CHEM121

Unit 6: Enzymes

Lecture 10

At the end of the lecture, students should be able to:

- Define the term enzyme
- Name and classify enzymes according to the:
    - type of reaction catalyzed
    - type of specificity
- Describe the effect that enzymes have on the activation energy of a reaction
- Discuss the role of the active site and the importance of enzyme specificity
- Describe the difference between the lock –and- key model and induced fit model of enzyme- substrate complex formation
- Predict and explain the effect of :
    - increasing enzyme and substrate concentration
    - temperature
    - pH

    on rates of an enzyme – catalyzed reaction
- Discuss the use of enzymes in medicine including elevated enzymes in blood for diagnosing the following conditions such as:
    - myocardial infraction
    - liver dysfunction
    - pancreatitis

1. Define the term enzyme

- ✓ Any of numerous proteins produced in living cells that accelerate or catalyze the metabolic processes of an organism.

OR

- ✓ Any of a group of complex proteins or conjugated proteins that are produced by living cells and act as catalysts in specific biochemical reactions.

- ✓ Enzymes are usually very selective in the molecules that they act upon, called **substrates**, often reacting with only a single substrate.
- ✓ The substrate binds to the enzyme at a location called the **active site** just before the reaction catalyzed by the enzyme takes place.
- ✓ Enzymes can speed up chemical reactions by up to a millionfold, but only function within a narrow temperature and pH range, outside of which they can lose their structure and become **denatured.**
- ✓ Enzymes are involved in such processes as the breaking down of the large protein, starch, and fat molecules in food into smaller molecules during digestion, the joining together of nucleotides into strands of DNA, and the addition of a phosphate group to ADP to form ATP.

- ✓ The names of enzymes usually end in the suffix - **ase.**

2. Name and classify enzymes according to the:

- ➢ **type of reaction catalyzed**
- ➢ **type of specificity**

(a) An enzyme is named based on:-
- what it reacts with
- how it reacts

(b) Generally add ***–ase*** ending, for example:-
Lactase – enzyme that reacts with lactose
Pyruvate decarboxylase – removes carboxyl (COO-) group from pyruvate

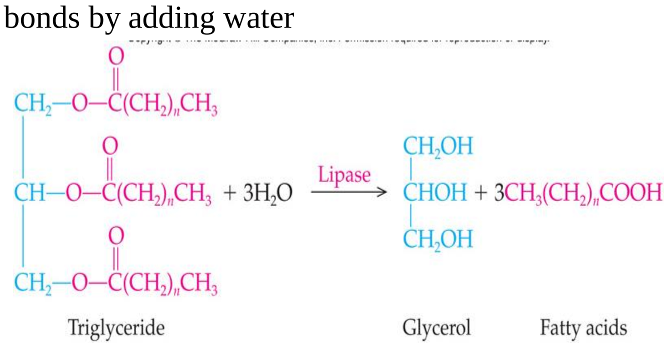
(c) Some names are historical - no direct relationship to substrate or reaction type
Catalase, Pepsin, Chymotrypsin, Trypsin

(d) Oxidoreductases catalyze redox reactions
- Reductases
- Oxidases

(e) Transferases transfer a functional group from one molecule to another
- Kinases transfer a phosphate group

(f) Lyases catalyze removal of groups to form double bonds or the reverse - break double bonds

(g) Hydrolases cleave bonds by adding water



(h) Isomerase – rearrange functional groups

(i) Ligase – join 2 molecules

Classes of Enzyme Specificity

Enzyme specificity: the ability of an enzyme to bind only one, or a very few, substrates thereby catalyzing only a single reaction

(a) Absolute: enzyme reacts with only one substrate
  - E.g. aminoacyl tRNA synthetase

(b) Group: enzyme catalyzes reaction involving any molecules with the same functional group
  - E.g. hexokinase

