Enzymology- An overview-2

Factors affecting Enzyme activity

-Numerous factors affect the reaction rate:

Temperature

-The reaction rate increases with temperature to a maximum level, then abruptly declines with further increase of temperature

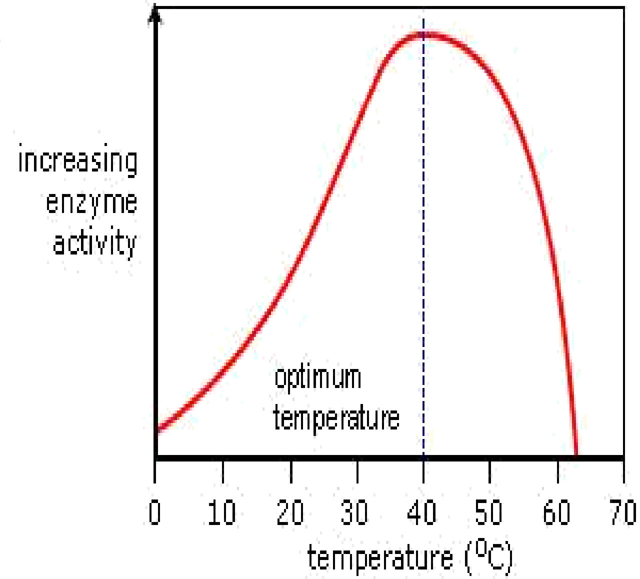
- Most animal enzymes rapidly become denatured at temperatures above 40^{o}C

-The optimal temperatures of the enzymes in higher organisms rarely exceed 50 °C

-The Q_{10}, or temperature coefficient, is the factor by which the rate of a biologic process increases for a 10 °C increase in temperature.

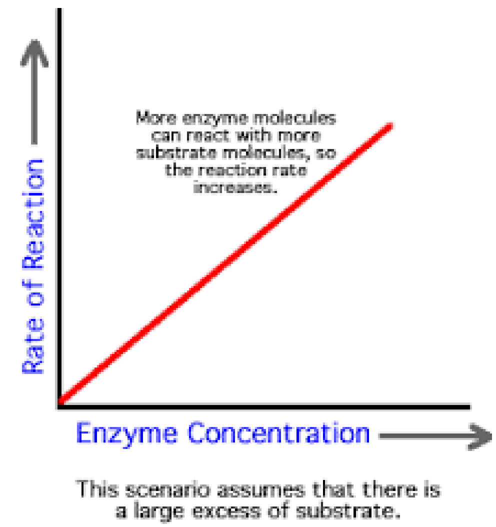
Effect of Temperature

- For mammals and other homoeothermic organisms, changes in enzyme reaction rates with temperature assume physiologic importance only in circumstances such as fever or hypothermia.



Effect of enzyme concentration

-As the amount of enzyme is increased, the rate of reaction increases.
- If there are more enzyme molecules than are needed, adding additional enzyme will not increase the rate.
- Reaction rate therefore increases then it levels off.



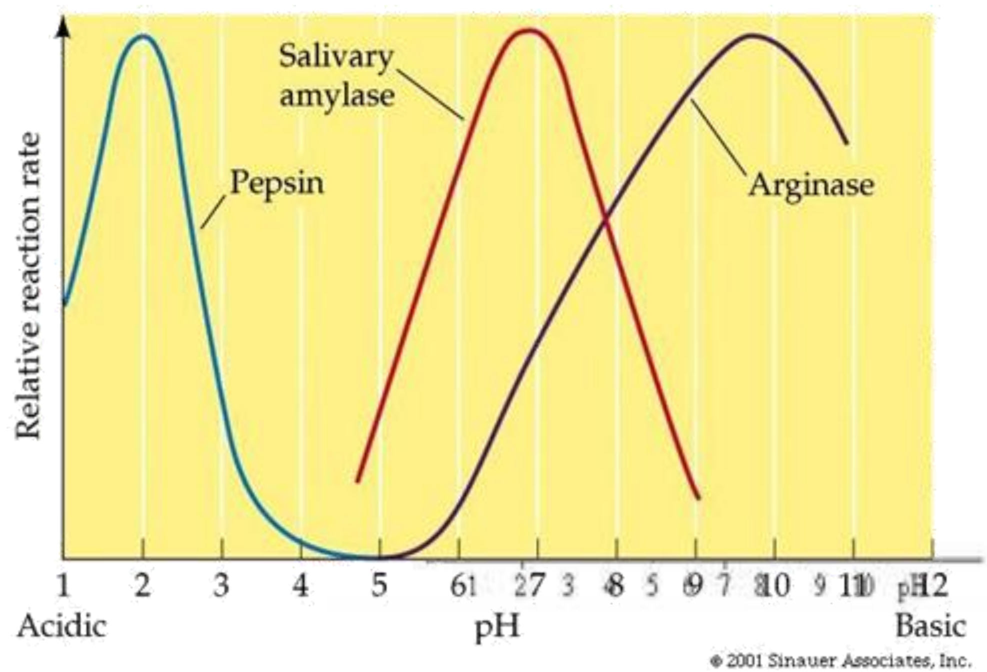
Effect of pH on enzyme activity

-The rate of almost all enzyme-catalyzed reactions exhibits a significant dependence on hydrogen ion concentration

- Most intracellular enzymes exhibit optimal activity at pH values between 5 and 9.

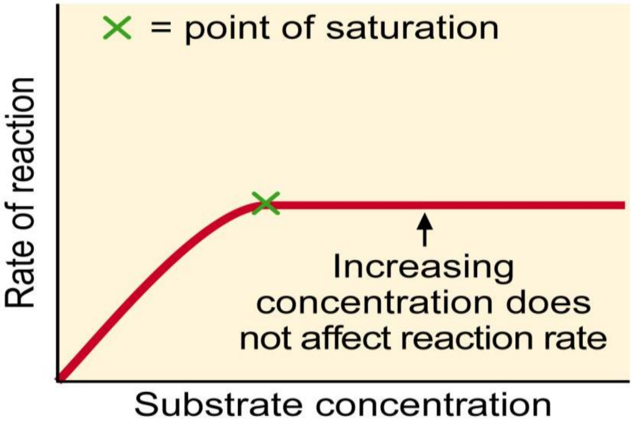
-The relationship of activity to hydrogen ion concentration reflects the balance between enzyme denaturation at high or low pH and effects on the charged state of the enzyme, the substrates, or both.

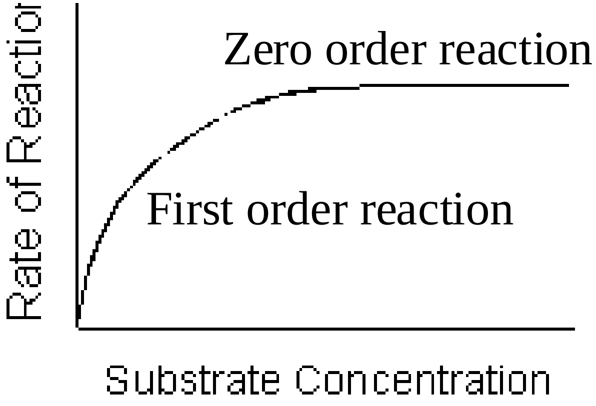
- Except for Pepsin, acid phosphatase and alkaline phosphatase, most enzyme have optimum pH between 5 to 9.



Effect of substrate concentration

- At lower concentrations, the active sites on most of the enzyme molecules are not filled because there is not much substrate.
- Higher concentrations cause more collisions between the molecules.
-The rate of reaction increases (First order reaction).
-The maximum velocity of a reaction is reached when the active sites are almost continuously filled.
- Increased substrate concentration after this point will not increase the rate.
- Reaction rate therefore increases as substrate concentration is increased but it levels off (Zero order reaction).





The shape of the curve that relates activity to substrate concentration is hyperbolic.

Enzyme kinetics

- It is the study of the chemical reactions that are catalyzed by enzymes.
- In enzyme kinetics, the reaction rate is measured and how get changes in response to changes in experimental parameters such as substrate concentration, enzyme concentration etc.
- This is the oldest approach to understanding enzyme mechanisms and remains the most important.
- The initial rate (or initial velocity), designated Vi, when [S] is much greater than the concentration of enzyme [E] can be measured by Michaelis–Menten kinetics. It is one of the simplest and best-known models of enzyme kinetics.

- **Note#** Michaelis-Menten equation, the rate equation for a one-substrate enzyme-catalyzed reaction.

Michaelis-Menten Kinetics

-The Michaelis-Menten equation is a quantitative description of the relationship between the rate of an enzyme-catalyzed reaction [V_i], the concentration of substrate [S] and two constants, V max and km (which are set by the particular equation).

-The symbols used in the Michaelis-Menten equation refer to the reaction rate [V_i], maximum reaction rate (V max), substrate concentration [S] and the Michaelis-Menten constant (km).

Michaelis-Menten equation

-The dependence of initial reaction velocity on [S] and Km may be illustrated by evaluating the Michaelis-Menten equation under three conditions.

$$v_1 = \frac{V_{max}[S]}{\{K_m + [S]\}}$$

1- When [S] is much less than km, the term km + [S] is essentially equal to km.
Since V max and km are both constants, their ratio is a constant (k).
In other words, when [S] is considerably below km, V max is proportionate to k[S].
The initial reaction velocity therefore is directly proportionate to [S].

2- When [S] is much greater than km, the term km + [S] is essentially equal to [S].

Replacing km + [S] with [S] reduces equation to

$$V_i = V_{max}$$

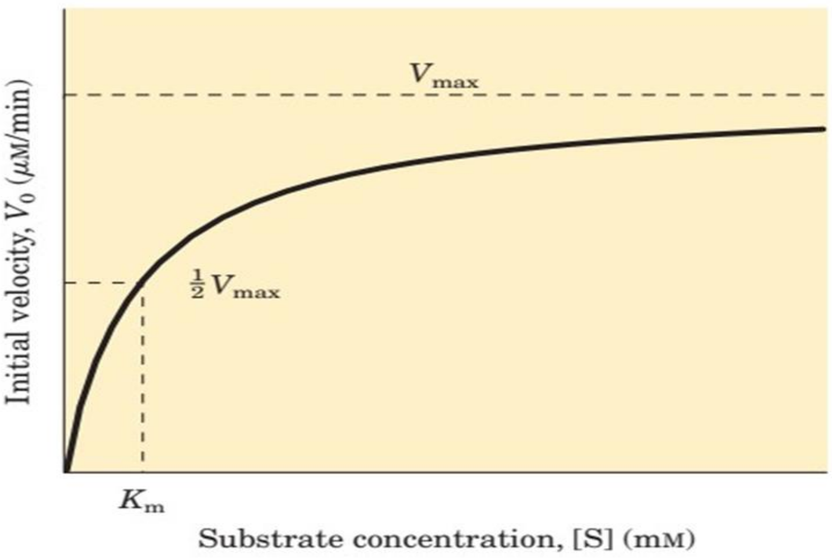
- Thus, when [S] greatly exceeds km, the reaction velocity is maximal (V max) and unaffected by further increases in substrate concentration.

3- When [S] = km

Equation states that when [S] equals km, the initial velocity is half-maximal.

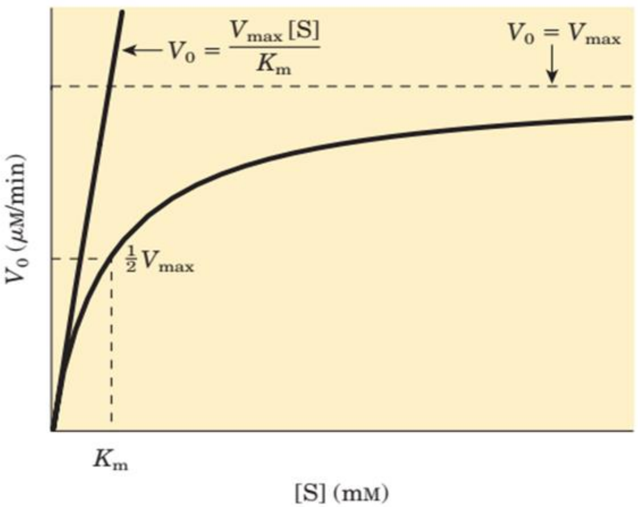
Equation also reveals that km is a constant and may be determined experimentally from—the substrate concentration at which the initial velocity is half-maximal.

Plot of substrate concentration versus reaction velocity



Graphical Representation of Michaelis-Menten equation

- The equation describes the kinetic behavior of all enzymes that Michaelis Menten kinetics.
- This equation practically determine the value of Km and Vmax and, also describe the analysis of inhibitor action.
- But double-reciprocal plot is more convenient procedure, to determine an approximate value of Km.



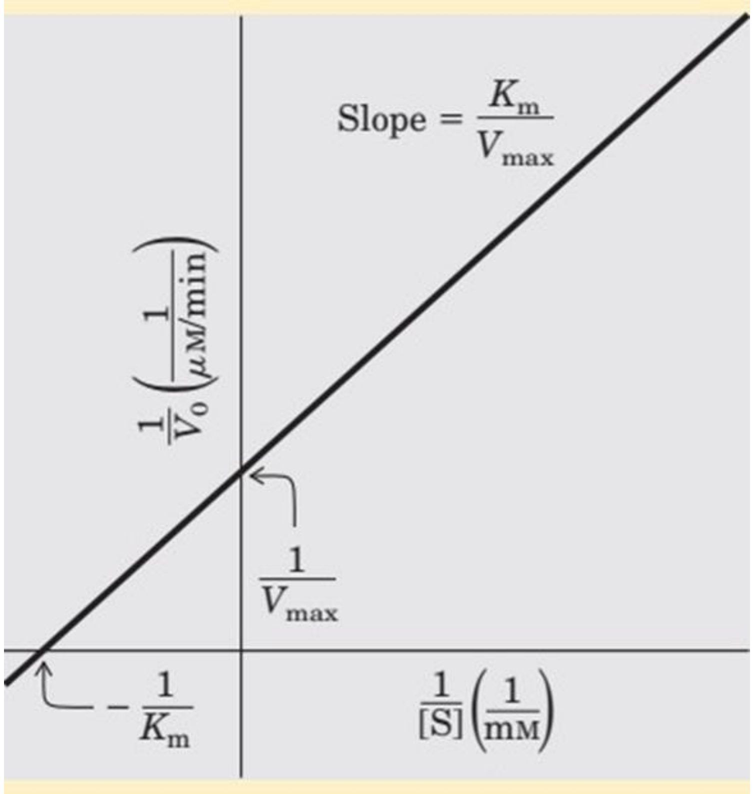
Lineweaver-Burk Plot

- A Linear Form of the Michaelis-Menten Equation is used to determine km & V max.

$$v_i = \frac{V_{max}[S]}{K_m + [S]} \quad \text{Invert} \quad \frac{1}{v_i} = \frac{K_m + [S]}{V_{max}[S]} \quad \text{factor} \quad \frac{1}{v_i} = \frac{K_m}{V_{max}[S]} + \frac{[S]}{V_{max}[S]} \quad \text{and simplify}$$

$$\frac{1}{v_i} = \left(\frac{K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$

- Lineweaver-Burk plot, has the great advantage of allowing a more accurate determination of Vmax, and Km.
- The double-reciprocal plot is very useful to determine the mechanism of enzymatic reaction
- This line has a slope of Km/Vmax, an intercept of 1/Vmax on the 1/V0 y -axis, and an intercept of -1/Km on the 1/[S] x-axis .



Km and its significance

-The Michaelis constant K_m is the substrate concentration at which V_i is half the maximal velocity (Vmax/2) attainable at a particular concentration of enzyme

- It is specific and constant for a given enzyme under defined conditions of time , temperature and pH

- K_m determines the affinity of an enzyme for its substrate, lesser the Km for is the affinity and vice versa, it is inversely proportionate to the affinity

- K_m value helps in determining the true substrate for the enzyme.

Enzymology- An overview-3

Enzyme Inhibition

- Inhibitors are chemicals that reduce the rate of enzymatic reactions.

-They are usually specific and they work at low concentrations.

-They block the enzyme but they do not usually destroy it.

- Many drugs and poisons are inhibitors of enzymes in the nervous system.

- Inhibitors of the catalytic activities of enzymes provide both pharmacologic agents and research tools for study of the mechanism of enzyme action

The effect of enzyme inhibition

- Irreversible inhibitors: combine with the functional groups of the amino acids in the active site, irreversibly.
- Reversible inhibitors: these can be washed out of the solution of enzyme by dialysis.

Classification: based on:

- Their site of action on the enzyme,
- Whether they chemically modify the enzyme,
- The kinetic parameters they influence.

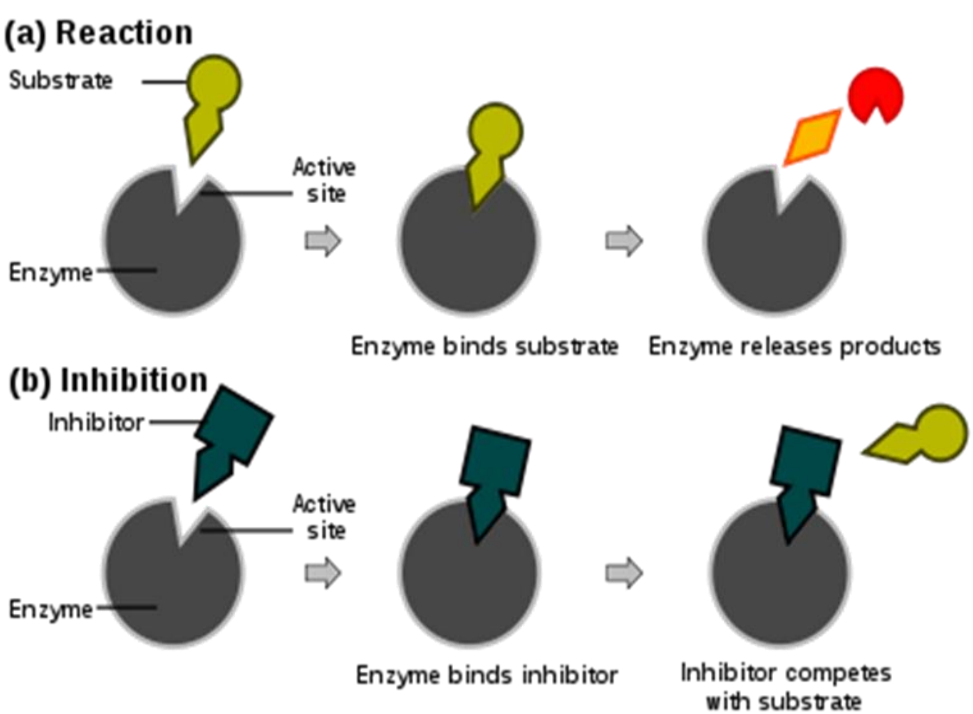
Types of enzyme inhibition

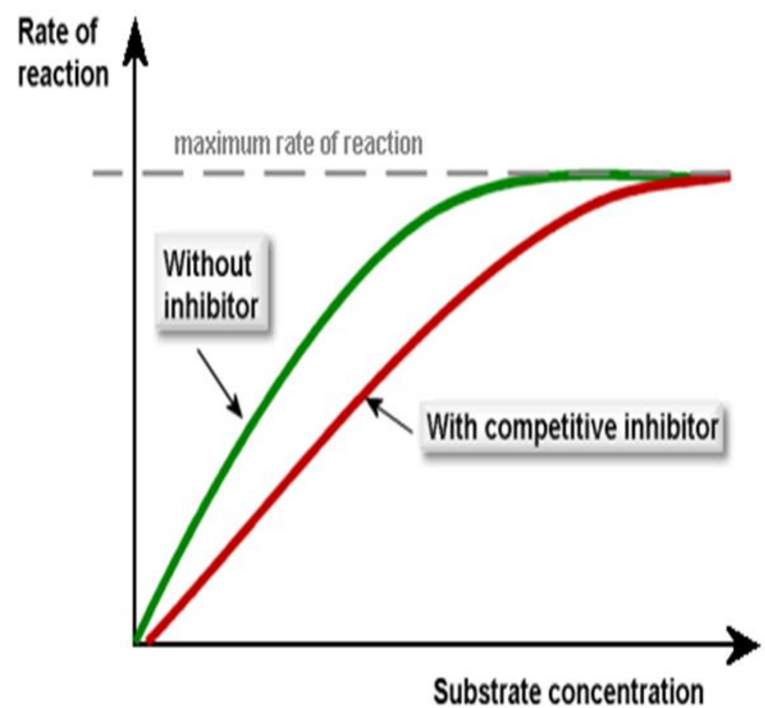
- Competitive inhibition
- Non Competitive inhibition
- Uncompetitive inhibition
- Suicidal inhibition
- Allosteric inhibition
- Feed back inhibition

Competitive enzyme inhibition

A competitive inhibitor

- Has a structure similar to substrate (structural Analog)
- Occupies active site
- Competes with substrate for active site
- Has effect reversed by increasing substrate concentration
- Vmax remains same but Km is increased



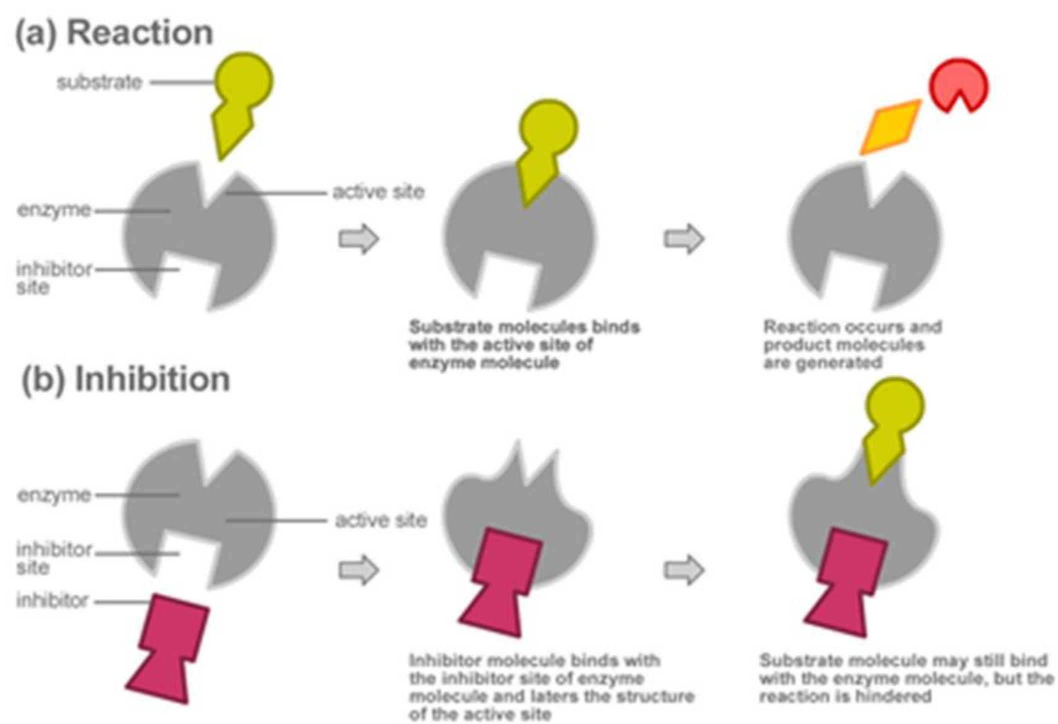


Clinical significance of competitive enzyme inhibitors

Drug	Enzyme Inhibited	Clinical Use
Dicoumarol	Vitamin K Epoxide Reductase	Anticoagulant
Sulphonamide	Pteroid Synthetase	Antibiotic
Trimethoprim	Dihydrofolate reductase	Antibiotic
Pyrimethamine	Dihydrofolate reductase	Antimalarial
Methotrexate	Dihydrofolate reductase	Anticancer
Lovastatin	HMG CoA Reductase	Cholesterol Lowering drug
Alpha Methyl Dopa	Dopa decarboxylase	Antihypertensive
Neostigmine	Acetyl Cholinesterase	Myasthenia Gravis

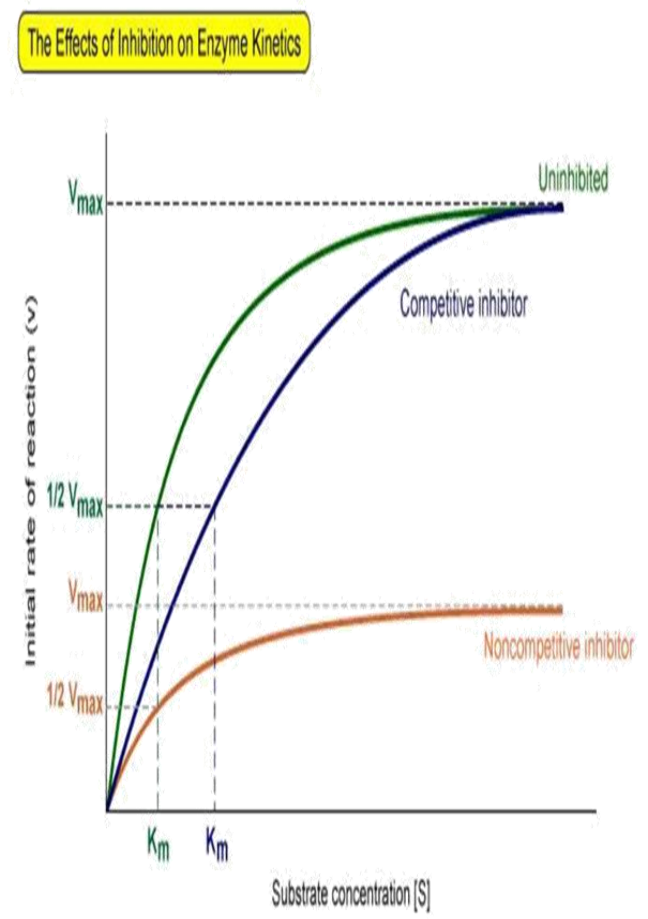
Non competitive enzyme inhibition

- Noncompetitive inhibitors bind enzymes at sites distinct from the substrate-binding site.
- Generally bear little or no structural resemblance to the substrate.
- Binding of the inhibitor does not affect binding of substrate.
- Formation of both EI and EIS complexes is therefore possible.
- The enzyme-inhibitor complex can still bind substrate, its efficiency at transforming substrate to product, reflected by V_{max}, is decreased.



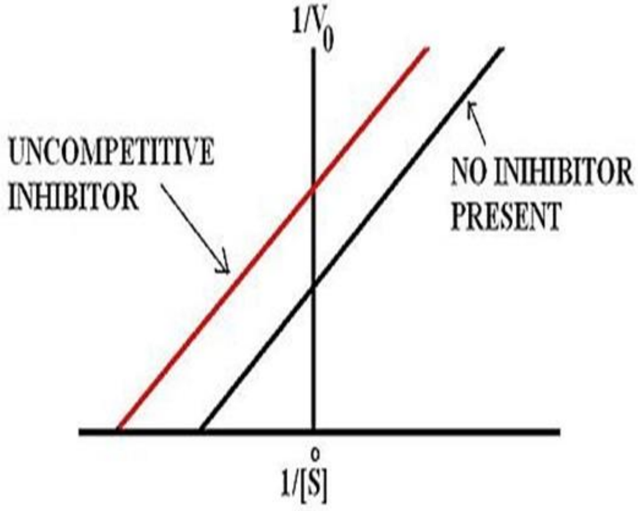
Examples of non competitive enzyme inhibitors

- Cyanide inhibits cytochrome oxidase.
- Fluoride inhibits enolase and hence glycolysis.
- Iodoacetate inhibits enzymes having SH groups in their active sites.
- BAL (British Anti Lewisite, dimercaprol) is used as an antidote for heavy metal poisoning
    - Heavy metals act as enzyme poisons by reacting with the SH groups, BAL has several SH groups with which the heavy metal ions bind and thereby their poisonous effects are reduced.

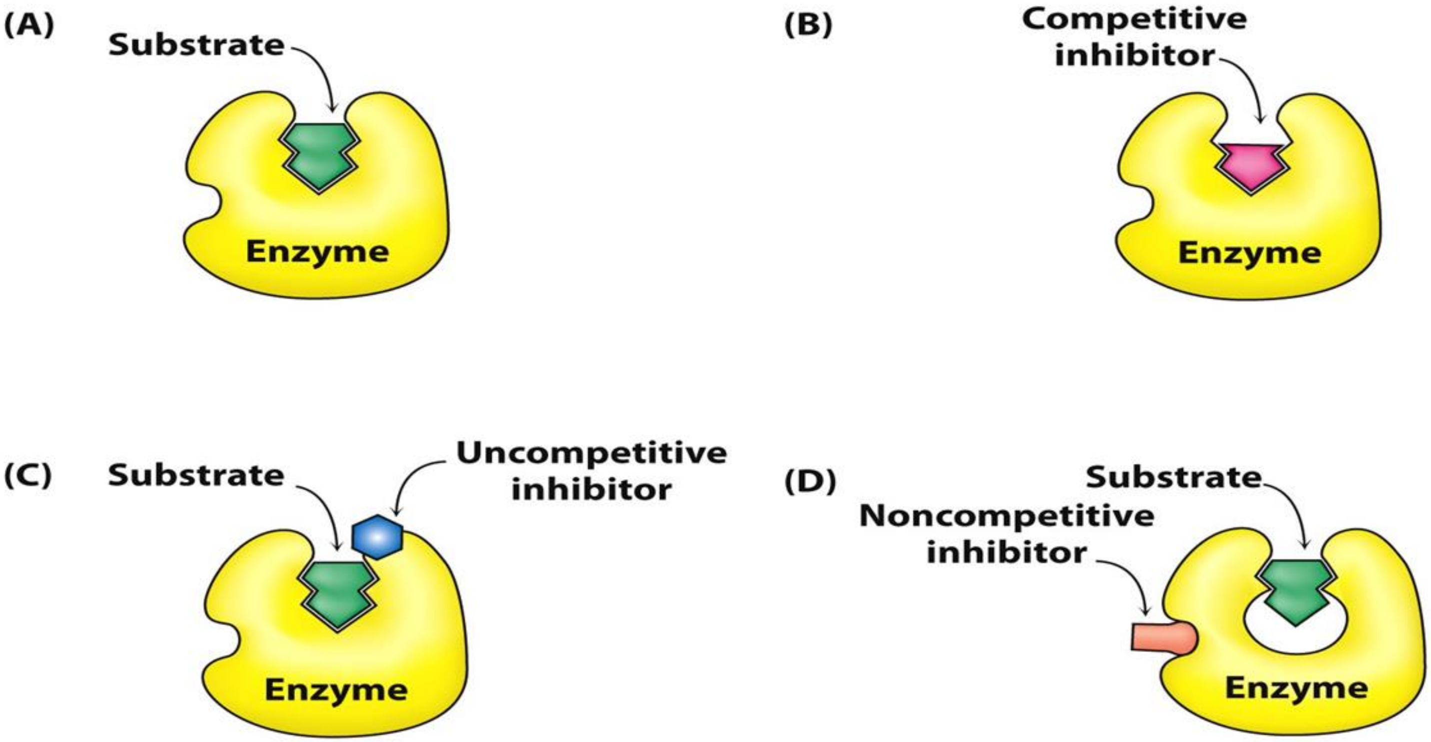


<u>Uncompetitive enzyme inhibition</u>

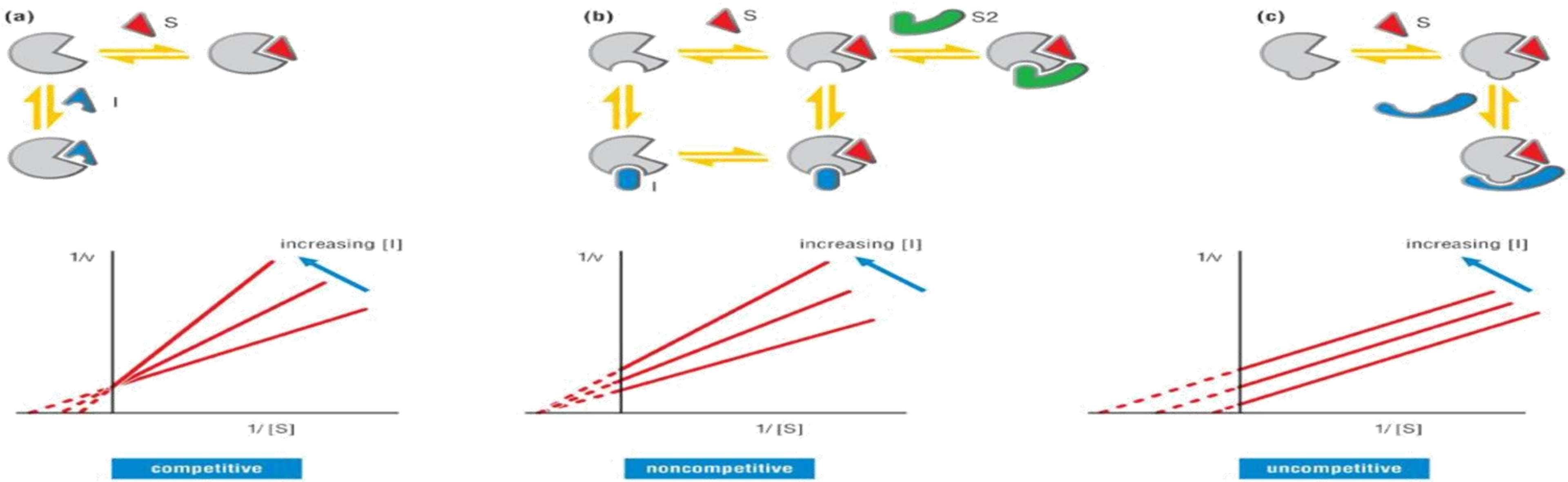
- Inhibitor binds to enzyme- substrate complex
- Both Vmax and Km are decreased
- Such as ; Inhibition of placental alkaline phosphatase (Regan isoenzyme) by phenylalanine



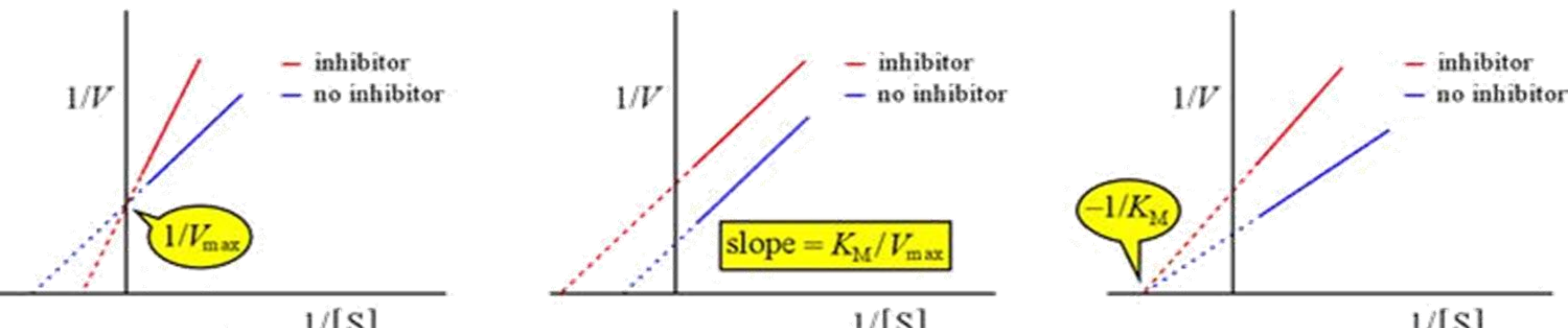
Competitive V/S non competitive V/S uncompetitive enzyme inhibition



From **Protein Structure and Function 2005-2006 Online Update**
by Gregory A Petsko and Dagmar Ringe



The Lineweaver-Burk plots for inhibition



Competitive inhibition	Uncompetitive inhibition	Noncompetitive inhibition
K_M increased	K_M reduced	K_M unaffected
V_{max} unaffected	V_{max} reduced	V_{max} reduced

Suicidal inhibition

- Irreversible inhibition

- Structural analog of the substrate is converted to more effective inhibitor with the help of enzyme to be inhibited.

-The new product irreversibly binds to the enzyme and inhibits further reaction.

- Such as ;

  Ornithine decarboxylase: is irreversibly inhibited by difluormethyl ornithine, as a result multiplication of parasite is arrested .

  Used against trypanosome in sleeping sickness

-Allopurinol is oxidized by xanthine oxidase to alloxanthine which is a strong inhibitor of xanthine oxidase

- Aspirin action is based on suicide inhibition

  Acetylates a serine residue in the active center of cyclo-oxygenase .

  Thus, PG synthesis is inhibited so inflammation subsides

- Disulfiram: used in treatment of alcoholism

  Drug irreversibly inhibits the enzyme aldehyde dehydrogenase preventing further oxidation of acetaldehyde which produces sickening effects leading to aversion to alcohol.

Allosteric inhibition

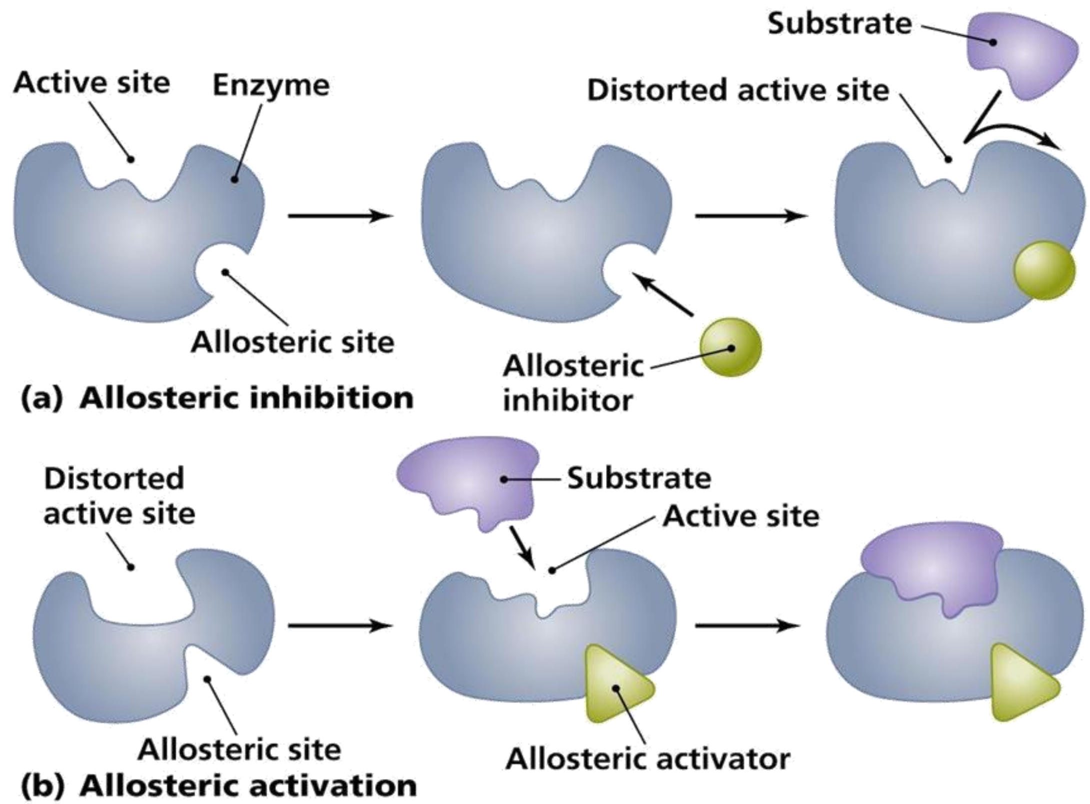
- Some enzymes have **other site** (allosteric site) similar but different from the active site which may or may not physically adjacent to the active site.

-This site binds an effector called the allosteric effector that may be an activator (positive modifier) or inhibitor (negative modifier).

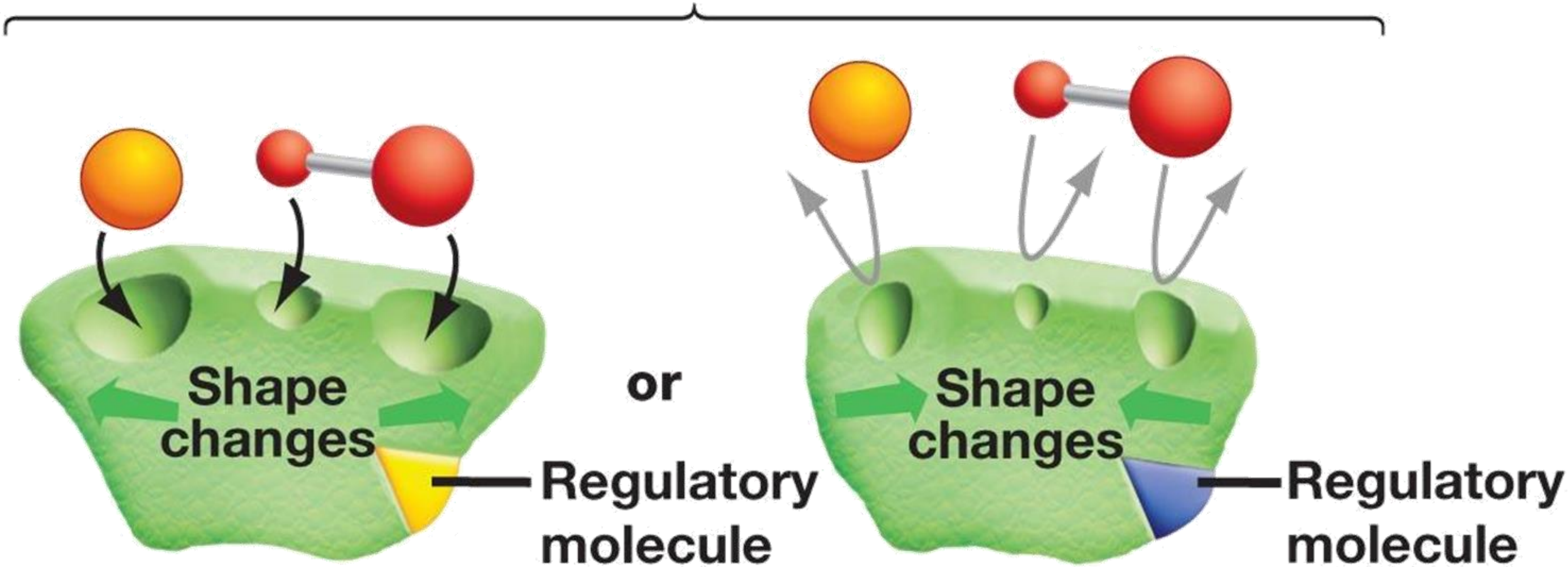
-The allosteric effector is usually a metabolite or a product resulting from the process of metabolism.

- Enzymes having these sites are called allosteric enzymes.

- Inhibitor is not a substrate analogue.
- Partially reversible, when excess substrate is added.
- Km is usually increased (K series enzymes).
- Vmax is reduced (V series enzymes).
- When the inhibitor binds the allosteric site, the configuration of the active site is changed so that the substrate can not bind properly.
- Most allosteric enzymes possess quaternary structure.



(b) Allosteric regulation



Allosteric activation

The active site becomes available to the substrates when a regulatory molecule binds to a different site on the enzyme.

Allosteric deactivation

The active site becomes unavailable to the substrates when a regulatory molecule binds to a different site on the enzyme.

Switching off

- When the inhibitor is present it fits into its site and there is a conformational change in the enzyme molecule.
- The enzyme's molecular shape changes.
- The active site of the substrate changes.
-The substrate cannot bind with the substrate and the reaction slows down.
-When the inhibitor concentration diminishes the enzyme's conformation changes back to its active form.
-This is not competitive inhibition but it is reversible

Example: Phosphofructokinase -1(PFK-1)

- It catalyzes phosphorylation of fructose-6-phosphate into fructose 1, 6 biphosphate
- It has an allosteric site for an ATP molecule (the inhibitor).

-When the level of ATP in the cell falls (↑ ratio of ADP to ATP) no ATP binds to the allosteric site of PFK-1, so, the enzyme’s conformation changes and the active site accepts substrate molecules causing activation of glycolysis.

-The respiration pathway accelerates and the level of ATP in the cell increases (↑ ratio of ATP to ADP) in the cell, ATP molecules can fit into the allosteric site of PFK-1 molecules.

-The enzyme’s conformation changes again and stops accepting substrate molecules in the active site

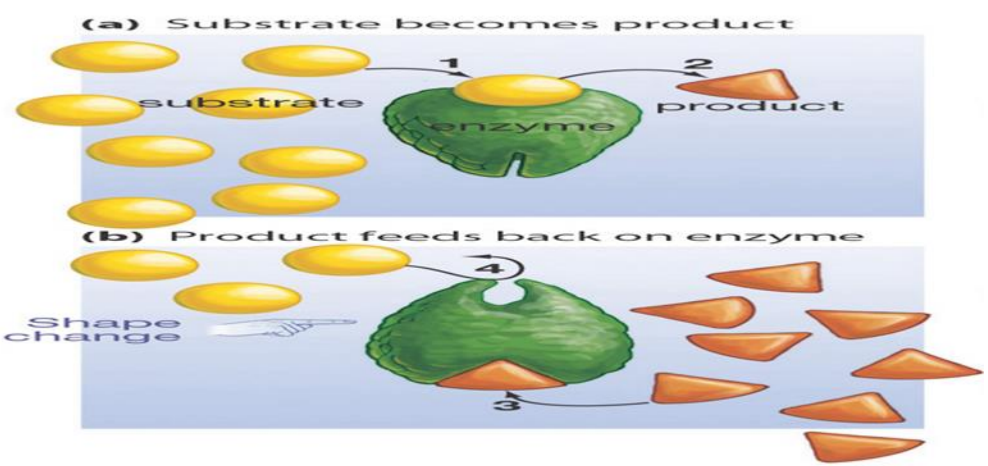
- Respiration slows down

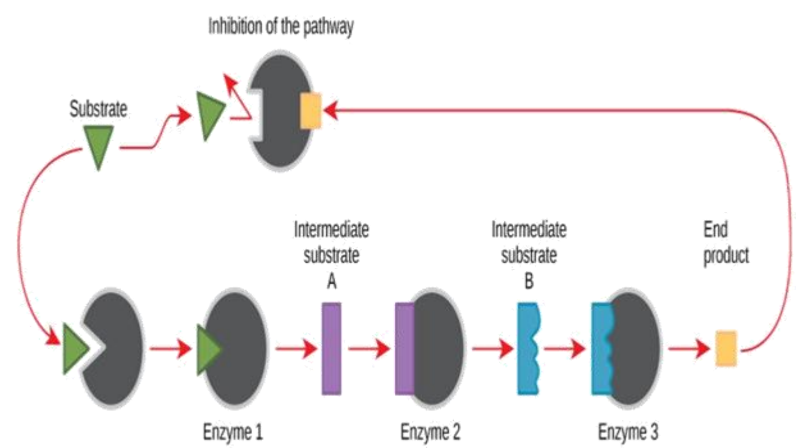
Feed back(end point) inhibition

- Cell processes consist of series of pathways controlled by enzymes. Each step is catalyzed by a different enzyme (**e_A**, **e_B**, **e_C** etc).

A B C D E F

- The first step (controlled by **e_A**) is often controlled by the end product (F), therefore negative feedback is possible (end products are controlling their own rate of production, no build up of intermediates (B,C, D and E).
- Usually such end product inhibition can affect allosterically.
- Accumulated product binds at a site other than the active site to bring about conformational changes, so as to inhibit the binding of the substrate and the reaction rate declines.





Enzymology- An overview-4

Regulation of enzyme activity

Several ways to regulate enzyme activity:

1. Modulation of enzyme activity:

    A- Covalent modification.

    B- Allosteric modulation.

2. Proteolytic cleavage of proenzymes.

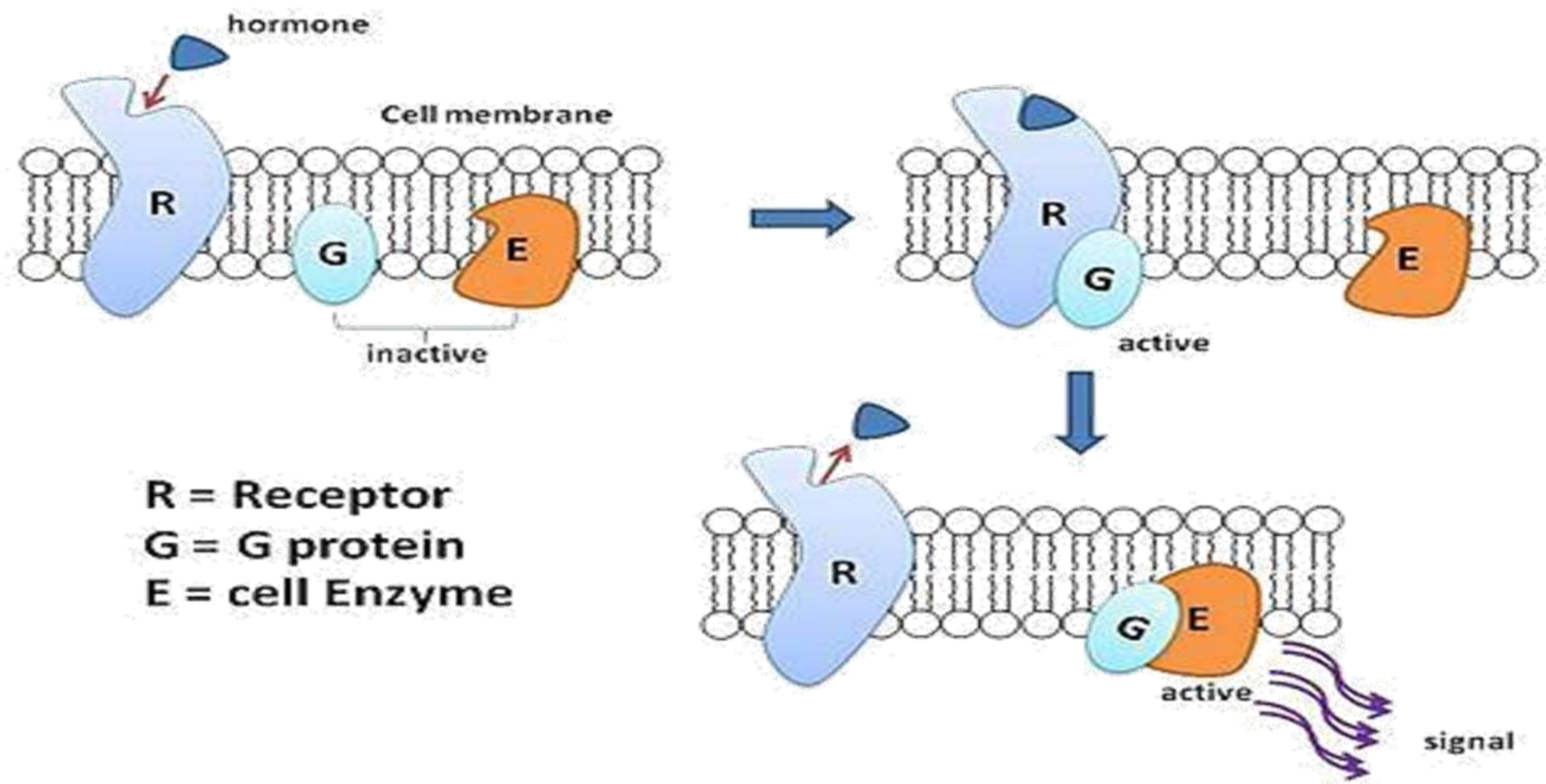
3. Compartmentation.

