind permanently to their target enzyme, "Permanently" here means over a time-scale which may be minutes for some bacterial enzymes, and months or years for enzymes found in cells in eukaryotes.

B. Competitive and non-competitive inhibitors:

1. Competitive inhibitors: This type of inhibition occurs when the inhibitors binds reversibly to the active site where the substrate normally would occupy. Therefore, both inhibitor and substrate compete for active site.

a. Here is structural similarity between substrate and inhibitor e.g. malonic and succinic acids.

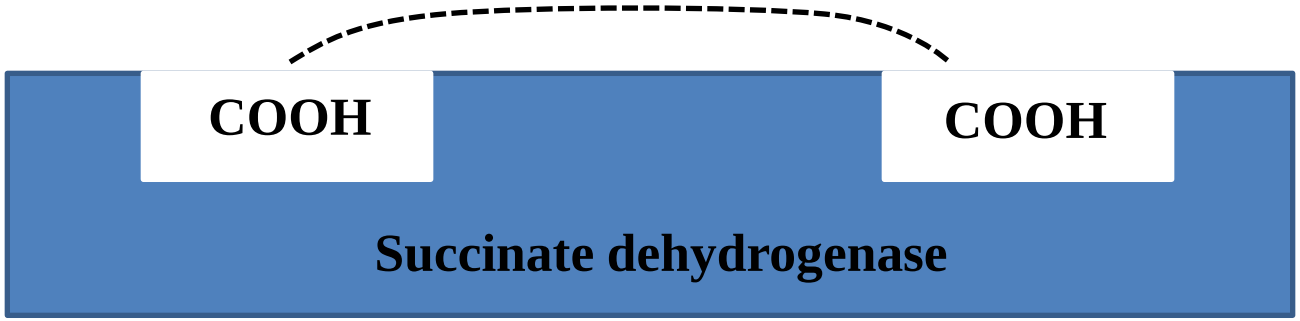


b. The combination between enzyme and substrate or inhibitor depends on:

1. Concentration of substrate
2. Concentration of inhibitor
3. Affinity of both inhibitor and substrate to the active site of the enzyme.

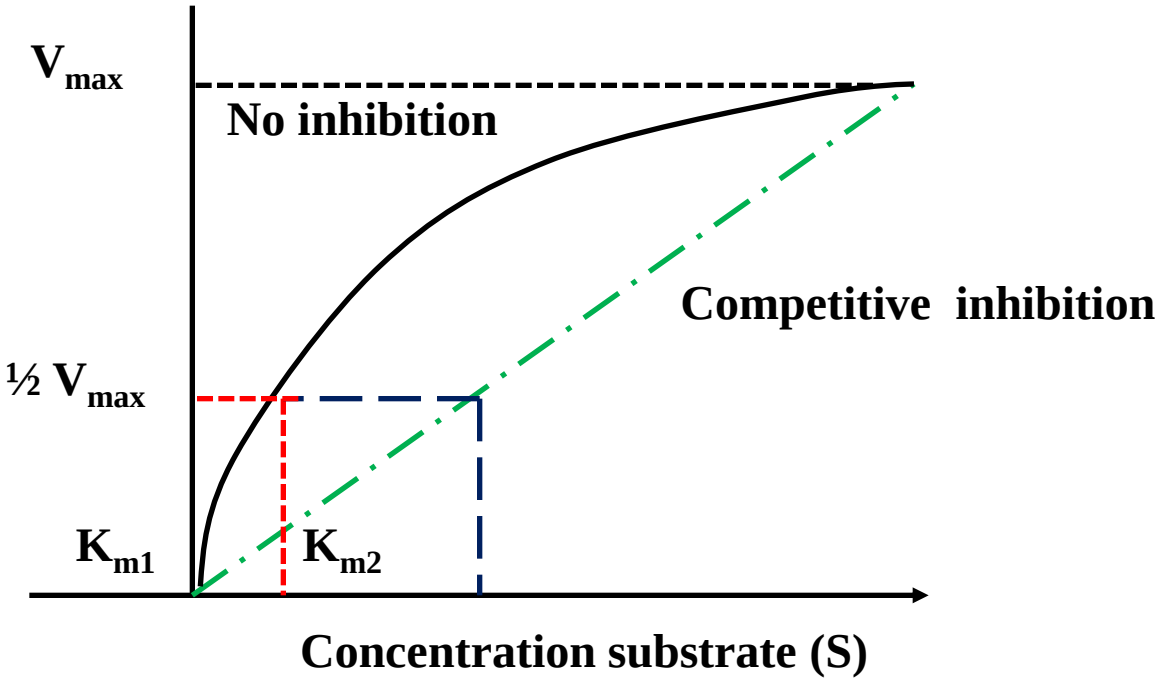
c. Competitive inhibition is reversible and can be removed by adding excess substrate.

