ENZYME INHIBITORS

Enzymology 3

إعداد
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Enzyme Inhibition

Definition and Properties of Inhibitors:

- Inhibitors are chemicals that:

1. Either reduce the rate of enzymatic reactions **Or**
2. Stop the rate of enzymatic reactions.

- Characteristics of enzyme inhibitors:

1. They are usually specific.
2. They work at low concentrations (large amount of inhibitors is not needed to achieve their purpose).
3. 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:

1. Pharmacologic agents (used in drugs).
2. Research tools for study of the mechanism of enzyme action.

Classification:

- We classify enzymes according to

1. The effect of enzyme inhibition (How strong does the inhibitor bind to enzyme).
2. Their site of action on the enzyme (Where does it bind).
3. Whether they chemically modify the enzyme (What is the conformational changes that happen when its binds).
4. The kinetic parameters they influence (Enzyme kinetic => K_m/V_{max}).

- The effect of enzyme inhibition:

1. **Irreversible inhibitors:**
   - Combine with the functional groups of the amino acids in the active site or in another site, **irreversibly** (by covalent or non-covalent strong bonds which can't be broken down).
   - Irreversible = the active form of the enzyme will be lost ⟶ the cell well need to synthesis another molecule of enzyme to do it work.
2. **Reversible inhibitors:**
   - Combine with the functional groups of the amino acids in the active site or in another site, **reversibly** (by non-covalent thus can be dissociate from their binding site).
   - They can be washed out of the solution of enzyme by dialysis.

Q: How does the inhibitor work?

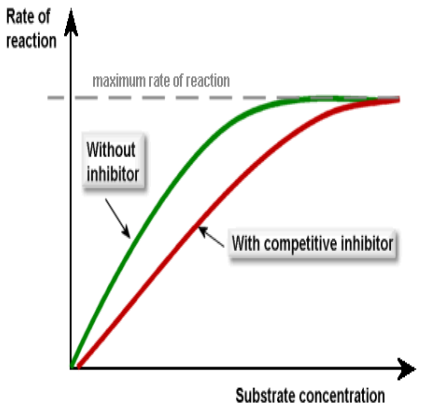
A: Generally, the inhibitor bind to the functional group in the site (active site or another site) → affect the stability of substrate in the active site → cause the substrate to be unstable → reduce the rate of the reaction.

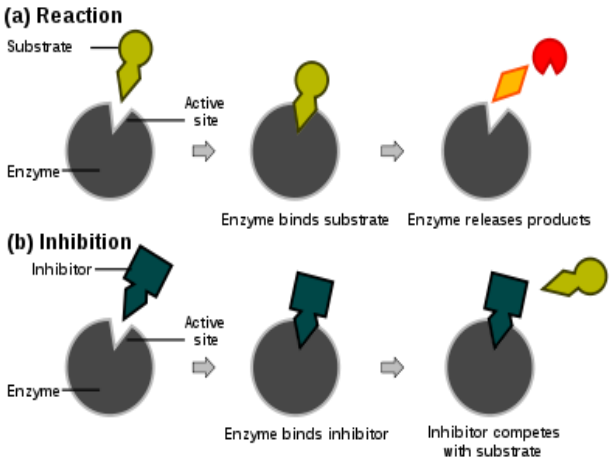
Types of enzyme inhibition:

1-	Competitive inhibition	Reversible inhibitor
2-	Non-Competitive inhibition	Reversible inhibitor
3-	Uncompetitive inhibition	Reversible inhibitor
4-	Suicidal inhibition	**Irreversible inhibitor**
5-	Allosteric inhibition	*Regulation mechanism*
6-	Feedback inhibition	*Regulation mechanism*

#Competitive enzyme inhibition:

- Has a structure similar to substrate (structural Analog) ➔ Occupies active site ➔ Competes with substrate for active site





- V_{max} remains same but K_m is increased

- **Inhibitor vs. Substrate :**
  - Generally substrate will bind to the active site because of the specificity of the enzyme
  - But if we increase the concentration of inhibitor [Inhibitor] the inhibitor will bind to the enzyme active site ***though*** *enzyme has the neither affinity nor specificity to the inhibitor*
  - By increasing the concentration of substrate again to be over the concentration of inhibitor *the inhibitor will leave the enzyme* (Competitive inhibitors have effect reversed by increasing substrate concentration) and vice versa.

