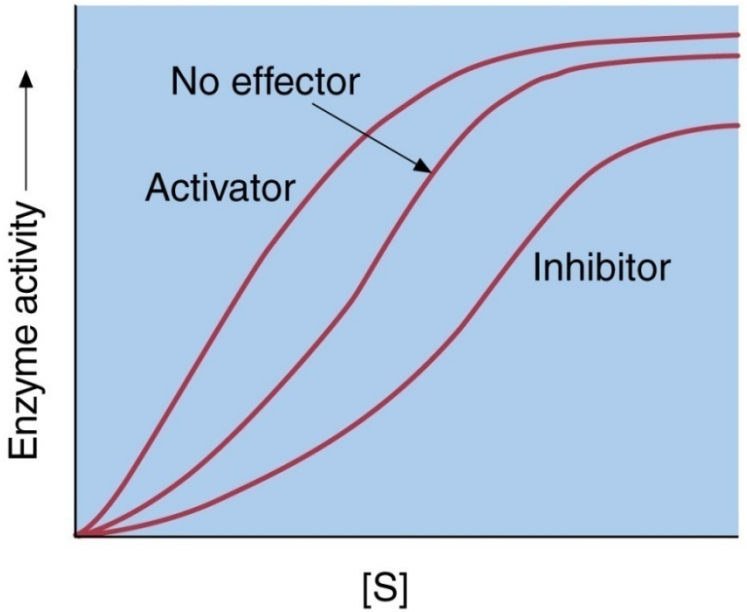
Chapter 6

Overview

Catalysis – most important function of proteins

- **Enzymes** – protein catalysts
  - Globular protein
  - Increase rate of metabolic processes
  - **Enzymes kinetics** – info on reaction rates & measure of affinity

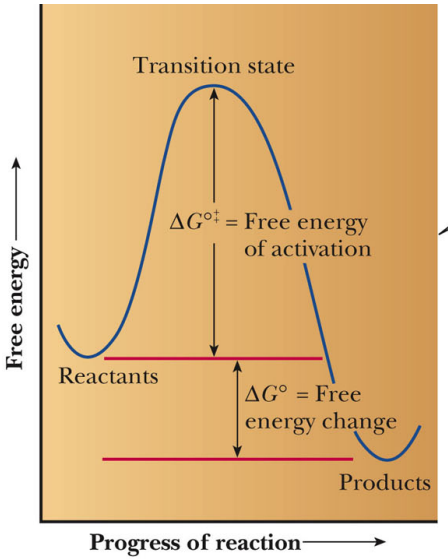


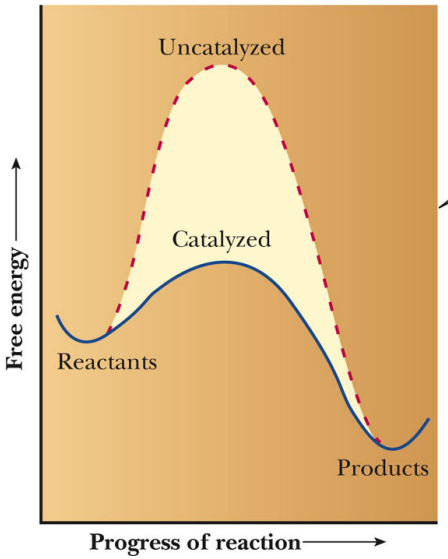
Key Characteristics:

- Increase reaction rate by lowering E_a
  - Obey laws of thermodynamics – no effect on K_{eq} values
  - Rate depends on ΔG^o of activation
    - Occurs when colliding molecules possess a minimum amount of energy called the **activation energy** (E_a)
  - Increase rate up to 10^{20}
  - Increases forward and reverse reactions equally
  - Most reactions require an initial energy input
- Present in low concentrations – not consumed
- Controlled by regulator mechanisms
- Active sites – bind transition state of reacting substrates
  - ✓ Very specific catalyzing only 1 stereoisomer while others catalyze a family of similar reactions
  - ✓ Negligible formation of side products

Lowering activation energy

- **Free energy of activation ($\Delta G^{o\ddagger}$)** -amount of energy to convert 1 mol of **substrate** (reactant) from the ground state to the **transition state**
- **Transition state** – highest energy, least stable
- **Free energy change (ΔG^o)** – energy released from reaction





Enzyme-catalyzed reaction

- **Substrate**, S, binds to enzyme forming ES complex
- **Active site**: the small portion of the enzyme surface
  - Frequently in a cleft or crevice
  - Optimally orient the substrate to achieve the transition state at a lower energy

- Binds by noncovalent forces
  - Hydrogen bonding, electrostatic attractions, van der Waals attractions
  - Essential amino acids in or near active site

$$E + S \leftarrow \rightarrow ES$$

Enzymes-substrate complex

Section 6.1: Properties of Enzymes

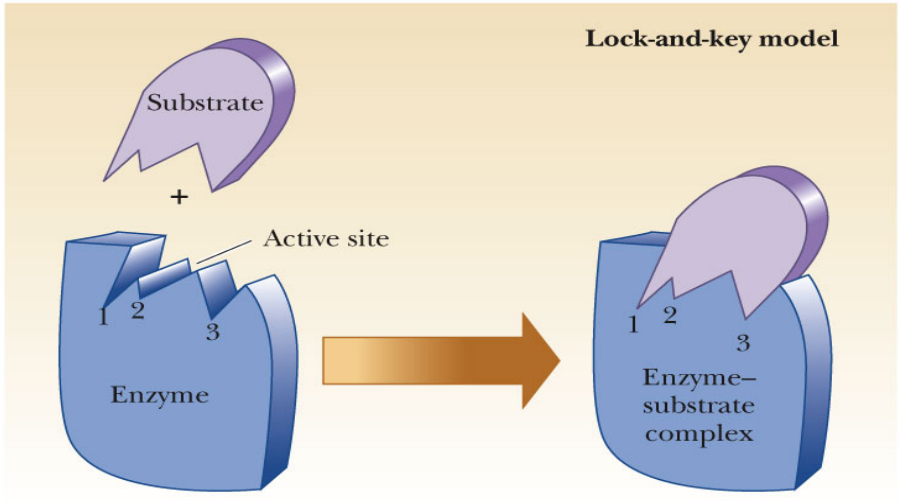
Two enzymatic models

- **Lock-and-key model:** substrate binds to portion of the enzyme with a complementary shape
- **Induced fit model:** binding of substrate induces a conformational change in the enzyme that results in a complementary fit

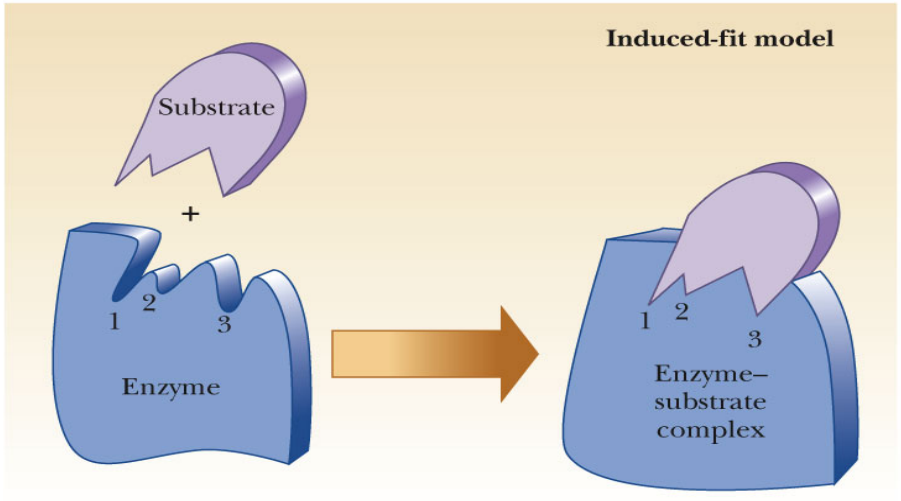
- Catalysis occurs in transition state
  - Bonds rearrange
    - Substrate close to atoms
    - Substrate in correct orientation

- Non-protein components
  - **Cofactors** – ions, Mg^{2+} or Zn^{2+}
  - **Coenzymes –** complex organic molecule
  - **Apoenzyme** - protein component lacking essential cofactor
  - **Holoenzyme –** enzymes with bound cofactors

