CHAPTER 6
Enzymes

Key topics about enzyme function:

– Physiological significance of enzymes
– Origin of catalytic power of enzymes
– Chemical mechanisms of catalysis
– Mechanisms of chymotrypsin and lysozyme
– Description of enzyme kinetics and inhibition
What are enzymes?

• Enzymes are catalysts
  • Increase reaction rates without being used up
• Most enzymes are globular proteins
  • However, some RNA (ribozymes and ribosomal RNA) also catalyze reactions
• Study of enzymatic processes is the oldest field of biochemistry, dating back to late 1700s
• Study of enzymes has dominated biochemistry in the past and continues to do so
Why biocatalysis over inorganic catalysts?

- Greater reaction specificity: avoids side products
- Milder reaction conditions: conducive to conditions in cells
- Higher reaction rates: in a biologically useful timeframe
- Capacity for regulation: control of biological pathways

- Metabolites have many potential pathways of decomposition
- Enzymes make the desired one most favorable
Enzymatic Substrate Selectivity

Example: Phenylalanine hydroxylase
Reaction Conditions Compatible with Life

- $37^\circ\text{C}$
- pH $\approx 7$
Six Classes of Enzymes: Defined by the Reactions Catalyzed

Figure 6-3
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Enzyme-Substrate Complex

- Enzymes act by binding substrates
  - The noncovalent enzyme substrate complex is known as the **Michaelis complex**
  - Description of chemical interactions
  - Development of kinetic equations

\[ v = \frac{k_{cat}[E][S]}{K_m + [S]} \]
Enzyme-Substrate Complex

Figure 6-1
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Enzymatic Catalysis

• Enzymes do not affect equilibrium ($\Delta G$)
• Slow reactions face significant activation barriers ($\Delta G^\dagger$) that must be surmounted during the reaction
• Enzymes increase reaction rates ($k$) by decreasing $\Delta G^\dagger$

$$k = \left(\frac{k_B T}{h}\right) \exp\left(\frac{-\Delta G^\dagger}{RT}\right)$$
Reaction Coordinate Diagram

Figure 6-2
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Enzymes Decrease $\Delta G^\ddagger$
# Rate Enhancement by Enzymes

## TABLE 6–5  Some Rate Enhancements Produced by Enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Rate (10^x)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclophilin</td>
<td>10^5</td>
</tr>
<tr>
<td>Carbonic anhydrase</td>
<td>10^7</td>
</tr>
<tr>
<td>Triose phosphate isomerase</td>
<td>10^9</td>
</tr>
<tr>
<td>Carboxypeptidase A</td>
<td>10^{11}</td>
</tr>
<tr>
<td>Phosphoglucomutase</td>
<td>10^{12}</td>
</tr>
<tr>
<td>Succinyl-CoA transferase</td>
<td>10^{13}</td>
</tr>
<tr>
<td>Urease</td>
<td>10^{14}</td>
</tr>
<tr>
<td>Orotidine monophosphate decarboxylase</td>
<td>10^{17}</td>
</tr>
</tbody>
</table>

---

*Table 6-5*

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How to Lower $\Delta G^\neq$

Enzymes organize reactive groups into close proximity and proper orientation

• Uncatalyzed bimolecular reactions
  two free reactants $\rightarrow$ single restricted transition state
  conversion is entropically unfavorable

• Uncatalyzed unimolecular reactions
  flexible reactant $\rightarrow$ rigid transition state conversion is entropically unfavorable for flexible reactants

• Catalyzed reactions
  Enzyme uses the binding energy of substrates to organize
  the reactants to a fairly rigid ES complex
  Entropy cost is paid during binding
  Rigid reactant complex $\rightarrow$ transition state conversion is entropically OK
Support for the Proximity Model

The rate of anhydride formation from esters and carboxylates shows a strong dependence on proximity of two reactive groups (work by Thomas C. Bruice’s group).
How to Lower $\Delta G^\neq$

Enzymes bind transition states best

• The idea was proposed by Linus Pauling in 1946
  – Enzyme active sites are complimentary to the transition state of the reaction
  – Enzymes bind transition states better than substrates
  – Stronger/additional interactions with the transition state as compared to the ground state lower the activation barrier

Largely $\Delta H^\neq$ effect
Illustration of TS Stabilization Idea: Imaginary Stickase

(a) No enzyme

(b) Enzyme complementary to substrate

(c) Enzyme complementary to transition state

Figure 6-5
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Catalytic Mechanisms

– acid-base catalysis: give and take protons
– covalent catalysis: change reaction paths
– metal ion catalysis: use redox cofactors, $pK_a$ shifters
– electrostatic catalysis: preferential interactions with TS
General Acid-Base Catalysis

When proton transfer to or from H₂O is faster than the rate of breakdown of intermediates, the presence of other proton donors or acceptors does not increase the rate of the reaction.

