CHY2026: General Biochemistry

UNIT 6: ENZYMES AND VITAMINS

Nature of Enzymes

- They are proteins
- Biological catalysts…Unlike inorganic catalysts

  (a) enzymes do not last indefinitely

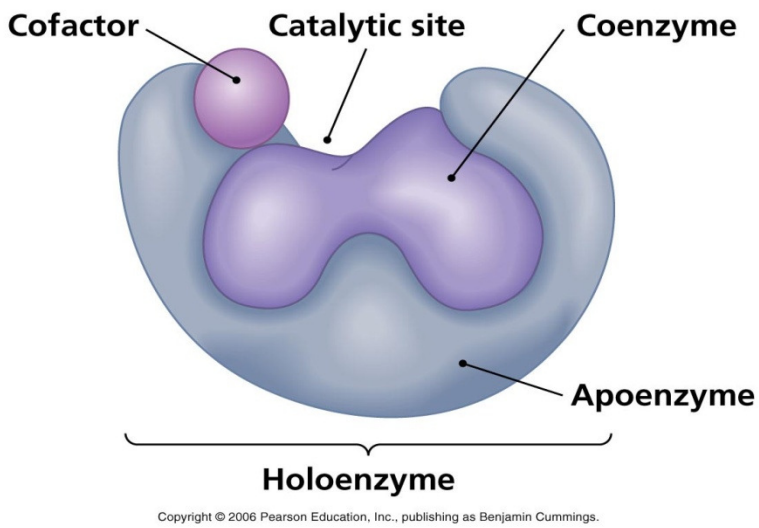
  (b) They are biological products
- They speed up biochemical reactions by lowering the activation energy necessary for a reaction to take place
- Differences between enzymes and vitamins

  (a) All enzymes are proteinaceous

  (b) Vitamins are not synthesized by animal cells
- Enzymes can be denatured and precipitated with salts, solvents and other reagents. They have molecular weights ranging from 10,000 to 2,000,000

Enzymes Cofactors

- These are non protein components of an enzyme that is essential to carrying out their functions **(cofactors)**
- The enzyme-cofactor complex is called a **haloenzyme**
- Enzyme portion without the cofactor is called an **apoenzyme**



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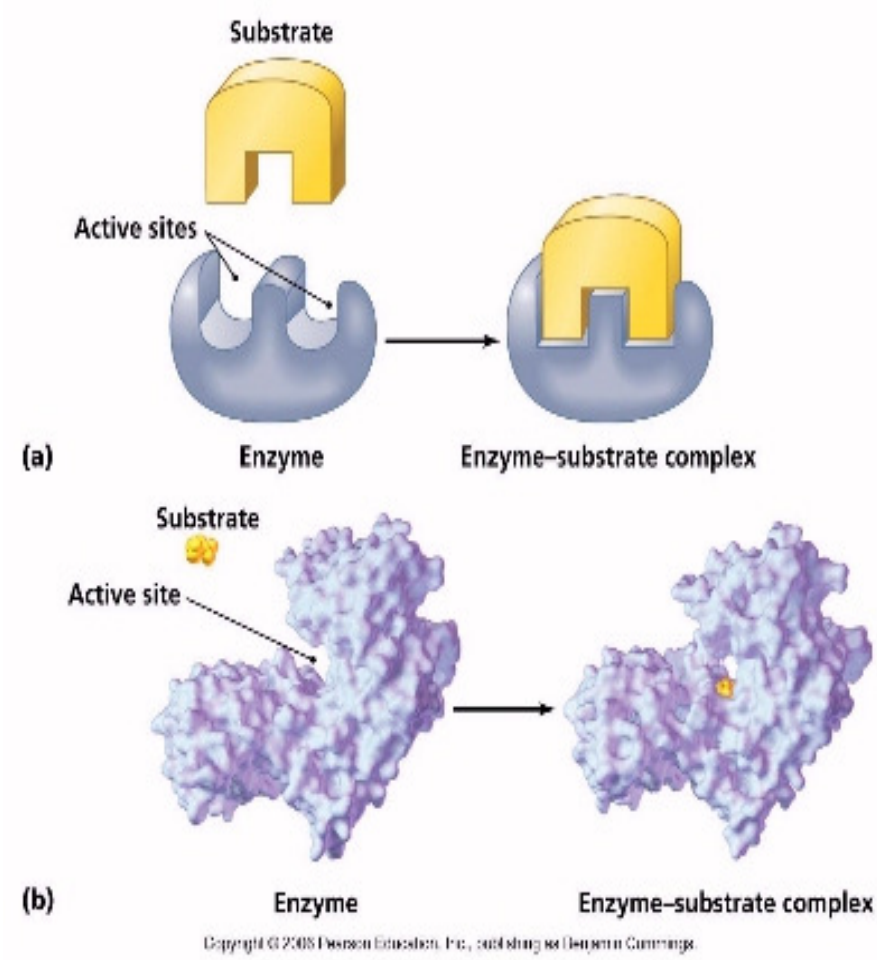
Enzymes Cofactors

- There are three groups of cofactors

1. Activators/ Inorganic ions - eg. Salivary amylase activity is increased in the presence of chloride ions (Cl^-)
2. Prosthetic groups – eg. Flavin adenine dinucleotide (FAD), haem and biotin
3. Coenzymes – eg. Adenosine triphosphate (ATP), nicotinamide adenine dinucleotide (NAD [oxidised form], NADH [reduced form])

Enzymes

- Their reactions may be **anabolic** - involved in synthesis or **catabolic** – involved in breakdown
- The name of the enzyme always end with the suffix –ase
- Enzymes bind to reactants **(substrate)**
- The area on the enzyme that binds to the substrate is called the **active site**



Nomenclature and Classification

(a) ***Substrate Acted Upon by the Enzyme***

The substance upon which enzymes act is called the SUBSTRATE

Enzymes can be named by adding the suffix –ase to the name of the substrate catalyzed

e.g. carbohydrates – carbohydrases

lipids – lipases

maltose - maltase

b) ***Type of Reaction Catalyzed***

Enzymes are highly specific as to the reaction they catalyze

The suffix –ase is added to the reaction

e.g. hydrolysis – hydrolases

isomerization – isomerases

transamination - transaminases

(c) Substance that is Synthesized

Substrate acted upon and type of reaction catalyzed can determine the name of the enzyme

e.g. dehydration of succinic acid – succinic dehydrogenase

Other classifications include:

