Chapter 14

Mechanisms of Enzyme Action

Biochemistry
by
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Outline of Chapter 14

1. What Are the Magnitudes of Enzyme-Induced Rate Accelerations?
2. What Role Does Transition-State Stabilization Play in Enzyme Catalysis?
3. How does Destabilization of ES Affect Enzyme Catalysis?
4. How Tightly Do Transition-State Analogs Bind to the Active Site?
5. What Are the Mechanisms of Catalysis?
6. What Can Be Learned from Typical Enzyme Mechanisms?

14.1 – What Are the Magnitudes of Enzyme-Induced Rated Accelerations?

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Enzyme</th>
<th>Uncatalyzed Rate, 1/min</th>
<th>Catalyzed Rate, 1/min</th>
<th>kcat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fru-1,6-P2 + 2H2O → 2fructose-1,6-P2</td>
<td>Fructose-1,6-phosphatase</td>
<td>2 × 10−10</td>
<td>21</td>
<td>1.0 × 108</td>
</tr>
<tr>
<td>Glucose-6-P + H2O → D-glucose + H3PO4</td>
<td>Fructose-1,6-phosphatase</td>
<td>1.0 × 10−10</td>
<td>1.4 × 107</td>
<td>7.2 × 107</td>
</tr>
<tr>
<td>2H2O + O2 → H2O2</td>
<td>Superoxide dismutase</td>
<td>7.2 × 10−8</td>
<td>55</td>
<td>1.4 × 107</td>
</tr>
<tr>
<td>CH3CO2H + Ca2+ → Ca(CH3CO2)2</td>
<td>Alkaline phosphatase</td>
<td>8.0 × 10−10</td>
<td>14</td>
<td>1.4 × 106</td>
</tr>
<tr>
<td>H2O2 + 2H+ + 2e− → 2H2O</td>
<td>Peroxidase</td>
<td>5 × 10−10</td>
<td>5 × 105</td>
<td>3 × 109</td>
</tr>
<tr>
<td>CO2 + H2O + DH2O → HCO3− + OH−</td>
<td>Alkaline phosphatase</td>
<td>0.12 × 10−10</td>
<td>1.5 × 10−5</td>
<td>1.5 × 106</td>
</tr>
<tr>
<td>CH3CO2H + NaOH → CH3COONa + H2O</td>
<td>Alkaline phosphatase</td>
<td>0.12 × 10−10</td>
<td>1.5 × 10−5</td>
<td>1.5 × 106</td>
</tr>
<tr>
<td>CO2 + H2O → HCO3− + H+</td>
<td>Carbonic anhydrase</td>
<td>2.7 × 10−5</td>
<td>4.5 × 106</td>
<td>4.5 × 107</td>
</tr>
<tr>
<td>CO2 + 2H2O → H2CO3 + OH−</td>
<td>Carbonic anhydrase</td>
<td>2.7 × 10−5</td>
<td>4.5 × 106</td>
<td>4.5 × 107</td>
</tr>
</tbody>
</table>
Enzymes are powerful catalysts. The large rate accelerations of enzymes (10^7 to 10^{15}) correspond to large changes in the free energy of activation for the reaction. All reactions pass through a transition state on the reaction pathway. The active sites of enzymes bind the transition state of the reaction more tightly than the substrate. By doing so, enzymes stabilize the transition state and lower the activation energy of the reaction.

H-O-H + Cl^- \rightleftharpoons H-O---H \rightleftharpoons Cl^- \rightleftharpoons HO^- + HCl

Reactant \hspace{1cm} \text{Transition state} \hspace{1cm} \text{Products}

- Transition state (10^{-13} sec)
- Intermediate (10^{-13} to 10^{-3} sec)

14.2 – What Role Does Transition-State Stabilization Play in Enzyme Catalysis?

The catalytic role of an enzyme is to reduce the energy barrier between substrate S and transition state. Rate acceleration by an enzyme means that the energy barrier between ES and EX‡ must be smaller than the barrier between S and X‡. This means that the enzyme must stabilize the EX‡ transition state more than it stabilizes ES. Enzymes bind the transition state structure more tightly than the substrate.

Figure 14.1 Enzymes catalyze reactions by lowering the activation energy. Here the free energy of activation for (a) the uncatalyzed reaction, \( \Delta G^\ddagger \), is larger than that for (b) the enzyme-catalyzed reaction, \( \Delta G^\ddagger_\text{E} \).
14.3 – How does Destabilization of ES Affect Enzyme Catalysis?

• The favorable interactions between the substrate and amino acid residues on the enzyme account for the intrinsic binding energy, \( \Delta G_b \).

• The intrinsic binding energy ensures the favorable formation of the ES complex.

• If uncompensated, it makes the activation energy for the enzyme-catalyzed reaction unnecessarily large and wastes some of the catalytic power of the enzyme.

What Roles Do Entropy Loss and Destabilization of the ES Complex Play?

Raising the energy of ES raises the rate

• For a given energy of EX\(^+\), raising the energy of ES will increase the catalyzed rate.

• This is accomplished by
  1. loss of entropy due to formation of ES
  2. destabilization of ES by
     • structural strain & distortion
     • desolvation
     • electrostatic effects
14.4 – How Tightly Do Transition-State Analogs Bind to the Active Site?

- Transition state exists only for about 10^{-13} sec, less than the time required for a bond vibration.
- The nature of the elusive transition state can be explored using transition state analogs.
- Transition state analogs are stable molecules, chemically and structurally similar to the transition state.
- Transition-state analogs are only approximations of the transition state itself and will never bind as tightly as would be expected for the true transition state.
14.5 – What Are the Mechanisms of Catalysis?

- Enzymes facilitate formation of near-attack conformations
- Covalent catalysis
- General acid-base catalysis
- Low-barrier hydrogen bonds
- Metal ion catalysis

Transition-State Analogs Make Our World Better

- Enzymes are often targets for drugs and other beneficial agents
- Transition state analogs often make ideal enzyme inhibitors (p424-425)
  - Enalapril and Aliskiren lower blood pressure
  - Statins lower serum cholesterol
  - Protease inhibitors are AIDS drugs
  - Juvenile hormone esterase is a pesticide target
  - Tamiflu is a viral neuraminidase inhibitor

Enzymes facilitate formation of near-attack conformations

- The enzyme active-site structure and dynamics have emerged from X-ray crystal structures and computer simulations of molecular conformation and motion at the active site
- This preorganization of active site allow it to select and stabilize substrate conformations in which the reacting atoms are in van der Waals contact and at an angle resembling the bond to be formed in the transition state
- such arrangements have been termed near-attack conformations (NACs)
- NACs are precursors to reaction transition states
Protein motions are essential to enzyme catalysis

- Proteins are constantly moving (p165) – bonds vibrate, side chains bend and rotate, backbone loops wiggle and sway, and whole domains move as a unit
- Enzymes depend on such motions to provoke and direct catalytic events
- Protein motions support catalysis in several ways:
  - Active site conformation changes can
  - Assist substrate binding
  - Bring catalytic groups into position
  - Induce formation of NACs
  - Assist in bond making and bond breaking
  - Facilitate conversion of substrate to product

Figure 14.7 NACs are characterized as having reacting atoms within 3.2 Å and an approach angle of ±15° of the bonding angle in the transition state.

Figure 14.8 The active site of liver alcohol dehydrogenase – a near-attack complex.
14.5 – What Are the Mechanisms of Catalysis?

**Covalent catalysis**

• Some enzyme reactions derive much of their rate acceleration from the formation of covalent bonds between enzyme and substrate

\[
BX + Y \rightarrow BY + X
\]

\[
BX + Enz \rightarrow E:B + X + Y \rightarrow Enz + BY
\]

• Most enzymes that carry out covalent catalysis have ping-pong kinetic mechanisms

The side chains of amino acids in proteins offer a variety of nucleophilic centers for catalysis, including amines, carboxylate, aryl and alkyl hydroxyls, imidazoles, and thiol groups

• These groups are readily attack electrophilic centers of substrates, forming covalently bonded enzyme-substrate intermediate

