Enzymology- An overview-2

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.

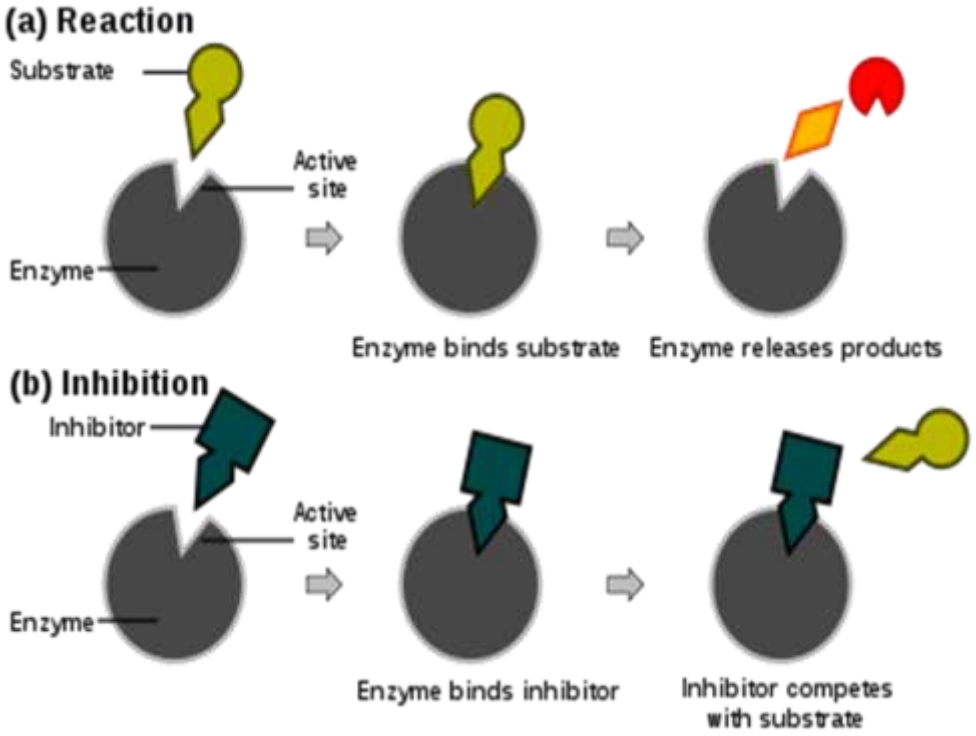
Types of enzyme inhibition

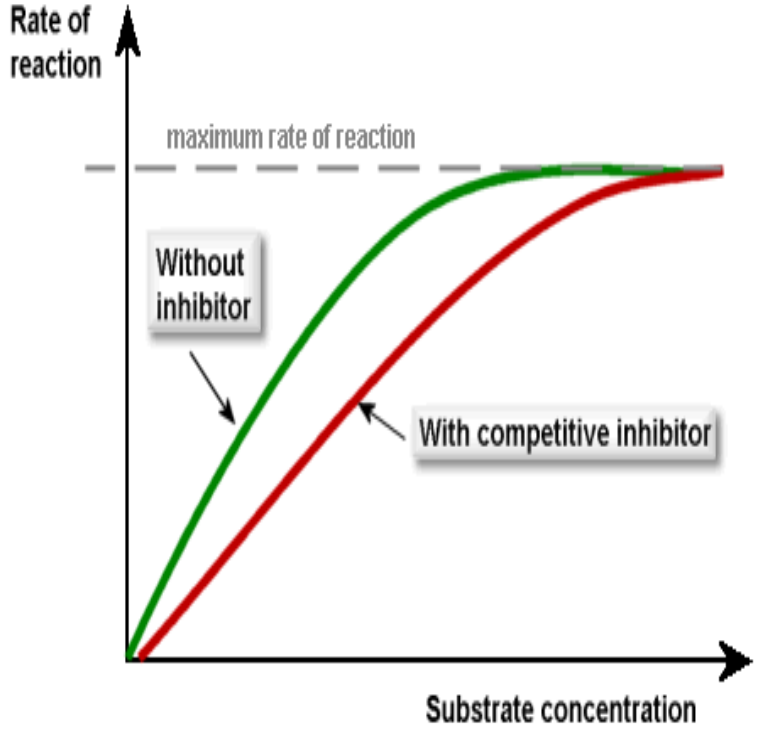
- Competitive inhibition
- Non Competitive inhibition
- Uncompetitive inhibition
- Suicidal inhibition
- Allosteric inhibition
- Feed back inhibition