d. The classic example of competitive and reversible inhibition is the of succinate dehydrogenase enzyme by malonic acid. The substrate is succinate acid.



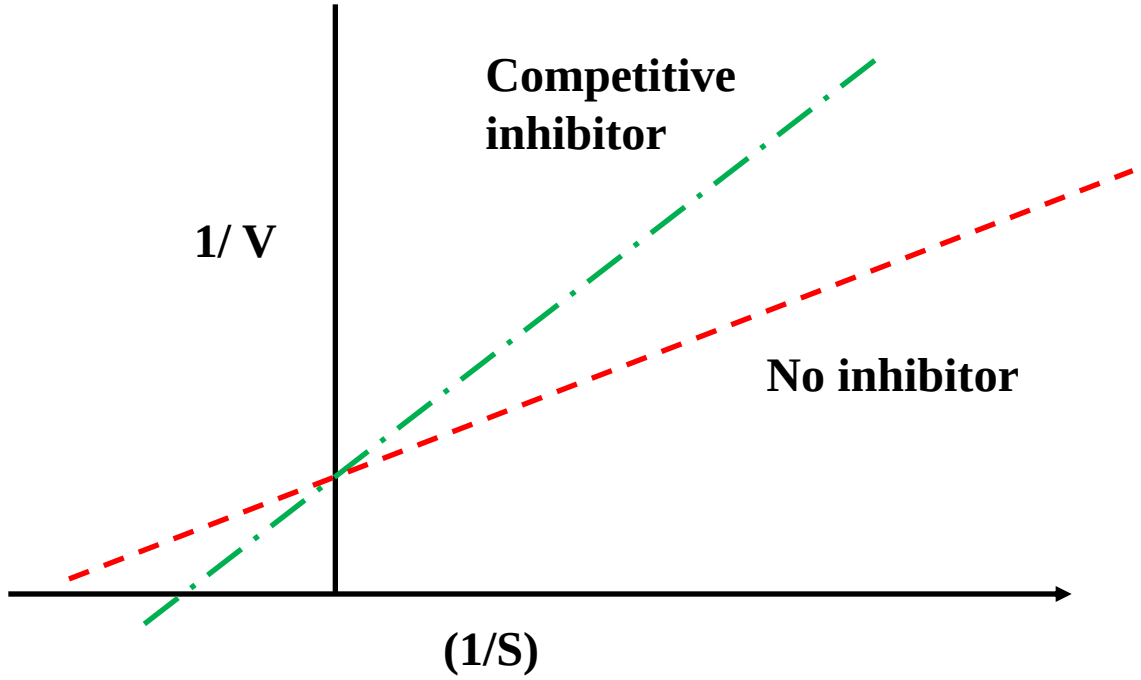
e. Effects of competitive inhibitor on V_{max}:
The effect of a competitive inhibitor is reversed by increasing substrate concentration. Therefore, at a sufficiently high substrate concentration, the reaction velocity reaches the V_{max} observed in the absence of inhibitor.

f. Effect of K_m: a competitive inhibition increases the K_m of substrate i.g. in the presence of a competitive inhibitor more substrate is needed to achieve ½ V_{max}



g. Effect on lineweaver-burk plot:

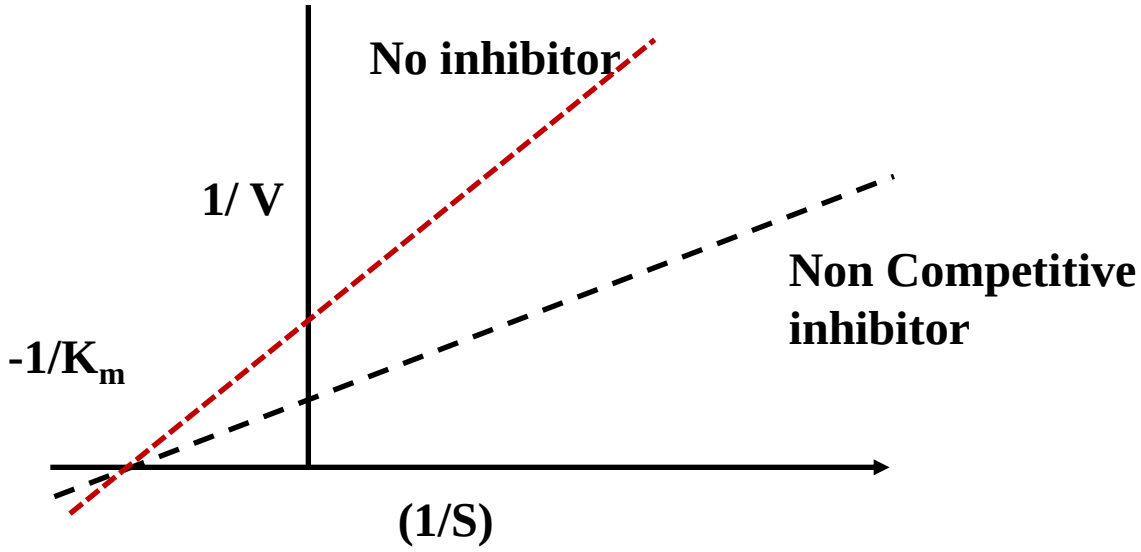
Competitive inhibition shows a characteristics lineweaver-burk plot in which the reciprocal plot of the inhibited and uninhibited reactions intersects on the Y axis and $1/V_{max}$ (V_{max} is unchanged). The inhibited and uninhibited reactions show different X axis intercepts, indicating that the apparent K_m is increased in the presence of the competitive inhibitor.



Lineweaver-burke plot of competitive and non competitive inhibition of an enzyme

2. Non competitive inhibitors: this type of inhibition occurs when the inhibitor and substrate bind the different sites on the enzyme.

a. The inhibitor can bind either free enzyme or the enzyme substrate complex.
b. There is no structural similarity between substrate and inhibitor.
c. The inhibitor binds to a site on the enzyme other than the active site.
d. Examples of non-competitive inhibitors are heavy metals as mercury and oxidizing agents.

