BCMB 3100 – Chapters 6, 7, 8

Enzymes –
Enzyme Mechanism

Mechanisms of Enzymes

- Energy diagrams
- Binding modes of enzyme catalysis
  - Chemical modes of enzyme catalysis
    - Acid-Base catalysis
    - Covalent catalysis
  - Binding modes of enzyme catalysis
    - Proximity effect
    - Transition state stabilization
- Transition state analogs
- Induced fit
- Serine Proteases
Energy diagrams show the progress of a reaction

___________: high energy, unstable state in which a molecule is best suited to undergo a chemical reaction; state in which chemical bonds are being broken and formed. Lifetime $\sim 10^{-14}$ to $10^{-13}$ sec

Energy diagram for a single-step reaction

Energy diagram for reaction with intermediate

• __________ occurs in the trough between the two transition states
• Lifetime $> \sim 10^{-14}$ to $10^{-13}$ sec
• In this case, the rate determining step in the forward direction is formation of the first transition state
Enzymatic catalysis of the reaction \[ A+B \rightarrow A-B \]

How do enzymes increase the rate of a reaction?

**Proximity effect:**
"proper" positioning of substrates

**Transition state stabilization:** lowers activation energy

Mechanisms of Enzymes

- Energy diagrams
- Chemical modes of enzyme catalysis (*setting the stage*)
  - Acid-Base catalysis
  - Covalent catalysis
- Binding modes of enzyme catalysis
  - Proximity effect
  - Transition state stabilization
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What is the enzyme active site?

A few polar residues and H₂O molecules are found at the otherwise hydrophobic active site of an enzyme

_____________: polar amino acids that undergo changes during enzymatic catalysis

Ionic side chains are involved in two types of chemical catalysis:

1. ______________
2. ______________

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### Catalytic functions of reactive groups of ionizable amino acids

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Reactive group</th>
<th>Net charge at pH 7</th>
<th>Principal functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartate</td>
<td>—COO⁻</td>
<td>—1</td>
<td>Cation binding; proton transfer</td>
</tr>
<tr>
<td>Glutamate</td>
<td>—COO⁻</td>
<td>—1</td>
<td>Cation binding; proton transfer</td>
</tr>
<tr>
<td>Histidine</td>
<td>Imidazole</td>
<td>Near 0</td>
<td>Proton transfer</td>
</tr>
<tr>
<td>Cysteine</td>
<td>—CH₃SH</td>
<td>Near 0</td>
<td>Covalent binding of acyl groups</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>Phenol</td>
<td>0</td>
<td>Hydrogen bonding to ligands</td>
</tr>
<tr>
<td>Lysine</td>
<td>—NH₃⁺</td>
<td>+1</td>
<td>Anion binding; proton transfer</td>
</tr>
<tr>
<td>Arginine</td>
<td>Guanidinium</td>
<td>+1</td>
<td>Anion binding</td>
</tr>
<tr>
<td>Serine</td>
<td>—CH₃OH</td>
<td>0</td>
<td>Covalent binding of acyl groups</td>
</tr>
</tbody>
</table>

Note: pKa of ionizable groups of amino acids in proteins vary from pKa of free amino acids.
### pKa Values of amino acid ionizable groups in proteins

<table>
<thead>
<tr>
<th>Group</th>
<th>pK&lt;sub&gt;a&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terminal $\alpha$-carboxyl</td>
<td>3-4</td>
</tr>
<tr>
<td>Side-chain carboxyl</td>
<td>4-5</td>
</tr>
<tr>
<td>Imidazole</td>
<td>6-7</td>
</tr>
<tr>
<td>Terminal $\alpha$-amino</td>
<td>7.5-9</td>
</tr>
<tr>
<td>Thiol</td>
<td>8-9.5</td>
</tr>
<tr>
<td>Phenol</td>
<td>9.5-10</td>
</tr>
<tr>
<td>$\varepsilon$-Amino</td>
<td>~10</td>
</tr>
<tr>
<td>Guanidine</td>
<td>~12</td>
</tr>
<tr>
<td>Hydroxymethyl</td>
<td>~16</td>
</tr>
</tbody>
</table>

Be sure to consider and study

### Mechanisms of Enzymes

- **Energy diagrams**
- **Chemical modes of enzyme catalysis**
  - Acid-Base catalysis
  - Covalent catalysis
- **Binding modes of enzyme catalysis**
  - Proximity effect
  - Transition state stabilization
- **Transition state analogs**
- **Induced fit**
- **Serine Proteases**
Chemical modes of enzyme catalysis

• Acid-Base catalysis
• Covalent catalysis

…………………. : acceleration of a reaction by transfer of a proton

B: = base (proton acceptor)
BH+ = conjugate acid (proton donor)

• A general base (B:) can act as a proton acceptor to remove protons from OH, NH, CH or other XH
• This produces a stronger nucleophilic reactant (X:−)

\[ \text{\textless}X-H :B \xleftrightarrow{} -\text{\textless}X^{-} \quad H \rightarrow B \text{\textgreater} \]
General base catalysis reactions (continued)

- A ____________ (B: ) can remove a proton from water and thereby generate the equivalent of OH⁻ in neutral solution

Proton donors can also catalyze reactions

- A ____________ (BH⁺) can donate protons

- A covalent bond may break more easily if one of its atoms is protonated (below)
**RECALL! Pingpong (double displacement) reactions**

_______: part or entirety of S forms covalent bond with E and then with second S

~20% of enzymes use covalent catalysis

- All or part of a substrate is bound ________ to the enzyme to form a ________________
- Group X can be transferred from A-X to B in two steps via the covalent ES complex X-E
  
  \[
  \begin{align*}
  A-X + E & \rightleftharpoons X-E + A \\
  X-E + B & \rightleftharpoons B-X + E
  \end{align*}
  \]

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**Sucrose phosphorylase exhibits covalent catalysis** (E.C. 2.4.1.7)

(Sucrose = glucose-fructose)

**Step one:** a glucosyl residue is transferred to enzyme

\[\text{Sucrose} + \text{Enz} \rightleftharpoons \text{Glucosyl-Enz} + \text{Fructose}\]

**Step two:** Glucose is donated to phosphate

\[\text{Glucosyl-Enz} + P_i \rightleftharpoons \text{Glucose 1-phosphate} + \text{Enz}\]

\[\text{Sucrose} + P_i \rightarrow \text{fructose} + \text{glucose-1-P}\]
The two inflection points approximate the pKₐ values of the two ionizable residues of an enzyme can give information about ionic residues at the active site. A simple bell-shaped curve can result from two overlapping titrations of active site amino acids.

- The two inflection points approximate the pKₐ values of the two ionizable residues

*Left side of curve suggests a catalytic amino acid that functions in conjugate base form. *Right side of curve suggests amino acid that must function in conjugate acid form.

Papain’s activity depends upon ionizable residues: **His-159** and **Cys-25**

(a) Ribbon model  
(b) Active site residues  
(N blue, S yellow)
Three ionic forms of papain. Only the upper tautomer of the middle pair is active.

- pKa of Cys in papain = 3.4!
- pKa or His in papain = 8.3!

Different enzymes have different pH curves Fig. 8.4
Fastest Reactions are Diffusion-Controlled Reactions: rates approach rate of diffusion: $10^8$ to $10^9$ M$^{-1}$s$^{-1}$; speed of binding of substrates to the enzyme

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>$k_{cat}/K_m$ (M$^{-1}$s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase</td>
<td>H$_2$O$_2$</td>
<td>$4 \times 10^7$</td>
</tr>
<tr>
<td>Carbonic anhydrase</td>
<td>CO$_2$</td>
<td>$1.2 \times 10^4$</td>
</tr>
<tr>
<td>Acetylcholinesterase</td>
<td>Acetylcholine</td>
<td>$1.6 \times 10^4$</td>
</tr>
<tr>
<td>Fumarase</td>
<td>Fumarate</td>
<td>$1.6 \times 10^4$</td>
</tr>
<tr>
<td>Triose phosphate isomerase</td>
<td>α-Glyceraldehyde 3-phosphate</td>
<td>$4 \times 10^8$</td>
</tr>
<tr>
<td>Superoxide dismutase</td>
<td>$\cdot$O$_2^-$</td>
<td>$2 \times 10^9$</td>
</tr>
</tbody>
</table>

*The ratio $k_{cat}/K_m$ is the apparent second-order rate constant for the enzyme-catalyzed reaction $E + S \rightarrow E + P$. For these enzymes, the formation of the ES complex can be the slowest step.*

A. Triose Phosphate Isomerase (TPI)

- TPI catalyzes a rapid aldehyde-ketone interconversion
Proposed mechanism for TPI

- General acid-base catalysis mechanism (4 slides)

When dihydroxyacetone phosphate binds, the carbonyl oxygen forms a hydrogen bond with the neutral imidazole group of His-95. The carboxylate group of Glu-165 removes a proton from C-1 of the substrate to form an enediolate intermediate.