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



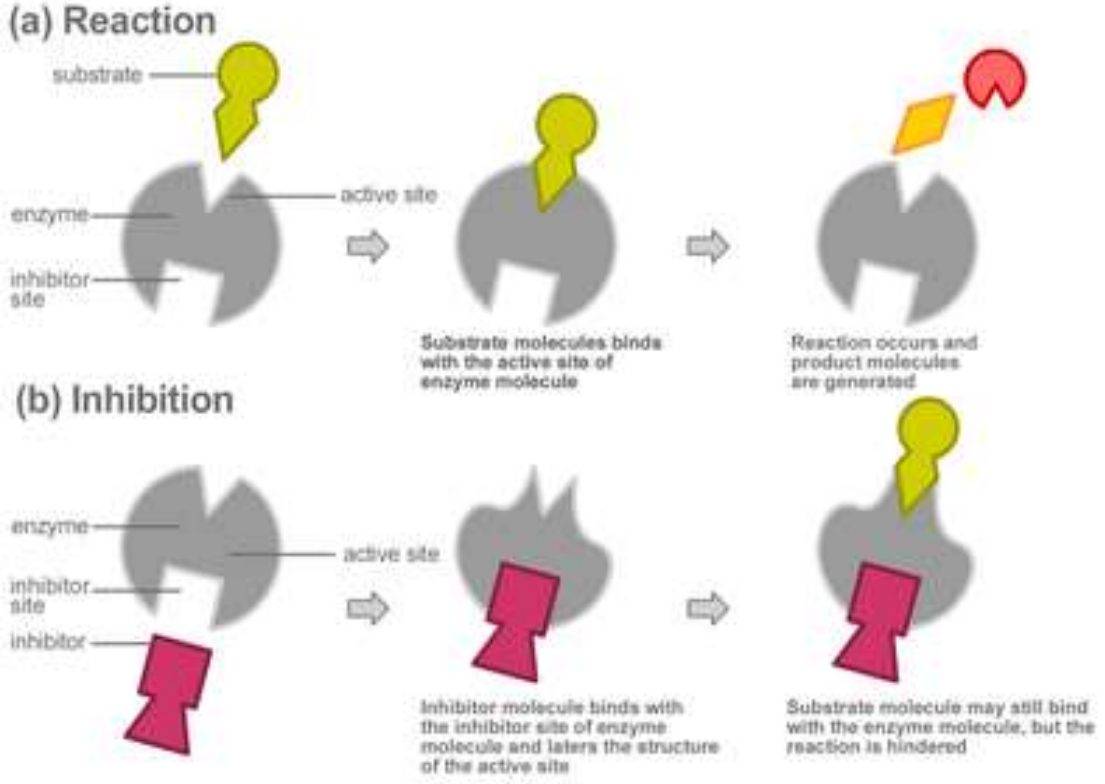


Clinical significance of competitive enzyme inhibitors

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

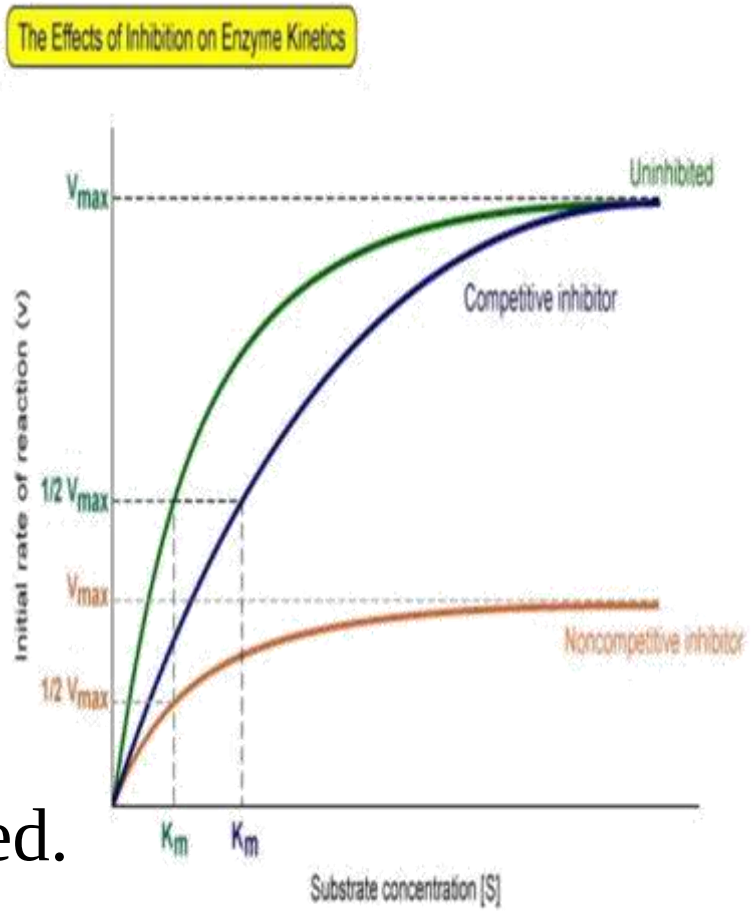
Non competitive enzyme inhibition

- 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.



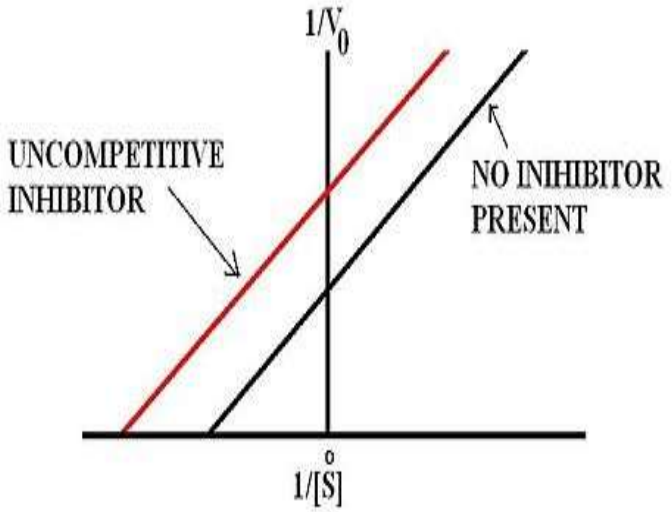
Examples of non competitive enzyme inhibitors

- 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.

