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 ~ $10^{-14}$ to $10^{-13}$ sec

Energy diagram for reaction with intermediate

• _______ occurs in the trough between the two transition states
• Lifetime > $10^{-14}$ 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 \[ A + B \rightarrow A-B \]

**Proximity effect:** “proper” positioning of substrates

**Transition state stabilization:** lowers activation energy

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**What is the enzyme active site?**

A few polar residues and H$_2$O 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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**Binding modes for enzymatic catalysis**

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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>$-\text{COO}^-$</td>
<td>-1</td>
<td>Carboxyl binding; proton transfer</td>
</tr>
<tr>
<td>Glutamate</td>
<td>$-\text{COO}^-$</td>
<td>-1</td>
<td>Carboxyl binding; proton transfer</td>
</tr>
<tr>
<td>Histidine</td>
<td>Imidazole</td>
<td>Near 0</td>
<td>Proton donor</td>
</tr>
<tr>
<td>Cysteine</td>
<td>$-\text{SH}$</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>$-\text{NH}_2^+$</td>
<td>+1</td>
<td>Amine binding; proton donor</td>
</tr>
<tr>
<td>Arginine</td>
<td>Guanidinium</td>
<td>+1</td>
<td>Amine binding</td>
</tr>
<tr>
<td>Serine</td>
<td>$-\text{CH}_2\text{OH}$</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

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**pKa Values of amino acid ionizable groups in proteins**

<table>
<thead>
<tr>
<th>Group</th>
<th>pKa</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>
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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-)

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 \( \text{BH}^+ \) can donate protons
• A covalent bond may break more easily if one of its atoms is protonated (below)

\[
\text{R}^+ + \text{OH}^- \xrightarrow{\text{slow}} \text{R}^- + \text{H}_2\text{O} \]

Sucrose phosphorylase exhibits covalent catalysis

(Sucrose = glucose-fructose)

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

*Sucrose + Enz \( \xrightarrow{\text{glucosyl-Enz + Fructose}} \)

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

Glucosyl-Enz + P\(_i\) \( \xrightarrow{\text{glucose 1-phosphate + Enz}} \)

pH-rate profile for papain

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 pK\(_a\) values of the two ionizable residues

\[
\begin{align*}
\text{Cys pK}_a \text{ in papain} &= 3.4 \text{ !} \\
\text{His pK}_a \text{ in papain} &= 8.3 \text{ !}
\end{align*}
\]
• 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>Enzymes with second-order rate constants near the upper limit</th>
<th>Substrate</th>
<th>$k_{cat}/K_m$ (M$^{-1}$s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catase</td>
<td>H$_2$O$_2$</td>
<td>4 x $10^9$</td>
</tr>
<tr>
<td>Carboxy anhydrase</td>
<td>CO$_2$</td>
<td>1.2 x $10^8$</td>
</tr>
<tr>
<td>Aspartate decarboxylase</td>
<td>Aspartate</td>
<td>1.6 x $10^9$</td>
</tr>
<tr>
<td>Fumarase</td>
<td>Fumarate</td>
<td>1.6 x $10^9$</td>
</tr>
<tr>
<td>Triose phosphate isomerase</td>
<td>α-Glycerol dehydro-5-phosphate</td>
<td>4 x $10^9$</td>
</tr>
<tr>
<td>Superoxide dismutase</td>
<td>-O$_2$</td>
<td>2 x $10^9$</td>
</tr>
</tbody>
</table>

*The ratio $k_{cat}/K_m$ in the apparent second order rate constant for the enzyme-catalyzed reaction $E+S\rightarrow E+S$; $E-P$ for the enzyme, 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)

TPI mechanism (continued)

- His-95 forms a strong hydrogen bond to the C-2 oxygen atom of the enediolate, and protonates this oxygen atom.

TPI mechanism (continued)

- Next, the imidazole group 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.
**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 ____________**

**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
**Transition-State (TS) Stabilization**

- increased interaction of E with S in transition-state (ES‡)
- 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) $\rightarrow 10^{-100}$
2. ______________

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

effective molarity: enhanced relative concentration of reactants due to binding to E
greater binding of transition states than S or P to E $\rightarrow$ lower activation energy $\rightarrow 10^4$-$10^5$

Enzyme rate accelerations $\sim 10^8$-$10^{12}$

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- Transition state analogs
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**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.

Inhibition of adenosine deaminase by a TS analog

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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
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

Glucose + ATP → Glucose 6-phosphate + ADP

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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
- ____________ 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 $\rightarrow$ rapid signal amplification

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

Chymotrypsin
Chymotrypsinogen

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 $\text{NH}_3^+$ 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 backbones of chymotrypsin (blue), trypsin (yellow), and elastase (green)

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

Chemistry.umeche.maine.edu/CHY431/Peptidase2/html

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

Substrate specificities are due to relatively small structural differences in active-site binding cavities.

Catalytic triad of serine proteases = Asp, His, Ser

- Imidazole ring (His-57) removes H from Ser-195 hydroxyl to make it a strong nucleophile (-CH₂O⁻)
- Buried carboxylate (Asp-102) stabilizes the positively-charged His-57 to facilitate serine ionization

α-Chymotrypsin mechanism (8 slides; 1)

Step (1): \( E + S \)

Binding of the substrate comprises Asp-102 and His-57. This strain is stabilized by formation of a low-barrier hydrogen bond. The mini-\( \phi \) of His-57 enables the mini-\( \phi \) ring to remain a proton from the hydrogen ionic group at Ser-195. The nucleophile oxygen of Ser-195 attacks the carbonyl carbon of the peptide bond to form a transesterification intermediate (di-\( \psi \)), which is believed to resemble the transition state.
(E-TI₁) (3)

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 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 from the vicinity of Gly-155 and Ser-195.

The imidazolylring of His-57 acts as an acid catalyst, donating a proton to the nitrogen of the substrate peptide bond, thus facilitating its cleavage.

(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. The peptide bond (P₁) with the new amino terminus leaves the active site, water enters.

(Acyl E + H₂O) (5)

Hydrolysis

Hydrolysis dissociation of the acyl-enzyme intermediate occurs when Asp-102 and His-57 act as a bicarbonate lyases and His-57 removes a proton from the water molecule to promote the nucleophile reaction, attacking the carbonyl group of the acyl-enzyme intermediate.

(E-TI₂) (6)

Tetrahedral intermediate

A second tetrahedral intermediate (E-TI₂) is formed and stabilized by the oxyanion hole.
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
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

- Carbocation formation

3. Cleavage reactions: less common when one electron remains with each product → two free radicals

- Free radical formation

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)

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### 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

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### Binding forces utilized for catalysis

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

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### 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)