TPI mechanism (continued)

His-95 forms a strong hydrogen bond to the C-2 oxygen atom of the enediolate, and protonates this oxygen atom.
Next, the imidazolate form of His-95 abstracts a proton from the hydroxyl group at C-1 and shuttles the proton between oxygen atoms, producing another unstable enediolate intermediate.

TPI mechanism (continued)

Intermediate 2

Glu-165

Enediolate intermediate

His-95

TPI mechanism (continued)

Intermediate 3

Glu-165 donates a proton to C-2, producing D-glyceraldehyde 3-phosphate.

Glu-165

Enediolate intermediate

His-95
Energy diagram for the TPI reaction

Enzyme with $E \rightarrow D$ mutation; 1000x slower, Km same

Wild type enzyme

Jeremy Knowles

How can the enzyme catalyze this reaction so quickly?

Proximity effect:

__________ in rate of a reaction due to binding of substrates at binding sites in the enzyme,
results in an __________ effective concentration of reactants,
results in more frequent formation of the __________

Jeremy Knowles
Excessive ES stabilization would create a “thermodynamic pit” and give little or no catalysis

• if E binds S too tightly (dashed profile), the activation barrier (2) could be similar to that of the uncatalyzed reaction (1)
• most Km values (substrate dissociation constants) indicate weak binding to enzymes

RECALL: Km usually $10^{-1}$ to $10^{-7}$M (100 mM to 0.1 µM)

Transition-State (TS) Stabilization

• increased interaction of E with S in transition-state (ES$^\dagger$)
• E distorts S, forcing it toward the transition state
• E must be complementary to transition-state in shape and chemical character
• E binds transition states $10^{10}$ to $10^{15}$ times more tightly than S
Basis for enzymatic catalysis

1. _____________ (e.g. acid-base & covalent catalysis) → 10-100 ↑

2. _____________

___________: "weak" binding (~0.1 M) of S to active site raises the effective concentration of S and favors more frequent transition states → 10^4-10^5 ↑

 effective molarity: enhanced relative concentration of reactants due to binding to E

________________: greater binding of transition states than S or P to E → lower activation energy → 10^4-10^5 ↑

Enzyme rate accelerations ~ 10^8-10^12

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• Transition state analogs
• Induced fit
• Serine Proteases
Wolfenden & Lienhard (1970s): showed that chemical analogs of ____________ are enzyme inhibitors

In Emil Fisher’s lock-and-key model for SE binding, the _____________. Binding of S to E distorts S to $\rightarrow$ transition state. The transition state must be stabilized for catalysis to occur.

Transition state analogs can $\rightarrow$ catalytic antibodies

Transition-state (TS) analogs

- **Transition-state analogs** are stable compounds whose structures resemble unstable transition states
- 2-Phosphoglycolate, a TS analog for the enzyme triose phosphate isomerase

2-Phosphoglycolate (transition-state analog) binds E 100X more tightly than either S does
Chapter 6  Mechanisms of Enzymes

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**Induced Fit:** substrate induced cleft closing  
(Daniel Koshland, 1950s)

- ___________ activates an enzyme by substrate-initiated conformation effect
- Induced fit is a substrate specificity effect, not a catalytic mode
- **Hexokinase** mechanism requires sugar-induced closure of the active site
- **Other examples:** pyruvate kinase, phosphoglycerate kinase, phosphofructokinase

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**Stereo views of yeast hexokinase**

- Yeast hexokinase contains 2 domains connected by a hinge region. Domains close on glucose binding.
  (a) Open conformation  
  (b) Closed conformation

\[ \text{Glucose} + \text{ATP} \rightarrow \text{Glucose 6-phosphate} + \text{ADP} \]
Chapter 6  Mechanisms of Enzymes

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What did you have for breakfast today?

