Lecture 16: Serine Proteases

Goals:

- Determine catalytic residues in proteins.
- 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

Proteases: The peptide bond is stable, but cleavage of the peptide bond releases energy, so the cleaved bond is more stable. Fortunately for life, in an uncatalyzed reaction, a peptide bond hydrolysis is expected to take ~10-100 years. However, peptide bonds need to be broken in milliseconds timeframe during biochemical reactions, which is made possible by proteases. Proteases are usually referred to by their key catalytic residues:

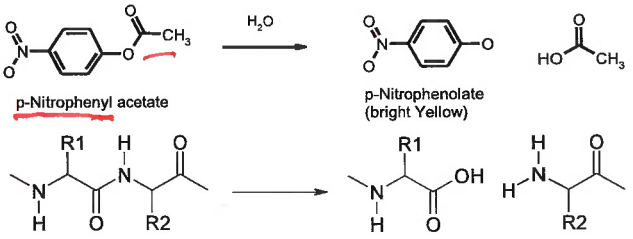
- Serine proteases (e.g. Trypsin)
- Thiol proetases (e.g. Papain)
- Aspartyl proteases (e.g. HIV protease)
- Metalloproteases (e.g. Zn protease)

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. They are usually produced in an inactive form (zymogen) and activated by proteolysis.

Proteases active in digestion include: Trypsin Chymotrypsin Elastase

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.

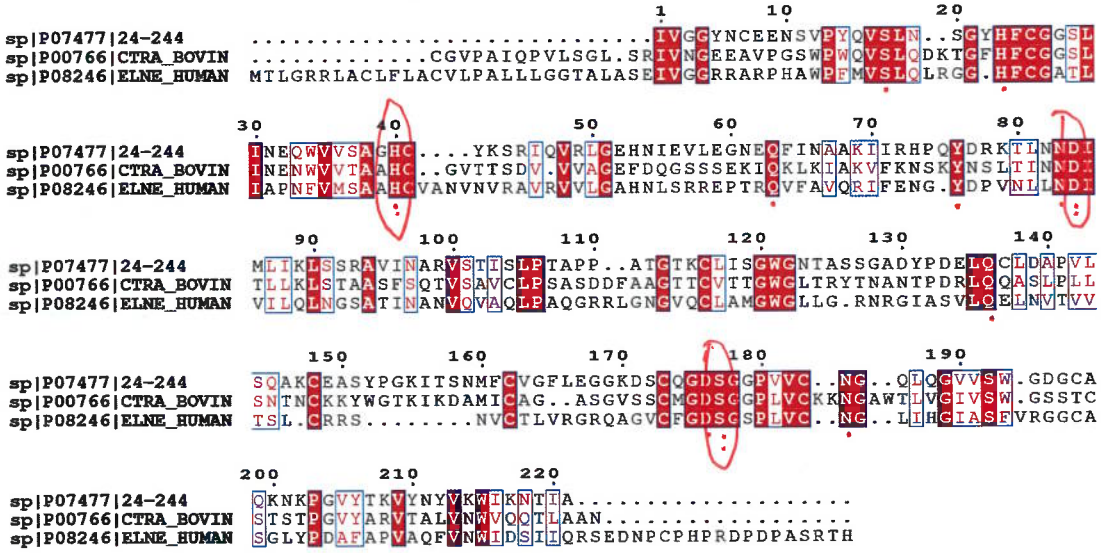


Peptide Hydrolysis:

How Catalytic Residues are Determined:

1. Sequence alignment between homologous enzymes (see below).
2. 3D Structure of enzyme-substrate (inhibitor) complex (clustered in active site)
3. Effects of pH on rates (to determine mechanism).
4. Alternations of residues (mutagenesis) – to test hypothesis on mechanism

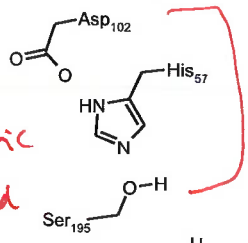
Alignment of trypsin, chymotrypsin, and elastase: Circle **conserved** polar residues that may be involved in catalysis (*found in all serine proteases*). Red blocks = conserved in all. Ignore Cys (C), non-polar residues (I, V, P, L, F, A), and G.