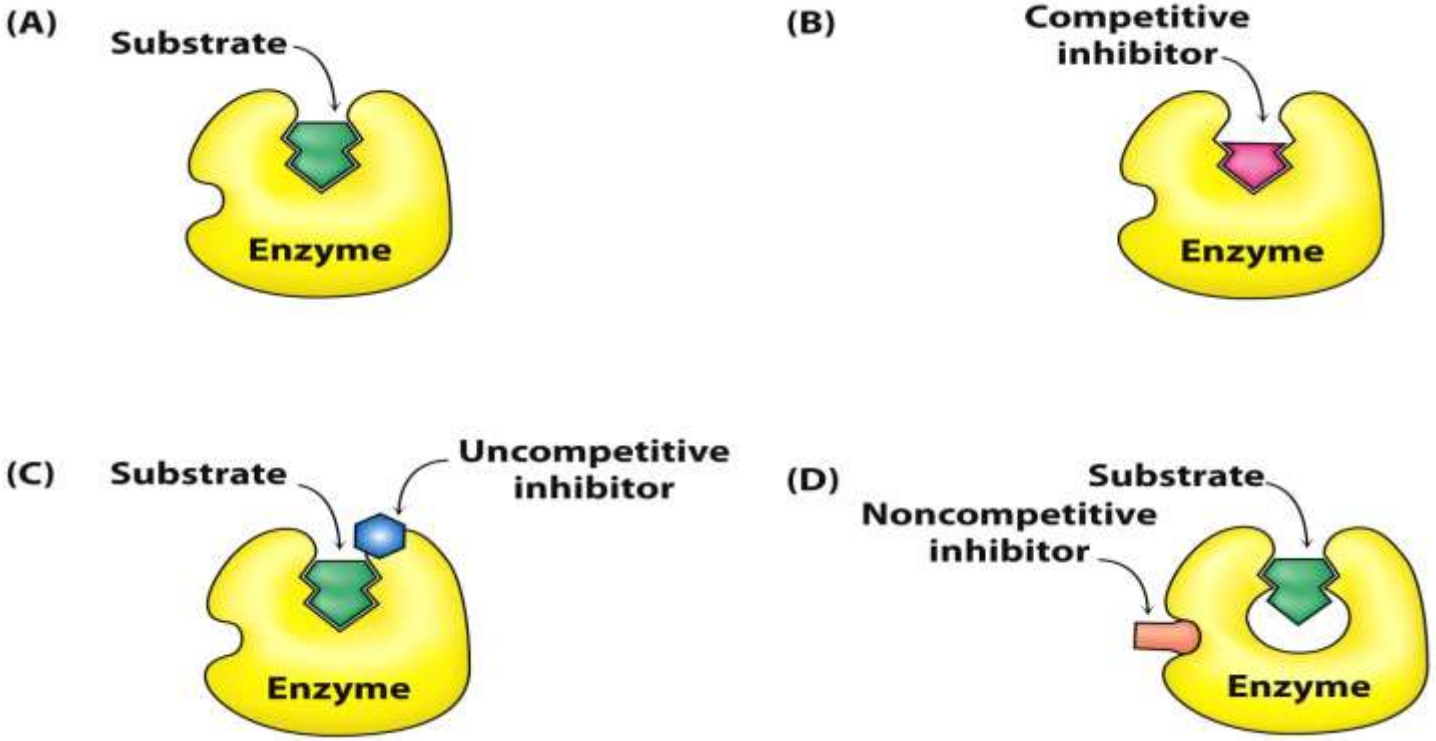


Uncompetitive enzyme inhibition

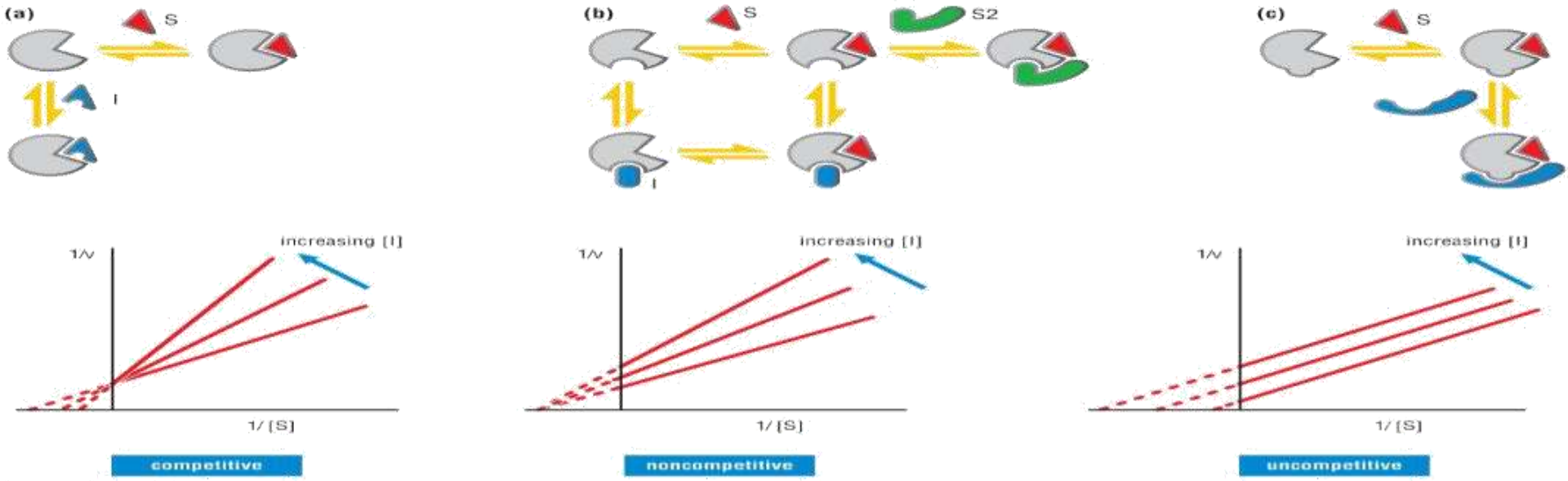
- Inhibitor binds to enzyme- substrate complex
- Both Vmax and Km are decreased
- Such as ; Inhibition of placental alkaline phosphatase (Regan isoenzyme) by phenylalanine



