Lecture 16: Introduction to Enzymes and Serine Proteases

Goals:
- Understand how enzymes stabilize the transition state
- Discuss some applications of enzymes
- Explain the role of key catalytic residues in serine proteases
- Describe enthalpy based transition state stabilization in serine proteases – oxyanion hole.
- Understand the structural basis of substrate specificity
- Understand the kinetic basic of substrate specificity

Enzyme-transition state complex is stabilized by direct interactions:
The enzyme transition-state complex is stabilized by direct interactions between the enzyme and the transition state. This reduces the free energy of the transition state due to $\Delta H^\circ$ (enthalpy).

Other Ways to Increase the Rate - Alternative Mechanism:
Membrane transport proteins – the “substrate” no longer goes through the lipid membrane, it goes through a protein channel instead.

Rates of enzyme catalyzed vs. uncatalyzed reactions:

<table>
<thead>
<tr>
<th></th>
<th>Uncatalyzed</th>
<th>Catalyzed</th>
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<tbody>
<tr>
<td></td>
<td>$S \rightleftharpoons X \rightleftharpoons P$</td>
<td>$S + E \rightleftharpoons ES \rightleftharpoons EX^\dagger \rightleftharpoons EP \rightleftharpoons E + P$</td>
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<tr>
<td>Rate of the reaction:</td>
<td>$v \propto \frac{[X^\dagger]}{[S]} = K_{EQ}$</td>
<td>$v_e \propto \frac{[EX^\dagger]}{[ES]} = K_{EQ}^e$</td>
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<tr>
<td>$K_{EQ} = e^{-\Delta G^\dagger/RT}$</td>
<td>$K_{EQ}^e = e^{-\Delta G^e^\dagger/RT}$</td>
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Proteases:
The peptide bond is stable due to its partial double bond character. In an uncatalyzed reaction, a peptide bond hydrolysis is expected to take ~10-100 years. However, peptide bonds need to be released in milliseconds timeframe during biochemical reactions, which is made possible by proteases. Proteases are usually referred to by their key catalytic residues:

- Serine proteases (Trypsin)
- Thiol proteases (Papain)
- Aspartyl proteases (HIV protease)
- Metalloproteases (Zn+)

Serine Proteases:
These enzymes play an important role in many processes, e.g. digestion of dietary protein, blood clotting cascade, and in several pathways of differentiation and development. Proteases active in digestion include:

<table>
<thead>
<tr>
<th>Trypsin</th>
<th>Chymotrypsin</th>
<th>Elastase</th>
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<tr>
<td>Lys, Arg</td>
<td>Phe, Tyr.</td>
<td>Argy, Lys.</td>
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Reactions Catalyzed:
Serine proteases can hydrolyze either esters or peptide bonds:

**Ester Hydrolysis:** The bright yellow color of the p-nitrophenolate ion provides a convenient way to monitor the rate of product formation.

Peptide Hydrolysis:

Catalytic Residues:
Key residues are Ser 195, His 57, and Asp 102. These three residues are called the catalytic triad. Serine is the nucleophile, for the first part of the reaction.

Nucleophile: group that is electron rich and can form bonds with electron deficient groups. In the case of the peptide bond (or ester) the electronegativity of the oxygen makes the carbonyl carbon electron deficient.

Catalytic Mechanism:
I. Substrate binds, forming (ES) complex.
II. Nucleophilic attack of the side chain oxygen of Ser 195 on the carbonyl carbon of the scissile bond (bond to be cleaved) forming a tetrahedral intermediate. Assist from His 57 (proton transfer from Ser 195). **Tetrahedral-intermediate (transition state) is stabilized by amides of Ser195 and Gly 193 – enthalpic stabilization of transition state.**
III: Breakage of the peptide bond with assistance from His 57 (proton transfer to the new amino terminus). Release of the first product.
IV: Acyl-intermediate: Note that the substrate is covalently attached to the active site Serine 195.
V: Nucleophilic attack of water on the acyl-enzyme intermediate with assistance of His 57 and formation of the tetrahedral intermediate.
VI: Decomposition of acyl intermediate and release of the second product. Enzyme is in the same form as in panel II!
Substrate Specificity: Why are certain substrates preferred (hydrolyzed at a faster rate)?
Serine proteases utilize all of the intermolecular forces that we have discussed to bind their substrates. In addition to general recognition of the peptide by H-bonds, a particular serine protease is specific for certain amino acids. The molecular nature of this specificity can be inferred from the structure of the active site:

![Trypsin and Chymotrypsin Diagram]

Kinetics & Specificity—“kinetic editing”:

$$S + E \rightleftharpoons ES \rightleftharpoons EX^+ \rightleftharpoons EP \rightleftharpoons E + P$$

- *Good substrate* — high $k_{cat}$, low $k_{off}$
- *Poor substrate* — high $k_{off}$

$[ES]$ will leave before catalysis occurs.