Catalytic Residues in Serine Protease:

Key residues are (numbers are given for the mature protein, numbers on alignment above are for the pre-protein, prior to cleavage).

- Ser 195: Nucleophile
- His 57: Activates nucleophile (serine and water)
- Asp 102: Facilitates activation of nucleophile.



These three residues are called the **catalytic triad**.

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 (see separate diagram, next page).

I. Substrate binds, forming (ES) complex, activation of Ser by proton transfer to His – stabilized by Asp(-)

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. Planer amide becomes tetrahedral. **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.

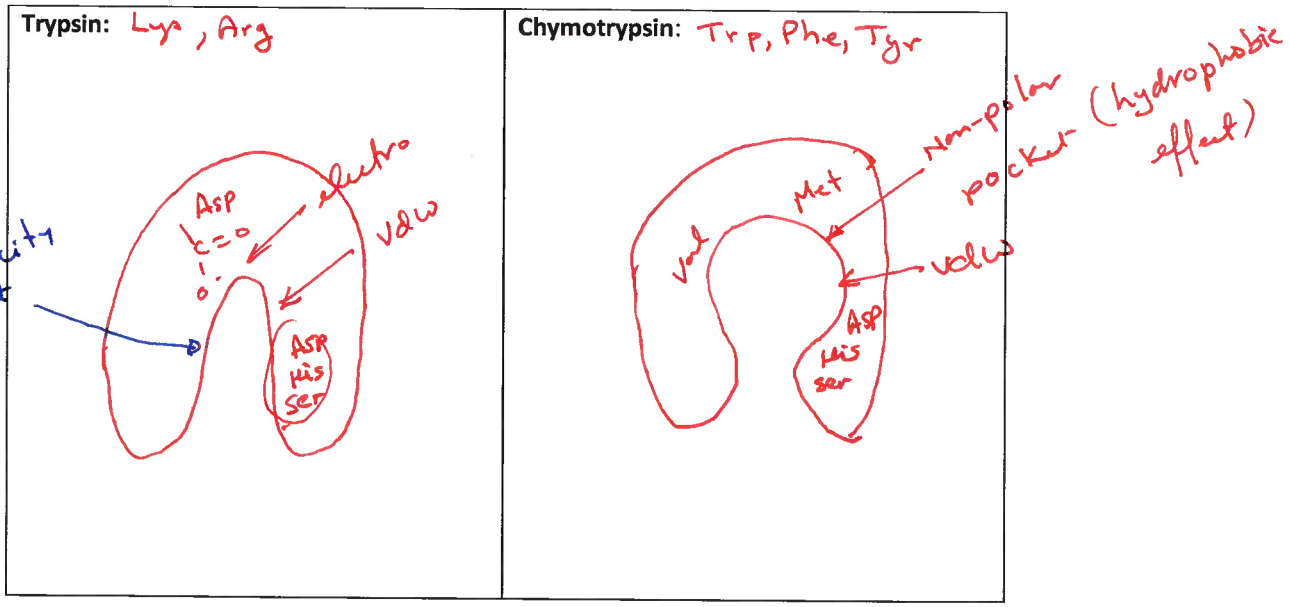
IV: Acyl-intermediate: Note that the remaining substrate is *covalently* attached to the active site Serine 195 via an ester linkage.

V: Nucleophilic attack of water on the acyl-enzyme intermediate with assistance of His 57/Asp 102 and formation of the second tetrahedral intermediate.