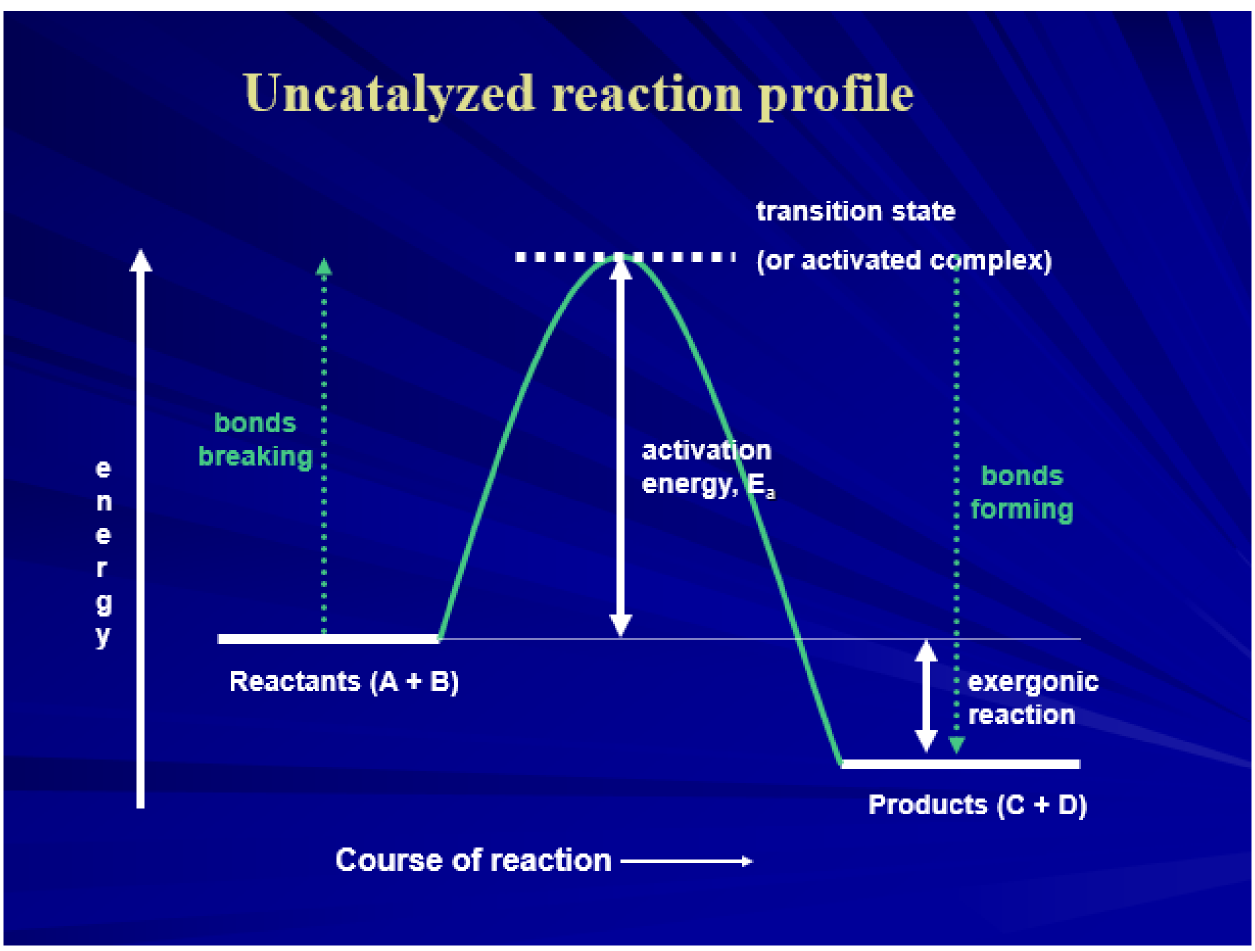
(c) Linkage: enzyme catalyzes the formation or break up of only certain category or type of bond
  - E.g. proteases such as trypsin hydrolyses peptide bonds

(d) Stereochemical: enzyme recognizes only one of two stereoisomers

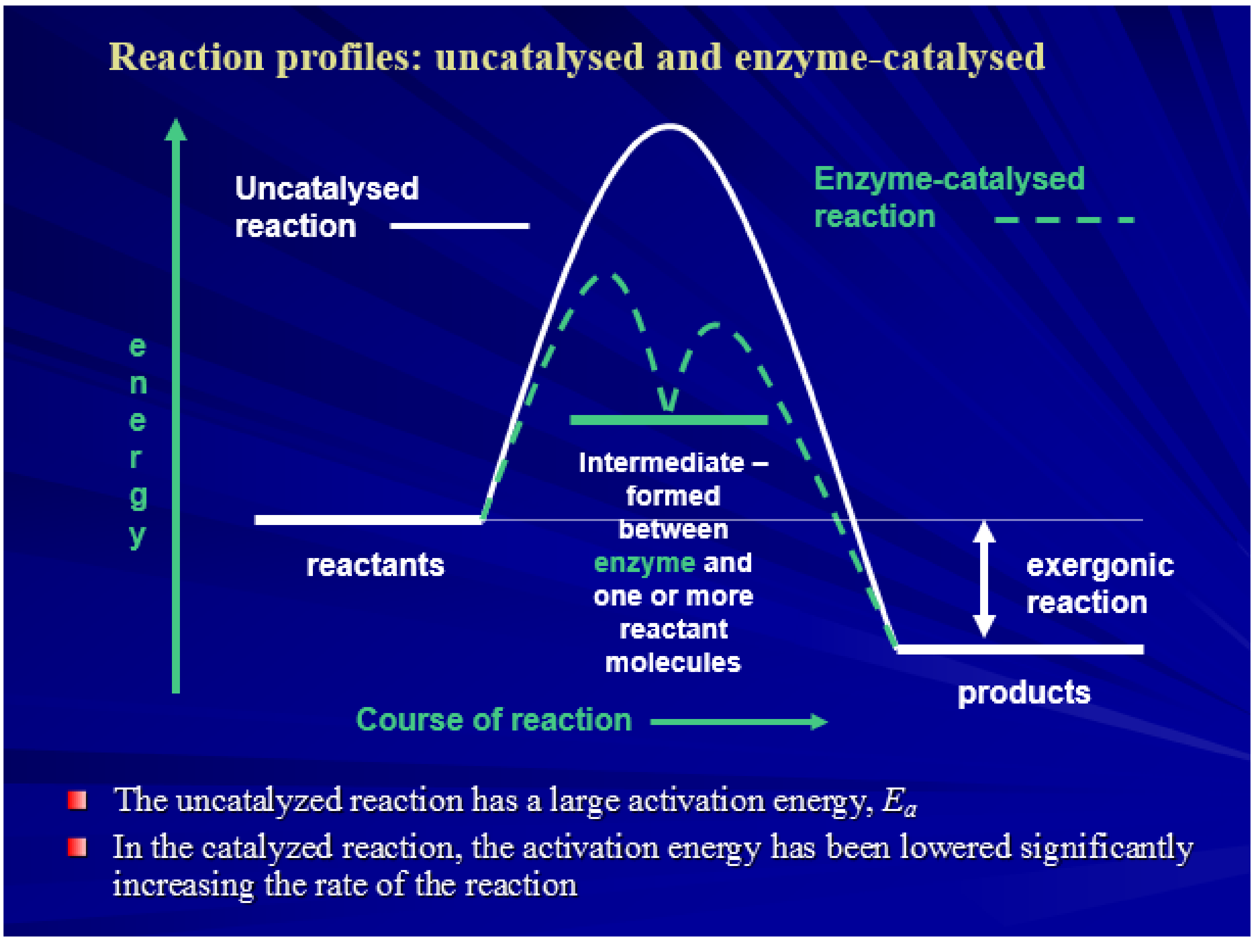
3. Describe the effect that enzymes have on the activation energy of a reaction

(a) How do enzymes speed up reactions? → lower activation energy of a reaction
  - When an enzyme and substrate react they form a reaction intermediate.
  - Formation of intermediate has a lower activation energy than the reaction between reactants without an enzyme catalyst

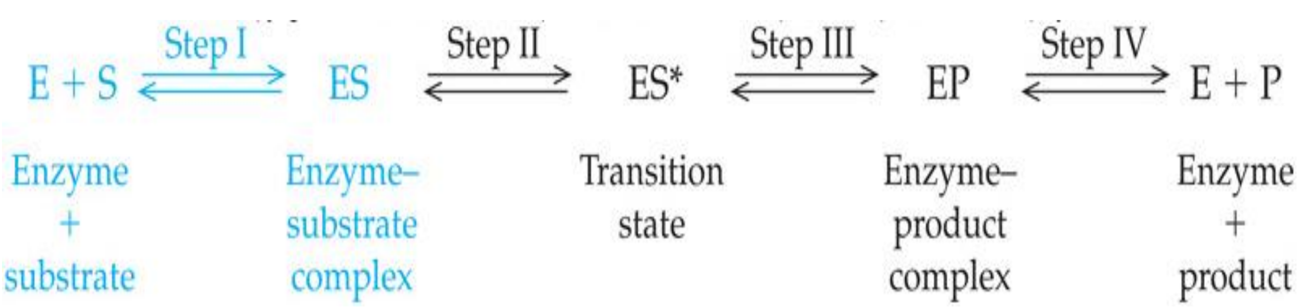
Uncatalyzed reaction profile



Reaction profiles: uncatalysed and enzyme-catalysed



(b) The 1st step involves formation of an enzyme-substrate complex (E-S)
(c) The part of the enzyme that binds with the substrate is called the active site
(d) Interaction of the enzyme and substrate → change in shape/position of substrate → new configuration is not energetically stable
(e) This 'new' state is called the transition state (E-S*)
(f) The transition state favors the conversion of substrate → product
(g) Enzyme-product (E-P) dissociates → product + unchanged enzyme



4. Discuss the role of the active site and the importance of enzyme specificity

(a) The part of the enzyme combining with the substrate is the active site

(b) The active site is where the substrate is broken down. It is the most important part of an enzyme

(c) Active sites characteristics include:
   - Pockets or clefts in the surface of the enzyme
   - Shape of active site is complimentary to the shape of the substrate
   - The enzyme attracts and holds the substrate using weak non-covalent interactions
   - Conformation of the active site determines the specificity of the enzyme

(d) The role of the active site is that it is the place where the actual chemical reaction takes place and that it creates a very specific environment that allows this chemical reaction to take place more easily (in biochemical terms; with a lower activation energy), thus catalyzing the reaction.