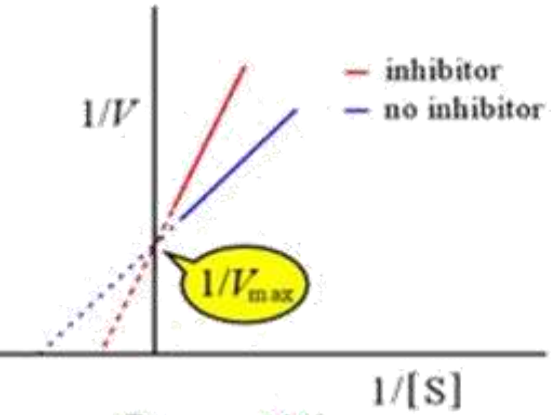
Competitive V/S non competitive V/S uncompetitive enzyme inhibition



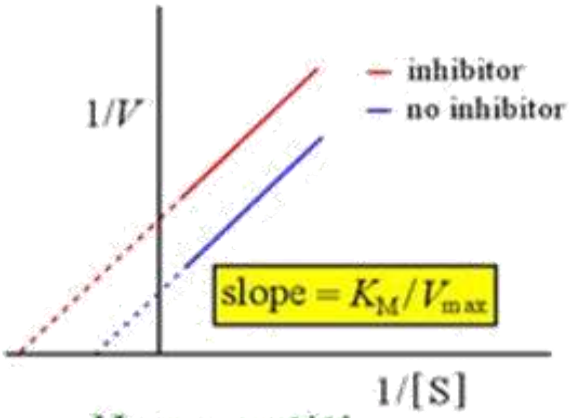
From Protein Structure and Function 2005-2006 Online Update
by Gregory A Petsko and Dagmar Ringe



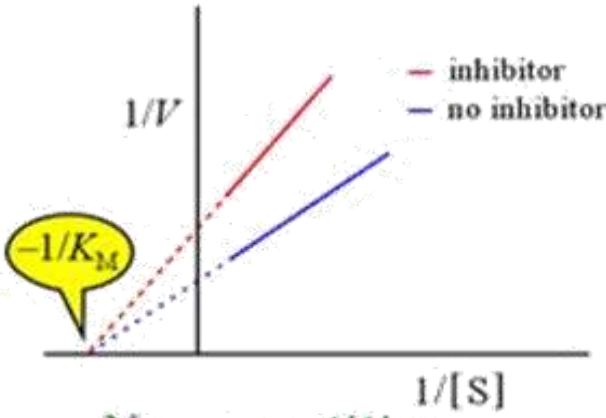
The Lineweaver-Burk plots for inhibition



Competitive inhibition
K_M increased
V_{max} unaffected



Uncompetitive inhibition
K_M reduced
V_{max} reduced



Noncompetitive inhibition
K_M unaffected
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

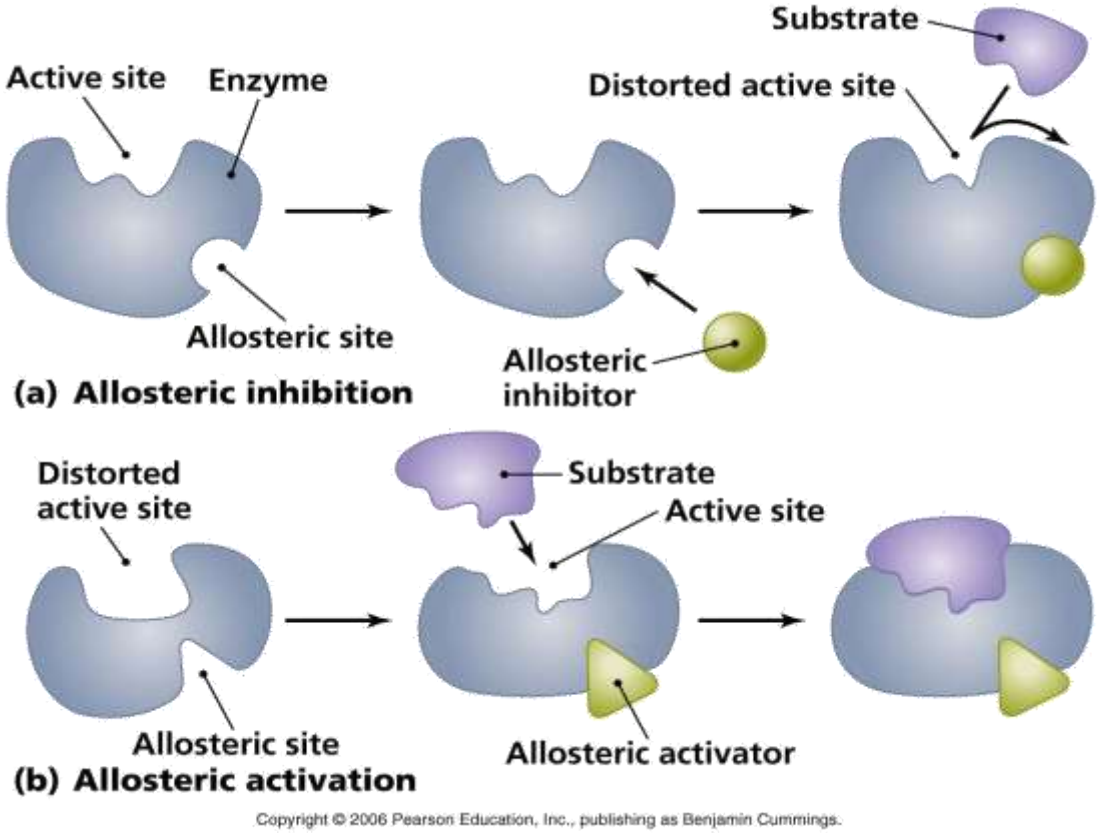
- Some enzymes have **other site** (allosteric site) similar but different from the active site which may or may not physically adjacent to the active site.