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Reacting Group</th>
<th>Covalent Intermediate</th>
</tr>
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<tbody>
<tr>
<td>Trypsin</td>
<td>Serine</td>
<td>Acyl-Ser</td>
</tr>
<tr>
<td>Chymotrypsin</td>
<td>Cysteine</td>
<td>Acyl-Cys</td>
</tr>
<tr>
<td>Glyceroldehydro-P dehydrogenase</td>
<td>Serine</td>
<td>Phospho-Ser</td>
</tr>
<tr>
<td>Phosphoglycerate mutase (page 447)</td>
<td>Serine</td>
<td>Phospho-His</td>
</tr>
<tr>
<td>Succinyl-CoA synthetase (page 576)</td>
<td>Histidine</td>
<td>Glutathione</td>
</tr>
<tr>
<td>Aldolase (page 545)</td>
<td>Lysine and other</td>
<td>Schiff base</td>
</tr>
<tr>
<td>Pyridoxal phosphate-enzyme (pages 408, 762, and 807)</td>
<td>Amino groups</td>
<td>Schiff base</td>
</tr>
</tbody>
</table>
14.5 – What Are the Mechanisms of Catalysis?

**General acid-base catalysis**
- Specific acid-base catalysis involves H⁺ or OH⁻ that diffuses into the catalytic center
- General acid-base catalysis involves acids and bases other than H⁺ and OH⁻
- These other acids and bases facilitate transfer of H⁺ in the transition state

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**Low-Barrier Hydrogen Bonds**
- The typical H-bond strength is 10-30 kJ/mol, and the O-O separation is typically 0.28 nm
- As distance between heteroatoms becomes smaller (<0.25 nm), H bonds become stronger
- Stabilization energies of LBHB may approach 60 kJ/mol in solution
- pKₐ values of the two electronegative atoms must be similar
- Energy released in forming an LBHB can assist catalysis

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**Low-barrier hydrogen bond (LBHB)**
- The typical strength of a hydrogen bond is 10 to 30 kJ/mol
- The typical H-bond strength is 10-30 kJ/mol, and the O-O separation is typically 0.28 nm
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14.5 – What Are the Mechanisms of Catalysis?

**Metal ion catalysis**

- Many enzymes require metal ions for maximal activity (metalloenzymes)
  1. Stabilizing the increased electron density or negative charge
  2. Provide a powerful nucleophile at neutral pH

\[ M^{2+} + \text{NucH} \rightarrow M^{2+}(\text{NucH}) \rightarrow M^{2+}(\text{NucH}) + H^+ \]

14.6 – What Can Be Learned from Typical Enzyme Mechanisms?

- **Serine proteases** and **aspartic proteases** are good examples
- Knowledge of the **tertiary structure** of an enzyme is important
- Enzymes are the catalytic machines that sustain life

**The Serine Proteases**

*Trypsin, chymotrypsin, elastase, thrombin, subtilisin, plasmin, TPA*

- Serine proteases are a class of proteolytic enzymes whose catalytic mechanism is based on an active-site serine residue
- Ser is part of a "catalytic triad" of Ser, His, Asp
- Serine proteases are homologous, but locations of the three crucial residues differ somewhat
- Enzymologists agree, however, to number them always as His-57, Asp-102, Ser-195

Figure 14.14 Thermolysin is an endoprotease with a catalytic Zn$^{2+}$ ion in the active site. The Zn$^{2+}$ ion stabilizes the buildup of negative charge on the peptide carbonyl oxygen, as a glutamate residue deprotonates water, promoting hydroxide attack on the carbonyl carbon.
Serine Protease Mechanism

A mixture of covalent and general acid-base catalysis

- Asp-102 functions only to orient His-57
- His-57 acts as a general acid and base
- Ser-195 forms a covalent bond with peptide to be cleaved
- Covalent bond formation turns a trigonal C into a tetrahedral C
- The tetrahedral oxyanion intermediate is stabilized by N-Hs of Gly-193 and Ser-195
Serine Protease Mechanism