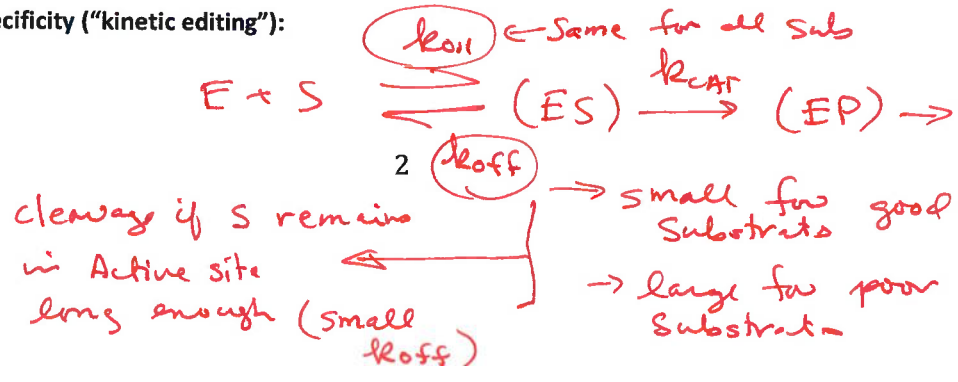
VI: Decomposition of acyl intermediate and release of the second product. Enzyme is in the same form as in panel I!

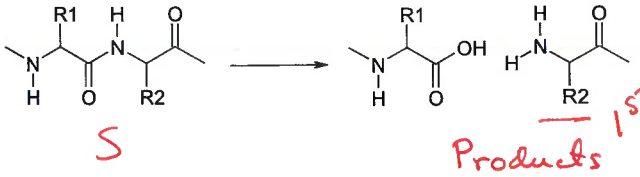
Substrate Specificity: Why are certain substrates preferred (hydrolyzed at a faster rate)?

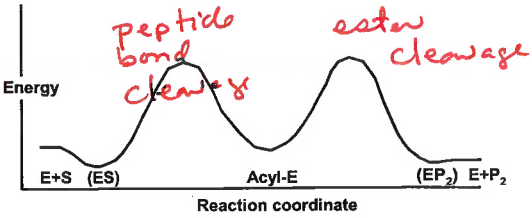
Serine proteases utilize all of the intermolecular forces that we have discussed to bind their substrates. In addition to general recognition of the peptide by H-bonds, a particular serine protease is specific for certain amino acids. The molecular nature of this specificity can be inferred from the structure of the active site:

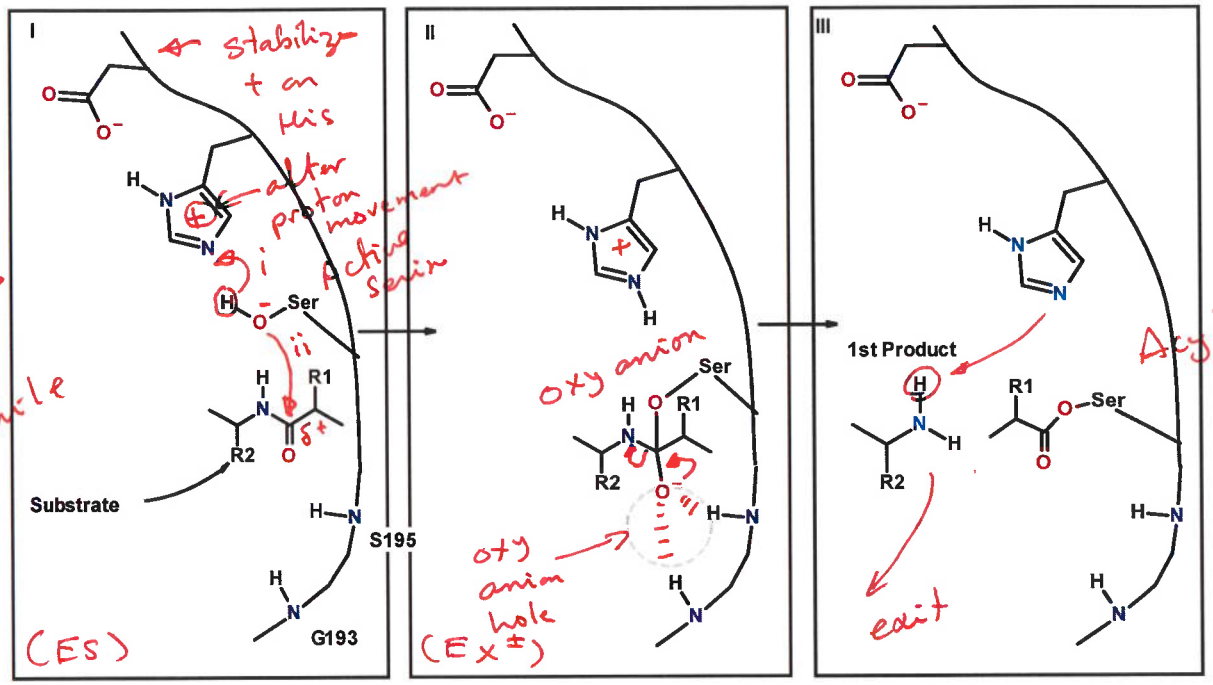


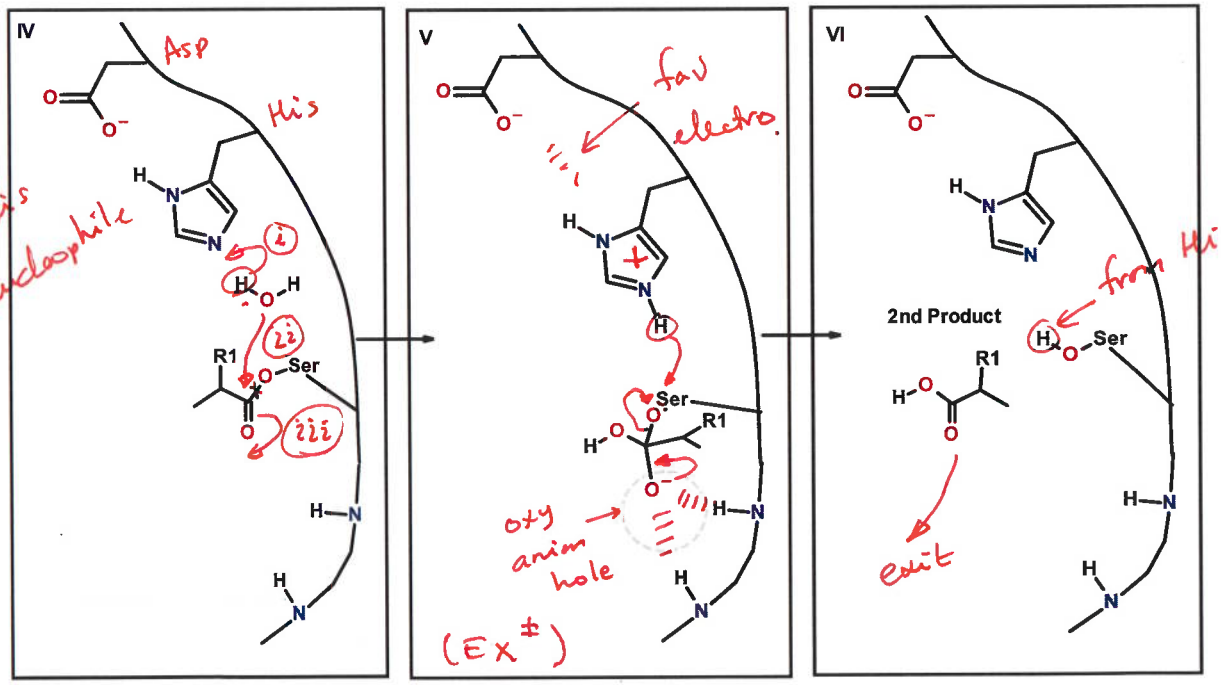
Kinetics & Specificity ("kinetic editing"):




R1
H
O
N
H
O
R2
S
R1
OH
H
N
O
R2
Products
1ST


peptide bond cleavage
ester cleavage
Energy
E+S
(ES)
Acyl-E
(EP2)
E+P2
Reaction coordinate


I
stabilize + on His
alter proton movement
i
ii
Ser
Substrate
S195
G193
(ES)
II
oxy anion
oxy anion hole
(Ex±)
III
1st Product
Acyl-E
exit
• peptide hydrolysis
• Ser nucleophile


IV
Asp
His
i
ii
iii
R1
Ser
V
fav electro.
Ser
R1
oxy anion hole
(Ex±)
VI
2nd Product
from His
exit
• ester hydrolysis
• H2O nucleophile