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

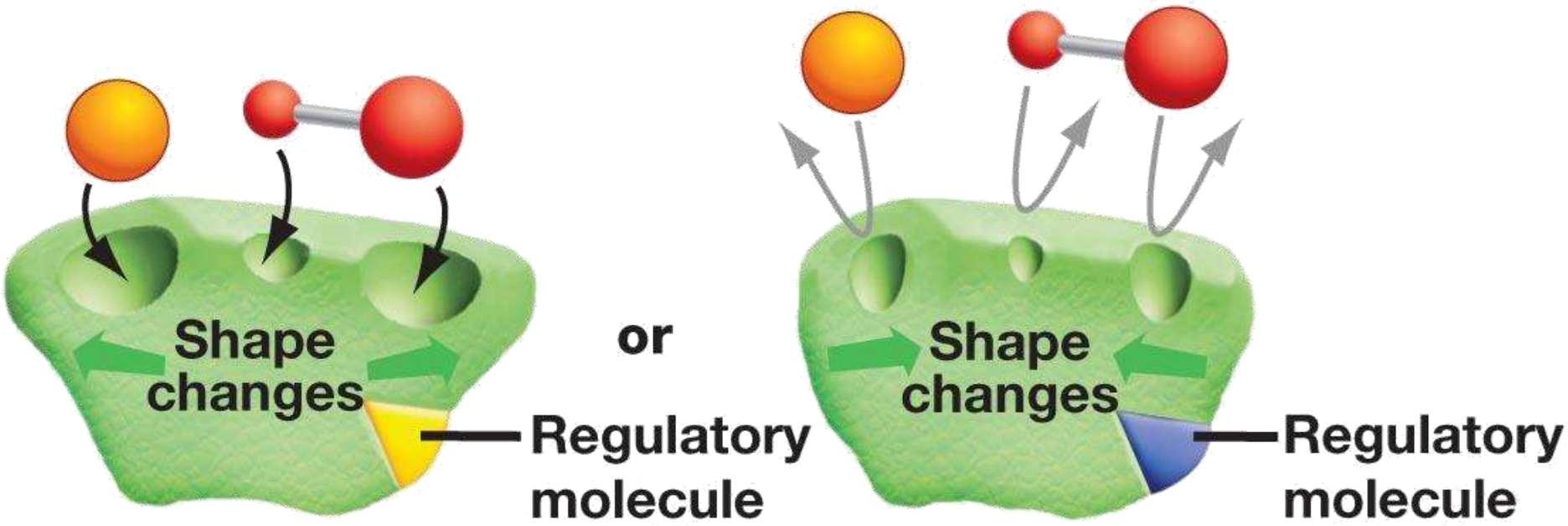
-The allosteric effector is usually a metabolite or a product resulting from the process of metabolism.

- 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 ($\mathbf{e_A}$, $\mathbf{e_B}$, $\mathbf{e_C}$ etc).

$$A \xrightarrow{e_A} B \xrightarrow{e_B} C \xrightarrow{e_C} D \xrightarrow{e_D} E \xrightarrow{e_E} F$$

-The first step (controlled by $\mathbf{e_A}$) 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.