(e) By having the substrate molecule(s) surrounded by a specific arrangement of amino acids lining the inside of the active site, chemical bonds are more easily broken or formed, allowing the reaction to proceed much more quickly than it would without the enzyme.

(f) The active site of an enzyme:
- is the site where the actual (bio)chemical reaction takes place
- confers specificity to the catalyzed reaction
- stabilizes the often energetically unfavourable chemical intermediates during the reaction

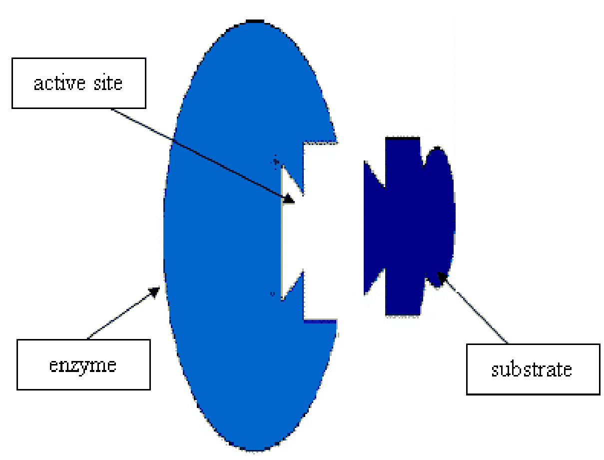
OR

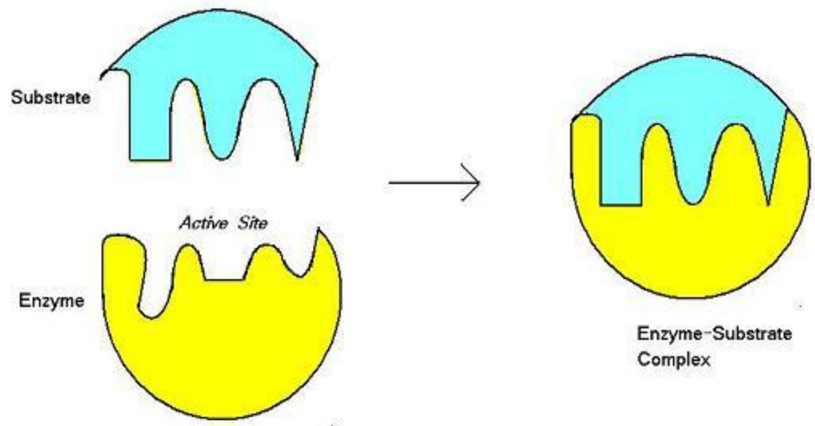
(g) The region on the surface of an enzyme to which a specific substrate or set of substrates binds.

(h) The properties of the active site are determined by the sequences of amino acids and the three-dimensional arrangement of the polypeptide chains of the enzyme.

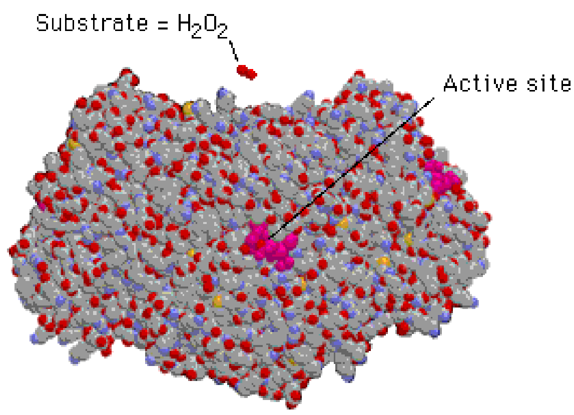
(i) The active site is not a point or plane but an intricate pocket or cleft – a 3-dimensional entity – structurally tailored to accept a particular substrate or group of substrates.

(j) The cleft or crevice excludes water from the active site, which would disrupt bond-breaking and -making processes, and is surrounded by non-polar amino acid residues, which give the active site a non-polar environment, important for both binding and catalysis.

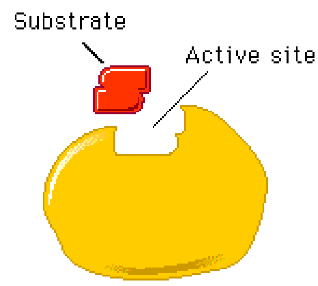




Induced-fit Model. - The enzyme active site forms a complementary shape to the substrate after binding.



Molecular model of catalase

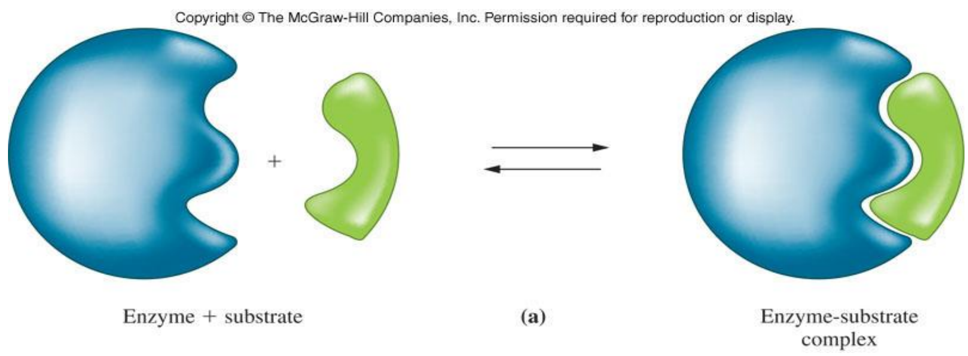


Schematic model of an enzyme

5. Describe the difference between the lock –and- key model and induced fit model of enzyme- substrate complex formation

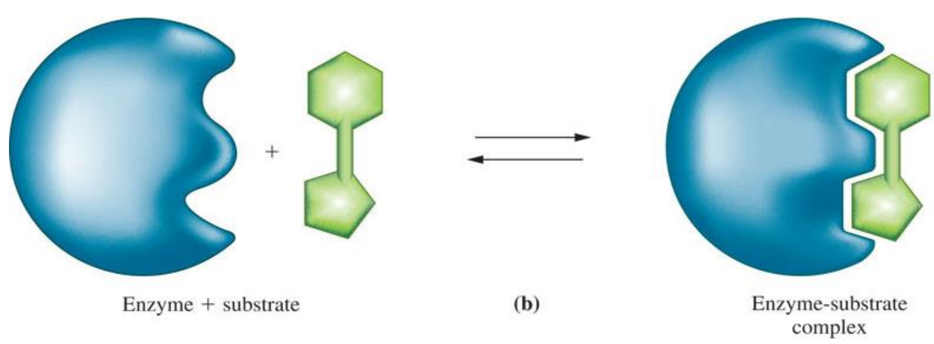
Lock and Key Enzyme Model

- In the lock-and-key model, the enzyme is assumed to be the lock and the substrate the key
  - The enzyme and substrate are made to fit exactly



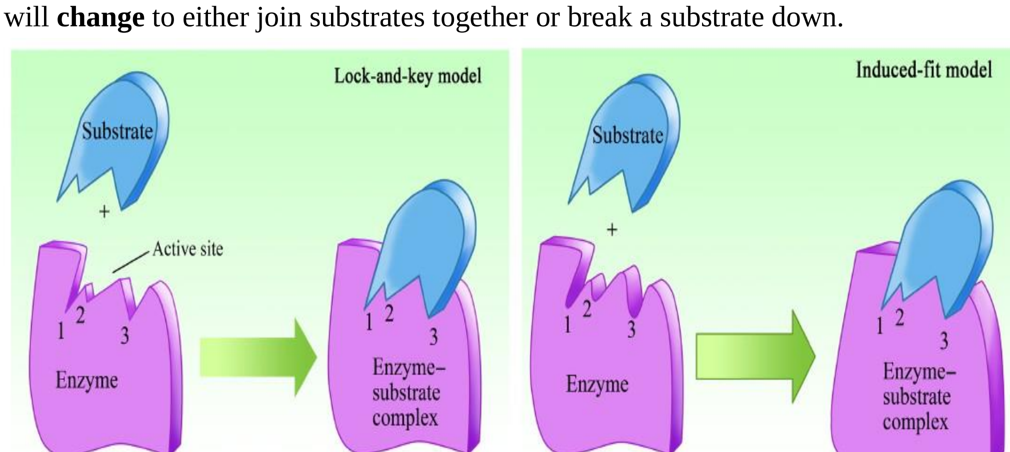
Induced Fit Enzyme Model

- The induced-fit model of enzyme action assumes that the enzyme active site is more a flexible pocket whose conformation changes to accommodate the substrate molecule



(a) The lock and key model means that the substrate must perfectly fit the enzyme, and the enzyme does not change.

(b) The induced fit model is different as when the substrate fits together with the enzyme, the enzyme itself will **change** to either join substrates together or break a substrate down.



A In the lock-and key model, the shape of the substrate and the confirmation of the active site are complementary to one another.

B In the linduced-fit model, the enzyme undergoes a confirmational change upon binding to substrate. The shape of the active site becomes complementary to the shape of the substrate only after the substrate binds to the enzyme.

<u>6. Predict and explain the effect of :</u>

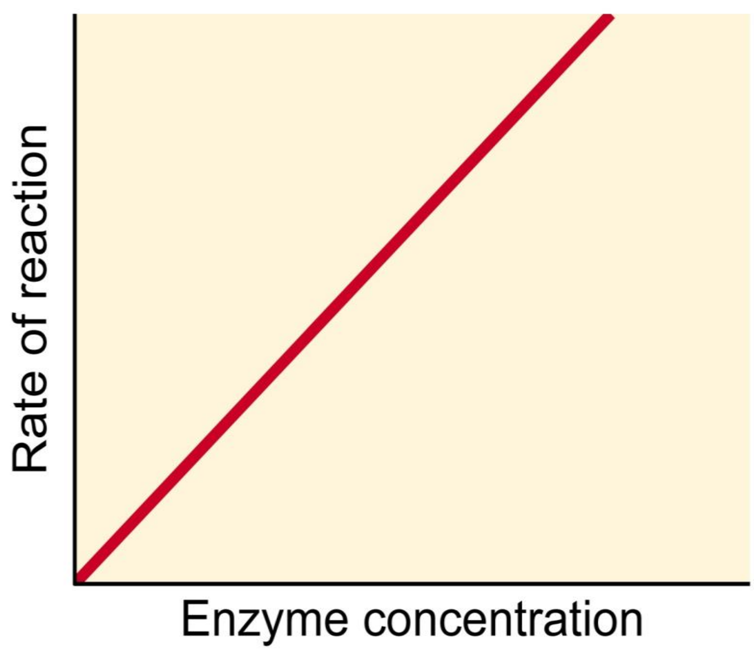
- **increasing enzyme and substrate concentration**
- **temperature**
- **pH**

<u>on rates of an enzyme – catalyzed reaction</u>

Factors Affecting Enzyme-Catalyzed Reactions

1. Enzyme concentration

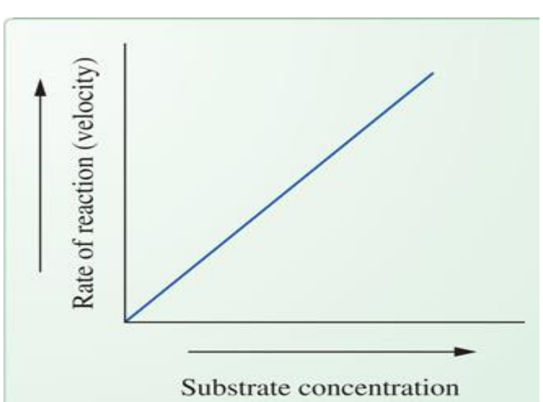
- Assuming a sufficient concentration of substrate is available, increasing enzyme concentration will increase the enzyme reaction rate.



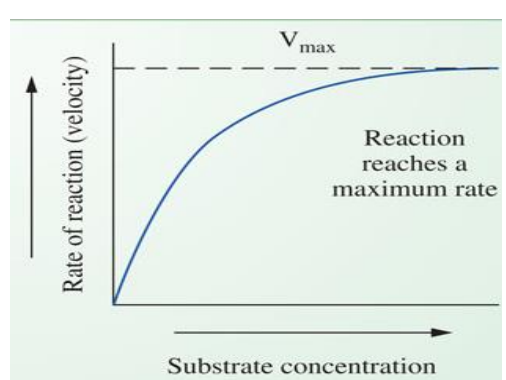
2. Substrate Concentration

- Rates of uncatalyzed reactions increase as the substrate concentration increases
- Rates of enzyme-catalyzed reactions show two stages
  - The first stage is the formation of an enzyme-substrate complex
  - This is followed by slow conversion to product
  - Rate is limited by enzyme availability

Uncatalyzed Reaction



Enzyme-Catalyzed Reaction

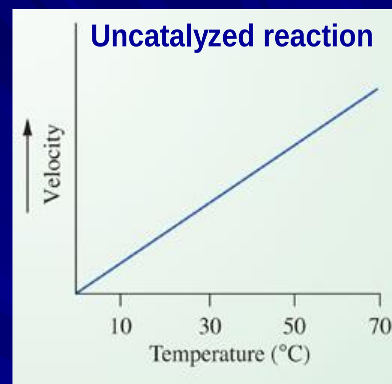


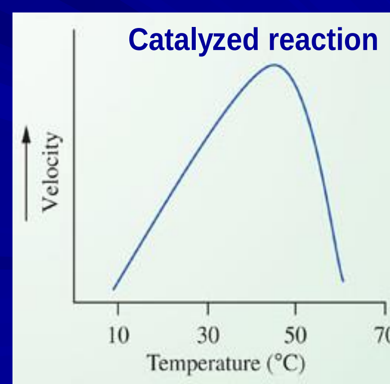
3. Temperature

Factors Affecting Enzyme-Catalyzed Reactions

3. Temperature

- The rate of an uncatalyzed reaction increases proportionally with temperature increase
- The rate of an enzyme catalyzed reaction also increases with increasing temperature however, an enzyme has an optimum temperature associated with maximal function
- Optimum temperature is usually close to the temperature at which the enzyme typically exists
  - 37°C for humans

