**Lecture 16: Enzyme Mechanism - Serine Proteases**



Reading: Horton: 6.7 Nelson and Cox: Chapter 6

**Key Terms:**

* **Transition state stabilization: Entropic**
* **Transition state stabilization: Enthaplic**
* **Acyl-enzyme intermediate**
* **Nucleophilic agent**
* **Catalytic triad (Ser, His, Asp)**
* **Substrate specificity**

Serine proteases play an important role in many processes, *e.g.* digestion of dietary protein, blood clotting cascade, and in several pathways of differentiation and development. They are generally produced as inactive **zymogens**, and auto-activate by peptide cleavage to form the active enzyme. Proteases active in digestion include:

Trypsin Chymotrypsin Elastase

**Reactions Catalyzed:**



Serine Proteases can hydrolyze *either* esters or peptide bonds:

*Ester Hydrolysis:*  Note that 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.

**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 I!



**Serine Proteases – Review**

Provide names and/or roles in the catalytic mechanism for each of the labeled items:



A:

B:

C:

D:

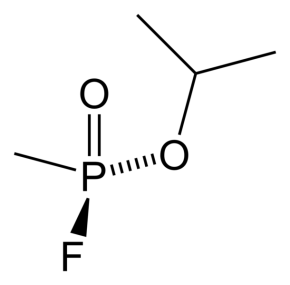
E:

**Kinetics & Specificity:**

**Substrate Specificity:**

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** cleaves after Lys and Arg residues: Asp189 in the active site of Trypsin interacts with the positive charge on Arg and Lys. | **Chymotrypsin** cleaves after aromatic (and large hydrophobic) residues: The active site of the enzyme contains a hydrophobic pocket, formed in part by Met192 of Chymotrypsin. |

**Esterases and Toxins**: The enzyme that degrades acetylcholine (a neurotransmitter associated with muscle contrations) is a serine esterase, with the same catalytic triad shown above. The nerve gas, **sarin**, inhibits this enzyme, interfering with breathing and other important functions .