ENZYMES: Binding & Catalysis

Lecture 17 (10/23/24)

A. Binding
B. Catalysis
C. Nomenclature
D. Catalysis
   1. Transition State Theory
   2. Catalytic strategies (What)
   3. Mechanistic strategies (How)

TODAY
•Reading: Ch5; 157
Ch12; 413-415

NEXT
•Reading: Ch5; 157-160
Ch6; 213-220
•Homework #18

E. Quantifying the Catalytic Power: Kinetics
   1. Review
   2. Enzyme Kinetics
   3. Initial rate (v_0) vs. [S] for enzymic reaction;
   4. ES complex
   5. Rate expression; Michaelis-Menten Kinetics (M-M)
   6. Meaning of rate expression (M-M eqn)
   7. Collection and manipulation of data
      a. Lineweaver-Burk; double reciprocal; $1/v_0$ vs. $1/[S]$
   8. Inhibition
      a. Irreversible: protein modification
      b. Reversible: Competitive, Uncompetitive, Noncompetitive

F. Active-site identification
   a. Determine mechanism-ordered *versus* random
   b. pH studies;
   c. Protein modification; Irreversible
   d. X-ray crystallography structure; cleft

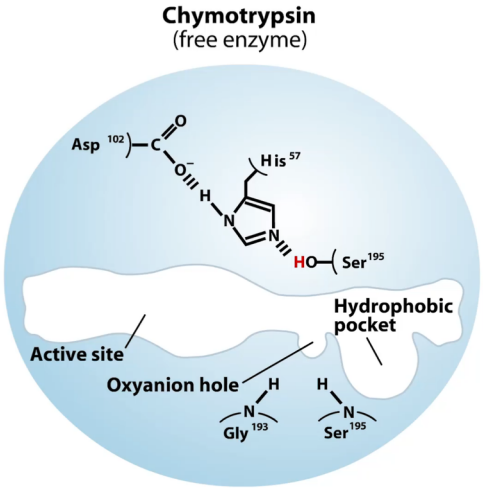
G. Energetics of Catalysis
   a. The $\Delta\Delta G^{\ddagger}$ is negative
   b. The $\Delta\Delta G^{\ddagger} = \Delta\Delta H^{\ddagger} - T\Delta\Delta S^{\ddagger}$; bonding effects &
      proximity/position effects
   a. Rate dependent on (**k**T/h)EXP($-\Delta G^{\ddagger}$/RT)
   b. Example of enzyme;
      I. Proline Racemase
      II. HIV protease; tetrahedral t.s. seen in two nM inhibitors (K_i < 1 nM); bioavailability

H. Enzyme Mechanisms
   a. Proteases
      1. Introduction; roles and types
         i. Roles: Serine:
         ii. Types: based on mechanism; Serine, Thiol, Carboxyl (acid), Metallo
   b. Serine Proteases
      1. Reaction & specificity
      2. Activation (zymogens); Ile a-amino pK_a impt.
      3. Active Site Determination
         i. esterase activity
         ii. burst kinetics => two-steps (Ping-pong bi bi)
         iii. protein modification
         iv. pH studies
         v. X-ray crystallography
      4. Proposed mechanism
         i. Catalytic triad (Ser-His-Asp) – highly conserved
         ii. Mechanism; tetrahedral intermediates and stabilize t.s with oxy-anion hole
         iii. Old; charge relay (but Ser-195 does not have the correct pK_a)
         iv. Low-barrier Hydrogen bonds- Role for Asp
      5. Specificity
         i. Chymotrypsin *versus* elastase
   c. Other protease mechanisms

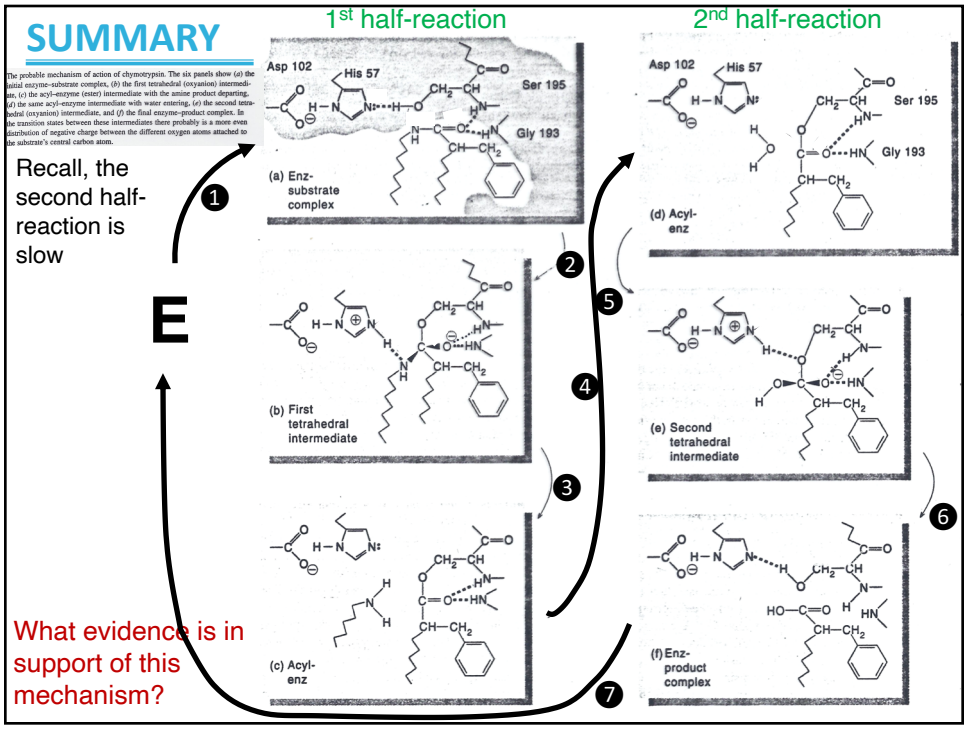
I. Enzyme Regulation
   a. Introduction
   b. Strategies
      a. Gene Regulation
      b. Covalent Modification
      c. Allosteric Control
   c. Covalent Modification
      1. Proteolysis
      2. Protein modification
         i. Phosphorylation
            a) Kinases
            b) Phosphatase

Chymotrypsin Mechanism

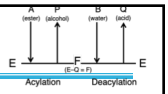
Animation



SUMMARY



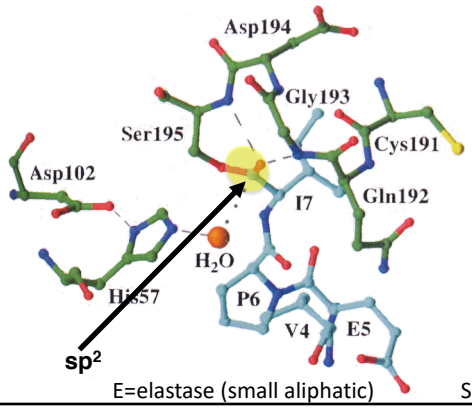
Chymotrypsin Mechanism

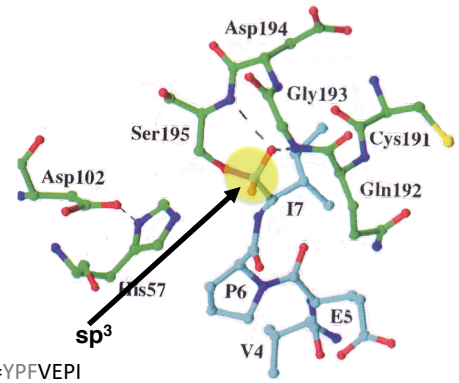


Recall, the second half-reaction is slow.
Perform X-ray crystallography but by trapping the enzyme in action!

If you protonate the His-57, it can't get beyond the acyl-enzyme intermediate.

If you de-protonate during turnover and stop the reaction, its built up at the slowest step (the His-57 proton is titrated and can't participate in breakdown of the tetrahedral intermediate).





E=elastase (small aliphatic) S=YPFVEPI

• *Enzyme mechanism and binding energy*

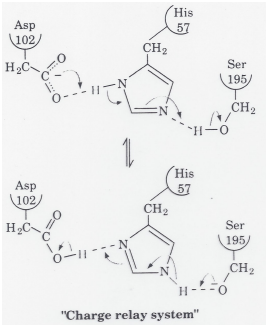
Chymotrypsin Mechanism

The "catalytic triad"

Asp102 – His57 – Ser195

The catalytic triad is found in all Serine Protease and Serine Esterases (e.g., acetylcholinesterase)

How does this work?