Kinetics

- The mechanism is based on studies of the hydrolysis of artificial substrates—simple organic ester

![Figure 14.19 Chymotrypsin cleaves simple esters, in addition to peptide bonds. P-Nitrophenylacetate has been used in studies of the chymotrypsin mechanism.](image)

Serine Proteases Display Burst Kinetics

![Figure 14.20 Burst kinetics in the chymotrypsin reaction.](image)

Serine Protease Mechanism

Kinetics

- In the chymotrypsin mechanism, the nitrophenylacetate combines with the enzyme to form an ES complex
- Followed by a rapid second step in which an acyl-enzyme intermediate is formed, with the acetyl group covalently bonded to the very reactive Ser-195

![Diagram of Serine Protease Mechanism](image)
Figure 14.23 A detailed mechanism for the chymotrypsin reaction. Note the low-barrier hydrogen bond (LBHB) in (c) and (g).

The Serine Protease Mechanism in Detail
Figure 14.21 The chymotrypsin mechanism: binding of a model substrate.

The Serine Protease Mechanism in Detail
Figure 14.21 The chymotrypsin mechanism: the formation of the covalent ES complex involves general base catalysis by His$^{57}$

The Serine Protease Mechanism in Detail
Figure 14.21 The chymotrypsin mechanism: His$^{57}$ stabilized by a LBHB.
The Serine Protease Mechanism in Detail

Figure 14.21 The chymotrypsin mechanism: collapse of the tetrahedral intermediate releases the first product.

(d)

C—N bond cleavage

Figure 14.21 The chymotrypsin mechanism: The amino product departs, making room for an entering water molecule.

(e)

Release of amino product

Figure 14.21 The chymotrypsin mechanism: Nucleophilic attack by water is facilitated by His57, acting as a general base.

(f)

Nucleophilic attack by water

Figure 14.21 The chymotrypsin mechanism: Collapse of the tetrahedral intermediate cleaves the covalent intermediate, releasing the second product.

(g)

Collapse of tetrahedral intermediate

Figure 14.21 The chymotrypsin mechanism: The amino product departs, making room for an entering water molecule.
The Serine Protease Mechanism in Detail

Figure 14.21 The chymotrypsin mechanism: Carboxyl product release completes the serine protease mechanism.

Figure 14.21 The chymotrypsin mechanism: At the completion of the reaction, the side chains of the catalytic triad are restored to their original states.

Transition-State Stabilization in the Serine Proteases

- The chymotrypsin mechanism involves two tetrahedral oxyanion intermediates
- These intermediates are stabilized by a pair of amide groups that is termed the “oxyanion hole”
- The amide N-H groups of Ser$^{195}$ and Gly$^{193}$ provide primary stabilization of the tetrahedral oxyanion

The “oxyanion hole”

The oxyanion hole of chymotrypsin stabilizes the tetrahedral oxyanion intermediate seen in the mechanism of Figure 14.21.
The Aspartic Proteases

- These enzymes are active at acidic pH
- possess two Asp residues at the active site and two Asps work together as general acid-base catalysts

<table>
<thead>
<tr>
<th>Table 14.1</th>
<th>Some Representative Aspartic Proteases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>Source</td>
</tr>
<tr>
<td>Pepsin</td>
<td>Stomach</td>
</tr>
<tr>
<td>Chymosin</td>
<td>Stomach</td>
</tr>
<tr>
<td>Carboxypeptidase D</td>
<td>Spleen, liver, and other animal tissues</td>
</tr>
<tr>
<td>Renin</td>
<td>Kidney</td>
</tr>
<tr>
<td>HIVprotease</td>
<td>AIDS virus</td>
</tr>
</tbody>
</table>

Aspartic Protease Mechanism

- pH dependence (fig 14.23)
- The aspartate carboxyl groups functioned alternately as general acid and general base
  - Deprotonated Asp acts as general base, accepting a proton from HOH, forming OH⁻ in the transition state
  - Other Asp (general acid) donates a proton, facilitating formation of tetrahedral intermediate

The Aspartic Proteases

- Most aspartic proteases have a tertiary structure consisting of two lobes (N-terminal and C-terminal) with approximate two-fold symmetry (fig 14.22)
- HIV-1 protease is a homodimer

Figure 14.23
pH-rate profiles for (a) pepsin and (b) HIV protease. (Adapted from Denburg, J., et al., 1968. The effect of pH on the rates of hydrolysis of three acylated dipeptidyl by pepsin.)
A Mechanism for the Aspartic Proteases

Figure 14.24 Mechanism for the aspartic proteases. LBHBs play a role in states E, ES, ET’, EQ’, and EP’Q.