2 Modes of E-S Complex Formation

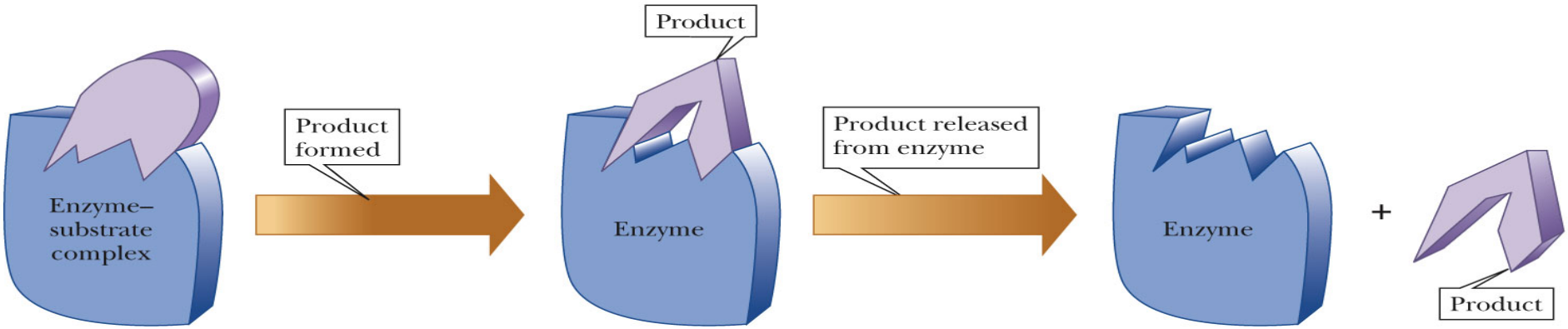


A In the lock-and-key model, the shape of the substrate and the conformation of the active site are complementary to one another.



B In the induced-fit model, the enzyme undergoes a conformational 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.

Product Formation



Section 6.2: Classification of Enzymes

1. **Oxidoreductases**

   oxidation-reduction reactions; oxidation state altered for 1 or more atoms; dehydrogenases, oxidases, reductases, hydroxylase, peroxidases

2. **Transferases**

   transfer molecular groups from donor molecule to acceptor molecule; transcarboxylases, transaminases

3. **Hydrolases**

   cleavage of bonds by addition of water; esterases, phosphataes

4. **Lyases**

   groups eliminated to form double bonds or added to double bonds; decarboxylases, hydratases

5. **Isomerases**

   intramolecular rearrangments; isomerases, mutases

6. **Ligases**

   bond formation between 2 substrate molecules; DNA ligase, synthetases

Section 6.3: Enzyme Kinetics

- **Enzyme kinetics** - quantitative study of enzyme catalysis; affinity for substrates and inhibitors
  - **Velocity** (rate) - change of a concentration of reactant or product per unit of time
  - **Initial velocity** (v_0) –beginning when concentration of S greatly exceeds E concentration, no P

For reaction: $A + B \rightarrow P$

- Rate of reaction is given by rate equation

$$\text{Rate} = -\frac{\Delta[A]}{\Delta t} = -\frac{\Delta[B]}{\Delta t} = \frac{\Delta[P]}{\Delta t}$$

- Rate of reaction at a given time

$$\text{rate} = K[A]^f[B]^g$$

  - **k is rate constant** - a proportionality constant
  - ***f,g*** reaction order for reactant
  - **[A]** – molar concentration

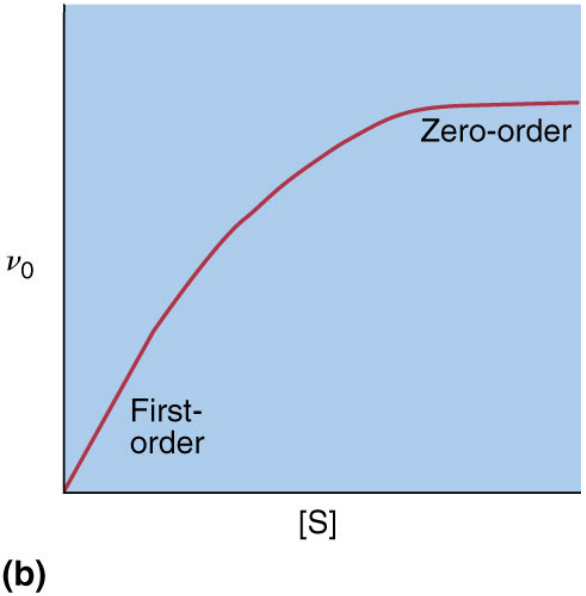


Figure 6.3b Enzyme Kinetic Studies

- **Order of reaction**: sum of exponents in the rate equation
  - Determined experimentally
- **First order** is unimolecular (no collisions required) (A → P)

$$\text{Rate} = k[A]^1$$

- **Second order** is bimolecular (A + B → P)

$$\text{Rate} = k[A]^1[B]^1$$

- Zero order – reactant independent; enzyme saturated
- Half-life -time for one-half of reactant molecules to be consumed

- **Michaelis-Menten Kinetics**
  - Simple reaction

    $S \rightarrow P$
  - Mechanism for an enzyme-catalyzed reaction

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

  - Michaelis constant K_m

$$K_m = \frac{k_{-1} + k_2}{k_1}$$

    - Lower the value of K_m, greater affinity of the enzyme for ES complex formation

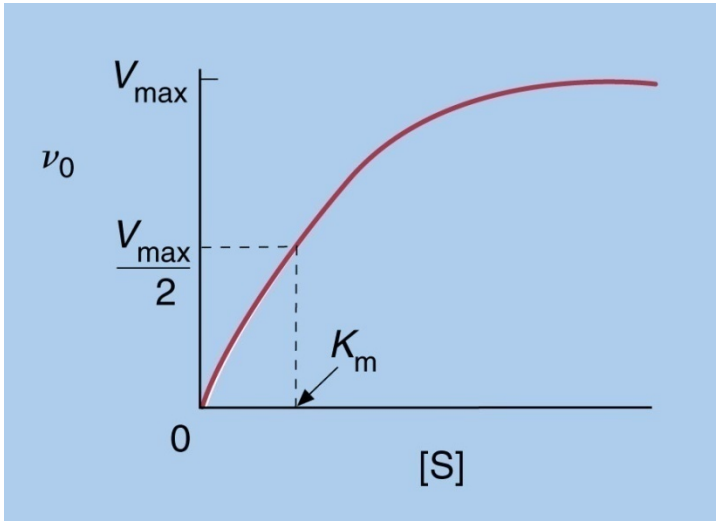


Figure 6.4 Initial Reaction Velocity v_0 and Substrate Concentration [S] for a Typical Enzyme-Catalyzed Reaction

- V_{max} - maximum velocity, E is saturated

$$v_0 = \frac{V_{max}[S]}{[S] + K_m}$$

Michaelis-Menten Equation

- **Turnover number,** k_{cat} - number substrate molecules converted to product per unit time
  - $K_{cat} = V_{max} / [E_t]$
- **Specificity constant,** (k_{cat}/K_m) – relationship between catalytic rate & substrate binding affinity

  Hi specificity constant = low K_m & hi K_{cat}

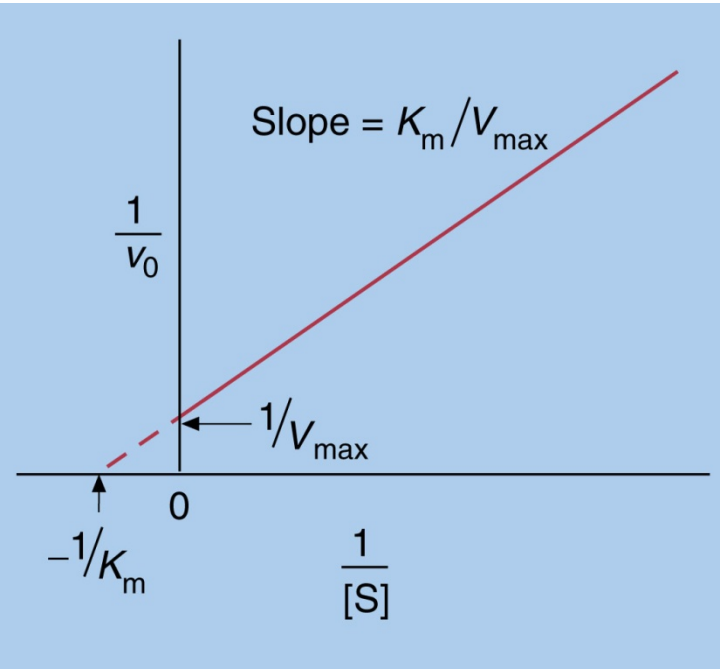


Figure 6.6 Lineweaver-Burk or Double-Reciprocal Plot

- Lineweaver-Burke plot has the form y = mx + b, formula for a straight line

$$\frac{1}{V} = \frac{K_M}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}}$$

$$y = m \cdot x + b$$

  - Slope of the line K_m/V_{max}
  - $1/V_{max}$ is the Y intercept
  - $-1/K_m$ is the X intercept

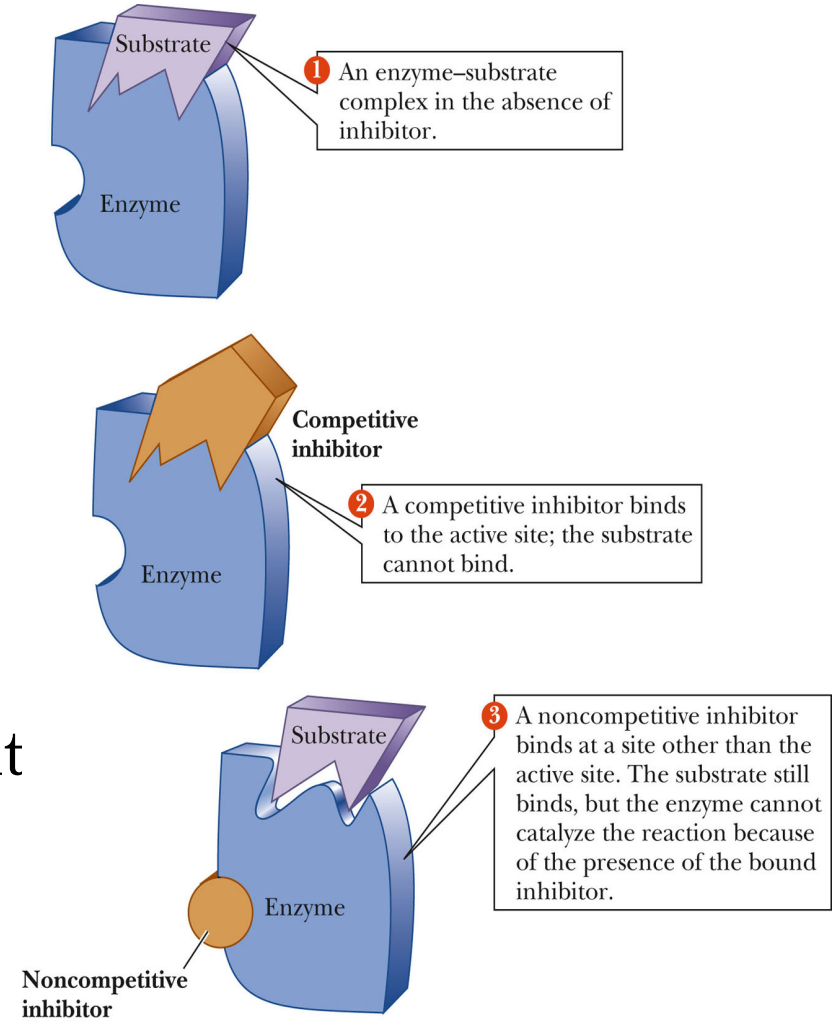
- **Multisubstrate Reactions** – 2 or more substrates
  - **Bisubstrate reactions**
    - **Sequential**—reaction cannot proceed until all substrates are bound to the enzyme active site
      - Ordered – 1st S must bind before 2nd
      - Random – substrates can bind in any order; product released in any order
    - **Double-Displacement Reactions**—first product is released before second substrate binds
      - Enzyme is altered by first phase of the reaction

- **Inhibitors** – substances that reduce enzyme activity
  - Drugs, antibiotics, food preservatives, poisons, metabolites of biochemical processes

- **Importance of inhibition**
  - Metabolic pathway regulation
  - Clinical therapies are based on enzyme inhibition
  - Functional properties & physical/chemical architecture

Section 6.3: Enzyme Kinetics

- **Reversible inhibitor**: substance binds causing inhibition, can be released
  - Competitive inhibitor: binds to the active (catalytic) site; blocks access
  - Noncompetitive inhibitor: binds to a site other than the active site; inhibits by changing conformation
  - Uncompetitive – binding site created after S is bound
- **Irreversible inhibitor**: substance causes permanent impairment
  - usually involves formation or breaking of covalent bonds to or on the enzyme



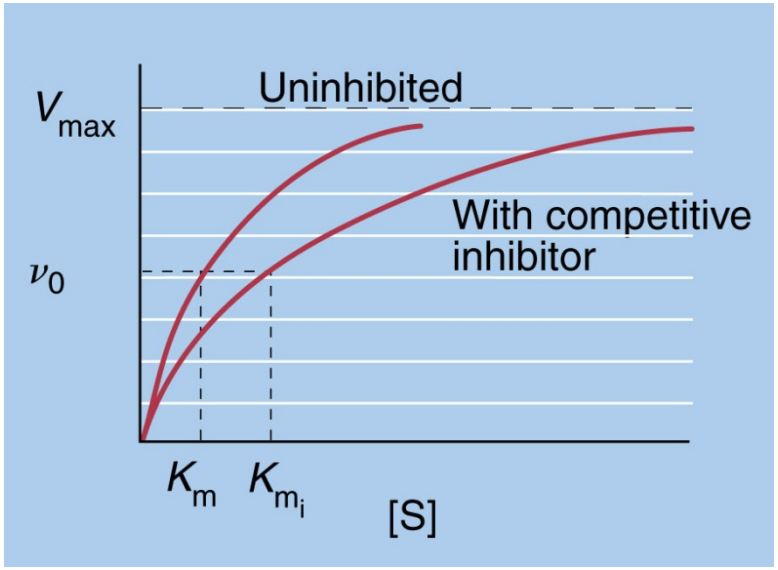


Figure 6.8 Michaelis-Menten Plot of Uninhibited Enzyme Activity Versus Competitive Inhibition

- **Competitive Inhibitors** bind reversibly to the enzyme at active site
  - Forms enzyme-inhibitor (EI) complex
  - Increasing S concentration overcomes competitive inhibition
    - ✓ Increases rate
  - V_{max} unchanged; K_{mI} increases