Without catalysis, unstable (charged) intermediate breaks down rapidly to form reactants.

When proton transfer to or from H₂O is slower than the rate of breakdown of intermediates, only a fraction of the intermediates formed are stabilized. The presence of alternative proton donors (HA) or acceptors (B⁺) increases the rate of the reaction.

Figure 6-8
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When proton transfer to or from \( \text{H}_2\text{O} \) is faster than the rate of breakdown of intermediates, the presence of other proton donors or acceptors does not increase the rate of the reaction.

Without catalysis, unstable (charged) intermediate breaks down rapidly to form reactants.

When proton transfer to or from \( \text{H}_2\text{O} \) is slower than the rate of breakdown of intermediates, only a fraction of the intermediates formed are stabilized. The presence of alternative proton donors (HA) or acceptors (B\(^{\pm}\)) increases the rate of the reaction.

Products

\[
\text{H} - \text{C} - \text{O} - \text{C} - \text{O}^- + \text{N} - \text{H} - \text{H}
\]
When proton transfer to or from \( \text{H}_2\text{O} \) is faster than the rate of breakdown of intermediates, the presence of other proton donors or acceptors does not increase the rate of the reaction.

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**Figure 6-8**

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When proton transfer to or from H$_2$O is faster than the rate of breakdown of intermediates, the presence of other proton donors or acceptors does not increase the rate of the reaction.

Without catalysis, unstable (charged) intermediate breaks down rapidly to form reactants.

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Products

![Chemical structure](image)
# Amino Acids in General Acid-Base Catalysis

<table>
<thead>
<tr>
<th>Amino acid residues</th>
<th>General acid form (proton donor)</th>
<th>General base form (proton acceptor)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glu, Asp</td>
<td>R—COOH</td>
<td>R—COO⁻</td>
</tr>
<tr>
<td>Lys, Arg</td>
<td>R⁺⁺⁺⁺ NH</td>
<td>R—NH₂</td>
</tr>
<tr>
<td>Cys</td>
<td>R—SH</td>
<td>R—S⁻</td>
</tr>
<tr>
<td>His</td>
<td>[Chemical structure]</td>
<td>[Chemical structure]</td>
</tr>
<tr>
<td>Ser</td>
<td>R—OH</td>
<td>R—O⁻</td>
</tr>
<tr>
<td>Tyr</td>
<td>[Chemical structure]</td>
<td>[Chemical structure]</td>
</tr>
</tbody>
</table>

*Figure 6-9*

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Covalent Catalysis

- A transient covalent bond between the enzyme and the substrate
- Changes the reaction Pathway
  - Uncatalyzed: \[ A \rightarrow^\mathrm{H_2O} B \rightarrow A + B \]
  - Catalyzed: \[ A \rightarrow B + X : \rightarrow A \rightarrow X + B \rightarrow A + X : + B \]
- Requires a nucleophile on the enzyme
  - Can be a reactive serine, thiolate, amine, or carboxylate
Metal Ion Catalysis

• Involves a metal ion bound to the enzyme

• Interacts with substrate to facilitate binding
  – Stabilizes negative charges

• Participates in oxidation reactions
Chymotrypsin uses most of the enzymatic mechanisms.
Active Site of Chymotrypsin with Substrate

Figure 6-19bcd
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Chymotrypsin Mechanism
Step 1: Substrate Binding

When substrate binds, the side chain of the residue adjacent to the peptide bond to be cleaved nestles in a hydrophobic pocket on the enzyme, positioning the peptide bond for attack.
Interaction of Ser^{195} and His^{57} generates a strongly nucleophilic alkoxide ion on Ser^{195}; the ion attacks the peptide carbonyl group, forming a tetrahedral acyl-enzyme. This is accompanied by formation of a short-lived negative charge on the carbonyl oxygen of the substrate, which is stabilized by hydrogen bonding in the oxyanion hole.

**Figure 6-22 part 2**
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Chymotrypsin Mechanism
Step 3: Substrate Cleavage

Instability of the negative charge on the substrate carbonyl oxygen leads to collapse of the tetrahedral intermediate; re-formation of a double bond with carbon displaces the bond between carbon and the amino group of the peptide linkage, breaking the peptide bond. The amino leaving group is protonated by His^{57}, facilitating its displacement.