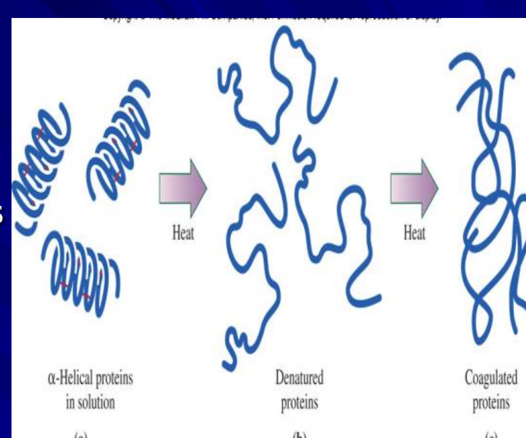




Factors Affecting Enzyme-Catalyzed Reactions

Temperature (Con't)

- Excessive heat can denature a enzyme making it completely nonfunctional
- Due to loss of 3Dshape by disrupting its H-bonds and other weak interactions
- Cold temperatures slow down enzyme activity but doesn't denature enzyme
- Cells cannot survive very high temperatures but there are some exceptions:
  - e.g. certain bacteria that live in hot springs thrive at temp near b.p. of water
  - possible because their amino acid sequence dictate structures that are stable at these seemingly impossible temperatures



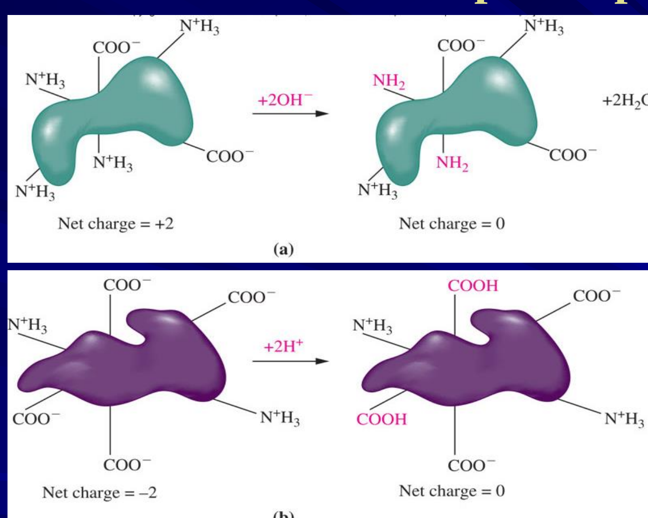
Denaturation of proteins is the loss of organized structure of a globular protein. Denaturation does not alter primary structure

4. pH

Factors Affecting Enzyme-Catalyzed Reactions

- Enzymes work best within a particular range of pH
- Extreme pH changes will denature the enzyme, destroying its catalytic ability
- Recall: some amino acids have side

Effect of pH on proteins



a) This protein has overall charge of 2+ but when base is added, some of the protonated amino groups lose the protons → equal # of + and - charges

b) This protein has overall charge of 2- but as acid is added, some of the COO^- groups are protonated → equal # of + and - charges

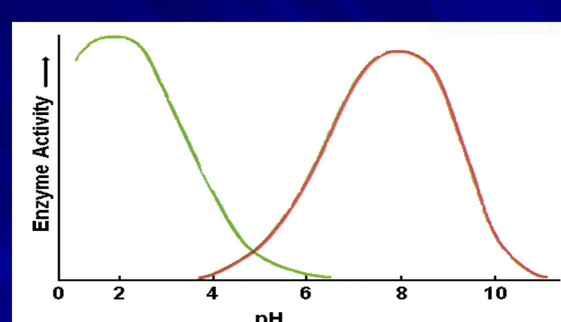
–**isoelectic point -** pH at which protein has a net charge of 0

–at isoelectric point protein has no net surface charge → no longer repel each other and are less soluble → tendency to clump together and precipitate out of solution

Factors Affecting Enzyme-Catalyzed Reactions

pH (Con't)

- Most cytoplasmic enzymes function best at pH 7
- Environments within the body in which enzymes function at a pH far from pH 7
  - Stomach: **Pepsin** - optimum pH 2
  - Small intestine: **Chymotrypsin** optimum pH 8
  - Lysosomes: **Hydrolytic enzymes** - optimum pH 4.8



