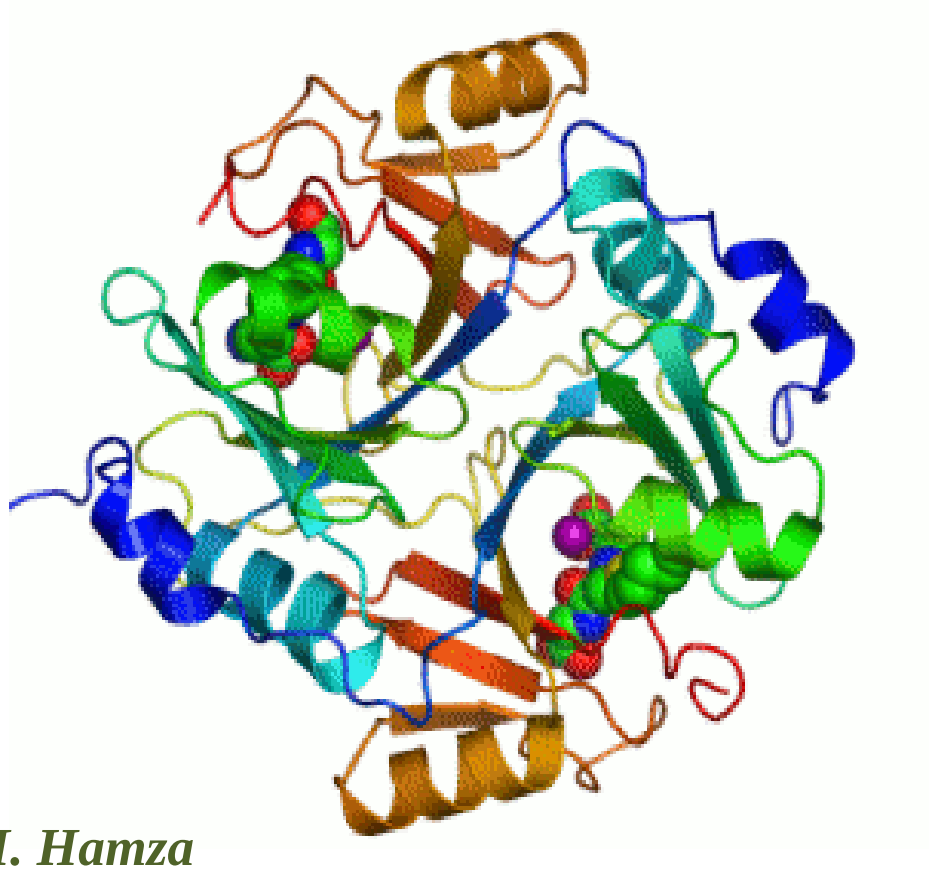
AL-RASHEED UNIVERSITY COLLEGE
DEPARTMENT OF MEDICAL LABORATORY TECHNIQUES



Enzymes

Lecture 2

Prepared By
Dr. Kutaiba I. Alzand & Dr. Rusul H. Hamza

الوحدة الأولى - المحاضرة الثانية - الزمن: 90 دقيقة
أهداف المحاضرة الثانية:

يتوقع في نهاية المحاضرة أن يكون الطالب قادراً على:

By the end of the lecture, the student should be able to:

1. Explain Energy Changes occurring during the reaction
2. Know the Alternate reaction pathway
3. Define free Energy of Activation
4. Know the factors that affect the velocity of an enzymatic reaction

موضوعات المحاضرة الثانية:

HOW ENZYMES WORK

- Energy changes occurring during the reaction
  - Free energy of activation:
  - Rate of reaction:
  - Alternate reaction pathway

FACTORS AFFECTING REACTION VELOCITY

- Substrate concentration
  - Maximal velocity
  - Hyperbolic shape of the enzyme kinetics curve
- Temperature
  - Increase of velocity with temperature
  - Decrease of velocity with higher temperature
- pH
  - Effect of pH on the ionization of the active site
  - Effect of pH on enzyme denaturation
  - The pH optimum varies for different enzymes

III. HOW ENZYMES WORK

The mechanism of enzyme action can be viewed from two different perspectives.

- The first treats catalysis in terms of energy changes that occur during the reaction, that is, enzymes provide an alternate, energetically favorable reaction pathway different from the uncatalyzed reaction.
- The second perspective describes how the active site chemically facilitates catalysis.

A. Energy changes occurring during the reaction

- Virtually all chemical reactions have an energy barrier separating the reactants and the products.
- This energy barrier, called the free energy of activation, is the energy difference between that of the reactants and a high-energy intermediate that occurs during the formation of product.
- For example, Figure 1.4 shows the changes in energy during the conversion of a molecule of reactant A to product B as it proceeds through the transition state (high-energy intermediate), T*:

$$A \leftrightarrows T^* \leftrightarrows B$$

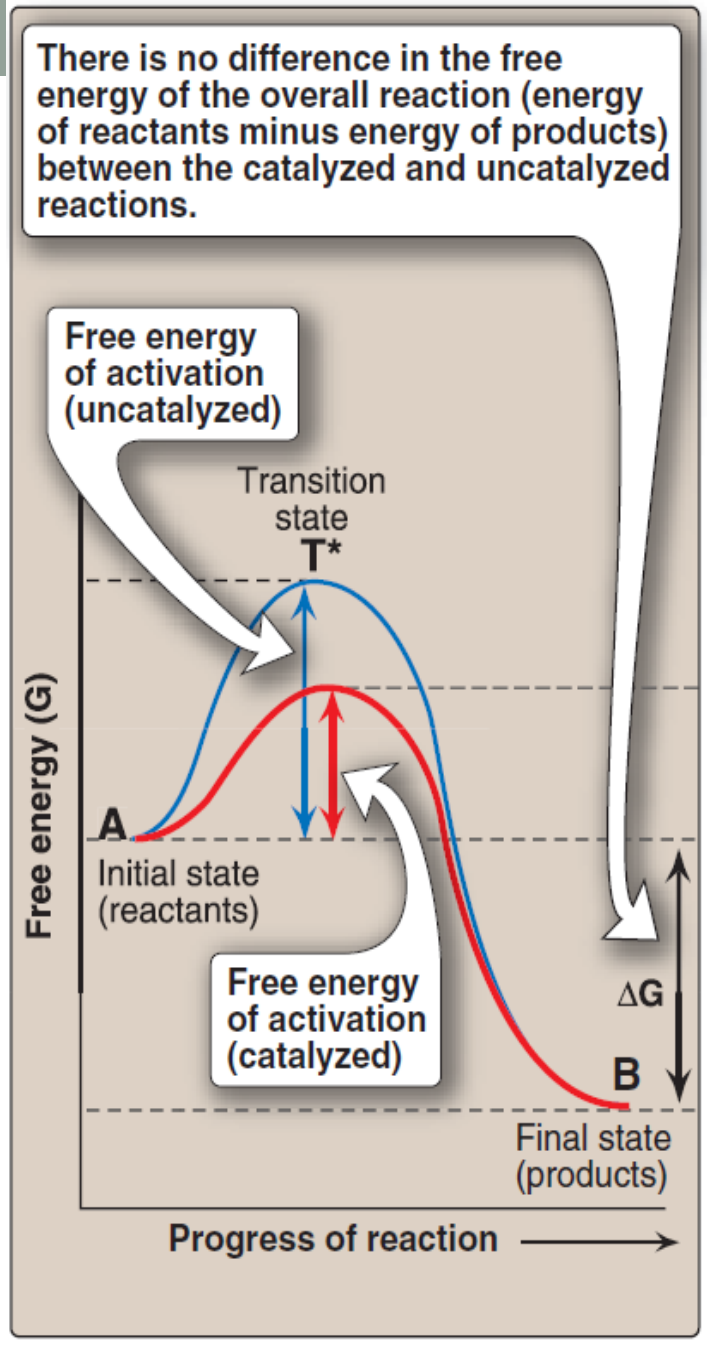


Figure 1.4 Effect of an enzyme on the activation energy of a reaction.

1. Free energy of activation:

- The peak of energy in Figure 1.4 is the difference in free energy between the reactant and T*, where the high-energy intermediate is formed during the conversion of reactant to product.
- Because of the high free energy of activation, the rates of uncatalyzed chemical reactions are often slow.

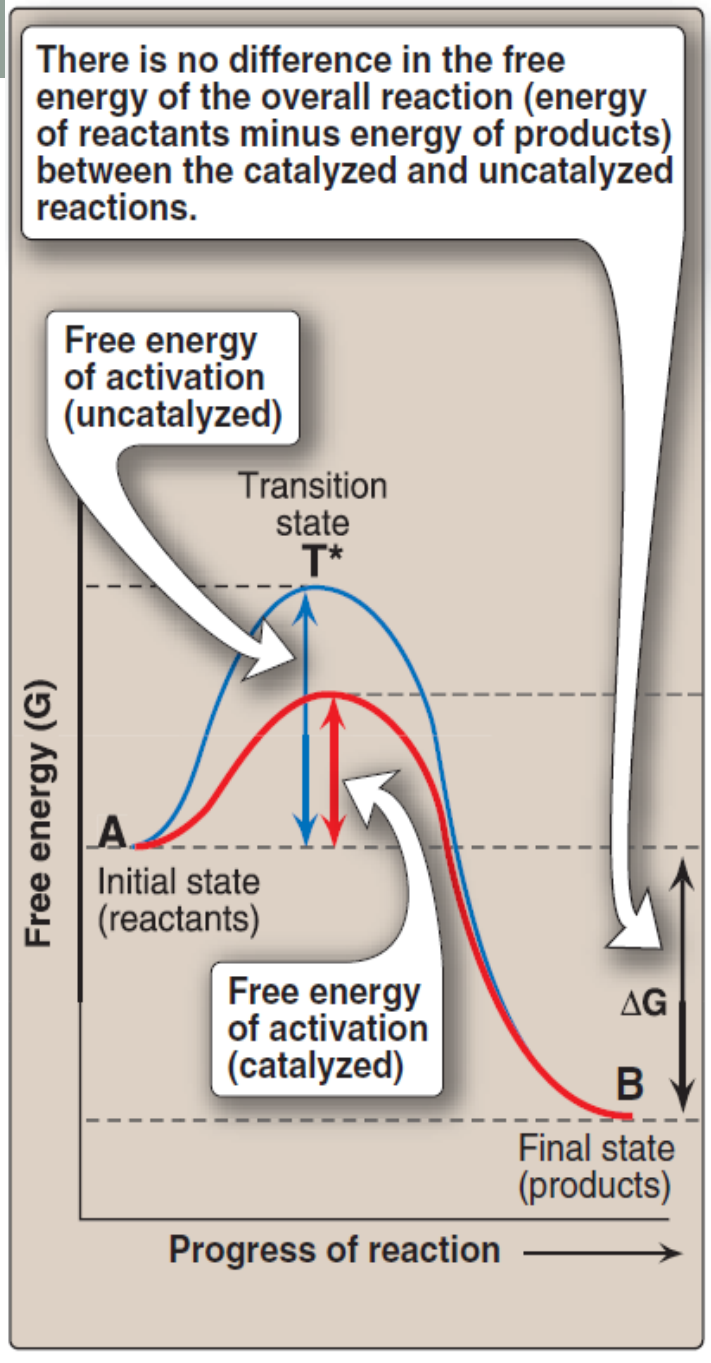


Figure 1.4 Effect of an enzyme on the activation energy of a reaction.

2. Rate of reaction:

- For molecules to react, they must contain sufficient energy to overcome the energy barrier of the transition state.
- In the absence of an enzyme, only a small proportion of a population of molecules may possess enough energy to achieve the transition state between reactant and product.
- The rate of reaction is determined by the number of such energized molecules.
- In general, the lower the free energy of activation, the more molecules have sufficient energy to pass through the transition state, and, thus, the faster the rate of the reaction.

3. Alternate reaction pathway:

- An enzyme allows a reaction to proceed rapidly under conditions prevailing in the cell by providing an alternate reaction pathway with a lower free energy of activation (Figure 1.4).
- The enzyme does not change the free energies of the reactants or products and, therefore, does not change the equilibrium of the reaction. It does, however, accelerate the rate with which equilibrium is reached.

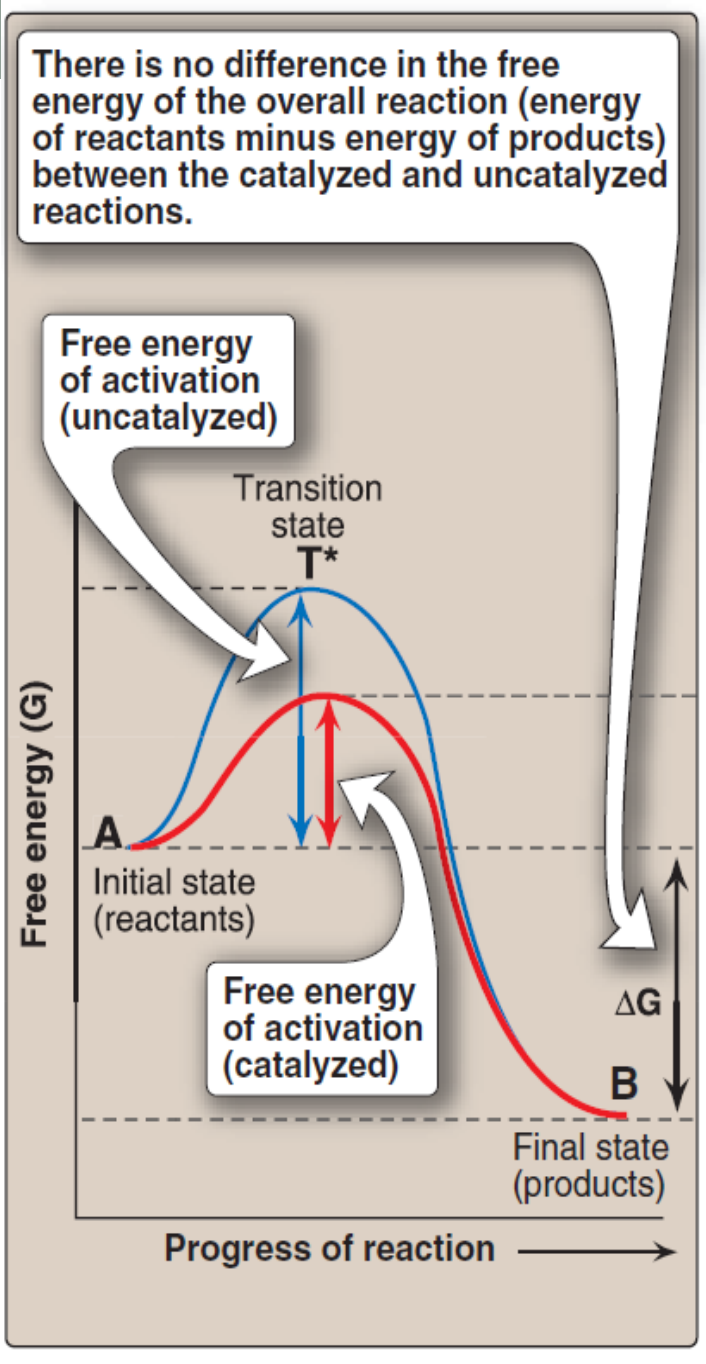
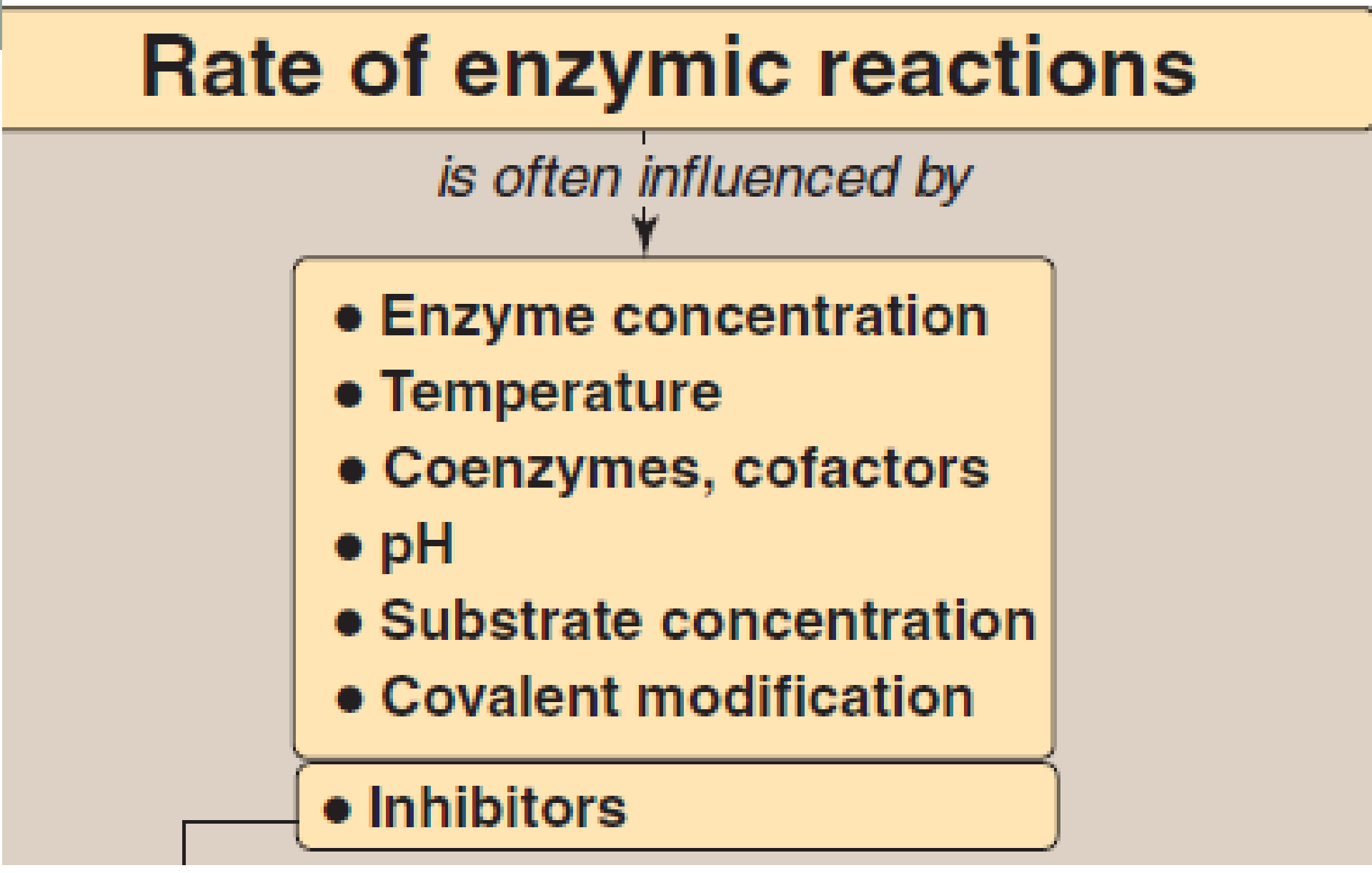


Figure 1.4 Effect of an enzyme on the activation energy of a reaction.

IV. FACTORS AFFECTING REACTION VELOCITY

- Enzymes can be isolated from cells, and their properties studied in a test tube (that is, *in vitro*).
- Different enzymes show different responses to changes in substrate concentration, temperature, and pH.
- This section describes factors that influence the reaction velocity of enzymes.
- Enzymic responses to these factors give us valuable clues as to how enzymes function in living cells (that is, *in vivo*).



- Inhibitor: An inhibitor is a substance that diminishes the rate of a chemical reaction; the process is called inhibition.

A. Substrate concentration

1. Maximal velocity:

- The rate or velocity of a reaction (v) is the number of substrate molecules converted to product per unit time; velocity is usually expressed as μmol of product formed per minute.

- The rate of an enzyme-catalyzed reaction increases with substrate concentration until a maximal velocity (V_{max}) is reached (Figure 1.5).

- The leveling off of the reaction rate at high substrate concentrations reflects the saturation with substrate of all available binding sites on the enzyme molecules present.

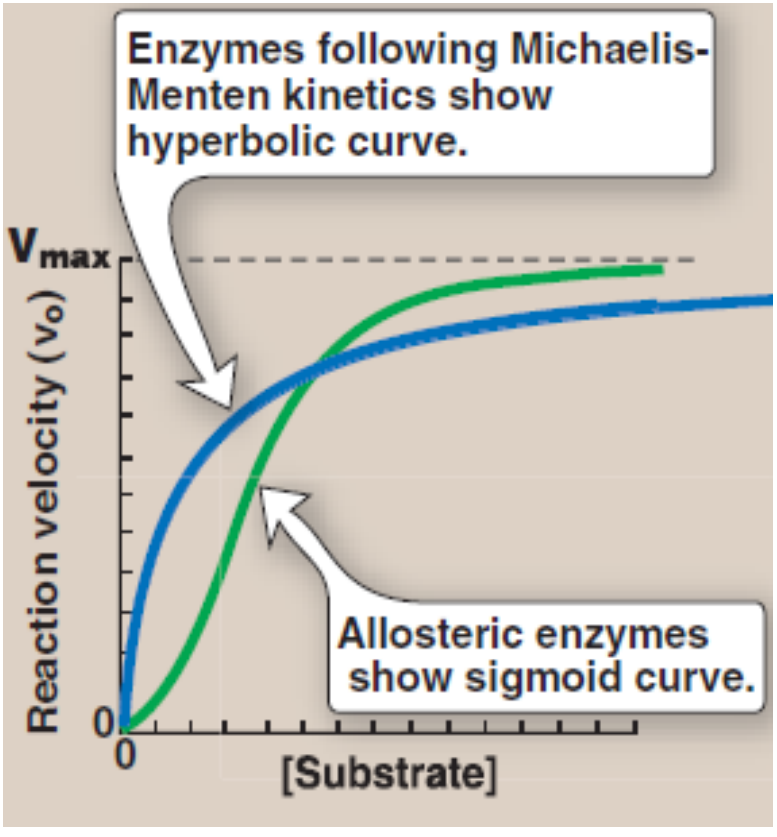
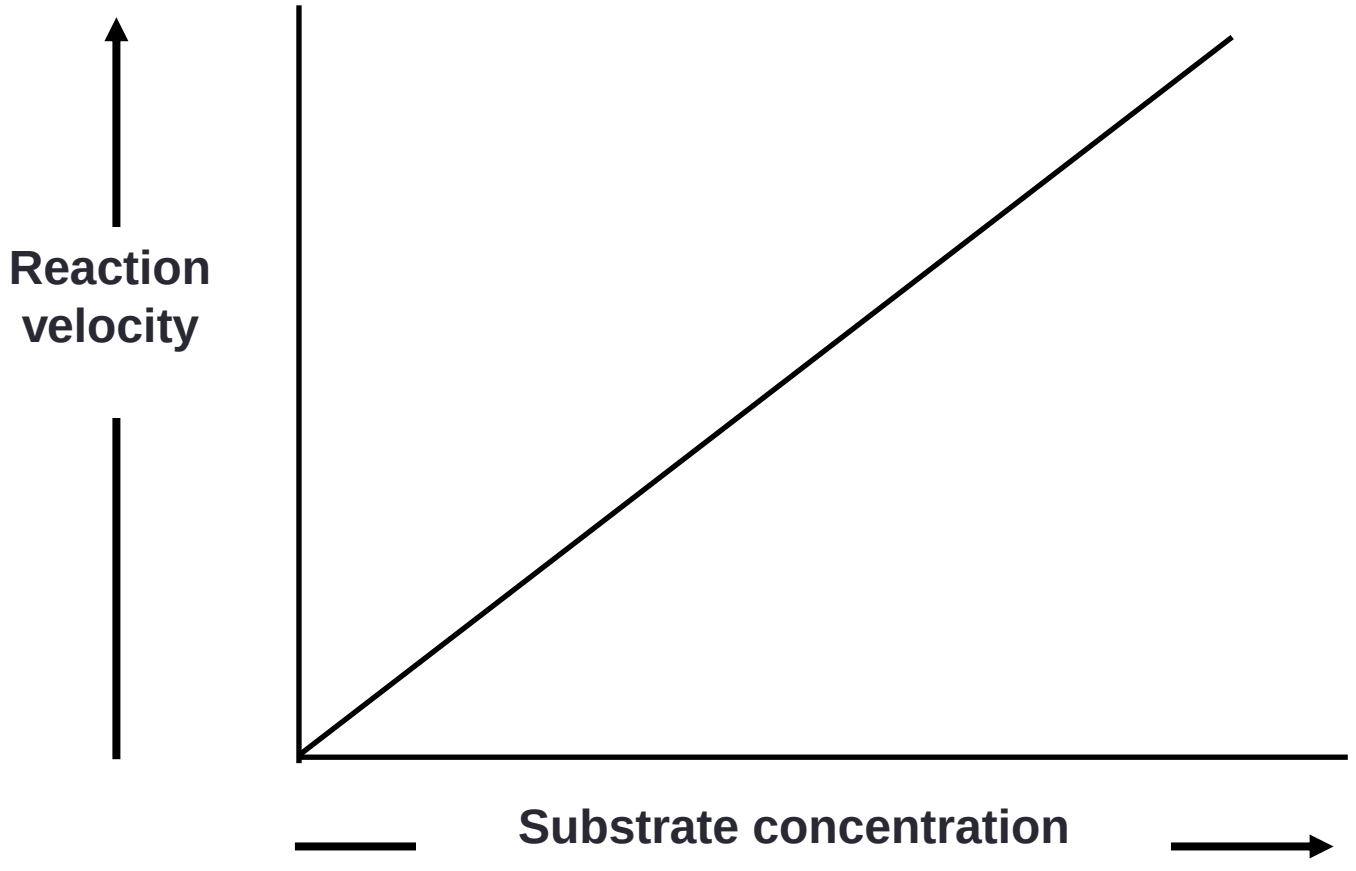


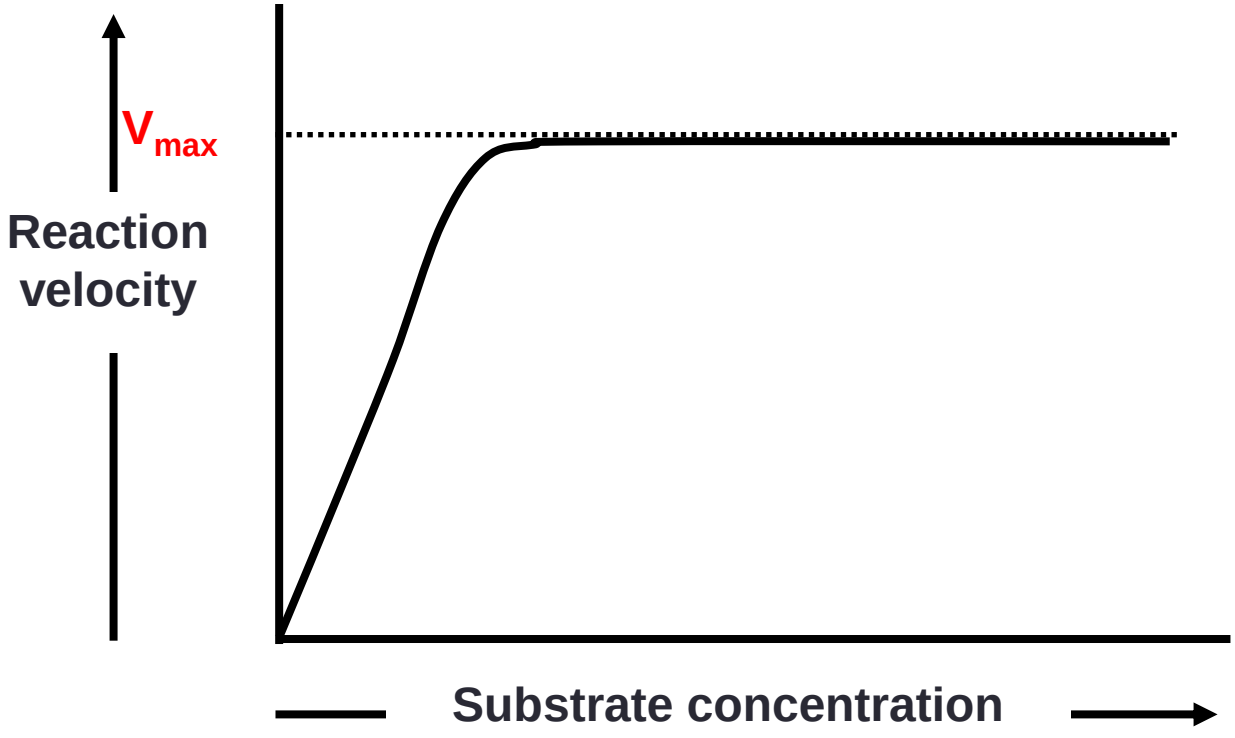
Figure 1.5 Effect of substrate concentration on reaction velocity.

Substrate concentration: Non-enzymic reactions



- The increase in velocity is proportional to the substrate concentration.

Substrate Concentration: Enzymic Reactions



- Faster reaction but it reaches a **saturation point** when all the enzyme molecules are occupied
- Alter the concentration of the **enzyme** then $\mathbf{V_{max}}$ will change too.

2. Hyperbolic shape of the enzyme kinetics curve:

- Most enzymes show Michaelis-Menten kinetics, in which the plot of initial reaction velocity (v_o) against substrate concentration ([S]), is hyperbolic.

- In contrast, allosteric enzymes do not follow Michaelis-Menton kinetics and show a sigmoidal curve that is similar in shape to the oxygen dissociation curve of hemoglobin.

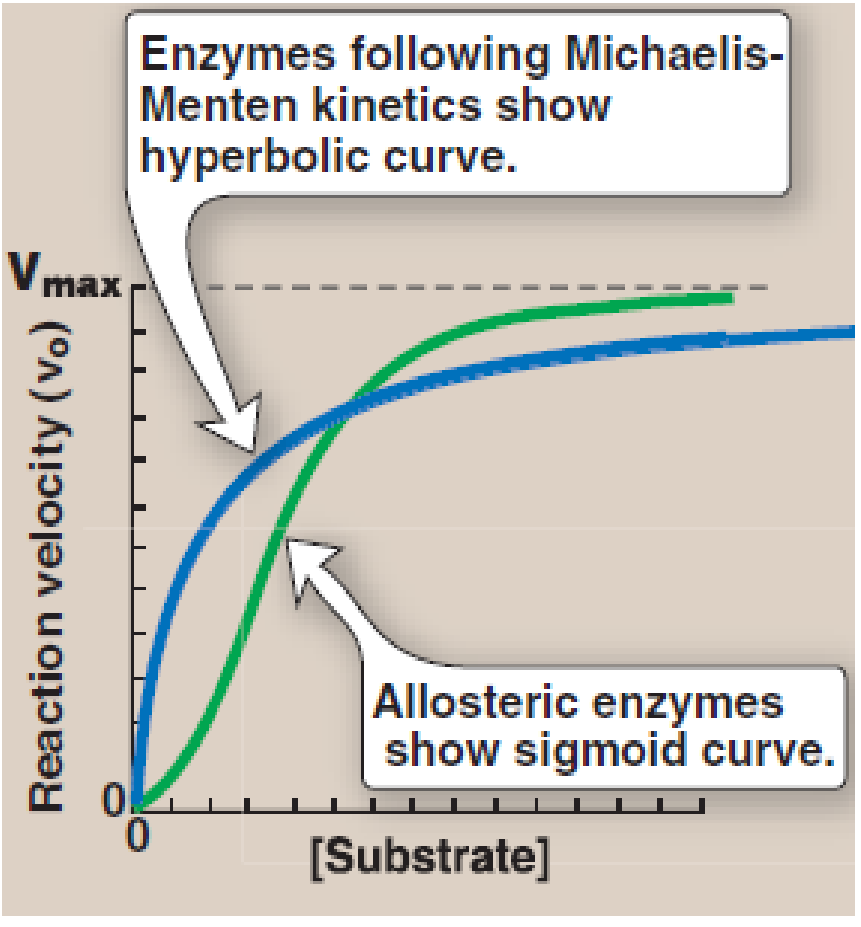


Figure 1.5 Effect of substrate concentration on reaction velocity.