Figure 6-22 part 3
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Chymotrypsin Mechanism

Step 4: Water Comes In

An incoming water molecule is deprotonated by general base catalysis, generating a strongly nucleophilic hydroxide ion. Attack of hydroxide on the ester linkage of the acyl-enzyme generates a second tetrahedral intermediate, with oxygen in the oxyanion hole again taking on a negative charge.

Figure 6-22 part 4
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Chymotrypsin Mechanism

Step 5: Water Attacks

Short-lived intermediate * (deacylation)

Acyl-enzyme intermediate

Collapse of the tetrahedral intermediate forms the second product, a carboxylate anion, and displaces Ser^{195}.

Figure 6-22 part 5
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Chymotrypsin Mechanism
Step 6: Break-off from the Enzyme

Enzyme-product 2 complex

Short-lived intermediate * (deacylation)

Collapse of the tetrahedral intermediate forms the second product, a carboxylate anion, and displaces Ser₁₉₅.

Figure 6-22 part 6
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Chymotrypsin Mechanism
Step 7: Product Dissociates
Peptidoglycan and Lysozyme

- Peptidoglycan is a polysaccharide found in many bacterial cell walls

- Cleavage of the cell wall leads to the lysis of bacteria

- Lysozyme is an antibacterial enzyme
Peptidoglycan and Lysozyme

Figure 6-27b
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General Acid-Base + Covalent Catalysis: Cleavage of Peptidoglycan by Lysozyme

X-ray structures of lysozyme with bound substrate analogs show that the C-1 carbon is located between Glu 35 and Asp 52 residues.
Cleavage of Peptidoglycan by Lysozyme: Two Successive $S_N2$ Steps Model

- Asp 52 acts as a nucleophile to attack the anomeric carbon in the first $S_N2$ step
- Glu 35 acts as a general acid and protonates the leaving group in the transition state
- Water hydrolyzes the covalent glycosyl-enzyme intermediate
- Glu 35 acts as a general base to deprotonate water in the second $S_N2$ step
A rearrangement produces a glycosyl carbocation. General acid catalysis by Glu\textsuperscript{52} protonates the displaced GlcNAc oxygen and facilitates its departure.

Asp\textsuperscript{52} acts as a covalent catalyst, directly displacing the GlcNAc via an \( S_N 2 \) mechanism. Glu\textsuperscript{35} protonates the GlcNAc to facilitate its departure.

Figure 6-28a part 1
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General base catalysis by Glu^{35} facilitates the attack of water on the glycosyl carbocation to form product.

Glu^{35} acts as a general base catalyst to facilitate the $S_N^2$ attack of water, displacing Asp^{52} and generating product.

Figure 6-28a part 2
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General base catalysis by Glu$^{35}$ facilitates the attack of water on the glycosyl carbocation to form product.

Glu$^{35}$ acts as a general base catalyst to facilitate the $S_N^2$ attack of water, displacing Asp$^{52}$ and generating product.
What is enzyme kinetics?

• Kinetics is the study of the rate at which compounds react

• Rate of enzymatic reaction is affected by:
  – enzyme
  – substrate
  – effectors
  – temperature
Why study enzyme kinetics?

- Quantitative description of biocatalysis
- Determine the order of binding of substrates
- Elucidate acid-base catalysis
- Understand catalytic mechanism
- Find effective inhibitors
- Understand regulation of activity
How to Do Kinetic Measurements

Experiment:
1) Mix enzyme + substrate
2) Record rate of substrate disappearance/product formation as a function of time (the velocity of reaction)
3) Plot initial velocity versus substrate concentration.
4) Change substrate concentration and repeat
Effect of Substrate Concentration

• Ideal rate: \[ v = \frac{V_{\text{max}}[S]}{K_m + S} \]

• Deviations due to:
  – limitation of measurements
  – substrate inhibition
  – substrate prep contains inhibitors
  – enzyme prep contains inhibitors
Effect of Substrate Concentration

Initial velocity, $V_o$ ($\mu M/min$)

$V_{max}$

$\frac{1}{2} V_{max}$

$K_m$

Substrate concentration, $[S]$ (mM)

Figure 6-11
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Saturation Kinetics: At high [S] velocity does not depend on [S].

\[ V_0 = \frac{V_{\text{max}} [S]}{K_m} \]

\[ V_0 = V_{\text{max}} \]

\[ V_0 (\mu \text{M/min}) \text{ vs. } [S] (\text{mM}) \]
Determination of Kinetic Parameters

Nonlinear Michaelis-Menten plot should be used to calculate parameters $K_m$ and $V_{max}$.