(d) Substance that is Synthesized

(e) Chemical composition of the Enzyme

N.B. The International Union of Biochemistry (IUB) takes into consideration the overall chemical reaction when naming enzymes

- There is 6 major classes of enzymes

1. ***Oxidoreductase:*** e.g. Dehydrogenase, oxidoreductase

- This group is comprised of enzymes which were once categorized dehydrogenases
- They catalyze electron transfer reactions and the transfer of hydrogen and oxygen atoms

  e.g. Alcohol + NAD $\rightarrow$ aldehyde/ketone + NADH + H^+

2. ***Transferases:*** e.g. Transaminase, kinase

- Enzymes that catalyze the transfer of a group (other than hydrogen) between a pair of substrate

  e.g. glucose + ATP $\rightarrow$ glucose-6-phosphate + ADP ***kinase***

3. ***Hydrolases:*** e.g. Estrases, digestive enzymes

- Enzymes catalyze the hydrolysis of their substrates by adding constituents of water across the bond they split

  e.g. sucrose $\rightarrow$ glucose + fructose ***sucrase***

4. ***Lyases:*** e.g. Decarboxylase, aldolases

- Enzymes catalyze the removal of groups from substrates by mechanisms other than hydrolysis

  e.g. L-malate $\rightarrow$ fumarate + H_2O ***fumarase***

5. ***Isomerase:*** e.g. Isomerase, epimerase

- Catalyze the interconversion of optical, geometric or position isomers by intramolecular rearangement of atoms or groups

  e.g. Glucose -6-phosphate $\rightarrow$ fructose – 6- phosphate ***phosphoglucoisomerase***

6. ***Ligases or Synthetases:*** e.g. They catalyze the linking together of two compounds utilizing the energy made available due to simultaneous breaking of the pyrophosphate bond in ATP or a similar compound

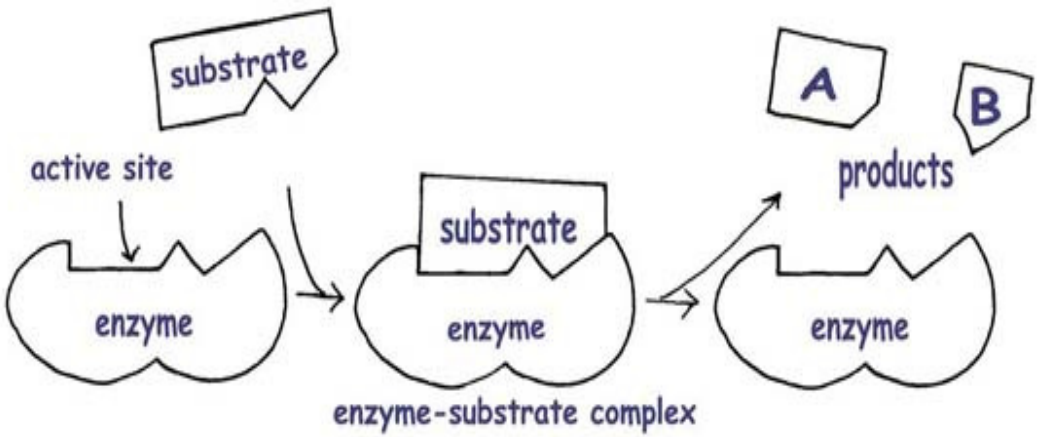
e.g. Succinyl Co A + GDP + Pi $\rightarrow$ succinate + Coenzyme A + GTP ***succinyl CoA synthetase***

Table 5.1 Enzyme Classification Based on Reaction Types

Class	Type of Reaction Catalyzed	Example
Hydrolase	Hydrolysis (catabolic)	Lipase—breaks down lipid molecules
Isomerase	Rearrangement of atoms within a molecule (neither catabolic nor anabolic)	Phosphoglucoisomerase—converts glucose 6-phosphate into fructose 6-phosphate during glycolysis
Ligase or polymerase	Joining two or more chemicals together (anabolic)	Acetyl-CoA synthetase—combines acetate and coenzyme A to form acetyl-CoA for the Krebs cycle
Lyase	Splitting a chemical into smaller parts without using water (catabolic)	Fructose 1,6-bisphosphate aldolase—splits fructose 1,6-bisphosphate into G3P and DHAP
Oxidoreductase	Transfer of electrons or hydrogen atoms from one molecule to another	Lactic acid dehydrogenase—oxidizes lactic acid to form pyruvic acid during fermentation
Transferase	Moving a functional group from one molecule to another (may be anabolic)	Hexokinase—transfers phosphate from ATP to glucose in the first step of glycolysis

Enzymes

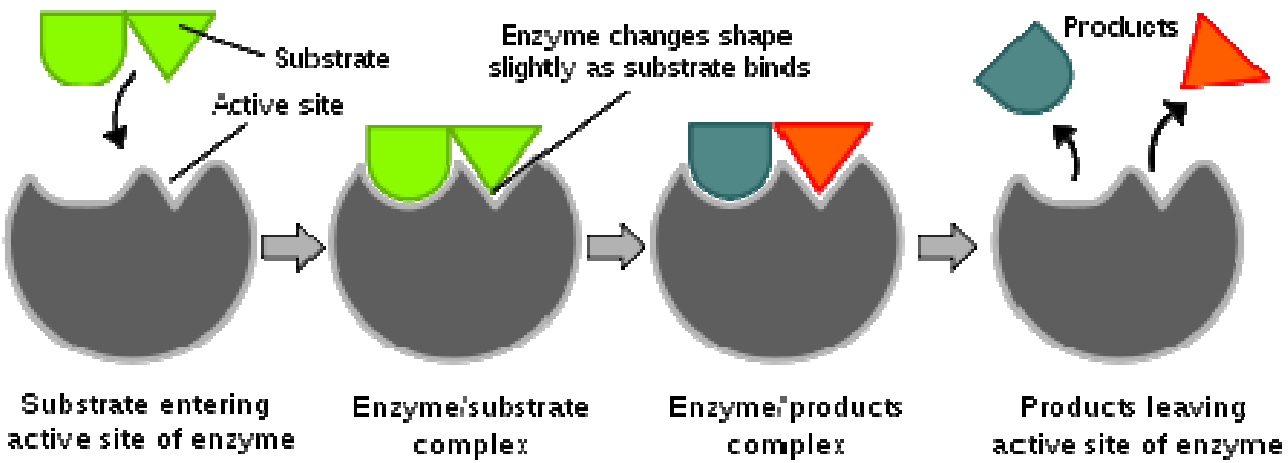
- The active site and the substrate have complementary shape enabling them to bind together (like pieces of a jig saw puzzle)
- The interaction is not rigid as would occur between a lock and a key (lock and key hypothesis – Fischer)



http://hsc.csu.edu.au/biology/core/balance/9_2_1/image1.jpg

Enzymes

- The enzyme has an internal flexibility and is able to undergo conformational changes, adjusting to the shape of the substrate (induced fit hypothesis – Koshland)



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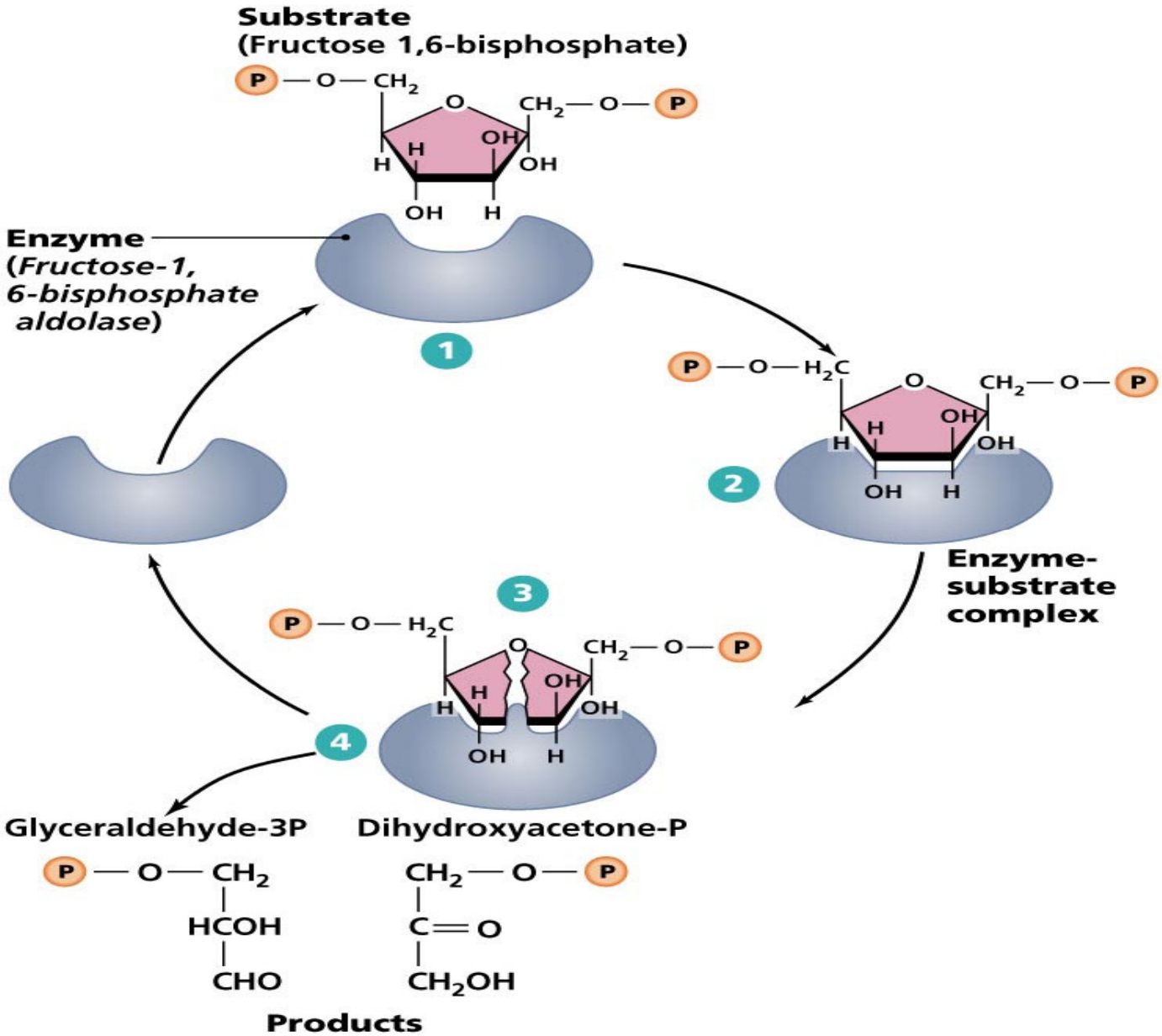
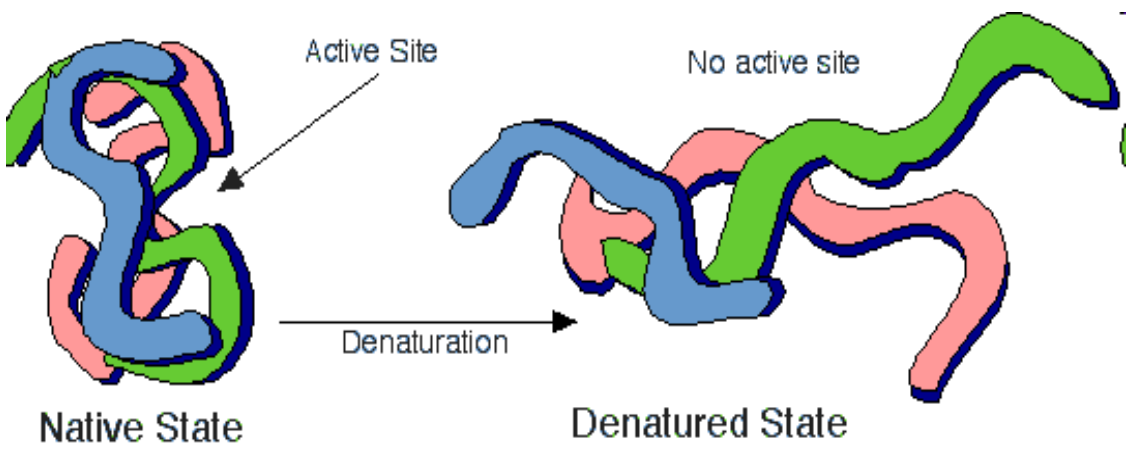