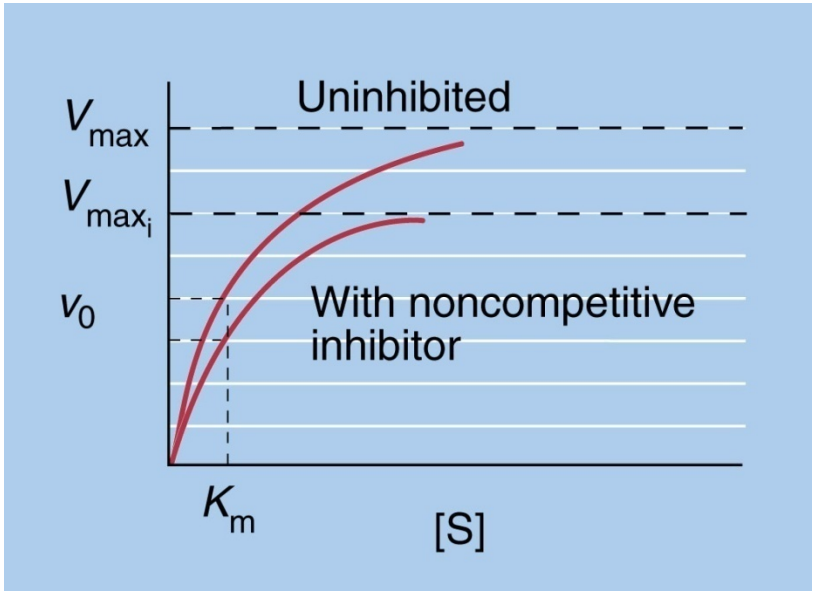
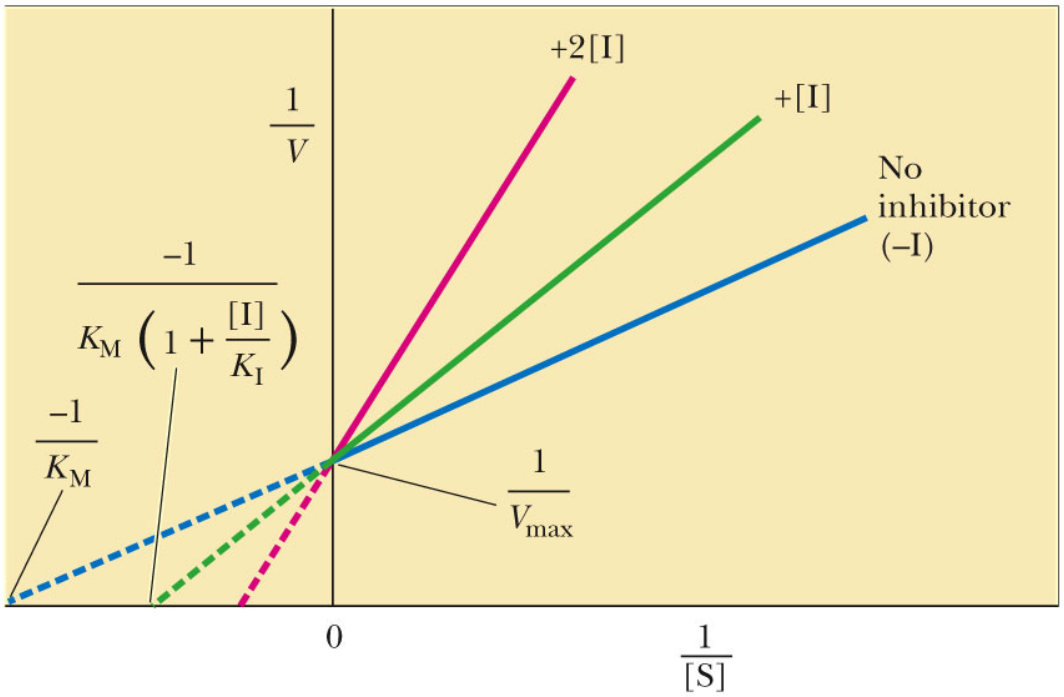
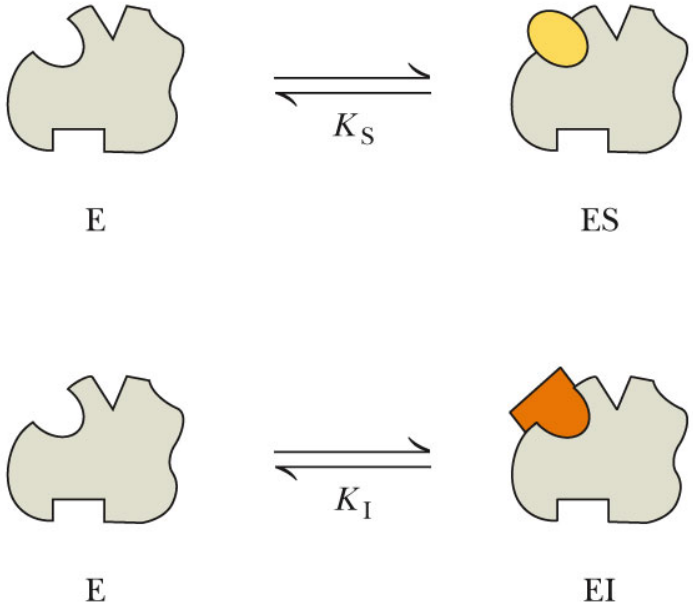


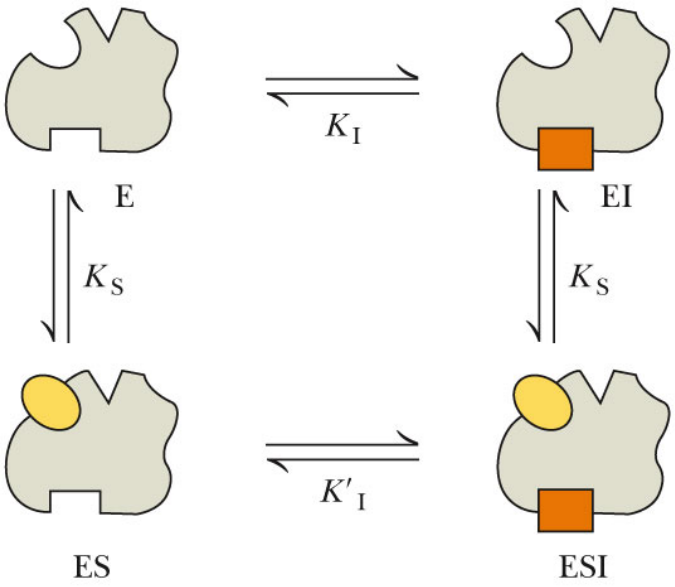
Figure 6.10 Michaelis-Menten Plot of Uninhibited Enzyme Activity Versus Noncompetitive Inhibition

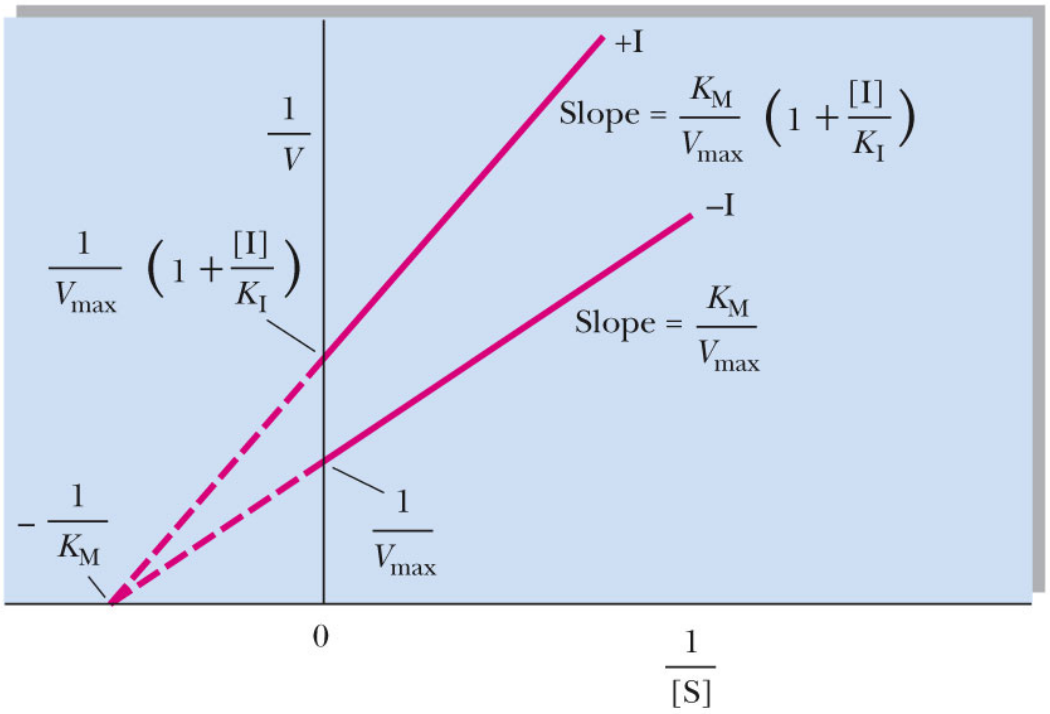
- **Noncompetitive Inhibitors** - bind reversibly at a site other than active site
  - Changes enzyme conformation
  - Increased substrate concentration partially reverses inhibition
  - V_{max} reduced; K_m unaffected





- **Competitive inhibition** increases K_m, not V_{max}





- **Pure noncompetitive** V_{max} lowered K_m unchanged

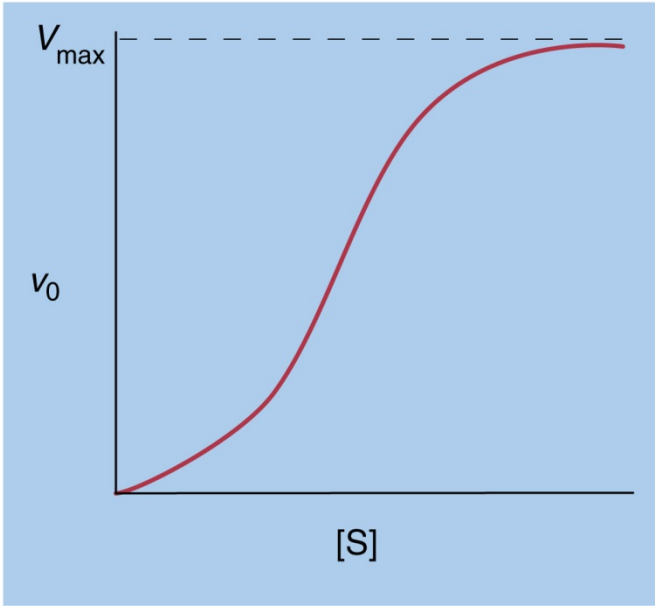


Figure 6.13 The Kinetic Profile of an Allosteric Enzyme

Allosteric enzyme: an oligomer whose biological activity is affected by other substances binding to it

- Substances change the enzyme's activity by altering the conformation(s) of its 3°-4°structure

Allosteric effector: a substance that modifies the behavior of an allosteric enzyme

- allosteric inhibitor – excess product
- allosteric activator – need product

- **Enzyme Kinetics, Metabolism**
  - Ultimate goal is understanding enzyme kinetics in living organisms
  - *In vitro* work does not always reflect *in vivo* reality
- **Macromolecular crowding** – cell interior highly heterogeneous; influences reaction rates and equilibrium constants in a non-linear way
  - Systems biologists are using computer modeling, *in vitro,* and *in vivo* data to overcome issues

- **Reaction mechanism** is a step-by-step description of a reaction
  - Electrons flow from a nucleophile to an electrophile

- One or more **intermediates** may form during the course of a reaction
  - **Free radicals** – highly reactive; at least 1 unpaired electron
  - **Carbocations** – electron deficient, positively charged carbon atom
  - **Carbanions** – nucleophilic carbon anions; 3 bonds & unshared electron pair

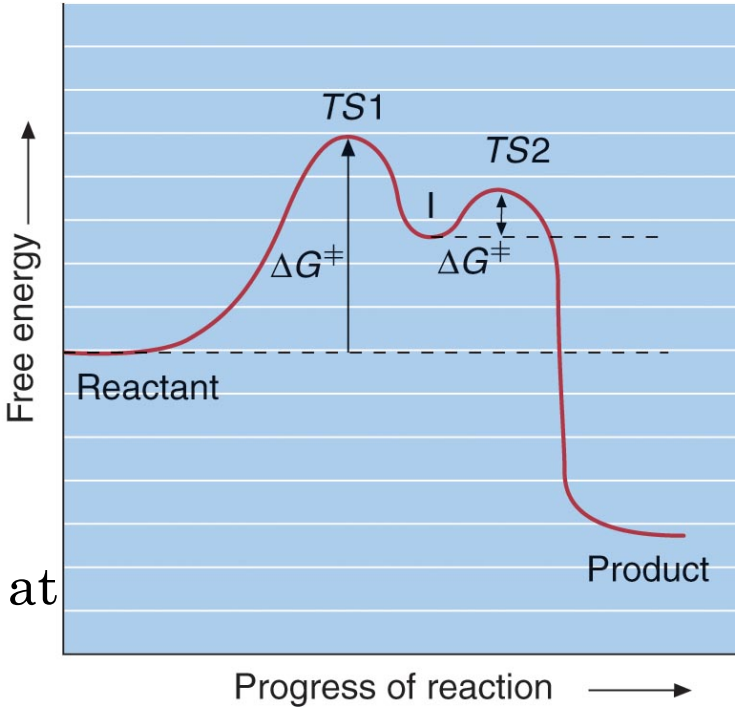
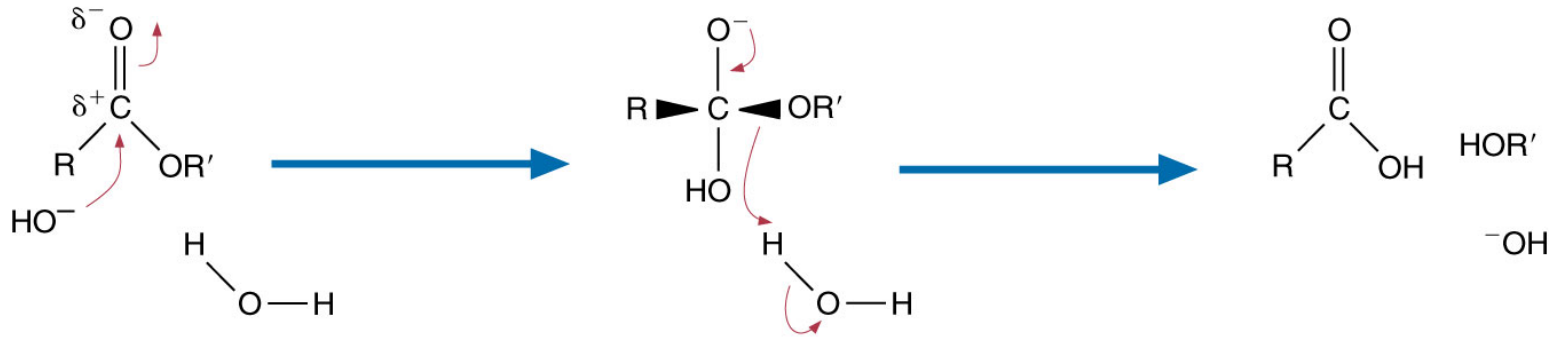


Figure 6.15 Energy Diagram for a Two-Step Reaction

Catalytic Mechanisms

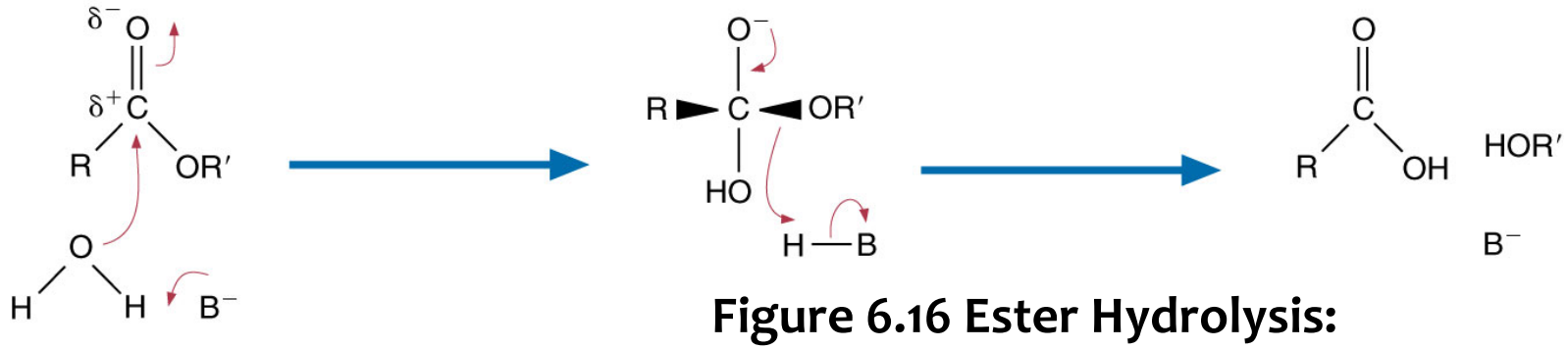
- **Proximity and Strain Effects** – substrate must come in close proximity to active site
- **Electrostatic Effects** – charge distribution may help position substrate



(a) Hydroxide ion catalysis

Figure 6.16 Ester Hydrolysis: Hydroxide Ion Catalysis

- **Acid-Base Catalysis**—proton transfer is an important factor in chemical reactions
  - Hydrolysis of an ester, for example, takes place better if the pH is raised
  - Hydroxide ion catalysis