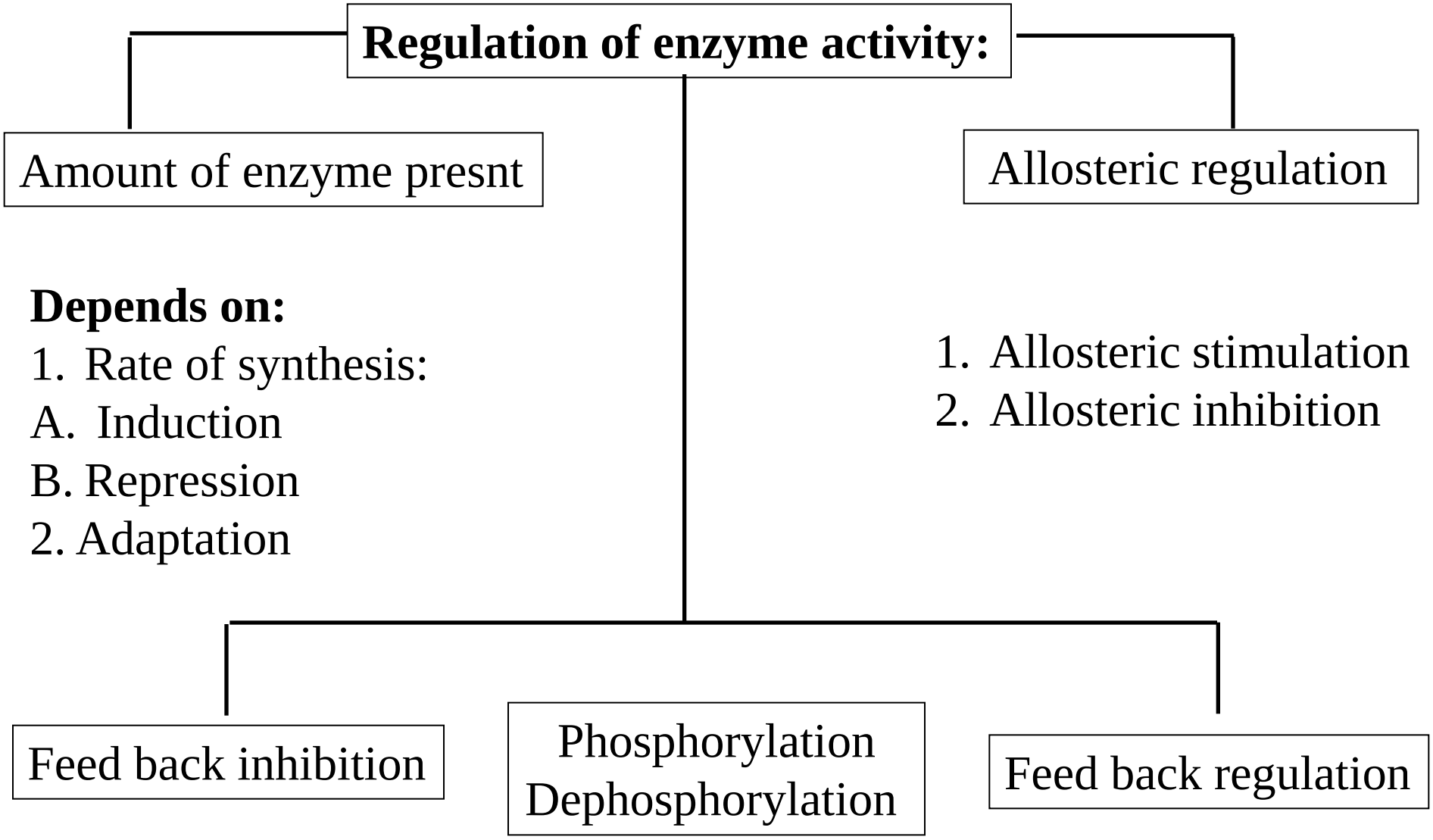


e. Effect of non-competitive inhibitors on V_{max}:
Non competitive inhibition can not be overcome by increasing the concentration of substrate. Thus, non-competitive inhibitors decrease the V_{max} of the reaction.

f. Effect on K_m: non-competitive inhibitors do not interfere with the biding of substrate to enzyme. Thus, the enzyme shows the same Km in the presence and absence of the non-competitive inhibitor.

g. Effect on Lineweaver-burk plot: non-competitive inhibition is readily differentiated from competitive inhibition by plotting 1/V versus 1/S and noting that V_{max} decreases in the presence of non-competitive inhibitor, whereas K_m is unchanged.

IX . Regulation of enzyme activity:



Enzyme activity is regulated by many mechanisms. This regulation helps to maintain constant intracellular environment in the face of changes in externals factors as changes in temperature or the presence or absence of water. Mechanisms regulating enzymes activity include:

A. Amount of enzyme present in the cell: this is determined by:

1. Rate of enzyme synthesis. Enzyme (protein) synthesis from amino acids may be stimulated by certain substances. These substances are called **inducers** and the process is called induction. Also enzyme synthesis may be inhibited by other substances which are called **repressors** and the process is called repression.

A. Inducers and induction: inducers may be substrates for enzymes, which might be compounds similar in structure to the substrates. Inducers usually stimulate DNA (gene) controlling enzyme synthesis.

B. Repressors and repression: repressor may be a product of metalboic pathway. It will inhibit DNA controlling enzyme synthesis. This is called feed back regulation.

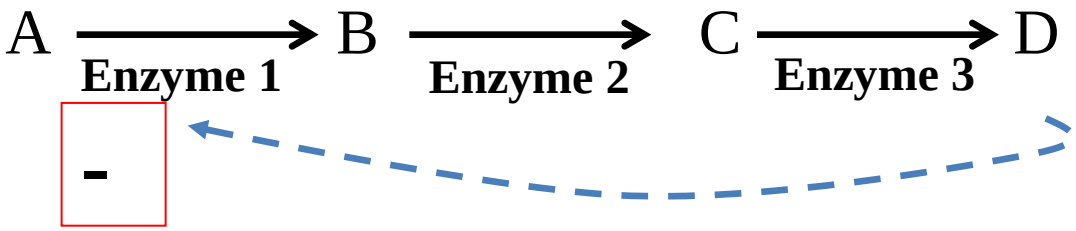
As a result of repression, the metabolic pathway stops, metabolic intermediates are removed and the enzyme biosynthesis again occurs. This is called derepression.

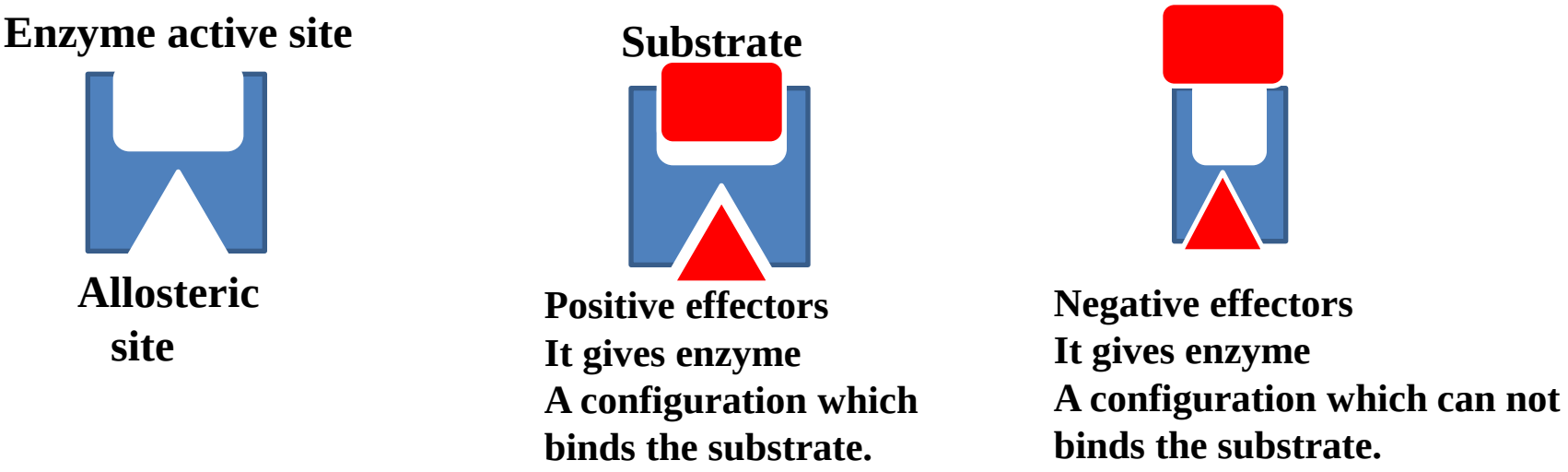
2. Adaptation: the amount of enzyme present in a cell can be increased or decreased according to the body needs. This process called **adaptation.**
For example, an increase in carbohydrate in diet will increase the liver enzymes involved in the process of carbohydrate metabolism. Starving causes decrease concentration of the enzyme.