HIV-1 Protease

A novel aspartate protease

- HIV-1 protease cleaves the polyprotein products of the HIV genome, producing several proteins necessary for viral growth and cellular infection
- This is a remarkable imitation of mammalian aspartic proteases
- HIV-1 protease is a homodimer - more genetically economical for the virus
- Active site is two-fold symmetric
- Two aspartate residues, Asp-25 and Asp-25’

Figure 14.26 HIV mRNA provides the genetic information for synthesis of a polyprotein. Proteolytic cleavage of this polyprotein by HIV protease produces the individual proteins required for viral growth and cellular infection.

Figure 14.27 (left) HIV-1 protease complexed with the inhibitor Crixivan (red) made by Merck. The flaps (residues 46-55 from each subunit) covering the active site are shown in green and the active site aspartate residues involved in catalysis are shown in white. (right) The close-up of the active site shows the interaction of Crixivan with the carboxyl groups of the essential aspartate residues.
Therapy for HIV?

Protease inhibitors as AIDS drugs

• If the HIV-1 protease can be selectively inhibited, then new HIV particles cannot form
• Several novel protease inhibitors are currently marketed as AIDS drugs
• Many such inhibitors work in a culture dish
• However, a successful drug must be able to kill the virus in a human subject without blocking other essential proteases in the body

Chorismate Mutase: A Model for Understanding Catalytic Power and Efficiency

• Direct comparison of enzyme-catalyzed reactions and their uncatalyzed counterparts is difficult
• Chorismate mutase has become a model for making this comparison, thanks to the efforts of a large number of enzyme mechanism researchers
• Chorismate mutase acts in the biosynthesis of phenylalanine and tyrosine in microorganisms and plants
• It involves a single substrate and catalyzes a concerted intramolecular rearrangement of chorismate to prephenate
• One C-O bond is broken and one C-C bond is formed

Protease inhibitor drugs used by AIDS Patients

Figure 14.28  The chorismate mutase reaction converts chorismate to prephenate in an intramolecular rearrangement.
Figure 24.28 A classic Claisen rearrangement. Conversion of allyl phenyl ether to 2-allyl alcohol proceeds through a cyclohexadienone intermediate, which then undergoes a keto-enol tautomerization.

The chorismate mutase reaction (and its uncatalyzed counterpart) occur via chair states

Figure 14.29 The critical H atoms are distinguished in this figure by blue and green colors.

Jeremy Knowles has shown that both the chorismate mutase and its uncatalyzed solution counterpart proceed via a chair mechanism. A transition state analog of this state has been characterized.

The structure of E. coli chorismate mutase

Figure 24.30 (a) the chorismate mutase homodimer (b)The active site, showing the bound transition-state analog.
**Transition state stabilization by electrostatic and hydrogen-bonding interactions**

Twelve electrostatic and hydrogen-bonding interactions stabilize the transition-state analog.

**The Chorismate Mutase Mechanism**

The carboxyvinyl group folds up and over the chorismate ring and the reaction proceeds via an internal rearrangement.

**The Chorismate Mutase Active Site Favors a Near-Attack Conformation**

Figure 14.33 Chorismate bound to the active site of chorismate mutase in a structure that resembles a near-attack complex. Arrows indicate hydrophobic interactions and red dotted lines indicate electrostatic interactions.

**Formation of a NAC is facile in the chorismate mutase active site**

Figure 14.34 Chorismate mutase facilitates NAC formation. The energy required to move from the NAC to the transition state is essentially equivalent in the catalyzed and uncatalyzed reactions.