Enzymes

- Enzymes are specific for a particular substrate
- When an enzyme-substrate is formed it is activated to form products
- The products formed cannot fit into the active site and escapes leaving the active site of the enzyme free

  N.B. When an enzyme is denatured, the active site no longer exists



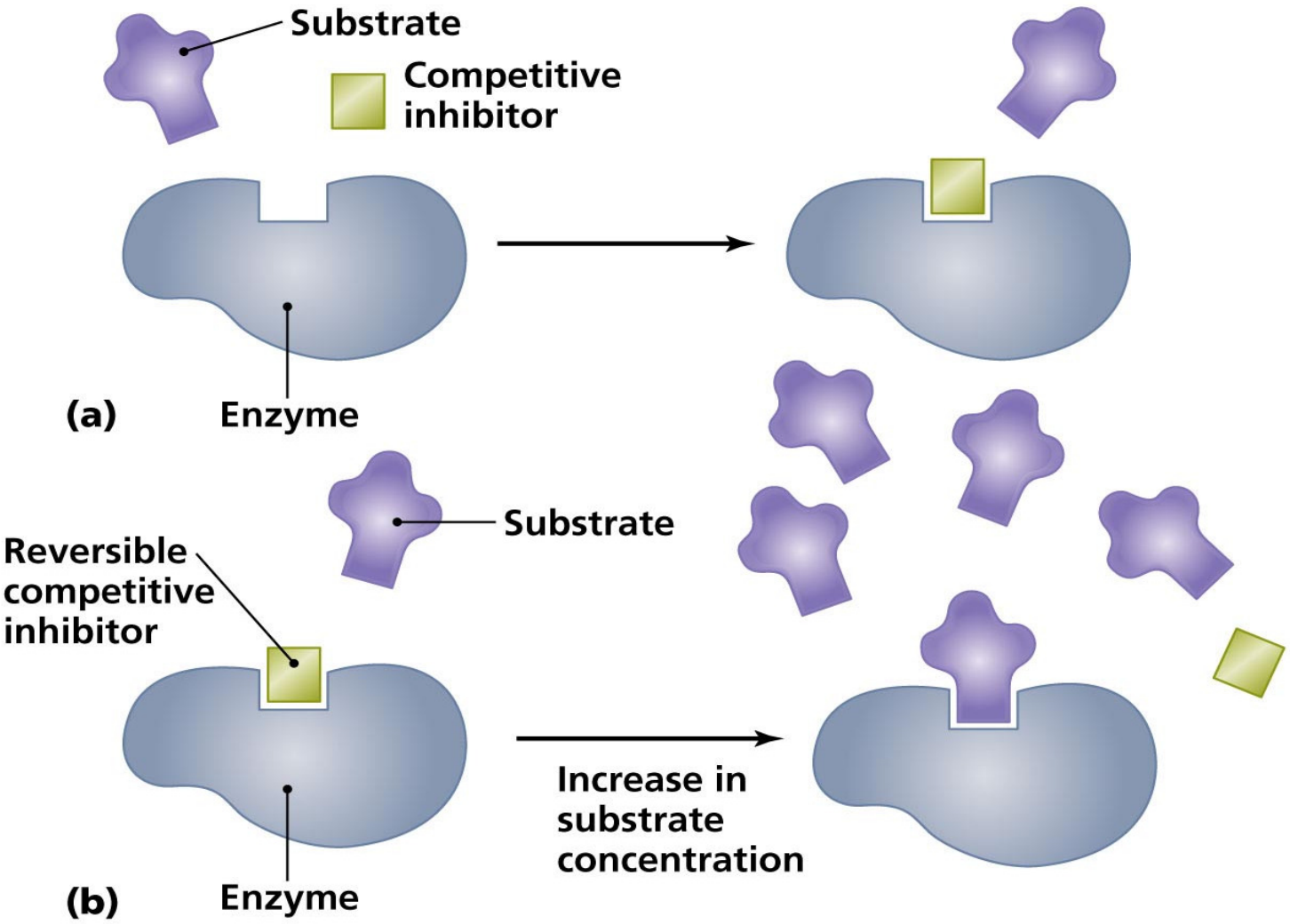
Competitive Inhibitors

- The inhibitor has a similar shape to the usual substrate for the enzyme, and competes with it for the active site
- However, once it is attached to the active site, no reaction takes place

Normally $E + S \rightleftharpoons E \cdot S\ \text{Complex} \longrightarrow E + P$

With inhibitor $E + I_c \rightleftharpoons E \cdot I_c\ \text{Complex}$

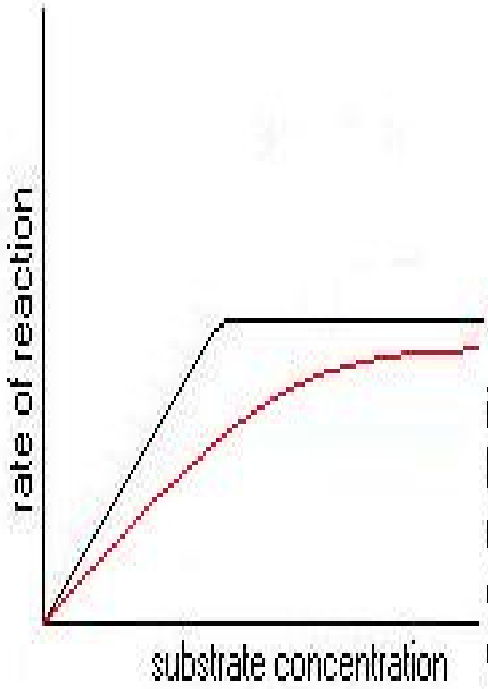
Competitive Inhibitors



Competitive Inhibitors

- The complex does not react any further to form products - but its formation is still *reversible*. It breaks up again to form the enzyme and the inhibitor molecule
- That is if you increase the concentration of the substrate, then the substrate can out-compete the inhibitor, and so the normal reaction can take place at a reasonable rate
- **Methanol poisoning** occurs because methanol is oxidized to formaldehyde and formic acid which attack the optic nerve causing blindness. Ethanol is given as an antidote for methanol poisoning because ethanol competitively inhibits the oxidation of methanol. Ethanol is oxidized in preference to methanol and consequently, the oxidation of methanol is slowed down so that the toxic by-products do not have a chance to accumulate.