(b) General base catalysis

Figure 6.16 Ester Hydrolysis: General Base Catalysis

- More physiological is the use of general bases and acids
- Side chains of many amino acids (e.g., histidine, lysine, and aspartate) can be used as general acids or bases
  - Depends on state of protonation, based on pK_a of functional groups

- **Covalent Catalysis**—formation of an unstable covalent bond with a nucleophilic group on the enzyme and an electrophilic group on the substrate

- **Roles of Amino Acids in Enzyme Catalysis**
  - Active sites of enzymes are lined with amino acids that create a microenvironment conducive to catalysis
  - Residues can be catalytic or noncatalytic
  - In order to participate in catalysis, the amino acid has to be charged or polar
    - For example: chymotrypsin (will discuss)
  - Noncatalytic side groups function to orient substrate or stabilize transition state

- **Role of Cofactors in Enzyme Catalysis**
  - **Metals**—important metals in living organisms are alkali metals (Na^+, K^+, Mg^{2+}, and Ca^{2+}) and transition metals (Zn^{2+}, Fe^{2+}, and Cu^{2+})
    - **Alkali metals** are usually loosely bound and play structural roles
    - **Transition metals** usually play a functional role in catalysis as part of a functional group
    - Metals are good Lewis acids and effective electrophiles

- **Coenzymes**—organic molecules that provide enzymes' chemical versatility
  - Contain functional groups that amino acid side chains do not
  - Can be tightly or loosely bound, structures are often changed by the catalytic process
  - Most are derived from vitamins
  - Three groups: electron transfer (NAD^+), group transfer (coenzyme A), and high-energy transfer potential (nucleotides)

Effects of Temperature

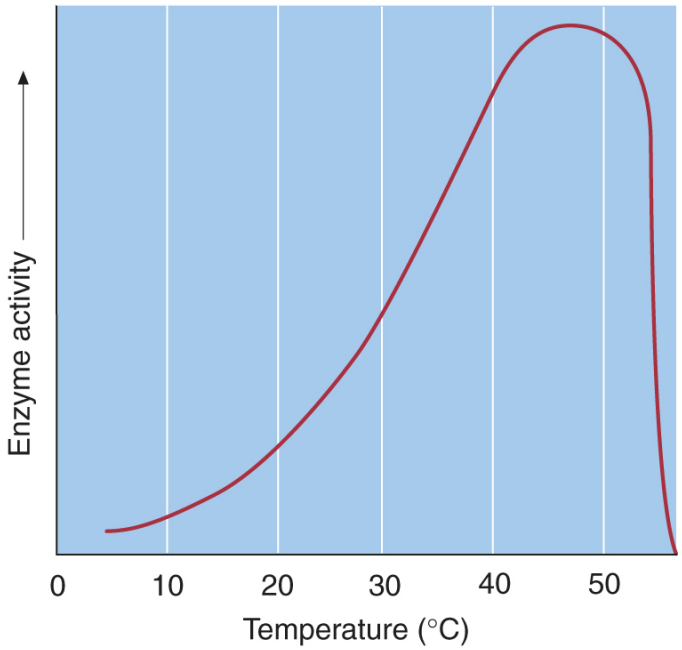


Figure 6.17 The Effect of Temperature on Enzyme Activity

- Higher the temperature, faster reaction rate; increased number of collisions
  - Enzymes are proteins denatured at high temperatures

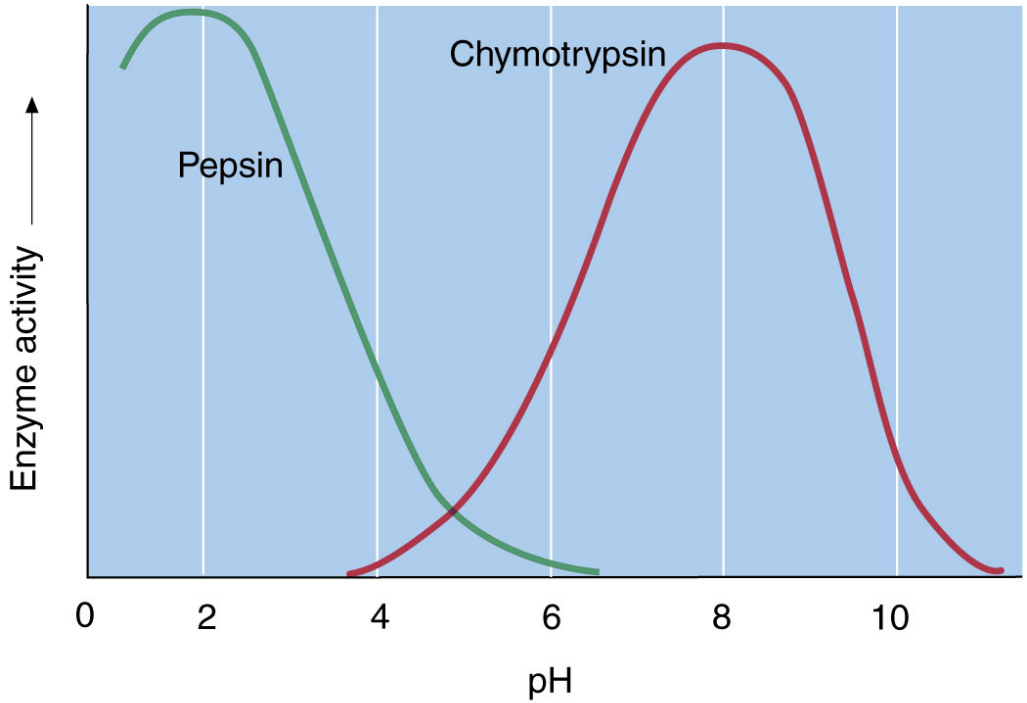


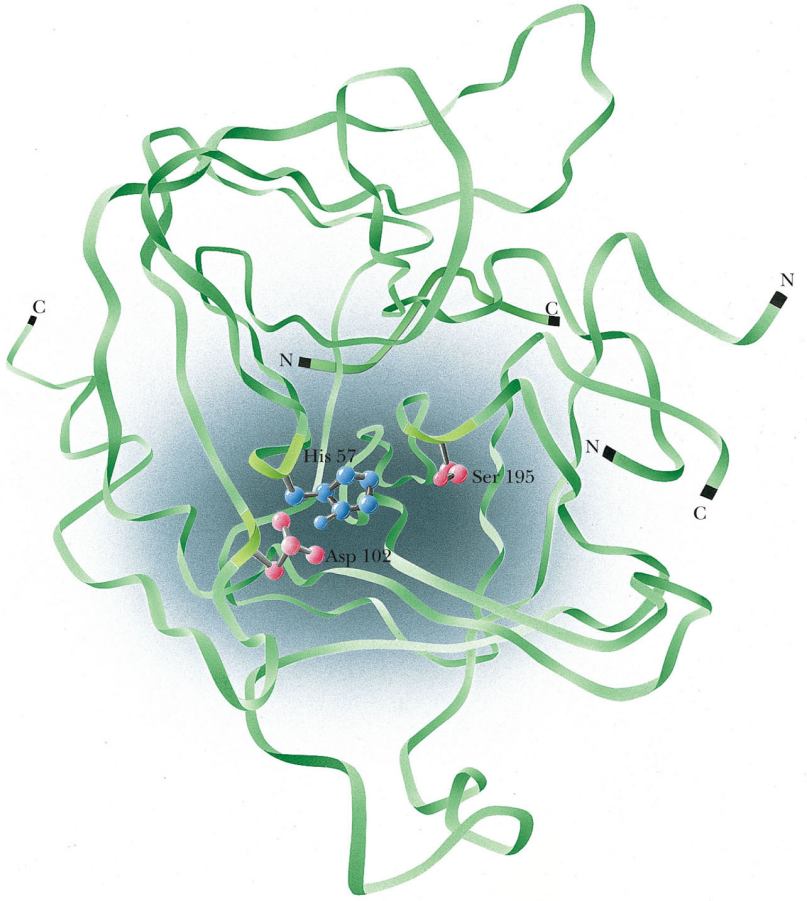
Figure 6.18 The Effect of pH on Two Enzymes