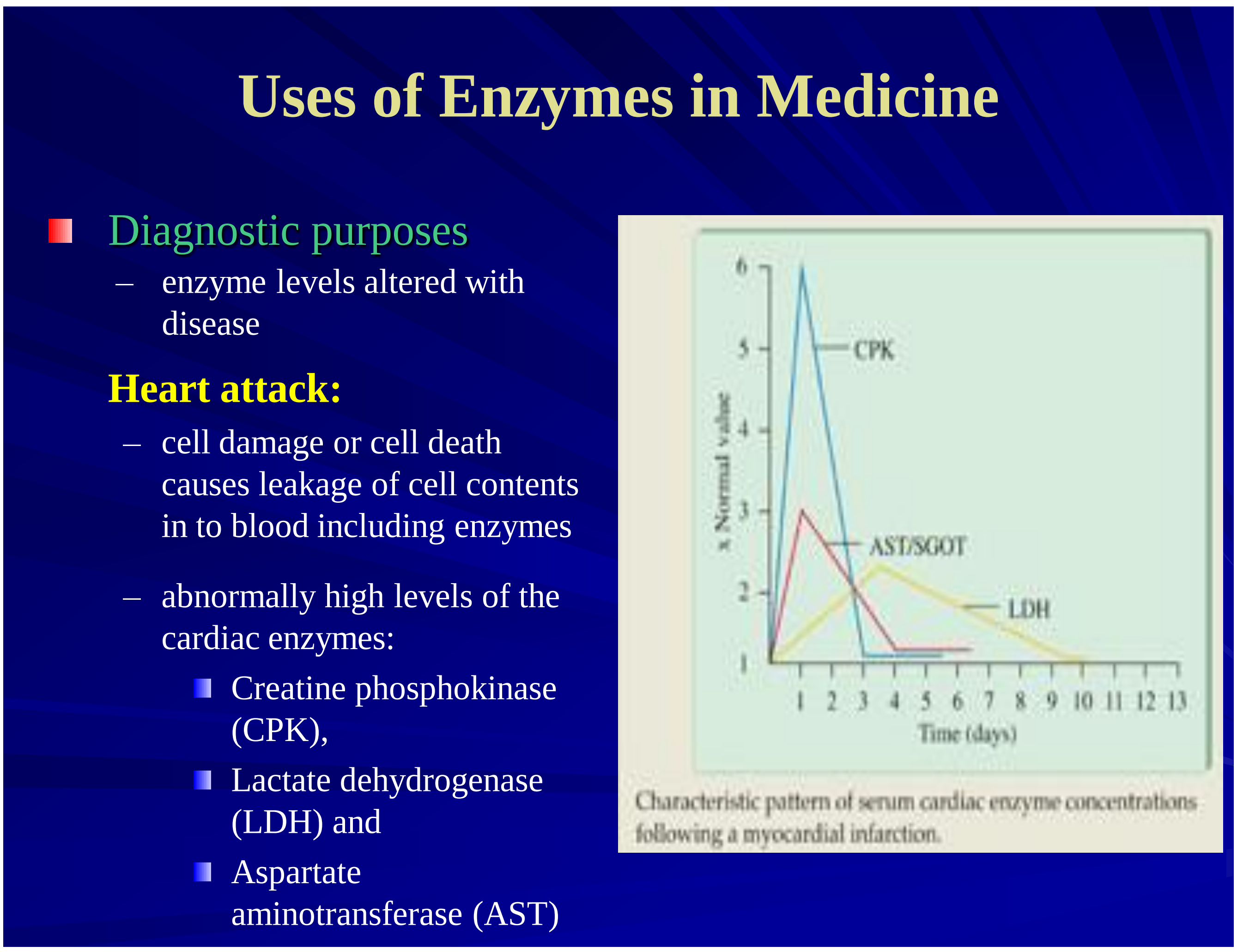
7. Discuss the use of enzymes in medicine including elevated enzymes in blood for diagnosing the following conditions such as:

- **myocardial infraction**
- **liver dysfunction**
- **pancreatitis**

USES OF ENZYMES IN MEDICINE

Drugs:
Administer b-galactosidase in form of tablet for treating lactose intolerance

Analytical reagents – enzyme used to measure another substance
- **Difficult to measure urea concentration in blood**
- **→urea converted to NH_3 via urease**
- **Blood urea nitrogen (BUN) measured**



Pancreatitis:

- Amylase
- Lipase

Liver disease e.g. cirrhosis and hepatitis

- Lactate dehydrogenase 2(LDH_2)
- Alanine aminotransferase (ALT)
- Asparatate aminotransferase (AST)