In the stomach gastric acid → pH 2; pepsin hydrolyzes proteins to peptides

The serine proteases are synthesized as zymogens in the pancreas, transported to the small intestine and activated by selective proteolysis
Properties of Serine Proteases

- Digestive serine proteases including ____________, ____________, and ____________ are synthesized and stored in the pancreas as zymogens (the zymogens are trypsinogen, chymotrypsinogen proelatase).
- ____________ are inactive enzyme precursors that must be covalently modified to become active.
- Storage of hydrolytic enzymes as ____________ prevents damage to cell proteins.
- Pancreatic zymogens are activated by ____________.
- The pancreatic zymogens are also regulated by enzyme inhibitors (e.g. trypsin inhibitor, Kd = 10^{-13} M !!)

Activation of some pancreatic zymogens

**Enzyme cascades → rapid signal amplification**

- Trypsinogen
- Enteropeptidase → Trypsin → Chymotrypsinogen → Proelastase → Elastase
- Chymotrypsin

**The protease zymogens are synthesized in the pancreas and activated in the duodenum.**

**Elastase** cleaves on the carbonyl side of aa with small, uncharged side chains.
The backbones of chymotrypsin (blue), trypsin (yellow), and elastase (green)

- Backbone conformations and active-site residues (red) are similar in these three enzymes.

Crucial difference between structures is absence of substrate binding pocket in the zymogen. Residues in yellow are ones that develop the pocket. Ile16 has just become the new N-terminal, producing an NH$_3^+$ that turns inward and interacts with the side-chain carboxyl of Asp194, forming an ion pair. This opens up the binding pocket. The three residues shown in red are the catalytic triad.
The effect of cleavage of chymotrypsin at between residues 13 & 14, 15 & 16, and 146 & 147 is to turn Asp194 outward, opening up the binding pocket. The catalytic site is essentially unchanged.

Binding sites of chymotrypsin, trypsin, and elastase

- Substrate specificities are due to relatively small structural differences in active-site binding cavities
Catalytic triad of serine proteases = Asp, His, Ser

α-Chymotrypsin mechanism (8 slides; 1)

Step (1): E + S

The noncovalent enzyme-substrate complex is formed, orienting the substrate for reaction. Interactions holding the substrate in place include binding of the $R_1$ group in the specificity pocket (shaded). The binding interactions position the carbonyl carbon of the scissile peptide bond (the bond susceptible to cleavage) next to the oxygen of Ser-195.

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**Catalytic triad of chymotrypsin**

- Imidazole ring (His-57) removes H from Ser-195 hydroxyl to make it a strong nucleophile (-CH$_2$O$^-$)
- Buried carboxylate (Asp-102) stabilizes the positively-charged His-57 to facilitate serine ionization
1. Substrate binding compresses Asp & His
2. Imidazole removes H+ from Ser
3. Nucleophilic O of Ser attacked Carbonyl of substrate
4. First tetrahedral intermediate formed

Binding of the substrate compresses Asp-102 and His-57. This strain is relieved by formation of a low-barrier hydrogen bond. The raised pKₐ of His-57 enables the imidazole ring to remove a proton from the hydroxyl group of Ser-195. The nucleophilic oxygen of Ser-195 attacks the carbonyl carbon of the peptide bond to form a tetrahedral intermediate (E-TI₁), which is believed to resemble the transition state.

1. Substrate C-O double bond changes to single bond
2. Negatively charge O moves to oxyanion hole and H-bonds to NH of Gly & Ser
3. Imidazolium of His donates H⁺ to N of scissile bond, yielding cleavage

When the tetrahedral intermediate is formed, the substrate C—O bond changes from a double bond to a longer single bond. This allows the negatively charged oxygen (the oxyanion) of the tetrahedral intermediate to move to a previously vacant position, called the oxyanion hole, where it can form hydrogen bonds with the peptide chain —NH groups of Gly-193 and Ser-195.

The imidazolium ring of His-57 acts as an acid catalyst, donating a proton to the nitrogen of the scissile peptide bond, thus facilitating its cleavage.
1. Carbonyl from remaining peptide forms covalent bond with enzyme yielding acyl-enzyme intermediate
2. Peptide product with new amino terminus leaves active site

(Acyl E + P₁) (4)

Acid-base & covalent catalysis

The carbonyl group from the peptide forms a covalent bond with the enzyme, producing an acyl-enzyme intermediate. After the peptide product (P₁) with the new amino terminus leaves the active site, water enters.