B. Temperature

1. Increase of velocity with temperature:

- The reaction velocity increases with temperature until a peak velocity is reached (Figure 1.6).
- This increase is the result of the increased number of molecules having sufficient energy to pass over the energy barrier and form the products of the reaction.

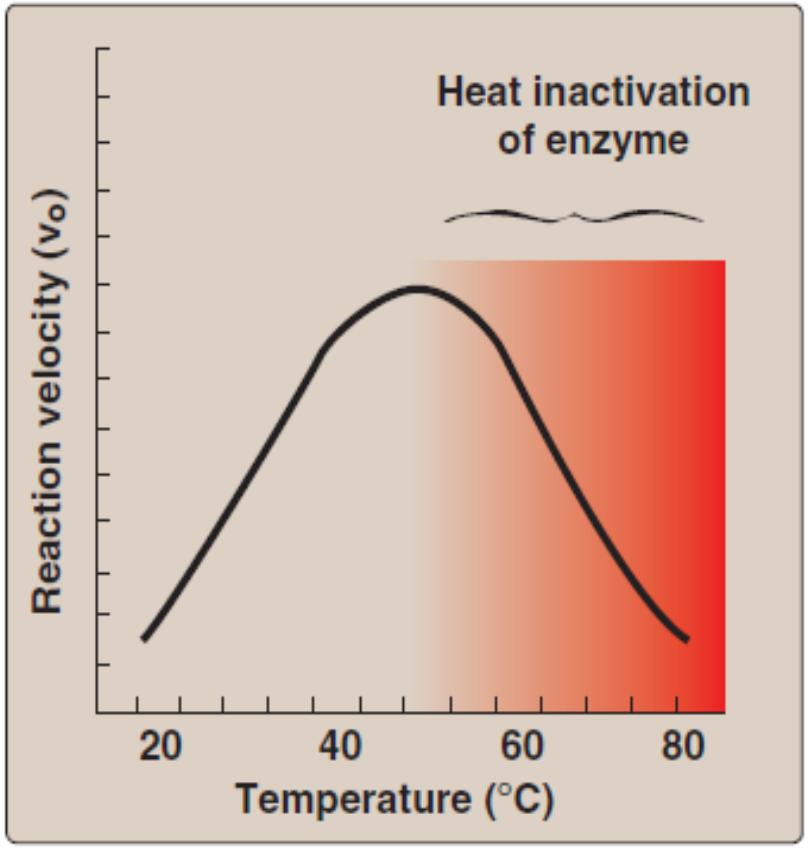


Figure 1.6 Effect of temperature on an enzyme catalyzed reaction.

2. Decrease of velocity with higher temperature:

- Further elevation of the temperature results in a decrease in reaction velocity as a result of temperature-induced denaturation of the enzyme (see Figure 1.6).

- The optimum temperature for most human enzymes is between 35 and 40°C.

- Human enzymes start to denature at temperatures above 40°C, but thermophilic bacteria found in the hot springs have optimum temperatures of 70°C.