4. Enzyme production.

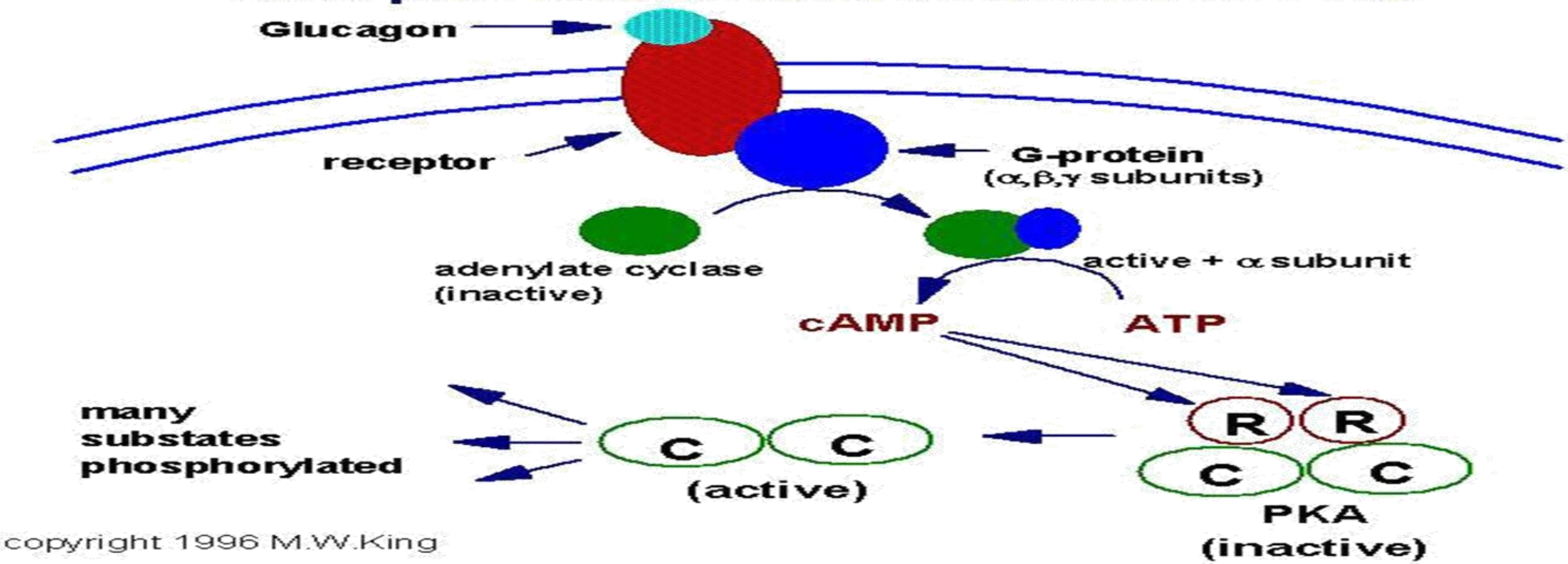
5. Feedback inhibition

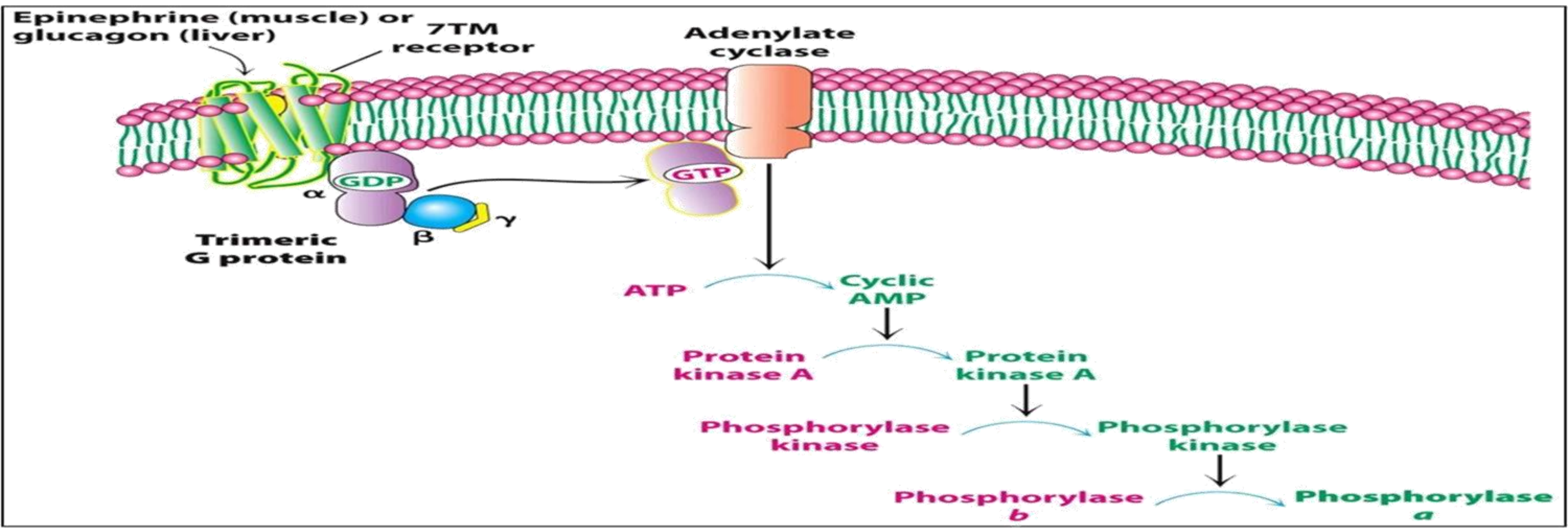
Usually by the addition of or lysis of phosphate (PO4) groups to and from enzymes.

- Some enzymes are active when phosphorylated, while, others are inactive when phosphorylated.



Receptor-Mediated Activation of PKA

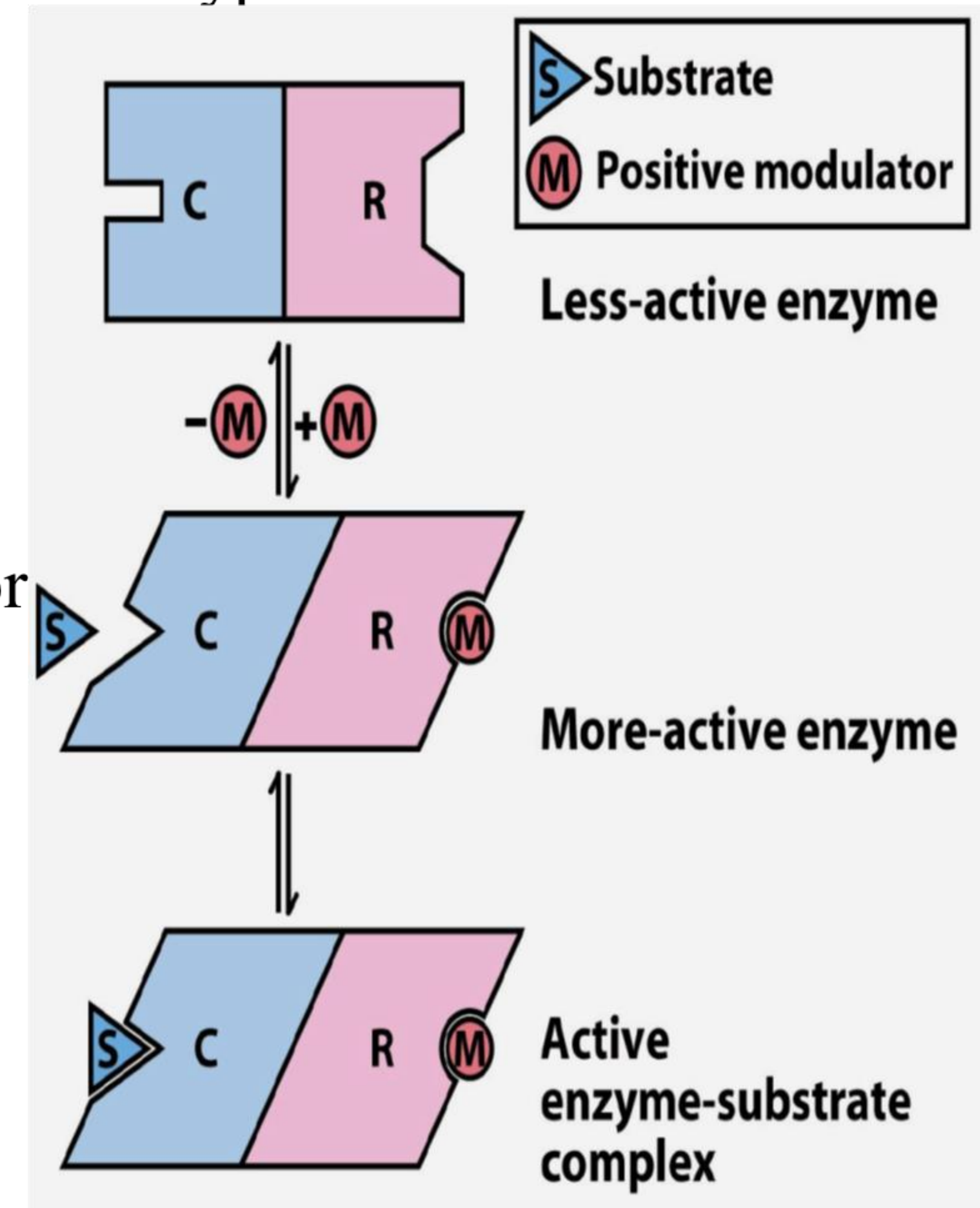


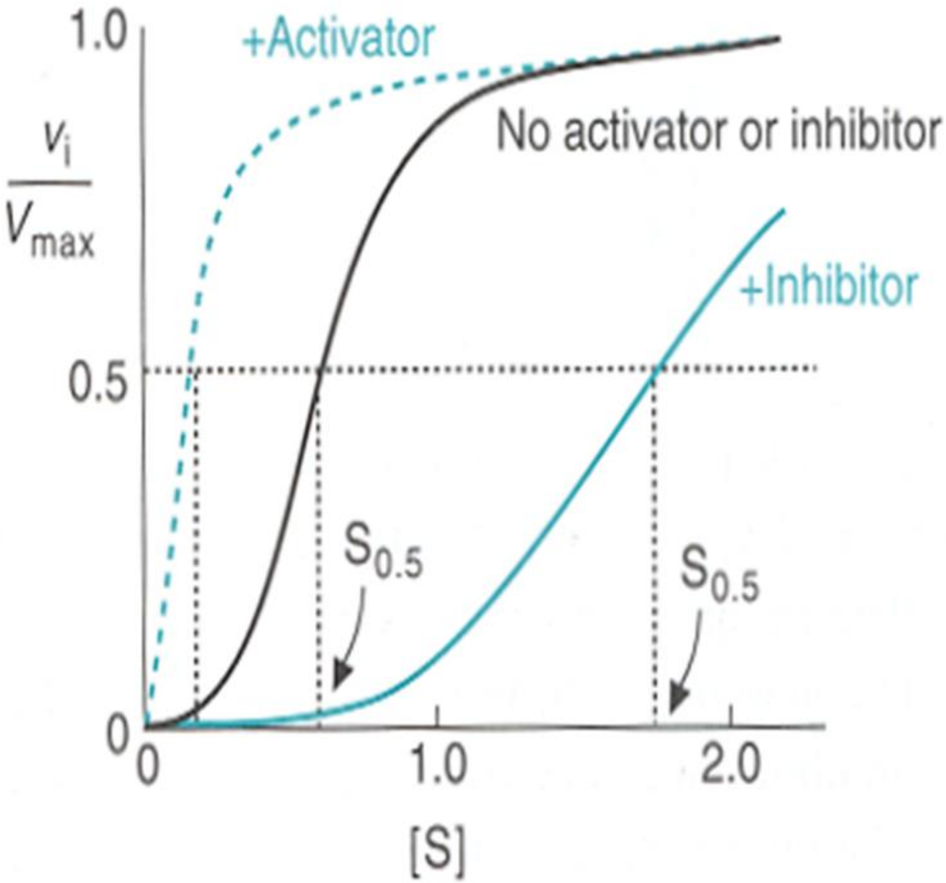


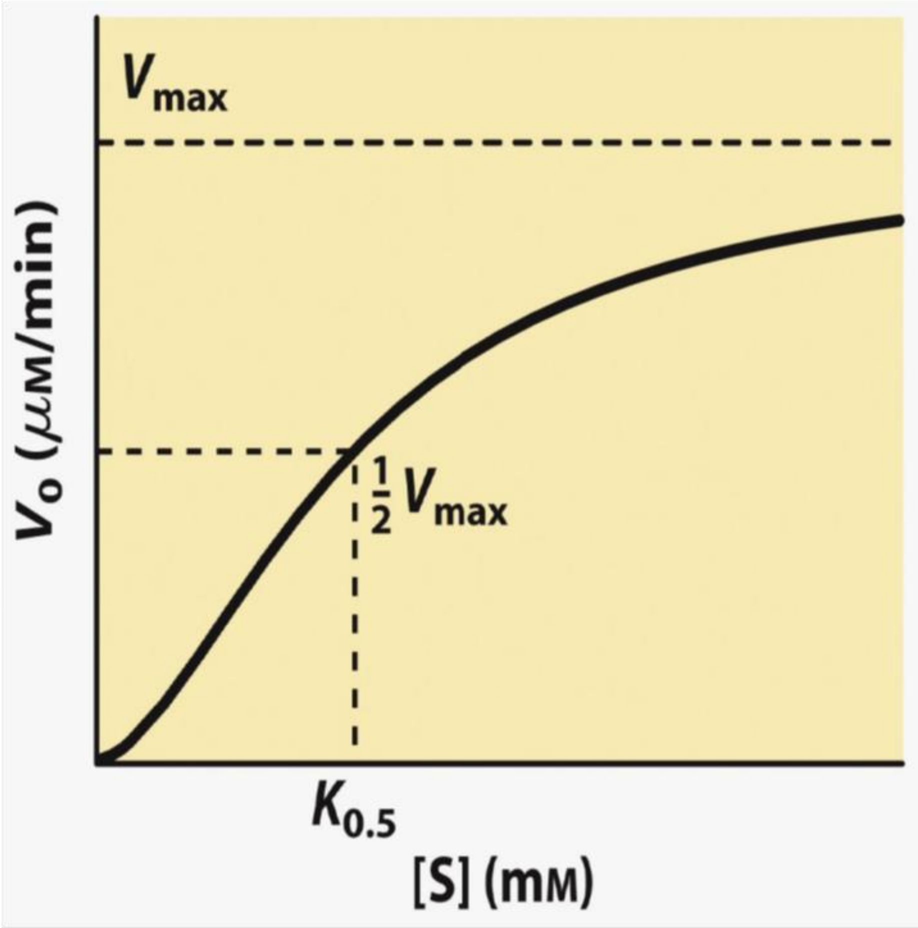
B- <u>Allosteric regulation</u>:

- Allosteric regulation is the term used to describe cases where an enzyme is functioning at one site, then, affected by binding of a regulatory molecule at another site.
- Allosteric regulation may either inhibit or stimulate an enzyme activity by changing the enzyme either to its active or inactive forms.
- The binding of an allosteric activator stabilizes its active form, while binding the allosteric inhibitor stabilizes the inactive form of the enzyme.
- End products are often inhibitors.
- Often allosteric modulators do not resemble the substrate or the product of the enzyme catalyzing the reaction.
- Allosteric modulators bind non-covalently to the enzyme at a site rather than the substrate binding site.

- Allosteric enzymes usually have quaternary structure
- Allosteric enzymes do not exhibit typical Michaelis- Menton kinetics.
- Instead, the curve is sigmoidal, which indicates that the binding of substrate to the enzyme changes (e.g. increases) the affinity of the enzyme for substrate.
- Some allosteric modulators alters the Km, the Vmax remains constant.
- The modulators are not altered by the enzyme.







Allosteric regulation gives sigmoidal curve

Effects of a positive (+) and a negative (-) modulator that alter the Km without altering the maximum velocity Vmax

2- <u>Proteolytic cleavage of proenzyme</u>:

- Zymogens activation: certain proteins are synthesized and secreted as inactive precursor proteins known as **proproteins**.

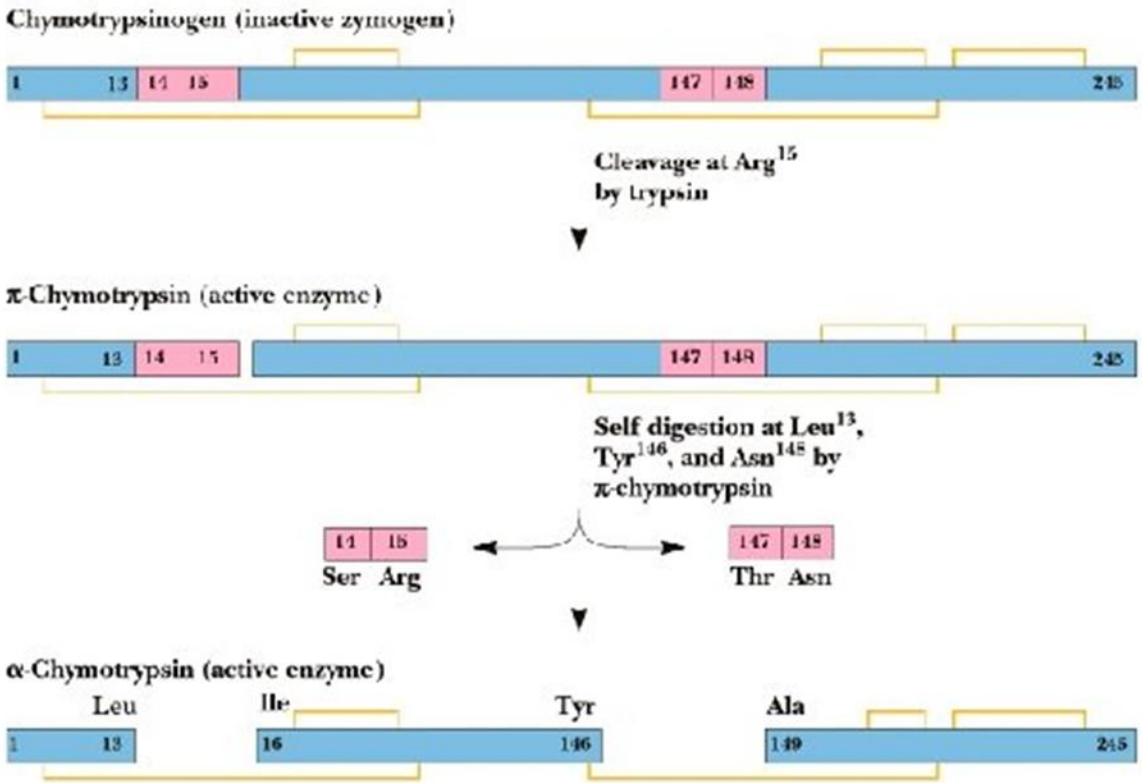
- The proproteins of enzymes are termed **proenzymes** or **zymogens**.

- Selective proteolysis converts a proprotein by one or more successive proteolytic "**clips**" to a form that exhibits the characteristic activity of the mature protein, such as , its enzymatic activity.

- The digestive enzymes pepsin, trypsin, and chymotrypsin (proproteins = pepsinogen, trypsinogen, and chymotrypsinogen, respectively), several factors of the blood clotting and blood clot dissolution cascades, are examples of Zymogen activation.

Proteolytic cleavage of proenzyme(zymogen)

Garrett & Grisham: Biochemistry, 2/e
Figure 15.4



Saunders College Publishing

Enzyme/substrate Compartmentation:

- Compartmentation **ensures metabolic efficiency** & **simplifies regulation**
- Segregation of metabolic processes into distinct subcellular locations like the cytosol or specialized organelles (nucleus, endoplasmic reticulum, Golgi apparatus, lysosomes, mitochondria, etc.) is another form of regulation

Plasma membrane	Amino acid transport systems, Na^+-K^+ ATPase
Cytosol	Glycolysis, glycogenesis and glycogenolysis, hexose monophosphate pathway, fatty acid synthesis, purine and pyrimidine catabolism, aminoacyl-tRNA synthetases
Mitochondria	Tricarboxylic acid cycle, electron transport and oxidative phosphorylation, fatty acid oxidation, urea synthesis
Nucleus	DNA and RNA synthesis
Endoplasmic reticulum (rough and smooth)	Protein synthesis, steroid synthesis, glycosylation, detoxification
Lysosomes	Hydrolases
Golgi apparatus	Glycosyl transferases, glucose-5-phosphatase, formation of plasma membrane and secretory vesicles
Peroxisomes	Catalase, D-amino acid oxidase, urate oxidase

4- **Enzyme production (hormonal regulation)**:

- Enzyme synthesis (transcription and translation of enzymes genes) can be induced or decreased by hormonal activity that controls the genes.
- This mechanism of enzyme regulation is slower than other mechanisms (**long-term regulation**), i.e. covalent and allosteric modulation of enzyme activity.
- Causes changes in the concentration of certain "inducible enzymes" (are adaptive, i.e. synthesized as needed by the cell). (Constitutive enzymes synthesis is at a constant rate).
- Induction occurs usually by the action of hormones, (e.g. steroid and thyroxine) and is exerted by changes in the expression of gene encoding the enzymes.
- More or less enzyme can be synthesized by hormonal activation or inhibition of the genes.

Example:

- Insulin induces increased synthesis of enzymes: glucokinase, glycogen synthase and PFK-1
- Insulin decreases the synthesis of several key gluconeogenic enzymes (amino acid glucose).

5- **<u>Feed back inhibition v/s feed back regulation</u>:**

- It is the regulation of a metabolic pathway by using end product as an inhibitor within the pathway to keep cells from synthesizing more product than necessary.
- Dietary cholesterol decreases hepatic synthesis of cholesterol, (feedback regulation not feedback inhibition).
- HMG-CoA reductase, the rate-limiting enzyme of cholesterol synthesis, is affected, but cholesterol does not feedback-inhibit its activity.

-Regulation in response to dietary cholesterol involves the effect of cholesterol or a cholesterol metabolite on the expression of the gene that encodes HMG-CoA reductase (enzyme repression).