1. Final substrate, H₂O enters binding pocket
2. His abstracts H⁺ from water
3. Nucleophilic O of OH⁻ reacts with carbonyl of enzyme-acyl intermediate

(Acyl E + H₂O) (5)

Hydrolysis

Hydrolysis (deacylation) of the acyl-enzyme intermediate starts when Asp-102 and His-57 again form a low-barrier hydrogen bond and His-57 removes a proton from the water molecule to provide an OH⁻ group to attack the carbonyl group of the ester.
1. His imidazolium ion donates H+
2. Formation of second tetrahedral intermediate
3. Stabilized by oxyanion hole

(E-TI₂) (6)

Tetrahedral intermediate

1. Second polypeptide product formed with a new carboxy terminus

(E-P₂) (7)

The second product (P₂)—a polypeptide with a new carboxy terminus—is formed.
1. Final polypeptide product leaves active site
2. Enzyme can now cleave a new polypeptide

\[(E + P_2)\] (8)

Additional material to aid in learning the material covered in the chapter
Review of Chemical Mechanisms

1. **Nucleophilic Substitution Reactions:** ionic reaction where both electrons stay with one atom → ionic intermediate + leaving group

   ionic reactions have **nucleophile** + **electrophile**

**Formation of tetrahedral intermediate**

**Direct displacement:** two molecules react to form a five group transition state

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**Two types of nucleophilic substitution reactions**

- **Formation of a tetrahedral intermediate**

- **Direct displacement**
2. **Cleavage reactions**: most common when both electrons stay with one atom

- formation of a carbanion (C retains both e-)
- formation of carbocation ion (C loses both e-)

**Carbanion formation**

\[ R_3 \text{C} \text{H} \longrightarrow R_3 \text{C}^{\ominus} + \text{H}^\oplus \]

Carbanion Proton

**Carbocation formation**

\[ R_3 \text{C} \text{H} \longrightarrow R_3 \text{C}^{\oplus} + \text{H}^{\ominus} \]

Carbocation Hydride ion

3. **Cleavage reactions**: less common when one electron remains with each product

→ two free radicals

**Free radical formation**

\[ R_1 \text{O} \text{OR}_2 \longrightarrow R_1 \text{O}^\cdot + \cdot \text{OR}_2 \]
4. Oxidation-reduction reactions

Oxidation
* addition of oxygen
* removal of hydrogen
* removal of electrons

• Electrons are transferred between two species
• Oxidizing agent gains electrons (is reduced)
• Reducing agent donates electrons (is oxidized)

Enzymes lower the activation energy of a reaction

(1) Substrate binding
• Enzymes properly position substrates for reaction (makes the formation of the transition state more frequent and lowers the energy of activation)

(2) Transition state binding
• Transition states are bound more tightly than substrates (this also lowers the activation energy)
Binding Modes of Enzymatic Catalysis

- Proper binding of reactants in enzyme active sites provides substrate specificity and catalytic power.

- Two catalytic modes based on binding properties can each increase reaction rates over 10,000-fold:
  1. **Proximity effect** - collecting and positioning substrate molecules in the active site.
  2. **Transition-state (TS) stabilization** - transition states bind more tightly than substrates.

Binding forces utilized for catalysis

1. Charge-charge interactions
2. Hydrogen bonds
3. Hydrophobic interactions
4. Van der Waals forces
A. The Proximity Effect

- Correct positioning of two reacting groups (in model reactions or at enzyme active sites):
  1. Reduces their degrees of freedom
  2. Results in a large loss of entropy
  3. The relative enhanced concentration of substrates (“effective molarity”) predicts the rate acceleration expected due to this effect

Reactions of carboxylates with phenyl esters

- Increased rates are seen when the reactants are held more rigidly in proximity (continued next slide)
B. Weak Binding of Substrates to Enzymes

- Energy is required to reach the transition state from the ES complex
- Excessive ES stabilization would create a “thermodynamic pit” and mean little or no catalysis
- Most $K_m$ values (substrate dissociation constants) indicate weak binding to enzymes
**Catalytic triad of serine proteases = Asp, His, Ser**

- Active-site Asp-102, His-57, Ser-195 are arrayed in a hydrogen-bonded network (O red, N blue)