Linearized double-reciprocal plot is good for analysis of two-substrate data or inhibition.
Lineweaver-Burk Plot: Linearized, Double-Reciprocal

\[
\text{Slope} = \frac{K_m}{V_{\text{max}}} 
\]

\[
\frac{1}{V_o} \text{ (\muM/min)} 
\]

\[
\frac{1}{V_{\text{max}}} 
\]

\[
\frac{1}{K_m} 
\]

\[
\frac{1}{[S]} \left( \frac{1}{\text{mM}} \right) 
\]
Derivation of Enzyme Kinetics Equations

• Start with a model mechanism
• Identify constraints and assumptions
• Carry out algebra ...
  – ... or graph theory for complex reactions

• Simplest Model Mechanism: $E + S \rightleftharpoons ES \rightarrow E + P$
  – One reactant, one product, no inhibitors
Identify Constraints and Assumptions

• Total enzyme concentration is constant
  – Mass balance equation for enzyme: $E_{\text{Tot}} = [E] + [ES]$
  – It is also implicitly assumed that: $S_{\text{Tot}} = [S] + [ES] \approx [S]$

• Steady state assumption

\[
\frac{d[ES]}{dt} = \text{rate of formation of } \text{ES} - \text{rate of breakdown of } \text{ES} = 0
\]

• What is the observed rate?
  – Rate of product formation

\[
\nu_{\text{net}} = \frac{dP}{dt} = k[ES]
\]
The final form in case of a single substrate is

\[ v = \frac{k_{\text{cat}} [E_{\text{tot}}] [S]}{K_m + [S]} \]

- \(k_{\text{cat}}\) (turnover number): how many substrate molecules can one enzyme molecule convert per second
- \(K_m\) (Michaelis constant): an approximate measure of substrate’s affinity for enzyme
- Microscopic meaning of \(K_m\) and \(k_{\text{cat}}\) depends on the details of the mechanism
Enzyme efficiency is limited by diffusion:

\[ \frac{k_{\text{cat}}}{K_M} \]

- Can gain efficiency by having high velocity or affinity for substrate
  - Catalase vs. acetylcholinesterase

### Table 6–8

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>( k_{\text{cat}} ) (s(^{-1}))</th>
<th>( K_m ) (M)</th>
<th>( \frac{k_{\text{cat}}}{K_m} ) (M(^{-1})s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholinesterase</td>
<td>Acetylcholine</td>
<td>(1.4 \times 10^4)</td>
<td>(9 \times 10^{-5})</td>
<td>(1.6 \times 10^8)</td>
</tr>
<tr>
<td>Carbonic anhydrase</td>
<td>(\text{CO}_2)</td>
<td>(1 \times 10^6)</td>
<td>(1.2 \times 10^{-2})</td>
<td>(8.3 \times 10^7)</td>
</tr>
<tr>
<td></td>
<td>(\text{HCO}_3^-)</td>
<td>(4 \times 10^5)</td>
<td>(2.6 \times 10^{-2})</td>
<td>(1.5 \times 10^7)</td>
</tr>
<tr>
<td>Catalase</td>
<td>(\text{H}_2\text{O}_2)</td>
<td>(4 \times 10^7)</td>
<td>(1.1 \times 10^0)</td>
<td>(4 \times 10^7)</td>
</tr>
<tr>
<td>Crotonase</td>
<td>Crotonyl-CoA</td>
<td>(5.7 \times 10^3)</td>
<td>(2 \times 10^{-5})</td>
<td>(2.8 \times 10^8)</td>
</tr>
<tr>
<td>Fumarase</td>
<td>Fumarate</td>
<td>(8 \times 10^2)</td>
<td>(5 \times 10^{-6})</td>
<td>(1.6 \times 10^8)</td>
</tr>
<tr>
<td></td>
<td>Malate</td>
<td>(9 \times 10^2)</td>
<td>(2.5 \times 10^{-5})</td>
<td>(3.6 \times 10^7)</td>
</tr>
<tr>
<td>(\beta)-Lactamase</td>
<td>Benzylpenicillin</td>
<td>(2.0 \times 10^3)</td>
<td>(2 \times 10^{-5})</td>
<td>(1 \times 10^8)</td>
</tr>
</tbody>
</table>

Two-Substrate Reactions

- **Kinetic mechanism**: the order of binding of substrates and release of products

- When two or more reactants are involved, enzyme kinetics allows to distinguish between different kinetic mechanisms
  - Sequential mechanism
  - Ping-Pong mechanism
(a) Enzyme reaction involving a ternary complex

Random order

\[
\begin{align*}
&\text{ES}_1 \\
&\text{ES}_2 \\
&\text{E} &\text{ES}_1\text{S}_2 &\rightarrow &\text{E} + \text{P}_1 + \text{P}_2 \\
&\text{ES}_1 &\text{ES}_2
\end{align*}
\]