B. **Allosteric regulation of enzyme activity:**

1. Allosteric enzymes generally catalyze the irreversible steps in metabolic pathway.
2. The term allosteric means (other sites). It indicates that molecules called effectors (also called modifiers or modulators) can bind non-covalently at a site other than active site.

a. Effectors are positive if they stimulate catalytic reaction and negative if they inhibit the reaction.
b. Effectors may be the end product of a metabolic pathway. If it inhibit the reaction (negative regulation), it is called: feed back inhibition.





Allosteric regulation of enzyme activity

3. Classes of allosteric enzymes: there are 2 classes of allosteric enzymes:

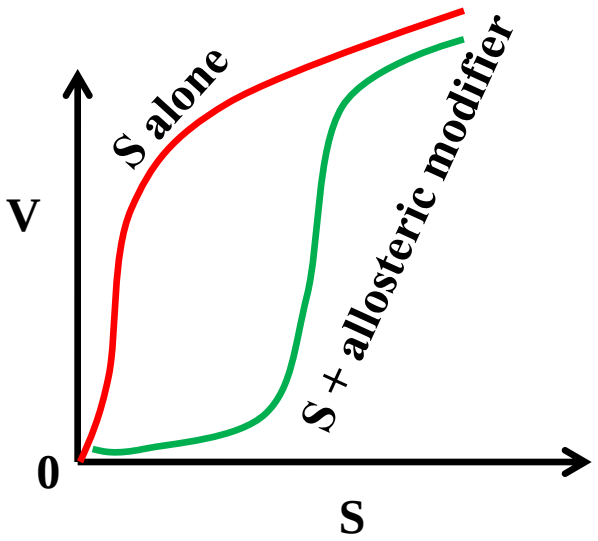
A. Homotropic enzymes: in which the substrate is also the effectors i.e. substrate bind to both active sites and allosteric site.

B. Heterotropic enzymes: in which the effector is another substrate than the original substrate, usually the end product of the pathway.

4. Effects of allosteric regulation on k_m and V_{max}:

Allosteric regulation of enzymes causes conformational changes at the catalytic site. This leads to:

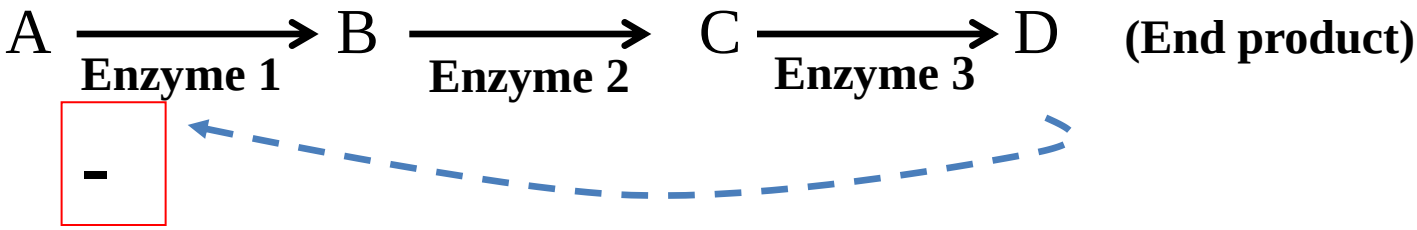
a. Altering K_m for a substrate and V_{max} for the overall reaction.
b. The substrate saturation curve for allostreic inhibition often are sigmoidal. Where the michaelis-menten equation does not apply.