    [Inhibitor] > [Substrate] ➔ the Inhibitor will bind
    [Substrate] > [Inhibitor] ➔ the Substrate will bind

Clinical significance of competitive enzyme inhibitors:

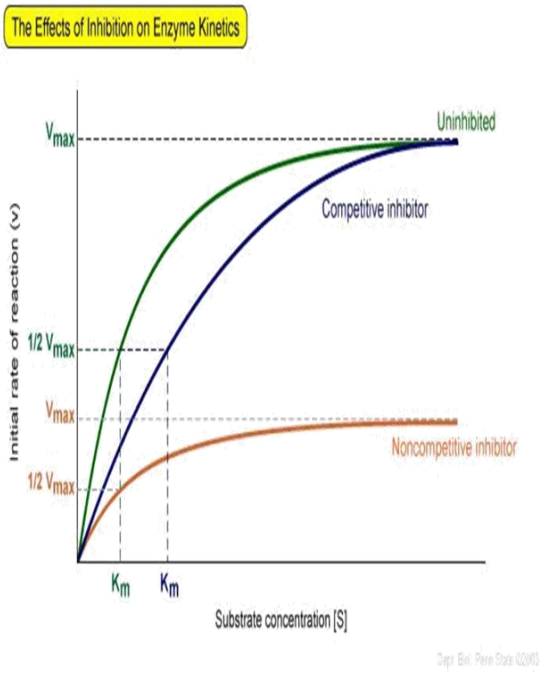
Drug	Enzyme Inhibited	Clinical Use
Dicoumarol	Vitamin K Epoxide Reductase	Anticoagulant
Sulphonamide	Pteroid Synthetase	Antibiotic
Trimethoprim	Dihydrofolate reductase	Antibiotic
Pyrimethamine	Dihydrofolate reductase	Antimalarial
Methotrexate	Dihydrofolate reductase	Anticancer
Lovastatin	HMG CoA Reductase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

Note: Trimethoprim + Pyrimethamine + Methotrexate inhibit the same enzyme (Dihydrofolate reductase) in different sites / uses.

Note: Lovastatin is a member of statins family ➔ they reduce cholesterol formation

#Noncompetitive enzyme inhibition:

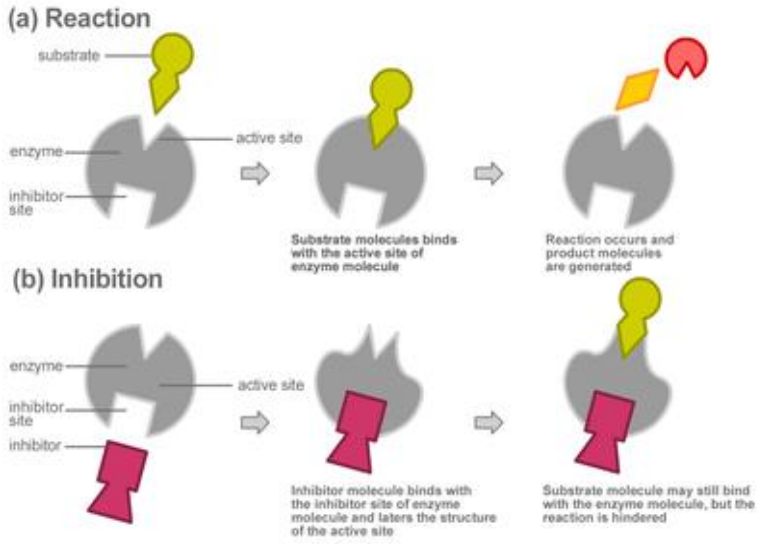
- Inhibitor Binds enzymes at sites distinct from the active site (No competition on the active site).
- Generally bear little or no structural resemblance to the substrate (No similarity).
- The inhibitor change the active site shape ➔ destabilization of the substrate ➔ lower the activity of enzyme ➔ decrease V_{max}
- **Substrate concentration has no affect** ➔ if we want to reverse the inhibition we must remove the inhibitor from the enzyme.
- Binding of the inhibitor does not affect binding of substrate ➔ therefore formation of both ***EI*** and ***EIS*** complexes is possible:
  #EI : enzyme-inhibitor complex
  #EIS : enzyme-inhibitor-substrate complex
- The enzyme-inhibitor complex can still bind substrate ➔ But its efficiency at transforming substrate to product is decreased.



V_{max} is decreased but K_m remains same

Noncompetitive Enzyme Inhibition

(Binding of the inhibitor does not affect binding of substrate)

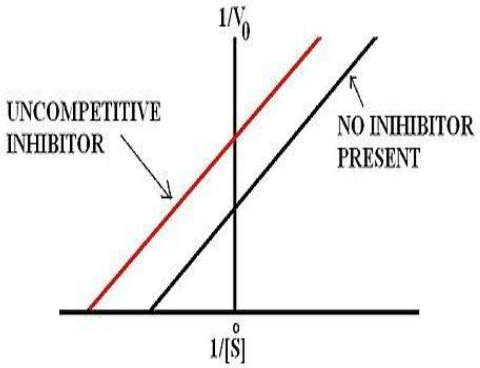


Examples of noncompetitive enzyme inhibitors:

1. Cyanide inhibits cytochrome oxidase.
2. Fluoride inhibits enolase and hence glycolysis.
3. Iodoacetate inhibits enzymes having SH groups in their active sites such as: *glyceraldehyde-3-phosphate dehydrogenase.*
   - **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.

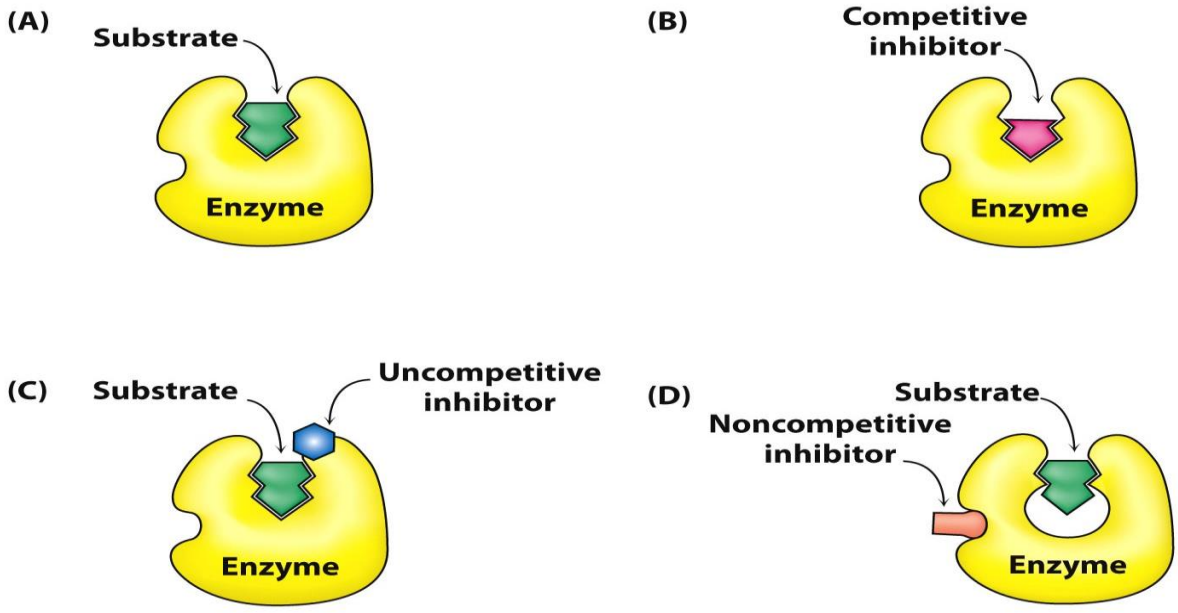
#Uncompetitive enzyme inhibition:

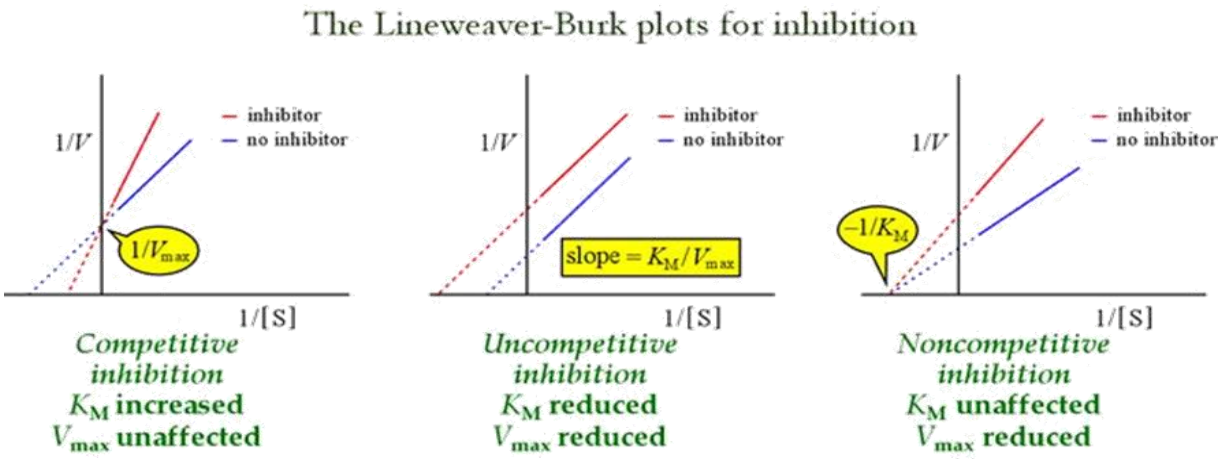
- Inhibitor binds to enzyme- substrate complex at another site of the enzyme that is close to the active site ➔ blocking the active site and preventing the release of the product ➔ Both V_{max} and K_m are decreased
- No similarity to the substrate
- No competition with the substrate
- Increasing substrate concentration has no effect on the inhibition
- Such as : Inhibition of placental alkaline phosphatase (Regan isoenzyme) by phenylalanine

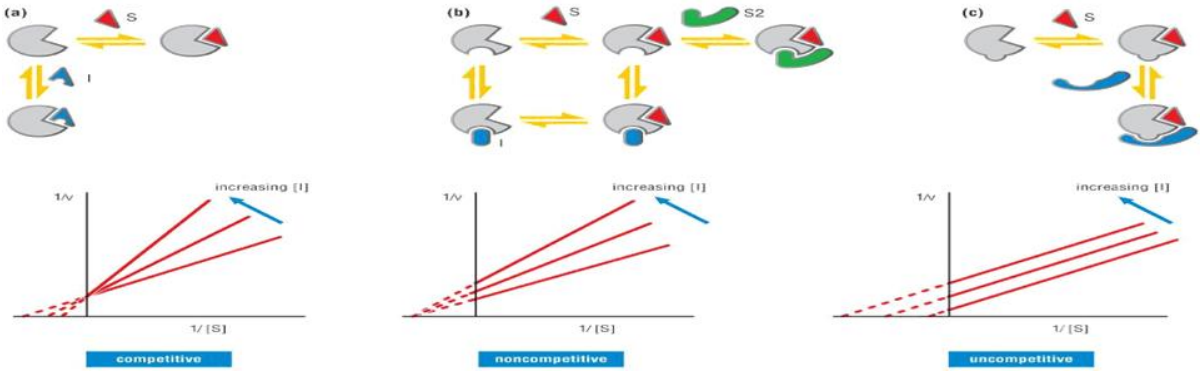


Both V_{max} and K_m are decreased

Competitive V/S noncompetitive V/S uncompetitive enzyme inhibition







- In competitive inhibitors: only 1 of the substrate or inhibitor bind to the enzyme
- In noncompetitive inhibitors: both the substrate and inhibitors bind in no specific order
- In uncompetitive inhibitors: the substrate bind first then enzyme- substrate complex

#Suicidal inhibition:

- Irreversible inhibition:
  1. The inhibitor is binding tightly (covalently)
  2. Can't be washed away
- The inhibitor in the beginning is a less effective inhibitor(Structural analog of the substrate) ➔ converted to more effective inhibitor with the help of enzyme to be inhibited (naive enzyme) ➔ The new product irreversibly binds to the enzyme and inhibits further reaction.

(**Clarification**➔ the suicidal doesn't block (kill) the enzyme, the enzyme kills itself (suicide) as it bind with the inhibitor and start catalysis➔ that's why we call this enzyme naive enzyme).

Examples of Suicidal inhibition:

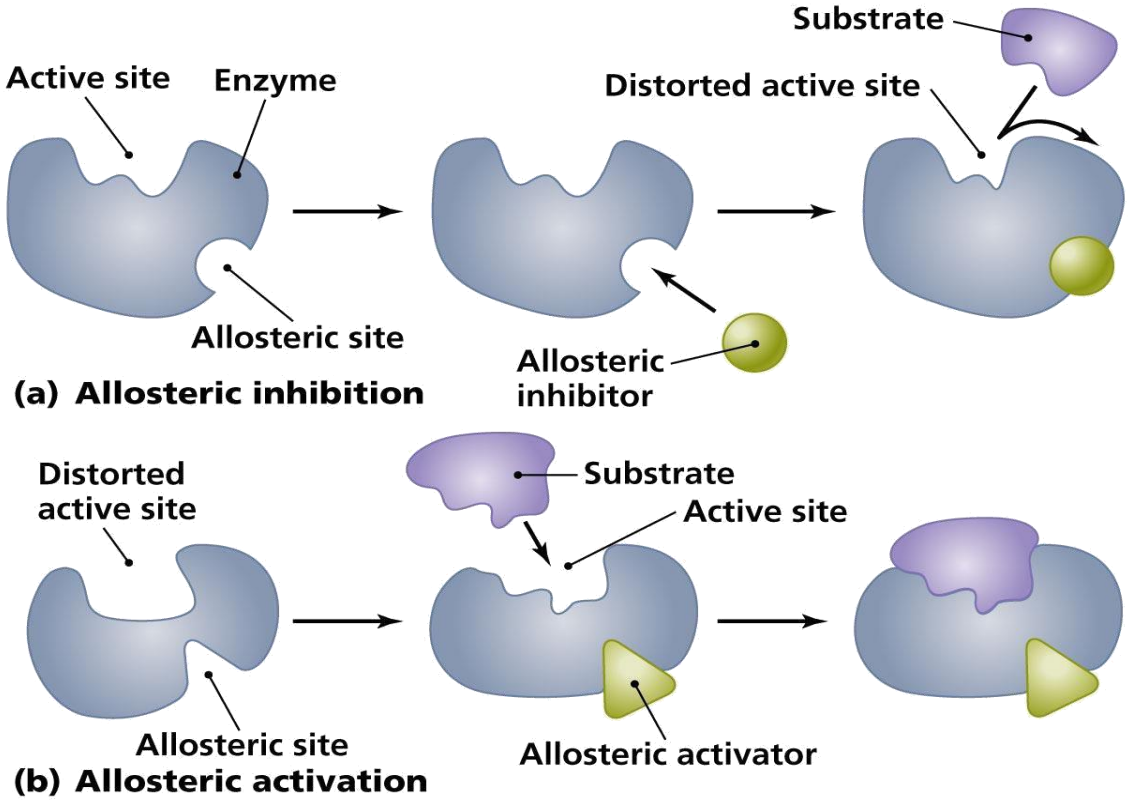
1. **difluormethyl ornithine:**
   - I. **Enzyme inhibited** ➔ Ornithine decarboxylase.
   - II. Usage ➔ against *trypanosome* in sleeping sickness (irreversibly inhibit (stop)/decrease multiplication of parasite) & decrease cell division.
2. **Allopurinol:**
   - I. **Enzyme inhibited** ➔ Xanthine oxidase enzyme.
   - II. Inhibition mechanism ➔ Xanthine Oxidase Enzyme *convert* Allopurinol by oxidation to alloxanthine "active form of allopurinol" which is a strong inhibitor of xanthine oxidase.
   - III. Usage ➔ against *gout disease* (which is a result of increase in production of uric acid).
3. **Aspirin:**
   - I. **Enzyme inhibited** ➔ cyclooxygenase (Synthesis a group of compounds that are called "*eicosanoids*")
   - II. Chemical structure ➔ acetylsalicylic acid ➔ so when we take any compound that contain acetylsalicylic acid it will be dissociated into: salicylic acid & acetic acid (active form of acetylsalicylic acid).
   - III. Usage ➔ *subsides (reduce) inflammation* **I.e.** *anti-inflammatory* **(**Acetic acid = Acetylates a serine residue in the active center of cyclooxygenase .Thus prostaglandins (PG) synthesis is inhibited/decreased so inflammation subsides).
4. **Disulfiram:**
   - I. **Enzyme inhibited** ➔ aldehyde dehydrogenase enzyme.
   - II. Usage ➔ treatment of *alcoholism* **(**irreversibly inhibits the enzyme aldehyde dehydrogenase reduce the production acetaldehyde which causes sickening effects leading to aversion to alcohol**).**

- Alcoholism:

  Ethanol ➔ aldehyde dehydrogenase enzyme in liver ➔ acetaldehyde ➔ effect (damage) the CNS ➔Oxidation (as a compensatory mechanism due to the effect on the CNS) ➔ acetic acid ➔ dissociate to H^+ shifting the pH ➔ inhibit enzymes ➔as another compensatory mechanism ➔ convert acetic acid to acetyl coenzyme A (the building unit for fatty acids and cholesterol) ➔ chronic alcoholism causes fatty liver ➔ then later on hypercholesterolemia

#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.
- Enzymes having these sites are called *allosteric enzymes*.
- This site binds an effector called the *allosteric effector* that may be:
  1) An activator (positive modifier) **Or**
  2) An inhibitor (negative modifier).
- The allosteric effector is usually a *metabolite* or a *product* resulting from the process of metabolism (not the substrate).
- **Allosteric inhibitor:**
  1) Is not a structure analogue of the substrate.
  2) Partially reversible, when excess substrate is added ➔ non-covalently bonded.
  3) When the inhibitor binds the allosteric site, *the configuration of the active site is changed* so that the substrate cannot bind properly.
  4) There are 2 types of allosteric enzymes:
     I. K series enzymes➔ K_m is usually increased ➔ reduce enzyme affinity to substrate
     II. V series enzymes➔ V_{max} is reduced
- Most allosteric enzymes possess **quaternary structure**.
- **The Curve** is *sigmoid shape* ➔ we can't apply "*Michaelis–Menten equation*" on it effectively.



Switching off:

- When the inhibitor is present it fits into its site and there is a conformational change in the enzyme molecule ➔
  i. The enzyme's molecular shape changes.
  ii. The active site of the substrate changes. ➔

  ➔ The enzyme cannot bind with the substrate and the reaction slows down. (Switching off)
- When the inhibitor concentration diminishes the enzyme's conformation changes back to its active form. (Switching on)
- **This is not competitive inhibition but it is *reversible*.**

Important Example: Phosphofructokinase (PFK-1)

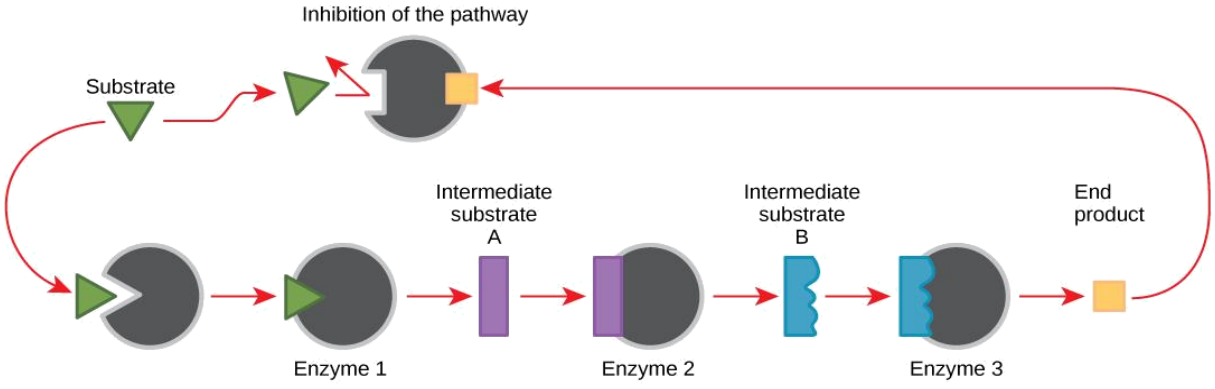
- **It catalyses:** phosphorylation of *fructose-6-phosphate* **into** *fructose 1, 6 biphosphate*.
- It has an allosteric site for an ATP molecule (the inhibitor).
- **Switching on:** When the level of ATP in the cell falls (↑ ratio of ADP to ATP)➔ no ATP binds to the allosteric site of PFK-1➔ so, the enzyme's conformation changes and the active site accepts substrate molecules➔ causing activation of glycolysis➔ The respiration pathway accelerates ➔
- **Switching off:** 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➔ stops accepting substrate molecules in the active site➔ respiration slows down.

#Feedback (end point) inhibition:

- Cell processes consist of series of pathways controlled by enzymes.

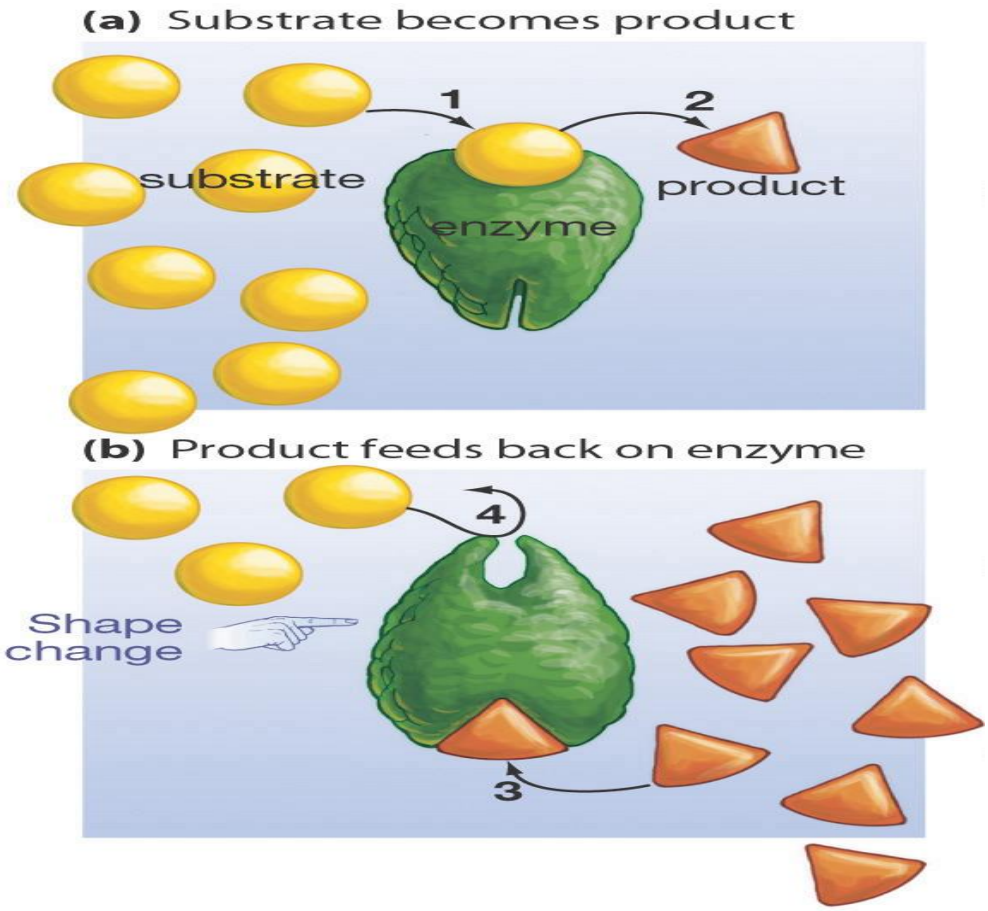
$$A \xrightarrow{e_A} B \xrightarrow{e_B} C \xrightarrow{e_C} D \xrightarrow{e_D} E \xrightarrow{e_E} F$$

- Each step is catalysed by a different enzyme (e_A, e_B, e_C, etc.).
- The first step (controlled by e_A) is often controlled by the end product (F).
- Therefore negative feedback is possible.
- **Negative feedback:** end product inhibit the key regulatory enzyme, thus cell can control (activate/inhibit) this metabolic pathway.



The cell itself undergo this type of inhibition according to its requirements

- The end products are controlling *their own rate of production*.
- There is <u>no build-up of intermediates</u> (B, C, D and E)
  - the inhibition should be accompanied by inability to synthesis intermediates ➔**Because** usually these intermediates cannot be utilized in another pathways ➔ their accumulation should be associated with more excretion and if they are not excreted the will cause *diseases*
- 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 ➔ the rate of reaction declines.
- Note: Good control points are committing steps (very negative activation energy (not easily reversed))



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