Competitive Inhibitors

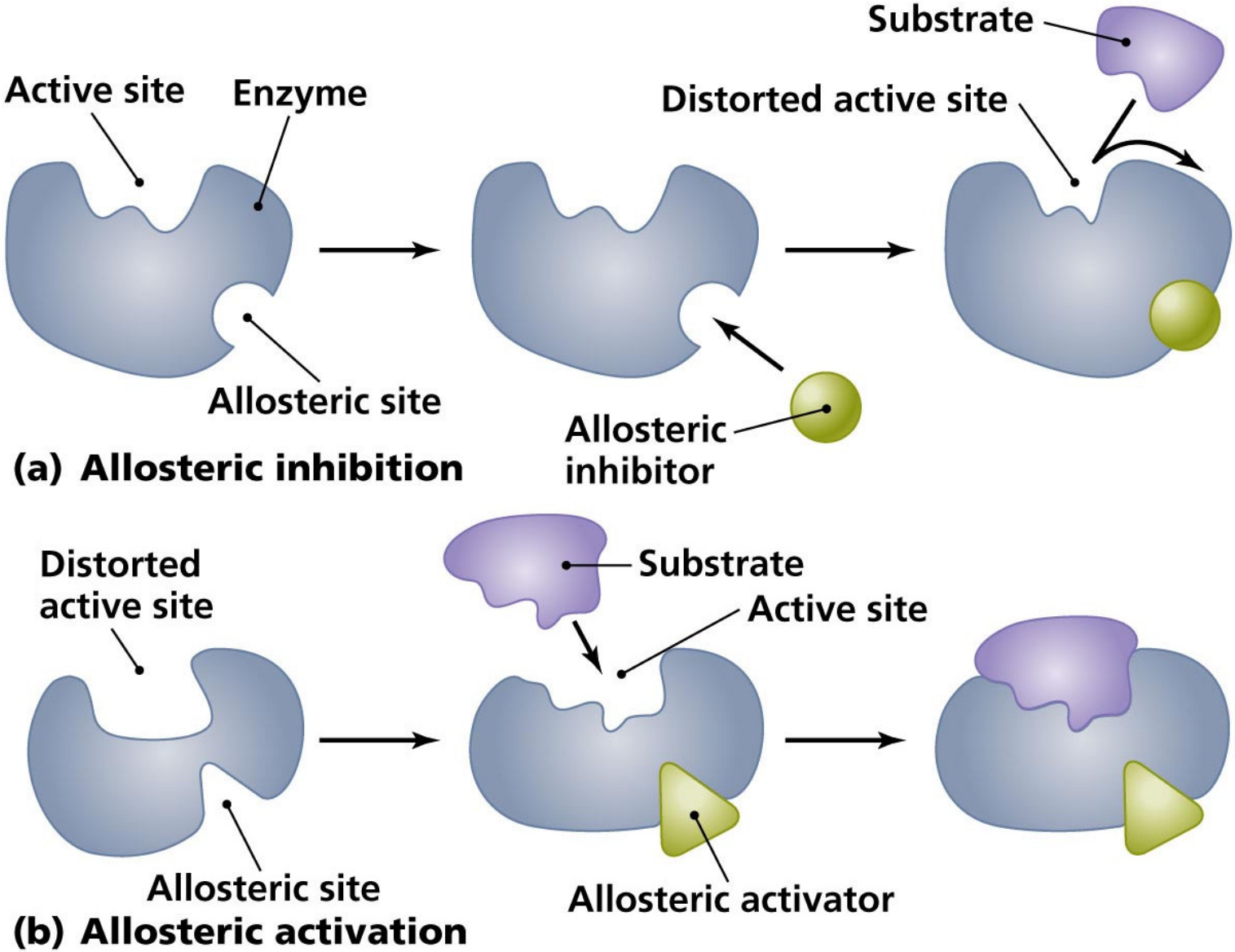


no inhibitor present

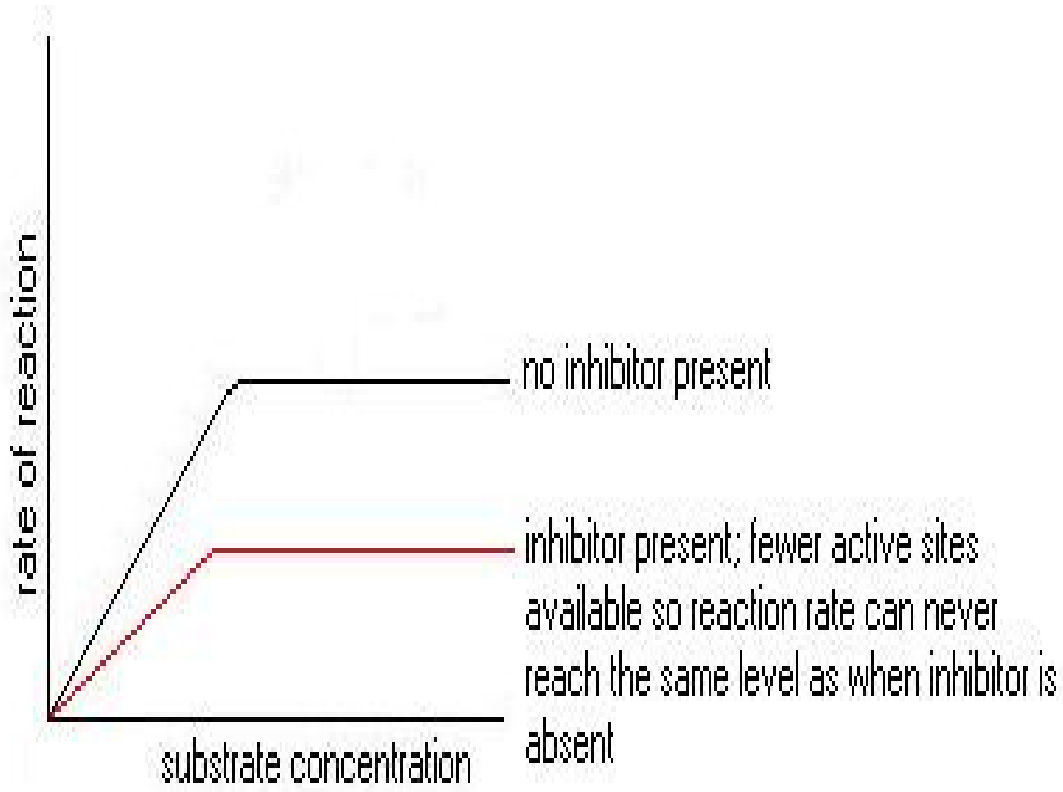
inhibitor present- akthough rate is decreased, at high substrate concentrations there is much more substrate than inhibitor so that substrate can compete effectively (the probability of an enzyme-substrate complex forming is high, even though inhibitor is present). The reaction rate is almost what it would be if inhibitor were absent

Non-competitive Inhibitors/ Allosteric Inhibitors

- A non-competitive inhibitor does not attach itself to the active site, but attaches somewhere else on the enzyme