Allosteric regulation of enzyme activity

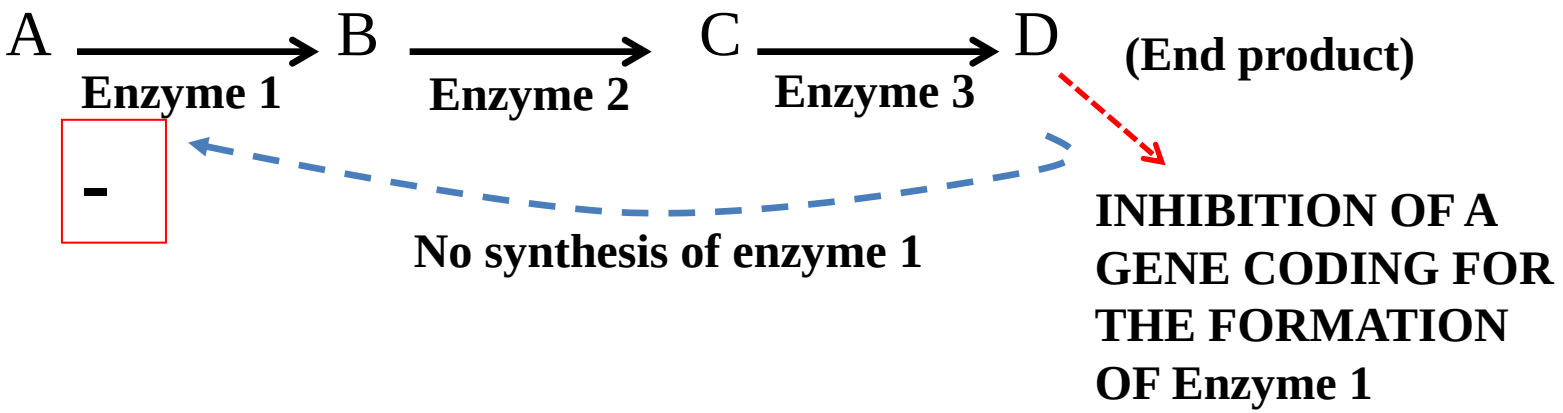
C. Feed back inhibition:

It means that the end product of a series of reaction directly inhibits the first enzyme of that series.



D. Feed back regulation:

It means the end product of a series of reaction has no inhibitory effect on the first enzyme. It rather affects the gene (s) that code for the formation of that enzyme., preventing its synthesis.

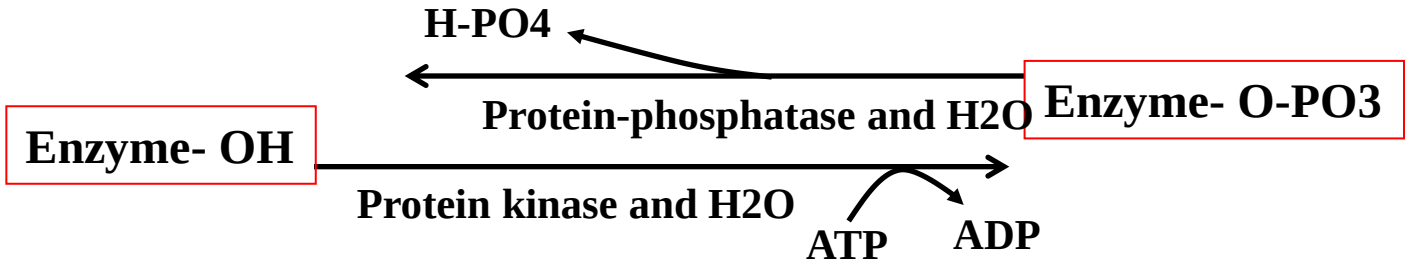


Both feed back inhibition and regulation aim to:

1. Production of any new substrate just according to the body needs.
2. Removal of any harmful effects of some end products e.g. lactic acid in anaerobic oxidation of glucose (glycolysis).