Ordered

\[
\begin{align*}
&E + S_1 &\text{ES}_1 &\text{ES}_1\text{S}_2 &\rightarrow &\text{E} + \text{P}_1 + \text{P}_2 \\
&S_2
\end{align*}
\]

(b) Enzyme reaction in which no ternary complex is formed

\[
\begin{align*}
&E + S_1 &\text{ES}_1 &\text{E'}\text{P}_1 &\rightarrow &\text{E'} + S_2 &\text{E'S}_2 &\rightarrow &\text{E} + \text{P}_2 \\
&P_1 &S_2
\end{align*}
\]
Sequential Kinetic Mechanism

• We cannot easily distinguish random from ordered
• Random mechanisms in equilibrium will give intersection point at y-axis
• Lineweaver-Burk: lines intersect
Ping-Pong Kinetic Mechanism

Lineweaver-Burk: lines are parallel
Enzyme Inhibition

Inhibitors are compounds that decrease enzyme’s activity

• Irreversible inhibitors (inactivators) react with the enzyme
  • One inhibitor molecule can permanently shut off one enzyme molecule
  • They are often powerful toxins but also may be used as drugs

• Reversible inhibitors bind to and can dissociate from the enzyme
  • They are often structural analogs of substrates or products
  • They are often used as drugs to slow down a specific enzyme

• Reversible inhibitor can bind:
  • to the free enzyme and prevent the binding of the substrate
  • to the enzyme-substrate complex and prevent the reaction
Competitive Inhibition

- Competes with substrate for binding
  - Binds active site
  - Does not affect catalysis

- No change in $V_{\text{max}}$; apparent increase in $K_M$
- Lineweaver-Burk: lines intersect at the y-axis
Competitive Inhibition

**Competitive inhibition**

$$E + S \rightleftharpoons ES \rightarrow E + P$$

$$+$$

$$I$$

$$K_i$$

$$EI$$

---

*Figure 6-15a*

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Competitive Inhibition

\[ \frac{1}{V_0} = \left( \frac{\alpha K_m}{V_{max}} \right) \frac{1}{[S]} + \frac{1}{V_{max}} \]

Box 6-2 figure 1
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Uncompetitive Inhibition

- Only binds to ES complex
  - Does not affect substrate binding
  - Inhibits catalytic function

- Decrease in $V_{\text{max}}$; apparent decrease in $K_M$
- No change in $K_M/V_{\text{max}}$
- Lineweaver-Burk: lines are parallel
Uncompetitive Inhibition

Uncompetitive inhibition

E + S ⇌ ES ⇌ E + P

I

K'_i

ESI

Figure 6-15b
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Uncompetitive Inhibition

\[
\frac{1}{V_0} = \left( \frac{K_m}{V_{max}} \right) \frac{1}{[S]} + \frac{\alpha'}{V_{max}}
\]

**Box 6-2 figure 2**

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**Mixed Inhibition**

- Binds enzyme with or without substrate
  - Binds to regulatory site
  - Inhibits both substrate binding and catalysis
- Decrease in $V_{\text{max}}$; apparent change in $K_M$
- Lineweaver-Burk: lines intersect left from the y-axis
- Noncompetitive inhibitors are mixed inhibitors such that there is no change in $K_M$
Mixed Inhibition

Mixed inhibition

E + S ⇌ ES ⇌ E + P

+ +
I I

$K_1$ $K_1'$

EI + S ⇌ ESI

Figure 6-15c
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Mixed Inhibition

\[
\frac{1}{V_0} = \left( \frac{\alpha K_m}{V_{max}} \right) \frac{1}{[S]} + \frac{\alpha'}{V_{max}}
\]

Box 6-2 figure 3
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Enzyme activity can be regulated

• Regulation can be:
  – noncovalent modification
  – covalent modification
  – irreversible
  – reversible
Noncovalent Modification: Allosteric Regulators

The kinetics of allosteric regulators differ from Michaelis-Menten kinetics.
Some Reversible Covalent Modifications

Figure 6-35
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Zymogens are activated by irreversible covalent modification

Figure 6-38
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The blood coagulation cascade uses irreversible covalent modification.
Some enzymes use multiple types of regulation
Chapter 6: Summary

In this chapter, we learned:

- why nature needs enzyme catalysis
- how enzymes can accelerate chemical reactions
- how chymotrypsin breaks down peptide bonds
- how to perform and analyze kinetic studies
- how to characterize enzyme inhibitors
- how enzyme activity can be regulated