- By attaching somewhere else it affects the structure of the enzyme *(conformational change at the active site)* and so the way the enzyme works
- Because there is no competition involved between the inhibitor and the substrate, increasing the substrate concentration will not help
- Some non-competitive inhibitors attach irreversibly to the enzyme, and therefore stop the enzyme from working permanently. Others attach reversibly

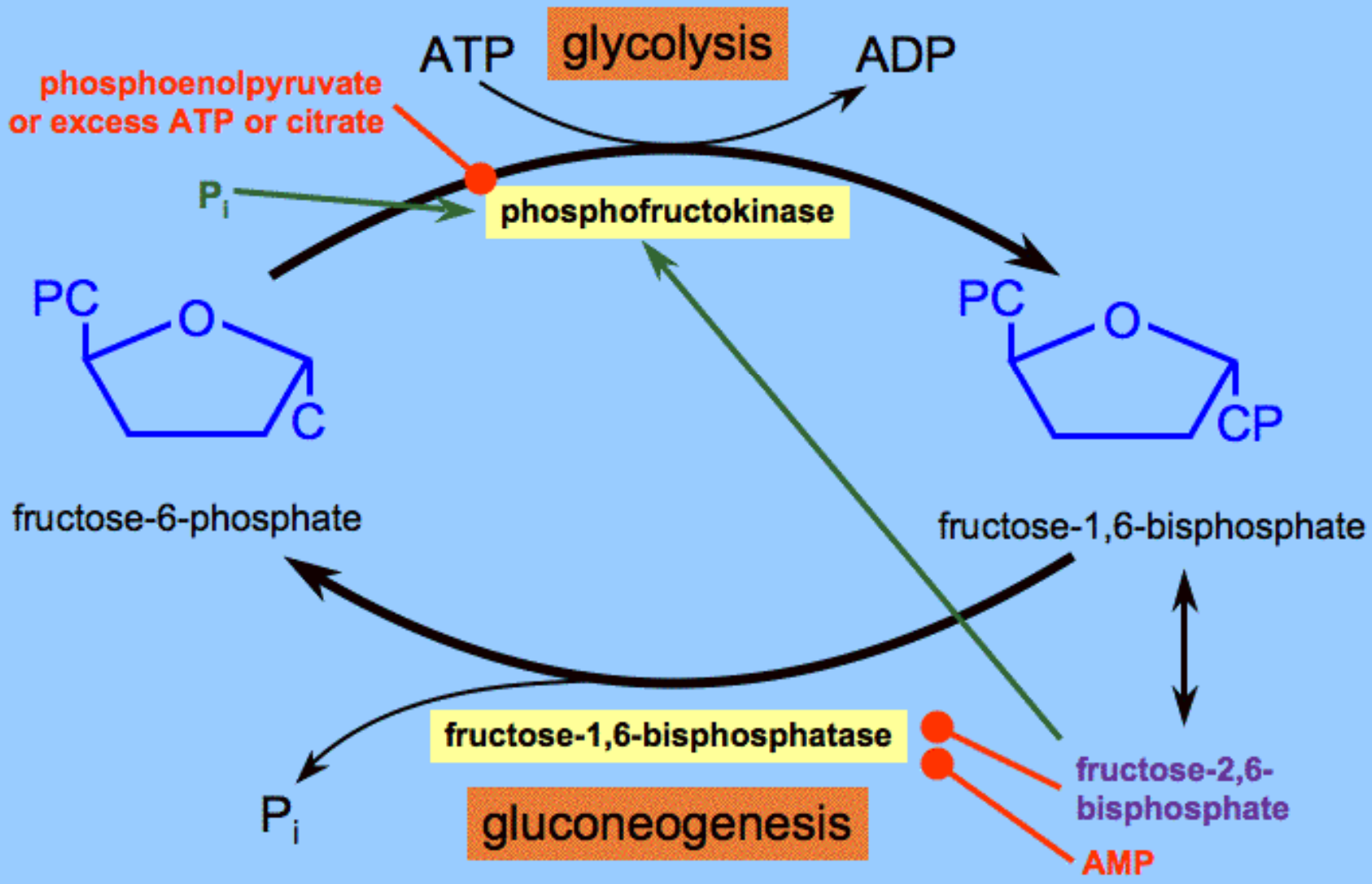


Non-competitive Inhibitors



Allosteric Regulation of Glycolysis and Gluconeogenesis

In addition to substrate/product feedback regulation:



Non-competitive inhibitors

- Since many enzymes contain sulfurhydrl (-SH), alcohol, or acid groups as part of their active sites, any chemical which can react with them acts as an irreversible inhibitor
- Heavy metals such as Ag^{+}, Hg^{2+}, Pb^{2+} have strong affinities for -SH groups

End Product Inhibition/ Negative Feedback

Consider the series of enzyme catalyzed reaction

$$A \rightarrow B \rightarrow C \rightarrow D$$

- If excess D (end-product) is formed, it non-competitively inhibits the enzyme that converts A to B (must be a non-competitive inhibitor otherwise the system wouldn't work at high concentrations of A). This effectively stops the production of B, and thus C and D.
- As no more D is being made, the excess D will eventually be used up. When this happens, the inhibition on the $A \rightarrow B$ reaction is lifted, and the system starts up again
- This is an example of **negative feedback** and is useful in ensuring that endless quantities of unnecessary end product are not produced

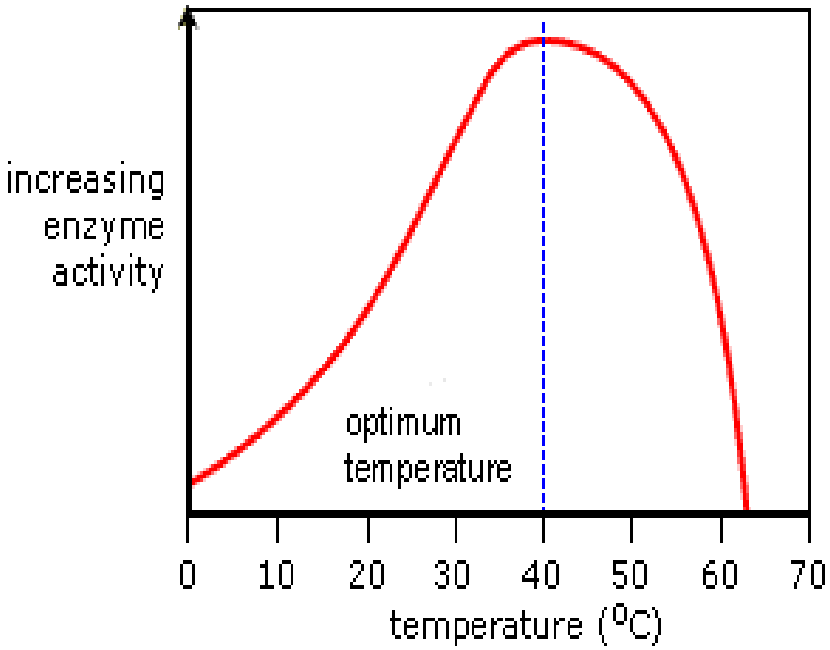
Factors Affecting Enzyme Kinetics

- The activity of an enzyme is affected by its environmental conditions
- Changing these alter the rate of reaction caused by the enzyme
- In nature, organisms adjust the conditions of their enzymes to produce an Optimum rate of reaction, where necessary, or they may have enzymes which are adapted to function well in extreme conditions where they live

Factors affecting Enzyme Kinetics: *Effect of Temperature*

- Little activity at low temperature
- Rate increases with temperature
- Enzymes are most active at optimum temperatures (usually 37°C in humans)
- Increasing temperature increases the Kinetic Energy that molecules possess
- Since enzymes catalyse reactions be randomly colliding with Substrate molecules, increasing temperature increases the rate of reaction, forming more product
- However, increasing temperature also increases the movement of enzyme molecules. This puts strain on the bonds that hold them together
- As temperature increases, more bonds, especially the Hydrogen and Ionic bonds, will break as a result of this strain

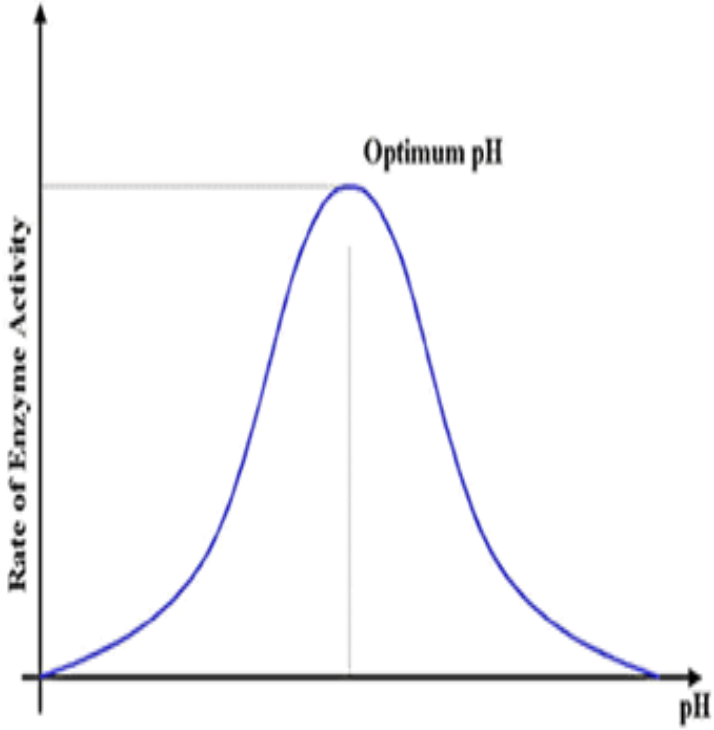
- Breaking bonds within the enzyme will cause the active site to change shape
- This change in shape means that the active site is less complementary to the shape of the substrate, so that it is less likely to catalyse the reaction. Eventually, the enzyme will become denatured and will no longer function

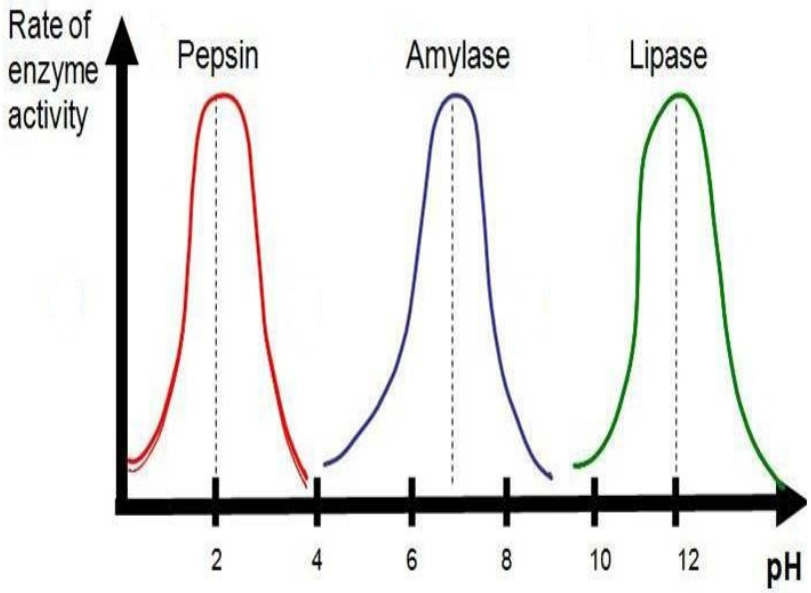


Factors affecting Enzyme Kinetics: *Effect of pH*

- Enzyme works best within a narrow pH range
- Each enzyme works best at particular pH, known as its optimum pH level
- At extreme pH levels, enzymes lose their shape and function and become denatured
- H^+ and OH^- ions are charged and therefore interfere with *Hydrogen* and *Ionic* bonds that hold the enzyme, since they will be attracted or repelled by the charges created by the bonds. This interference causes a change in shape of the enzyme, and importantly, its active site
- Any change in pH above or below the optimum will quickly cause a decrease in the rate of reaction, since more of the enzyme molecules will have active sites whose shapes are not, or at least less, complementary to the shape of their substrate

- Small changes in pH above or below the optimum do not cause a permanent change to the enzyme, since the bonds can be reformed. However, extreme changes in pH can cause enzymes to denature and permanently loose their function





Factors Affecting Enzyme Kinetics: *Effect of concentration*

- Changing the Enzyme and Substrate concentrations affect the rate of reaction of an enzyme catalysed reaction
- Controlling these factors in a cell is one way that an organism regulates its enzyme activity and so its metabolism
- Changing the concentration of a substance only affects the rate of reaction if it is the limiting factor: that is, it the factor that is stopping a reaction from preceding at a higher rate
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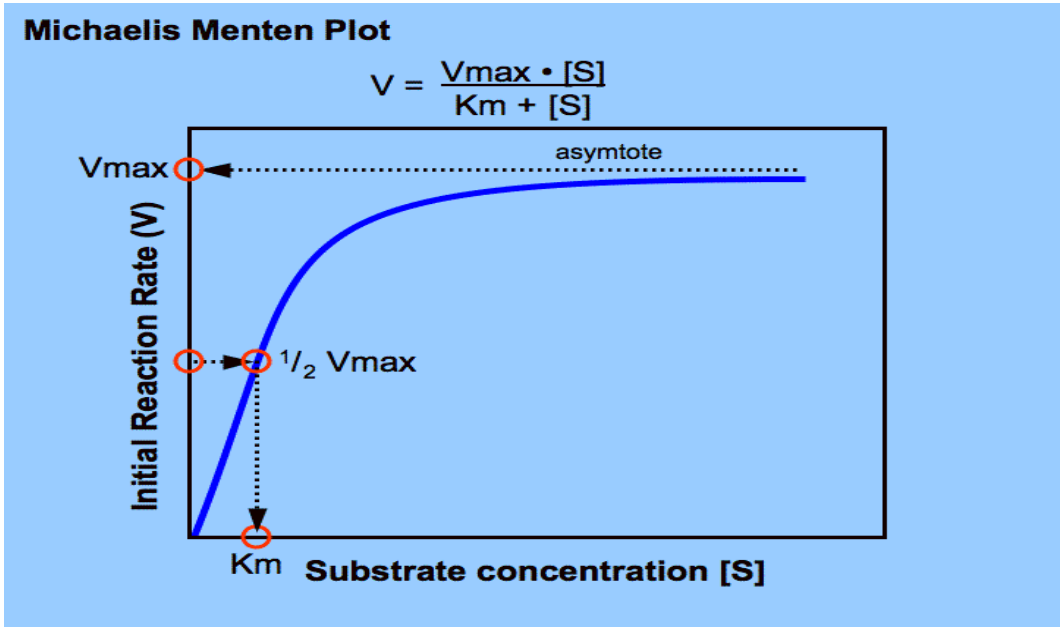
(a) Substrate Concentration

- Increasing substrate concentration increases the rate of reaction. This is because more substrate molecules will be colliding with enzyme molecules, so more product will be formed
- However, after a certain concentration, any increase will have no effect on the rate of reaction, since substrate concentration will no longer be the limiting factor
- The enzymes will effectively become saturated, and will be working at their maximum possible rate



(b) ***Enzyme Concentration***

- Increasing Enzyme Concentration will increase the rate of reaction, as more enzymes will be colliding with substrate molecules
- However there will be no effect when the enzyme concentration is now the limiting factor

Industrial Importance of Enzyme

- Enzymes can often replace chemicals or processes that present safety or environmental issues. For example, enzymes can:

1. Replace acids in the starch processing industry and alkalis or oxidizing agents in fabric desizing
2. Reduce the use of sulfide in tanneries
3. Replace pumice stones for "stonewashing" jeans
4. Allow for more complete digestion of animal feed leading to less animal waste
5. Remove stains from fabrics. Clothes can be washed at lower temperatures, thus saving energy. Enzymes can be used instead of chlorine bleach for removing stains on cloth. The use of enzymes also allows the level of surfactants to be reduced and permits the cleaning of clothes in the absence of phosphates
6. Enzymes also contribute to safer working conditions through elimination of chemical treatments during production processes. For example, in starch, paper and textile processing, less hazardous chemicals are required when enzymes are used.