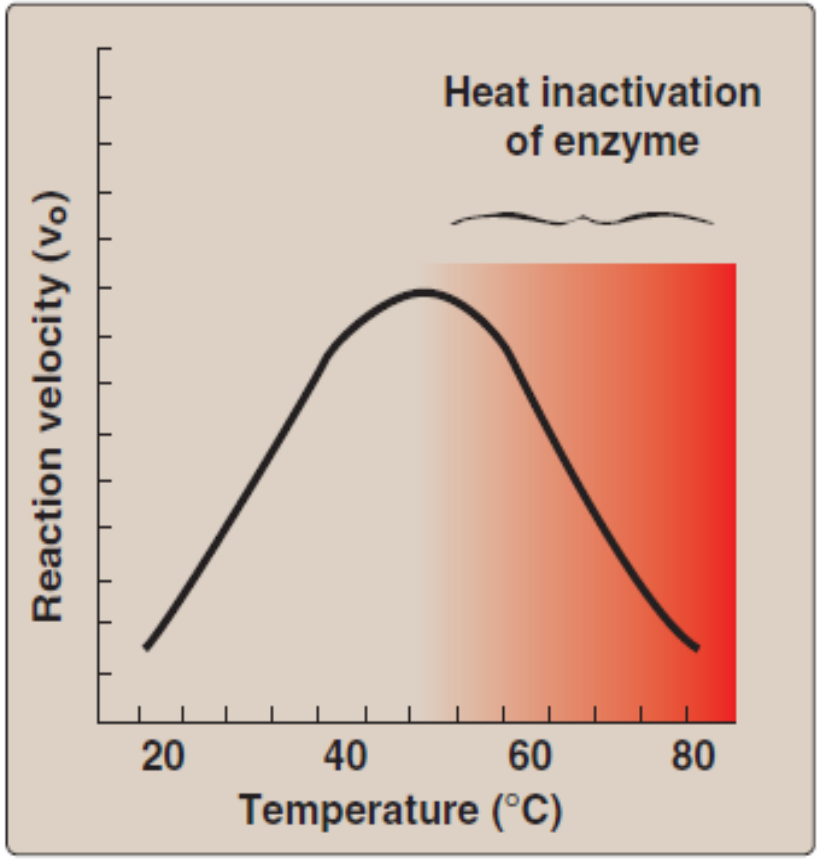
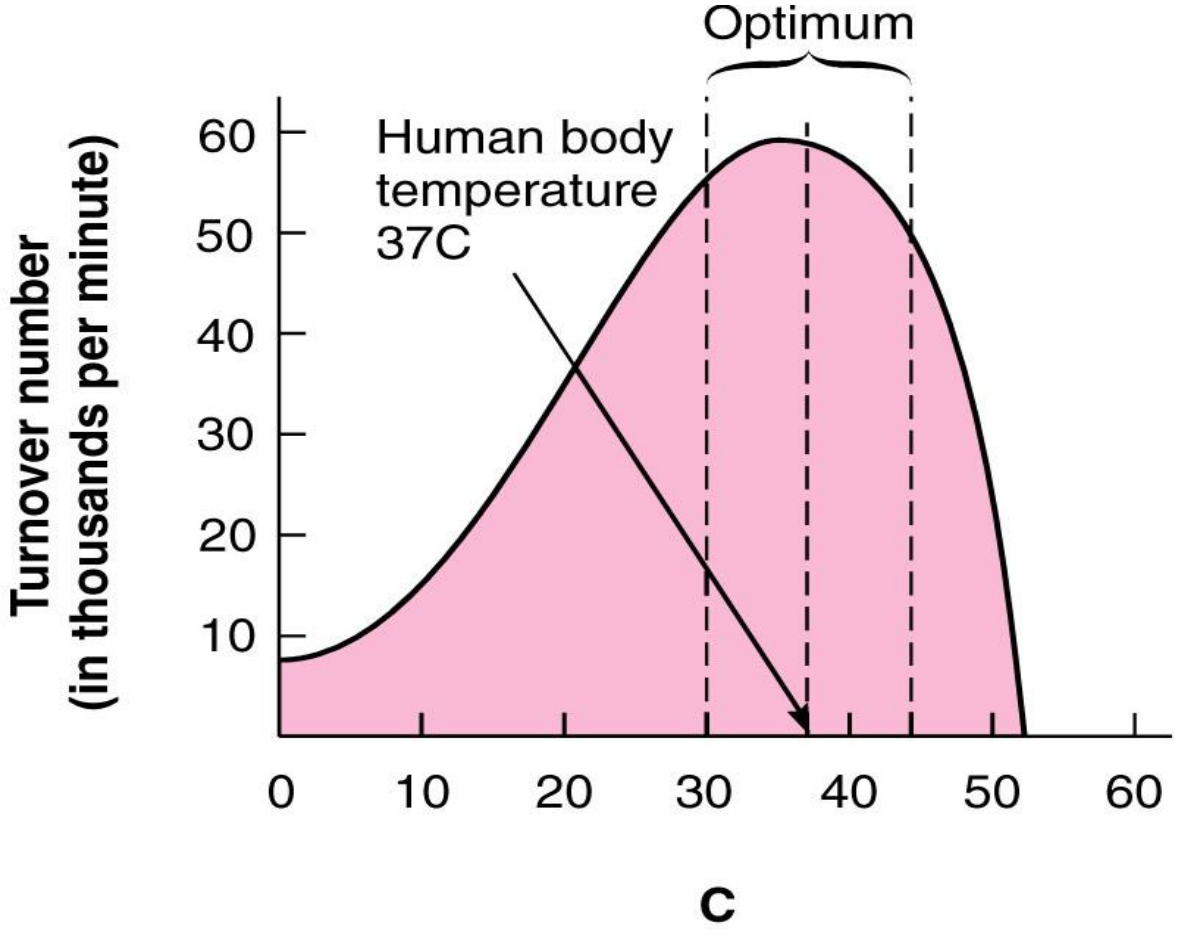


Figure 1.6 Effect of temperature on an enzyme catalyzed reaction.

Optimum Temperature

- ***Optimum temperature*** is the temperature at which enzymatic reaction occur fastest.



C. pH

1. Effect of pH on the ionization of the active site:

- The concentration of H^+ affects reaction velocity in several ways.
- First, the catalytic process usually requires that the enzyme and substrate have specific chemical groups in either an ionized or un-ionized state in order to interact.
- For example, catalytic activity may require that an amino group of the enzyme be in the protonated form ($–NH^{3+}$). At alkaline pH, this group is deprotonated, and the rate of the reaction, therefore, declines.

2. Effect of pH on enzyme denaturation:

- Extremes of pH can also lead to denaturation of the enzyme, because the structure of the catalytically active protein molecule depends on the ionic character of the amino acid side chains.

3. The pH optimum varies for different enzymes:

- The pH at which maximal enzyme activity is achieved is different for different enzymes, and often reflects the $[H^+]$ at which the enzyme functions in the body.

- For example, pepsin, a digestive enzyme in the stomach, is maximally active at pH 2, whereas other enzymes, designed to work at neutral pH, are denatured by such an acidic environment (Figure 1.7).