- **Effects of pH**
  - Catalytic activity is related to ionic state of the active site
  - Changes in ionizable groups could change structure of enzyme
  - **pH optimum – narrow range where enzyme functions**

Chymotrypsin—serine protease

- Serine proteases have a triad of amino acids in their active site (e.g., Asp 102, His 57, and Ser 195)
  - Hydrolyzes peptide bonds adjacent to aromatic amino acids

- Results of x-ray crystallography show the definite arrangement of amino acids
- Folding of backbone, mostly in antiparallel pleated sheet array, positions the essential amino acids around the active-site pocket



- Serine oxygen is nucleophile
- Attacks carbonyl group of peptide bond

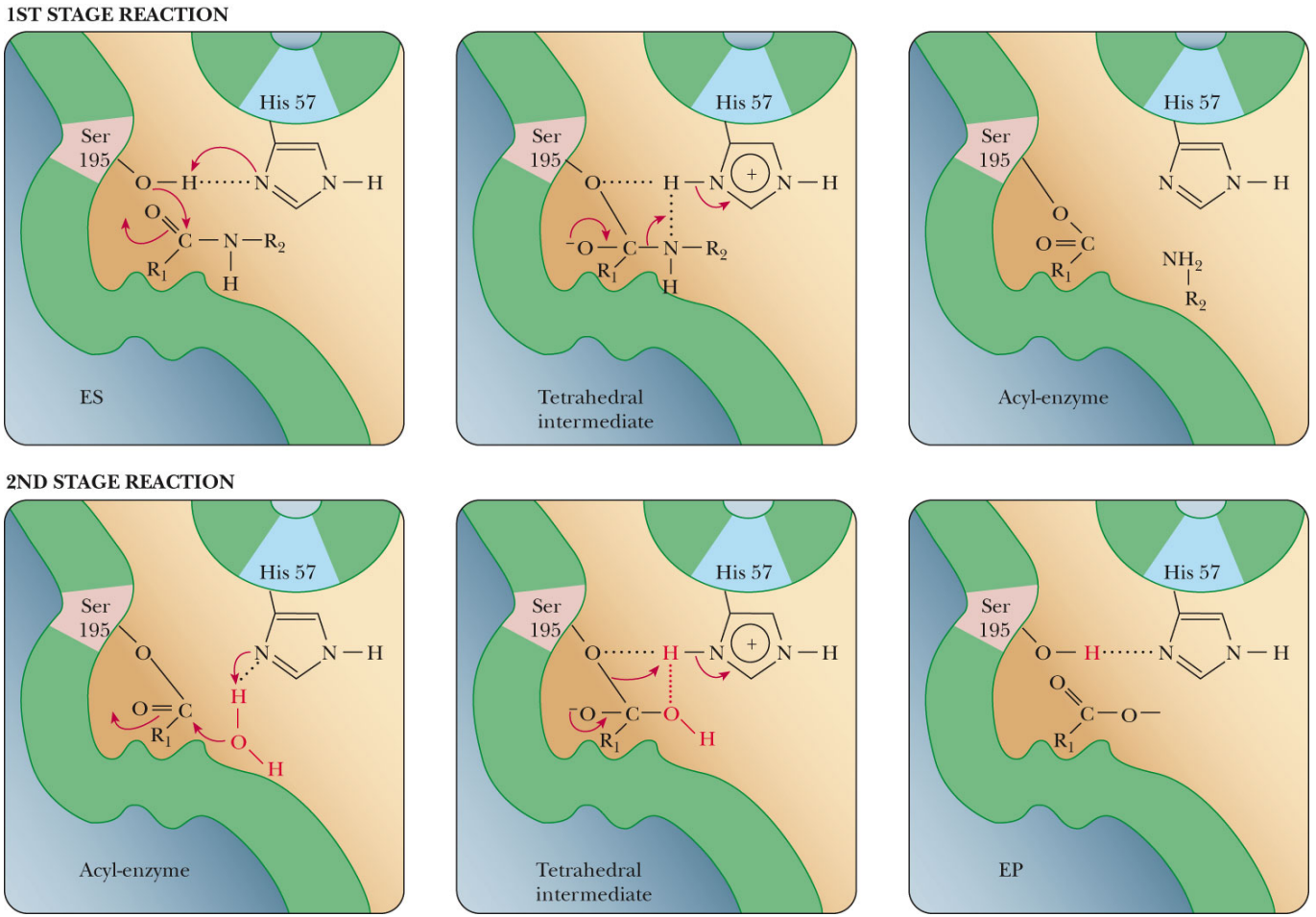
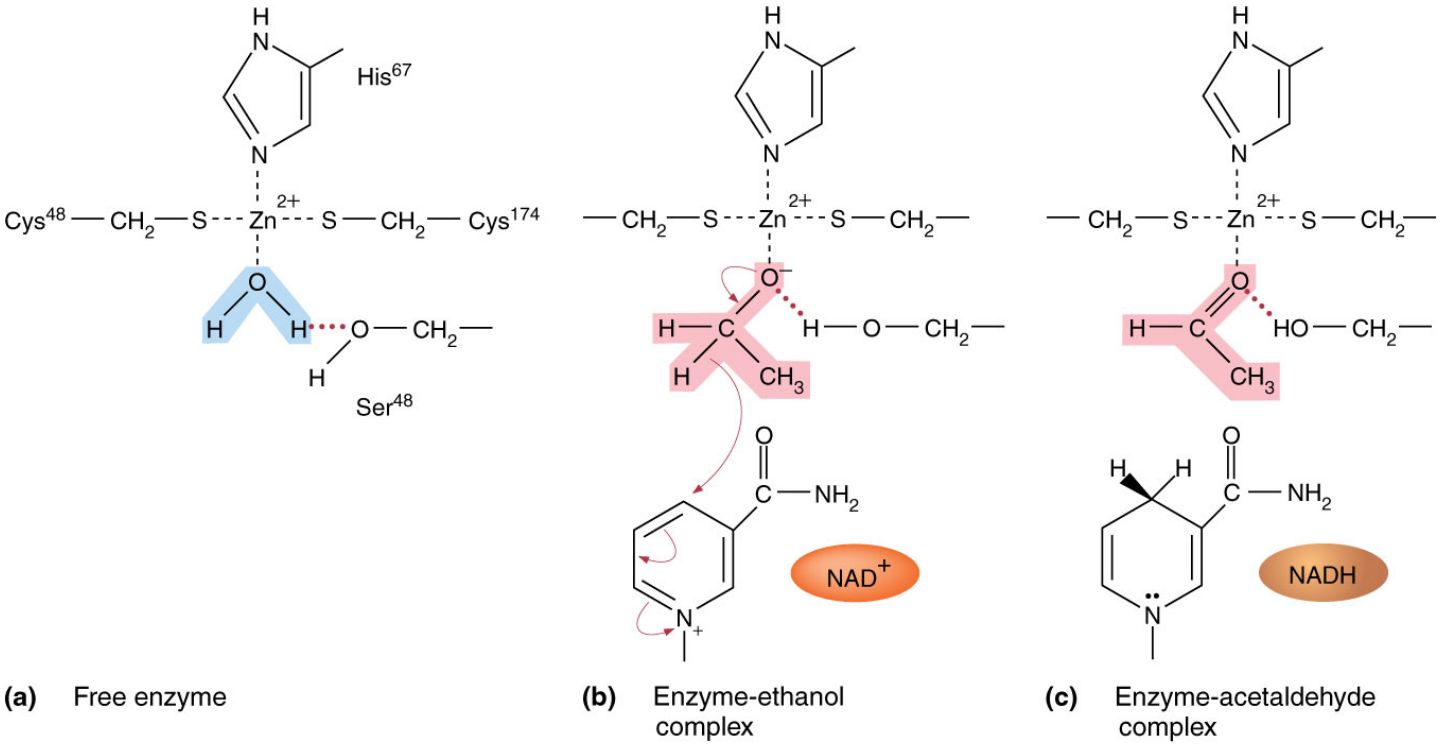


