Enzyme Catalysis-Serine Proteases

Concepts to be learned
• Activation Energy
• Transition State
• Example: Proteases
• Requirements for proteolysis
• Families of proteases
• Protein Folds used by proteases for catalysis
Catalysis

• Enzyme: increases rate of chemical reaction, decreases activation energy

• How?
  – Binding to the transition state of the substrate (L. Pauling 1946)

Reaction Path:

\[ \text{Residues of Enzyme} \rightarrow \text{Substrate} \rightarrow \text{Product} \]
Linus Pauling, 1901–1994
Enzymes accelerate chemical reactions by decreasing the activation energy.
How to Lower $\Delta G^\neq$
Enzymes bind transition states best

- The idea was proposed by Linus Pauling in 1946
  - Enzyme active sites are complementary 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
Covalent Catalysis

- A transient covalent bond between the enzyme and the substrate
- Changes the reaction Pathway

- Uncatalyzed: \[
\begin{align*}
A - B & \rightarrow A + B \\
\end{align*}
\]

- Catalyzed: \[
\begin{align*}
A - B + X : & \rightarrow A - X + B \rightarrow A + X : + B \\
\end{align*}
\]

- Requires a nucleophile on the enzyme
  - Can be a reactive serine, thiolate, amine, or carboxylate
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⁺H</td>
<td>R—NH₂</td>
</tr>
<tr>
<td>Cys</td>
<td>R—SH</td>
<td>R—S⁻</td>
</tr>
<tr>
<td>His</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ser</td>
<td>R—OH</td>
<td>R—O⁻</td>
</tr>
<tr>
<td>Tyr</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 6-9
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Metal Ion Catalysis

• Involves a metal ion bound to the enzyme

• Interacts with substrate to facilitate binding
  – Stabilizes negative charges

• Participates in oxidation reactions
Enzymes accelerate chemical reactions by decreasing the activation energy.
Hydrolysis of Peptide Bonds

\[
\text{polypeptide} \quad \overset{\text{scissile bond}}{\longrightarrow} \quad \text{peptide 1} + \text{peptide 2}
\]
Serine Proteases

• Peptide bond cleavage by forming tetrahedral transition states:
  - First Stage: Acylation
    • “Acyl-enzyme intermediate” formed
  - Second Stage: Deacylation
    • “Acyl-enzyme intermediate” is hydrolyzed by water
Chymotrypsin uses most of the enzymatic mechanisms.
Chymotrypsin
Two anti-parallel $\beta$ domains
Serine Proteases

- Rx: Peptide Bond Cleavage
- 4 Requirements
  - Catalytic triad
    - Ser, His, Asp
    - Ser forms a covalent bond with substrate → specific reaction path
    - His: accepts H\(^+\) from Ser, thereby facilitates bond formation, and stabilizes negatively charged transition state
    - Asp\(^-\): stabilizes positive charge of His\(^+\), increases rate ~10,000
  - Oxyanion binding site
    - Stabilizes transition state, forms 2 H-bonds to a negative oxygen of the substrate
  - Substrate specificity pocket
    - Recognition/identity (trypsin; chymotrypsin)
  - Non-specific binding site for polypeptide substrates
Aclylation and Deacylation of the Acyl-Enzyme Intermediate
Serine Proteases

- Rx: Peptide Bond Cleavage
- 4 Requirements
  - Catalytic triad
    - Ser, His, Asp
    - Ser forms a covalent bond with substrate → specific reaction path
    - His: accepts H⁺ from Ser, thereby facilitates bond formation, and stabilizes negatively charged transition state
    - Asp⁻: stabilizes positive charge of His⁺, increases rate ~10,000
  - Oxyanion binding site
    - Stabilizes transition state, forms 2 H-bonds to a negative oxygen of the substrate
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Tetrahedral Transition State
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.
Chymotrypsin Mechanism

Step 2: Nucleophilic 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.
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\textsubscript{195}.

Figure 6-22 part 5
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Chymotrypsin Mechanism

Step 6: Break-off from the Enzyme

Figure 6-22 part 6
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Chymotrypsin Mechanism

Step 7: Product Dissociates

Dissociation of the second product from the active site regenerates free enzyme.

Figure 6-22 part 7
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Chymotrypsin

• 2 domains
• Each domain: antiparalleled \( \beta \) -barrel, six \( \beta \) -strands

\[ \begin{align*}
4 (1-4) & \quad 2 (5,6) \\
\text{Greek Key Motif} & \quad \text{\( \beta \) -hairpin} \\
\text{Loop 3-4} & \quad \text{Loop 5-6}
\end{align*} \]

• Active Site: 2 loop regions from each domain
• Substrate specificity pocket- Aromatics
  – Trypsin: R or K
  – Elastase: Pocket blocked small uncharged
Chymotrypsin
Two anti-parallel $\beta$ domains
Specificity Pocket

Chymotrypsin

Gly 226
Ser 189

Gly 216

Trypsin

Gly 226
Asp 189

Gly 216

Elastase

Thr 226
Val 216

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Bacterial Subtilisin: $\alpha,\beta$ type  
(J. Kraut, UCSD)

- 4 $\alpha$ helices surrounding 5 parallel $\beta$-strands
- Active site:
  - C-end of the central $\beta$-strands
  - Catalytic triad: S,H, D

**Carboxypeptidase:** (catalysis by induced electronic strain on substrate)

**Zn$^{2+}$ Protease**
- Glu 270 directly attacks the carbonyl carbon of the scissile bond to form a “covalent mixed-anhydride intermediate”
- Zn$^{2+}$ binding $\rightarrow$ polarizes the carbonyl
- “environment”, non-polar, induced dipole
- Facilitates hydrolysis by water
Subtilisin
Active Site of Subtilisin
Serine Proteases

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    • “Acyl-enzyme intermediate” formed
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Enzyme Catalysis-Serine Proteases

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