E. **Covalent modification: phosphorylation/ dephosphrylation**:

1. Some enzymes may be regulated by covalent modification, most frequently by the addition or removal of phosphate group from the enzyme (through OH group of serine, threonine or tyrosine residues of the enzyme).
2. Phosphorylaytion reactions are catalyzed by a family of enzymes called protein kinase. It utilizes ATP as a phosphate donor. Phosphate groups are cleaved from phosporylated enzymes by the reaction of phosphoproteins-phosphatase.
3. Depending on the specific enzyme. The phophorylated form may be more or less active than the unphosphorylated enzyme. For example, phosphorylation of glycogen phosphorylase increases the activity, whereas the addition of phosphate to glycogen synthase results in a less active enzyme (carbohydrate metabolism).



X. Isoenzymes:

Definition: isoenzymes are fractions of one enzyme having the same catalytic activity, but differ chemical and immunological structure.

Example:

Lactic dehydrogenase enzyme (LD) i.e. contains 4 polypeptide chains.

There are 5 isoenzymes of lactate dehydrogenase enzyme.

LDI_1, LDI_2, LDI_3, LDI_4 and LDI_5.

Diagnostic importance of isozymes: Determination of different isozymes helps in diagnosis of diseases e.g.

1. Serum LDI1 increase in certain heart diseases (myocardical infection).
2. Serum LDI5 increases in certain liver disease (infective hepatitis).

XI. Enzyme synthesis:

A. Enzymes are synthesized by different tissues i. g.

- pancreas produces lipase, trypsin and amylase
- the stomach produce rennin, pepsinogen etc.

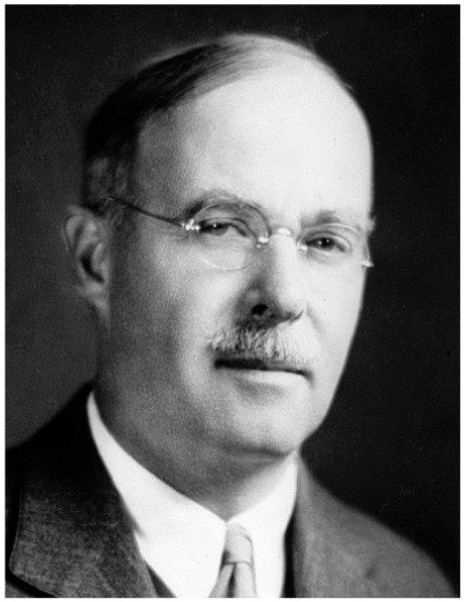
B. As they are proteins in nature, their synthesis occurs in ribosoms under the control of DNA i.e. each enzyme has its own gene in DNA for its production.

C. The steps of enzyme synthesis include:

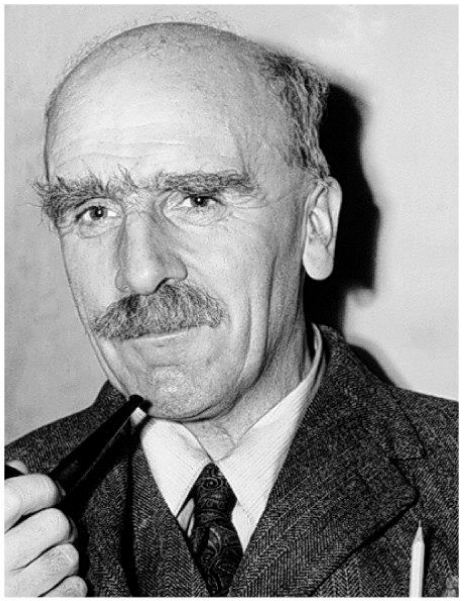
1. Activation of amino acids
2. Transcription of mRNA
3. Translation of gene code

The main founders and pioneers of chemistry and microbiology

Eduard Buchner
1860–1917



James Sumner
1887–1955



J. B. S. Haldane
1892–1964

History of Biochemistry is the History of Enzyme

1850 Louis Pasteur …….Fermentation (sugar → alcohol)

1892 J.B.S. Haldane ……. enzyme-substrate interaction

1897 Eduard Buchner ……. yeast extract → enzyme

1926 James Sumner ……. Urease crystallization → protein