Figure 6.20 Alcohol Dehydrogenase



- **Alcohol Dehydrogenase**—catalyzes the reversible oxidation of alcohols to aldehydes or ketones
  - Requires Zn^{+2}, Cys 48, Cys 174, His 67
  - Uses NAD^+ as a hydride ($H:^-$) ion acceptor

Section 6.5: Enzyme Regulation

- Enzyme regulation is necessary for:
  - **Maintenance of ordered state** – timely production of substances
  - **Conservation of energy** – acquire nutrients to meet energy needs
  - **Responsiveness to environmental changes** – increase/decrease in rates in response to environmental changes
- Four methods of control
  - **genetic control** - – synthesis in response to changing metabolic needs
  - **covalent modification** (e.g. phosphorylation) – interconversion between active/inactive forms
  - **allosteric regulation** – binding of effectors
  - **compartmentation** – physical separation of processes

- **Genetic Control**
  - Happens at the DNA level; **repression** or **induction** of enzyme synthesis

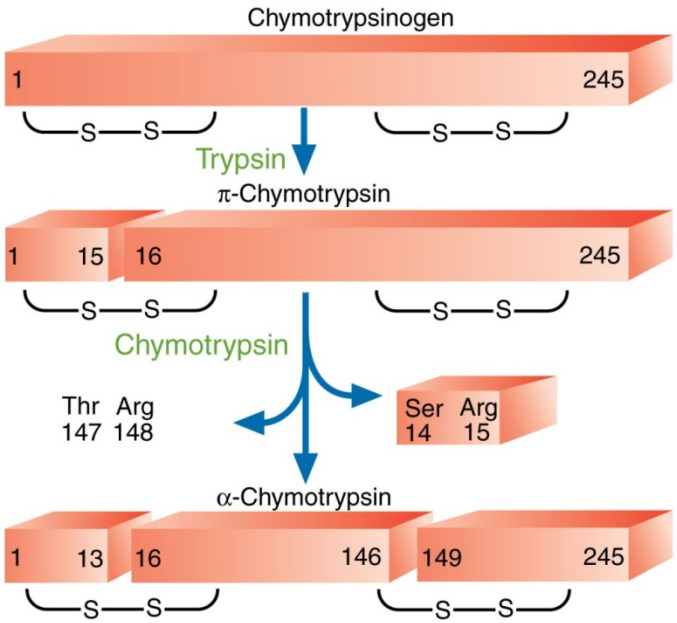


Figure 6.21 The Activation of Chymotrypsinogen

- **Covalent Modification**
  - Regulation – active to inactive form
  - Types -phosphorylation, methylation, acetylation, nucleotidylation
  - Inactive precursors - stored as **proenzymes** or **zymogens**

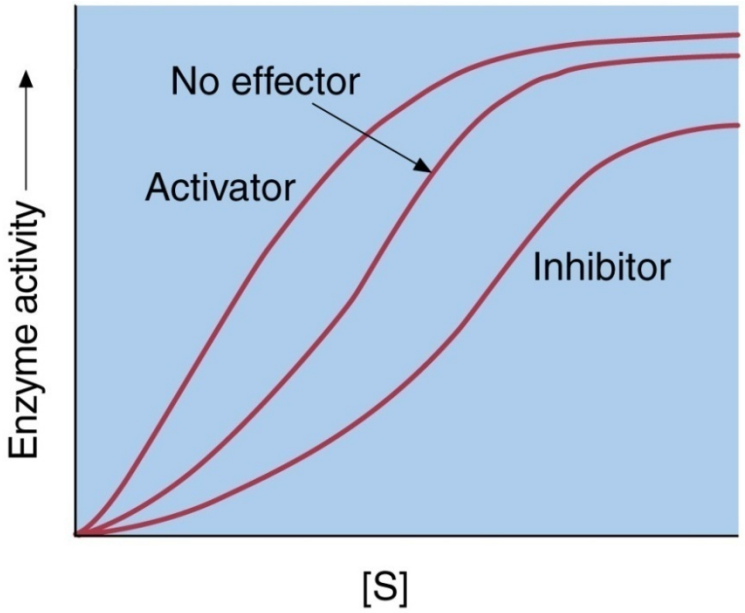
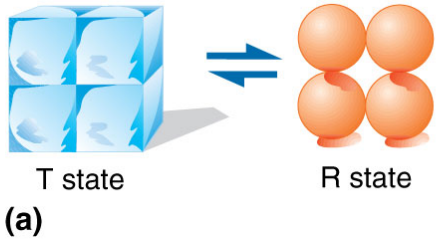


Figure 6.22 The Rate of an Enzyme-Catalyzed Reaction as a Function of Substrate Concentration

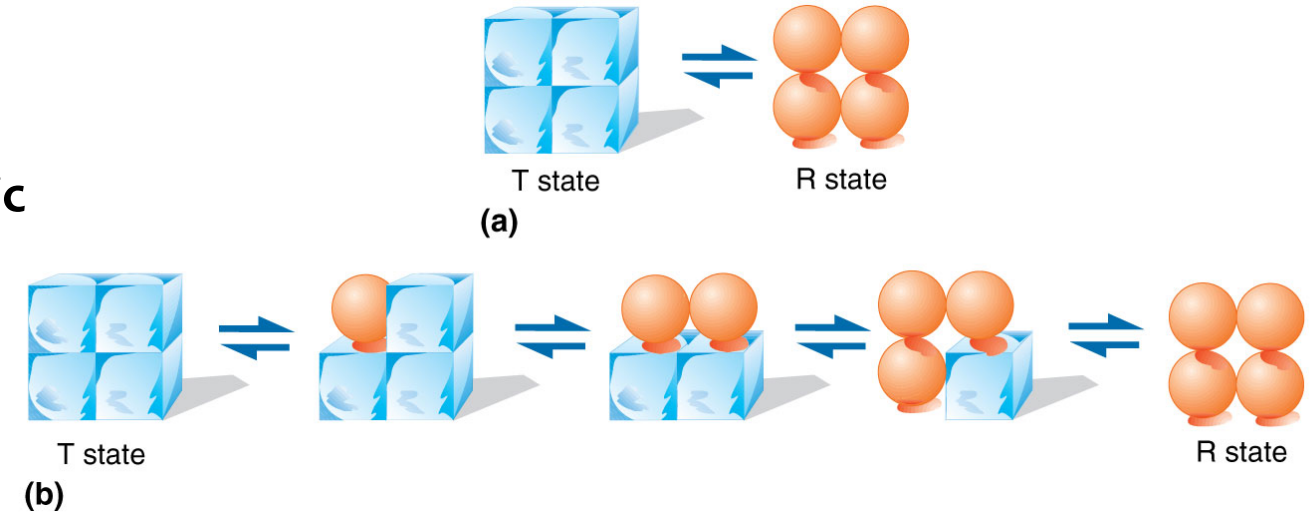
- **Allosteric Regulation**
  - Regulated by the binding of effectors at **allosteric sites**
    - Sigmoidal curve indicates
  - If effectors are substrates, then it is **homotropic**
  - If the ligand is different, then it is **heterotropic**

Figure 6.23a Allosteric Interaction Models



- Most allosteric enzymes are multisubunit enzymes
  - Shift between conformations: T (taut) low binding; R (relaxed) tight binding
- **Concerted** model, all subunits are changed at once from **taut** (T) to **relaxed** (R) or vice versa
  - Activator shifts the equilibrium in favor of the R form; inhibitor shifts in favor of the T form
- Supported by **positive cooperativity** where binding of one ligand increases subsequent binding
  - It is not supported by **negative cooperativity**

Figure 6.23 Allosteric Interaction Models



- Sequential model binding of the ligand to one subunit
  - Triggers a conformational change - passed to subsequent subunits
  - More complex model that allows for intermediate formations
  - Accounts for both positive and negative cooperativity
- Neither model perfectly accounts for all enzyme behavior

Section 6.5: Enzyme Regulation

- **Compartmentation** - Compartments created by cellular infrastructure regulate biochemical reactions
  - Physical separation makes separate control possible
  - Solves several problems:
    - **Divide and control** – coordinated regulation preventing wasteful dissipation of resources
    - **Diffusion barriers** – microenvironments to concentrate both E and S
    - **Specialized reaction conditions** – environments with unique properties; low pH for hydrolytic reactions
    - **Damage control** – segregation of toxic reaction products protects other cellular components