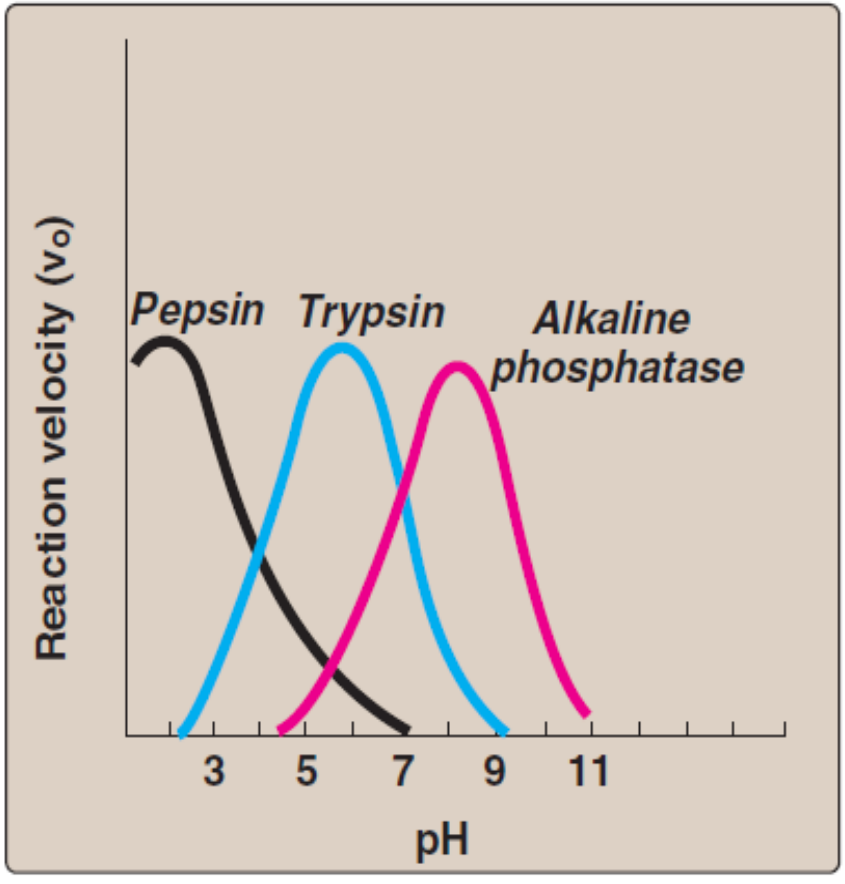
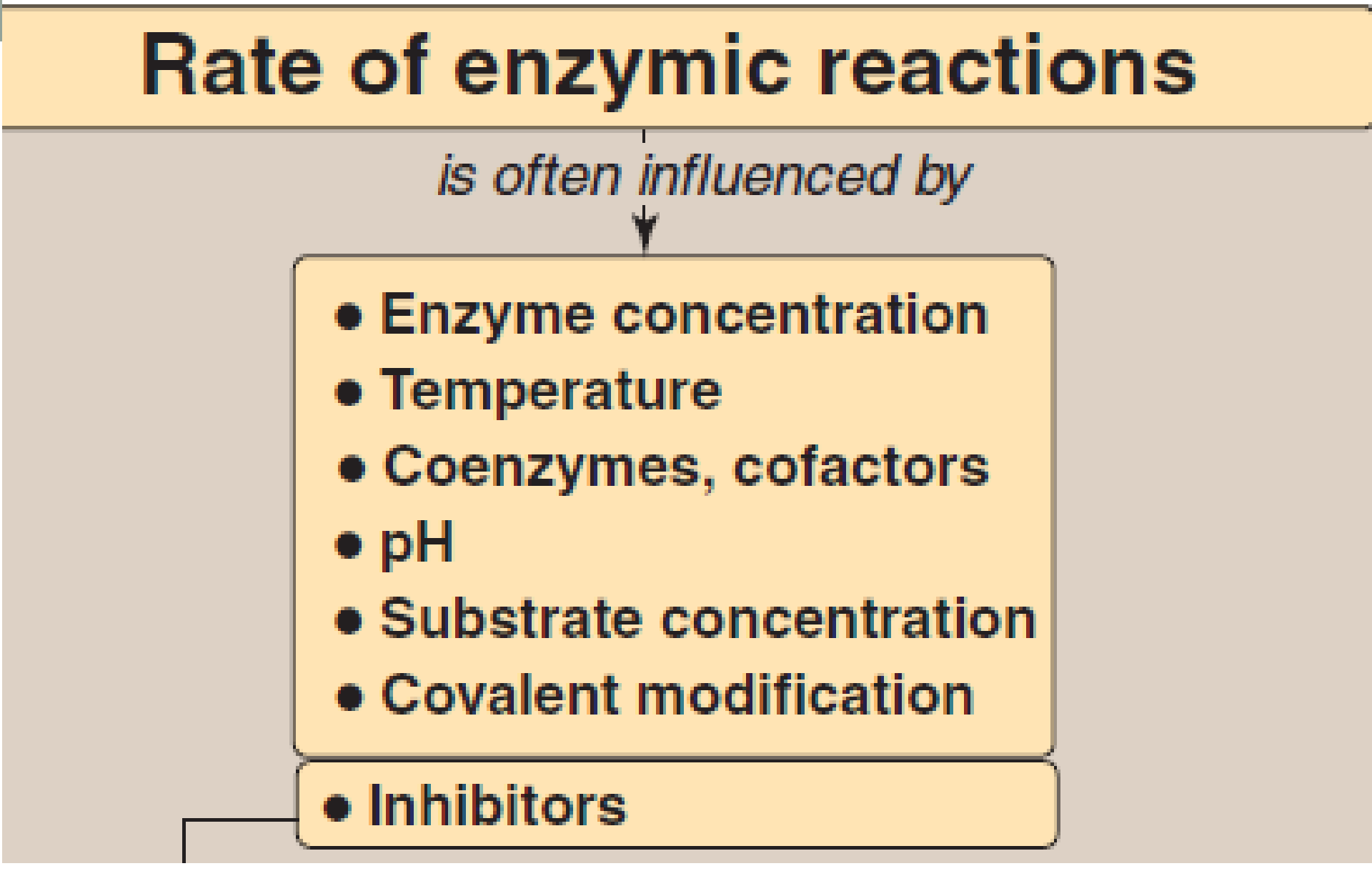


Figure 1.7 Effect of pH on enzyme-catalyzed reactions.



- Inhibitor: An inhibitor is a substance that diminishes the rate of a chemical reaction; the process is called inhibition.

نشاط (1/2/1) تمرين متعدد الخيارات

Multiple Choice Questions (MCQ)

Choose the ONE correct answer.

1. Enzymes are potent catalysts because they:
   - A. are consumed in the reactions they catalyze.
   - B. are very specific and can prevent the conversion of products back to substrates.
   - C. drive reactions to completion while other catalysts drive reactions to equilibrium.
   - D. increase the equilibrium constants for the reactions they catalyze.
   - E. lower the activation energy for the reactions they catalyze.
2. The role of an enzyme in an enzyme-catalyzed reaction is to:
   - A. bind a transition state intermediate, such that it cannot be converted back to substrate.
   - B. ensure that all of the substrate is converted to product.
   - C. ensure that the product is more stable than the substrate.
   - D. increase the rate at which substrate is converted into product.
   - E. make the free-energy change for the reaction more favorable.
3. Which one of the following statements is true of enzyme catalysts?
   - A. They bind to substrates, but are never covalently attached to substrate or product.
   - B. They increase the equilibrium constant for a reaction, thus favoring product formation.
   - C. They increase the stability of the product of a desired reaction by allowing ionizations, resonance, and isomerizations not normally available to substrates.
   - D. They lower the activation energy for the conversion of substrate to product.
   - E. To be effective they must be present at the same concentration as their substrates.

نشاط (1/ 2/2) تمرين التطابق

Match each term to its definition

1	Oxidoreductases	A	Catalyze formation of bonds between carbon and O, S, N coupled to hydrolysis of high energy Phosphates.
2	Transferases	B	Catalyze racemization of optical or geometric isomers.
3	Hydrolases	C	Catalyze cleavage of C–C, C–S and certain C–N bonds.
4	Lyases	D	Catalyze cleavage of bonds by addition of water.
5	Isomerases	E	Catalyze transfer of C-, N-, or P containing groups.
6	Ligases	F	Catalyze oxidation-reduction reactions.

Answer:

1. ……..... 2. …… 3. …… 4……

5…… 6……..