For this to work, all pK_a values should be similar. In other words, pK_a value of Asp slightly higher than that of Ser

Asp102 – His57 – Ser195

pK_a values as amino acids are:	3.5	6.0	15.0
The measured pK_a values are:	7.0	7.0(12.0)*	15.0

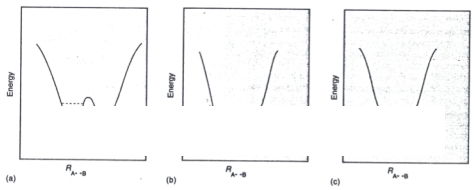
*only transiently

• *Enzyme mechanism and binding energy*

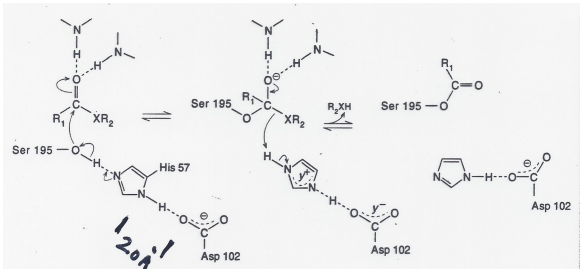
Chymotrypsin Mechanism

Low-barrier H-bond in the Transition State?

What is a Low-Barrier H-bond?



Where is the LBHB in the triad?



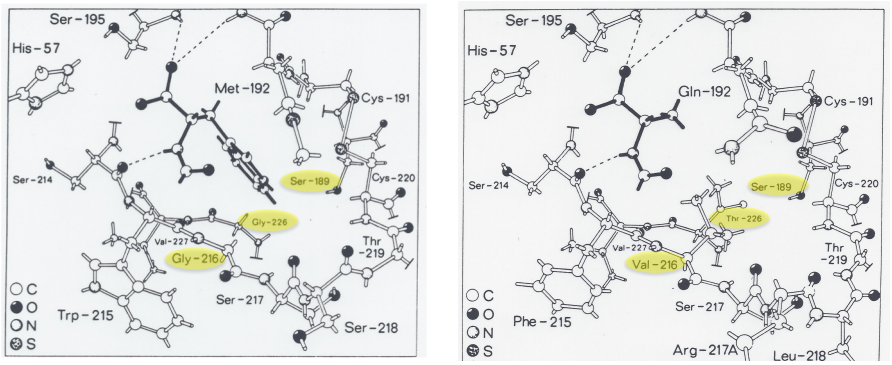
2.8 Å
2.0 Å
1.5 Å

What is significant about LBHBs is that they are 4-5x stronger than normal 2.8 Å H-bonds (~20 kcal/mole)

Enzymes

Substrate Specificity

Chymotrypsin vs. Elastase

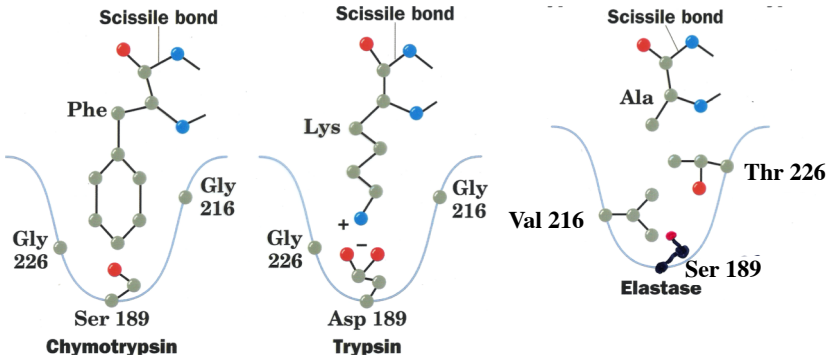


Trypsin is much like Chymotrypsin except for a Asp-189 instead of Ser-189.

Enzymes

Substrate Specificity

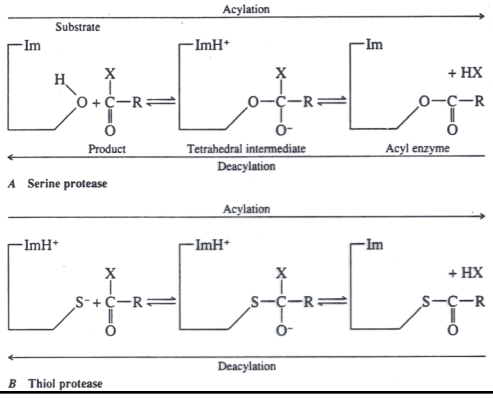
Chymotrypsin vs. Trypsin vs. Elastase



Enzymes

Class of Protease	Examples
Serine	Trypsin, Chymotrypsin, Elastase
Thiol	Papain, Cathepsin B, Caspases
Acid	HIV protease, Pepsin, Cathepsin D, Renin, Chymosin
Metal	Carboxylpeptidase A, Thermolysin

Very similar with acylation/deacylation half-reactions



Serine Protease

Cysteine (Thiol) Protease

Enzymes

Class of Protease	Examples
Serine	Trypsin, Chymotrypsin, Elastase
Thiol	Papain, Cathepsin B, Caspases
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Metal	Carboxylpeptidase A, Thermolysin