Lecture 16: Enzyme Mechanism: Serine Proteases

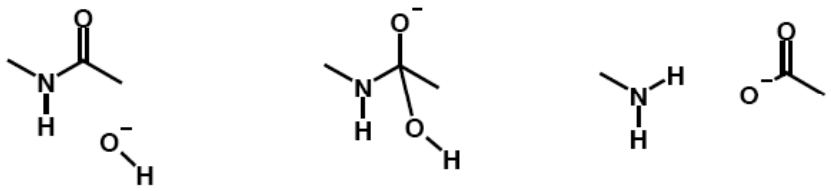
Assigned reading in Campbell: Chapter 7.5-7.7

Key Terms:

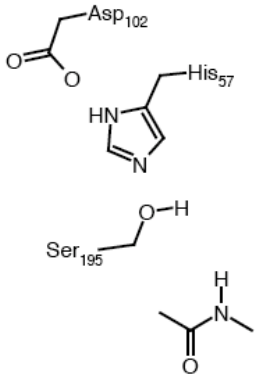
- **Transition State stabilization (enthalpic & entropic)**
- **Regulation of Serine proteases: zymogens**
- **Covalent Catalysis**
- **Acyl-enzyme intermediate**
- **Nucleophilic agent**
- **Preferential binding of transition state complex**
- **Catalytic triad (Ser, His, Asp)**
- **Substrate specificity**

16.1 The transition state of the enzyme substrate complex is stabilized in two ways:

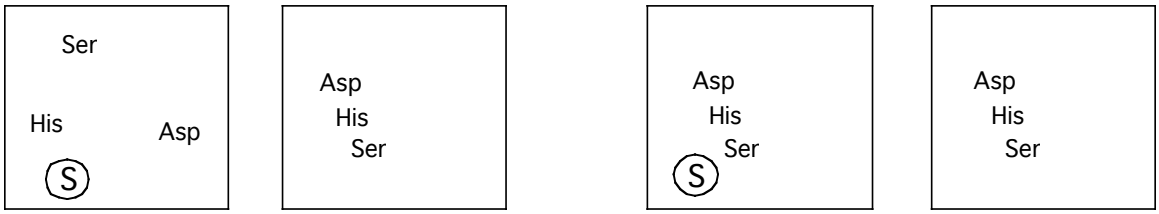
1. **Enthalpic** - The enzyme transition-state complex is stabilized by direct interactions (e.g. H-bonds, electrostatic interactions) between the enzyme and the transition state. This reduces the free energy of the transition state due to ΔH (enthalpy).



2. **Entropic** – Formation of the transition state requires a precise geometric arrangement of sidechain groups. In the case of a reaction occurring in solution, this would require considerable ordering of these chemical groups, i.e., a reduction in the entropy of the system, which is unfavorable.



In an enzyme, these groups are already in the correct position because of the way the protein folded, therefore there is no loss of entropy in the complex. For example, the serine proteases utilize a serine, histidine, and aspartic acid to catalyze peptide bond hydrolysis. Consider the following changes in entropy between the initial state and the transition state:



16.2 Serine Proteases:

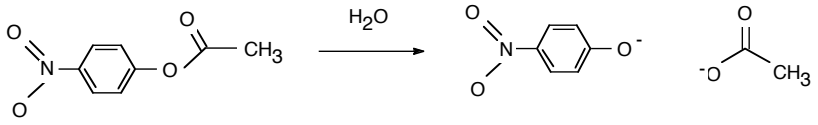
Diverse role of Serine Proteases:

Serine proteases play important roles 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.

16.2 Mechanism

Serine proteases can hydrolyze *either* esters or peptide bonds utilizing mechanisms of **covalent catalysis** and **preferential binding of the transition state** and by providing **suitable reactive groups** near the peptide bond that is cleaved.

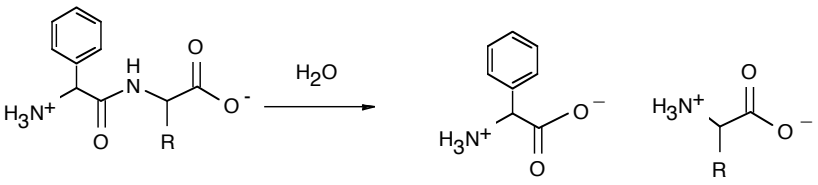
Ester hydrolysis:
Note that the bright yellow color of the p-nitrophenolate ion provides a convenient way to monitor the rate of product formation.



p-Nitrophenyl acetate

p-Nitrophenolate
(bright yellow)

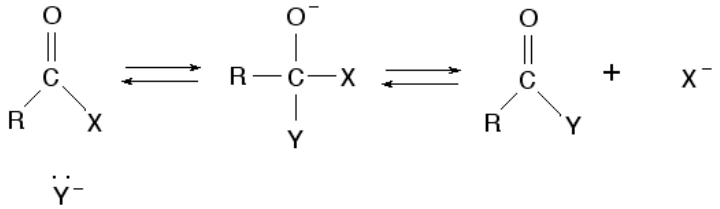
Peptide hydrolysis:
(e.g. Chymotrypsin)



16.3 **Catalytic Mechanism: key features**

- **Nucleophilic attack:** a species that is electron rich (has a negative charge or an unshared electron pair) forms a bond with an electron deficient species. In the case of the peptide bond (or ester) the electronegativity of the oxygen makes the carbonyl carbon electron deficient.

Example of a nucleophilic attack that is also used by serine proteases:



- **Formation of an acyl-enzyme intermediate:** Upon cleavage of the peptide bond, the acyl group of the substrate becomes covalently bound to the enzyme before it is transferred to a molecule of water.

16.4 Substrate Specificity:

Serine proteases utilize all of the intermolecular forces that we have discussed to bind their substrates. In particular, the peptide bond to be cleaved often forms one or more hydrogen bonds with the enzyme. In addition to general recognition of the peptide, 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: Asp 189 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 Met 192 of Chymotrypsin.

- Elastase cleaves after small residues, especially glycine and alanine. Val and Thr residues in the active site form a shallow binding pocket.

Steps in catalytic cycle:

1. Substrate binds
2. 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. Assistance from His 57 (proton transfer from Ser 195) and Asp 102. Tetrahedral intermediate or transition state is stabilized by amides of Ser 195 and Gly 193.
3. Breakage of the peptide bond with assistance from His 57 (proton transfer to the new amino terminus). Release of the first product.
4. Acyl-intermediate: Note that the substrate is covalently attached to the active site Ser 195.
5. Nucleophilic attack of water on the acyl-enzyme intermediate with assistance of His 57 and formation of the tetrahedral intermediate.

Decomposition of acyl-intermediate and release of the second product. Enzyme is in the same form as at the start!

Mechanism of Serine Proteases: