Chem 452 - Lecture 5
Catalytic Strategies
Part 1

Question of the Day. What type of substrate sequencing, **order sequential**, **random sequential**, or **ping-pong**, is displayed by the enzyme chymotrypsin?

Introduction

- Enzymes exhibit both catalytic power and specificity
- We will consider closely, four examples.

Introduction

- Chymotrypsin (1gct) 3.4.21.1
  - A Hydrolase, which cleaves peptide bonds in proteins
- Carbonic anhydrase (1ca2) 4.2.1.1
  - A Lyase, which adds water to CO_2.
- EcoRV (1rvb) 3.1.21.4
  - A Hydrolase, which cleave phosphodiester bonds in DNA
- Myosin motor domain ATPase (1fmv & 1fmw) 3.6.4.1
  - An enzyme that couples the hydrolysis of ATP to the mechanical motion.

Introduction

- The Enzyme Commission "names" for enzymes

TABLE 8.8 Six major classes of enzymes

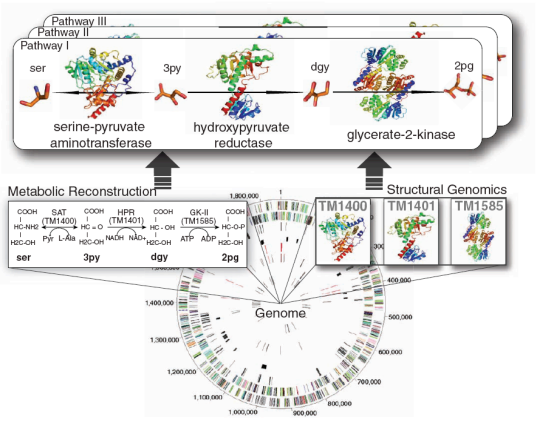
Class	Type of reaction	Example	Chapter
1. Oxidoreductases	Oxidation–reduction	Lactate dehydrogenase	16
2. Transferases	Group transfer	Nucleoside monophosphate kinase (NMP kinase)	9
3. Hydrolases	Hydrolysis reactions (transfer of functional groups to water)	Chymotrypsin	9
4. Lyases	Addition or removal of groups to form double bonds	Fumarase	17
5. Isomerases	Isomerization (intramolecular group transfer)	Triose phosphate isomerase	16
6. Ligases	Ligation of two substrates at the expense of ATP hydrolysis	Aminoacyl-tRNA synthetase	30

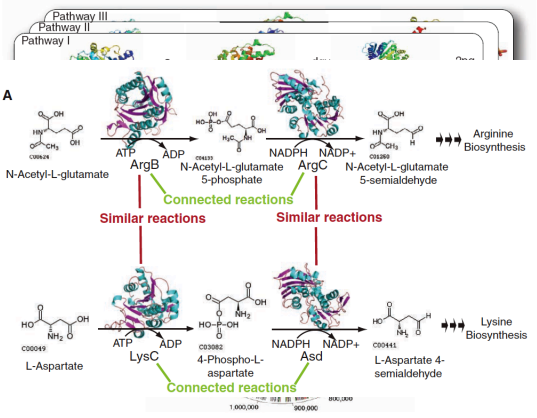
Introduction

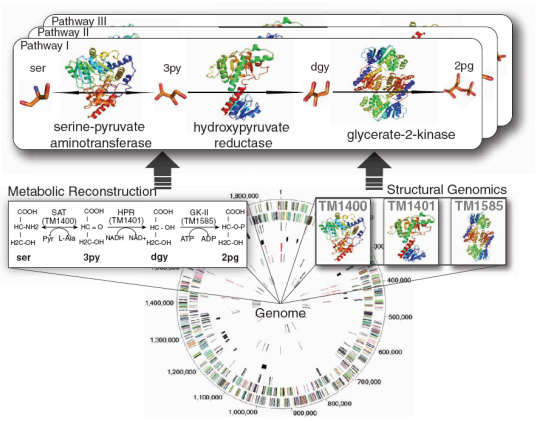
- These case studies will provide examples of
  - Generating powerful nucleophiles at neutral pH values.
  - Achieving high absolute reaction rates
  - Specificity for substrate selection
  - Specificity for products produced

Introduction

- Structural and mechanistic comparisons of enzyme action are sources of insight into the evolutionary history of enzymes.
  - Metabolic Reconstruction
    - Zhang, Ying et al., "Three-Dimensional Structural View of the Central Metabolic Network of Thermotoga maritima" Science 2009, 325, 1544-1549







Introduction

- Some Basic Catalytic Principles
  - Covalent Catalysis
  - General Acid/Base Catalysis
  - Catalysis by Approximation (Juxtaposition, or the proximity effect)
  - Metal Ion Catalysis
  - Transition state stabilization

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  - Metal
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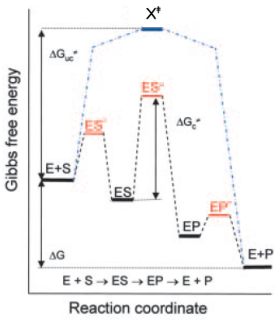

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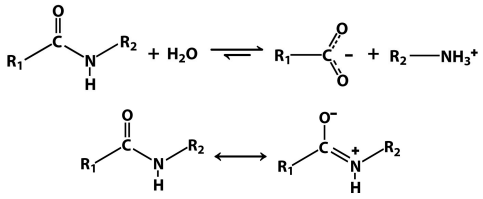
Chymotrypsin

- The hydrolysis of the peptide bond is thermodynamically favorable, but kinetically unfavorable.

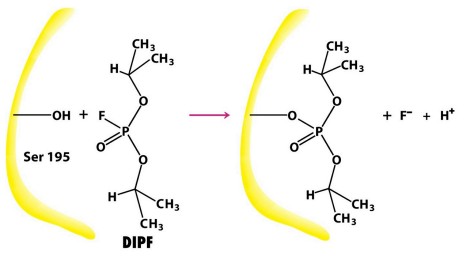


Chymotrypsin

- Chymotrypsin overcomes this by producing a powerful alkoxide nucleophile, in situ.
  - This is an example of **covalent catalysis**

Chymotrypsin

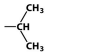
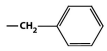
- DIPF selectively reacts with Ser 195 in chymotrypsin.



Chymotrypsin

- Chymotrypsin cleaves peptide bonds to the carboxy side of large non polar amino acid residues.

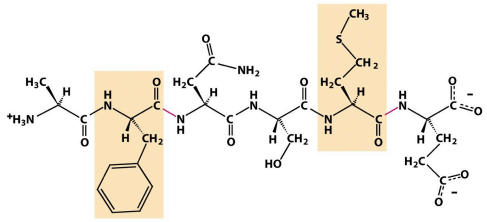
TABLE 8.6 Substrate preferences of chymotrypsin

Amino acid in ester	Amino acid side chain	k_{cat}/K_M (s^{-1} M^{-1})
Glycine	—H	1.3×10^{-1}
Valine		2.0
Norvaline	$-CH_2CH_2CH_3$	3.6×10^{2}
Norleucine	$-CH_2CH_2CH_2CH_3$	3.0×10^{3}
Phenylalanine		1.0×10^{5}

Source: After A. Fersht, *Structure and Mechanism in Protein Science: A Guide to Enzyme Catalysis and Protein Folding* (W. H. Freeman and Company, 1999), Table 7.3.

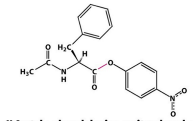
Chymotrypsin

- Chymotrypsin cleaves peptide bonds to the carboxy side of large non polar amino acid residues.

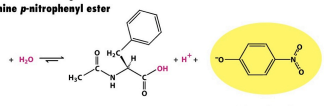


Chymotrypsin

- The chymotrypsin reaction can be followed using a **chromogenic** substrate.



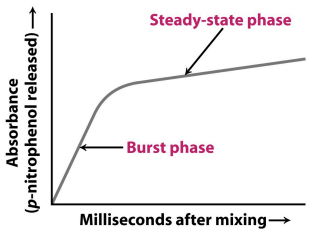
N-Acetyl-L-phenylalanine *p*-nitrophenyl ester



p-Nitrophenolate

Chymotrypsin

- Stop-flow kinetics experiments suggest a covalently bound intermediate is involved.



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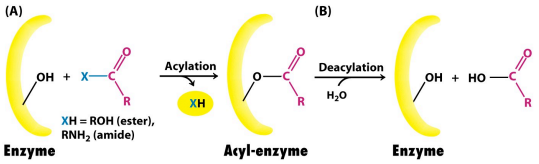
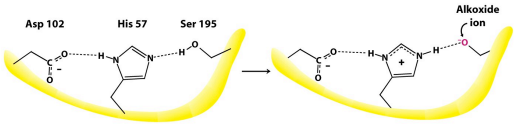


Chymotrypsin

- The reactive Ser 195 is part of a **catalytic triad**.



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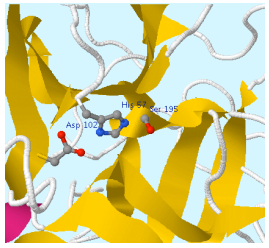

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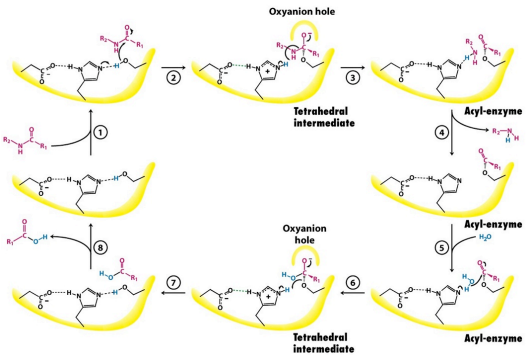
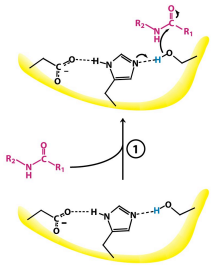
Chymotrypsin Catalytic Cycle



Chymotrypsin Catalytic Cycle

- Step 1: Substrate binding



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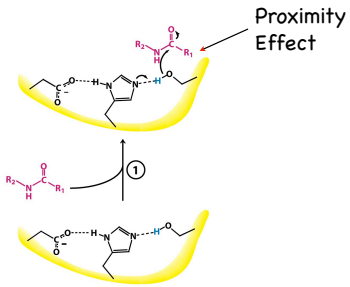

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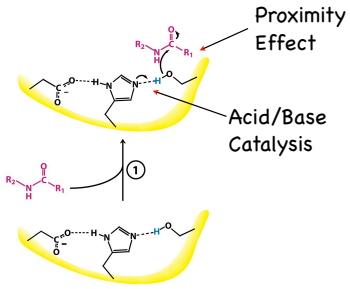
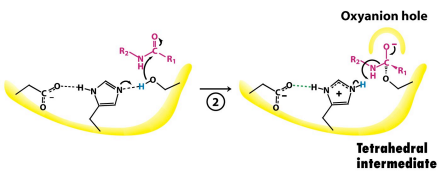


Chymotrypsin Catalytic Cycle

- Step 2: Transition state formation



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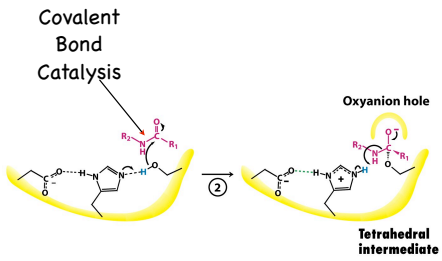

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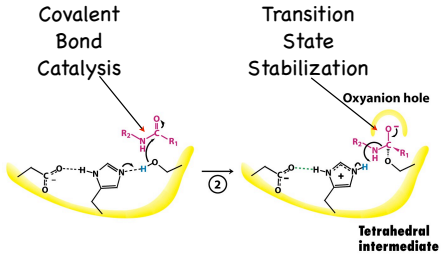
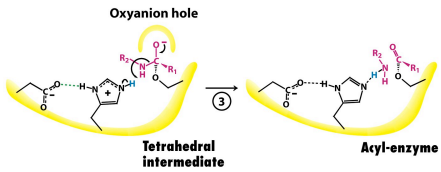


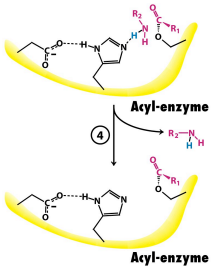
Chymotrypsin Catalytic Cycle

- Step 3: Peptide bond cleavage



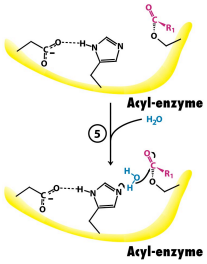
Chymotrypsin Catalytic Cycle

- Step 4: Release of first product (C-terminal half of the peptide)



Chymotrypsin Catalytic Cycle

- Step 5: Binding of the second substrate (H_2O)



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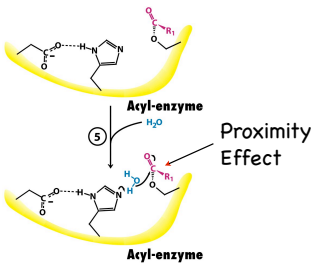

Chymotrypsin Catalytic Cycle

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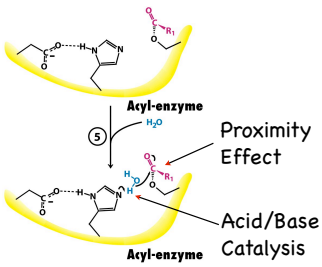
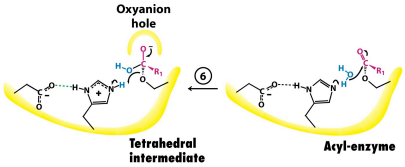


Chymotrypsin Catalytic Cycle

- Step 6: Transition state formation



Chymotrypsin Catalytic Cycle

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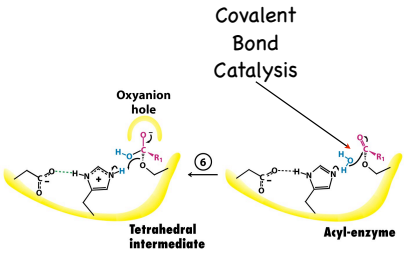

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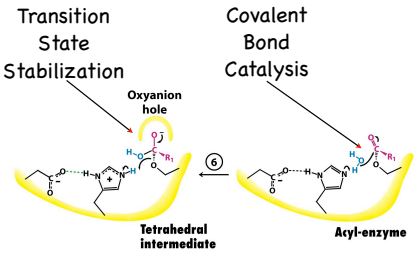
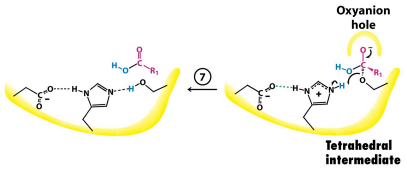


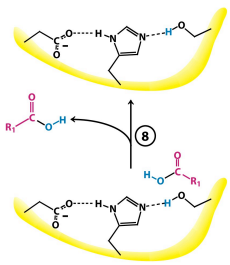
Chymotrypsin Catalytic Cycle

- Step 7: Ester bond cleavage



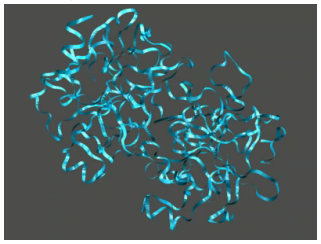
Chymotrypsin Catalytic Cycle

- Step 8: Release of second product (N-terminal half of the peptide)



Chymotrypsin Catalytic Cycle

- Putting it all together:
  - Step-by-Step through the catalytic cycle



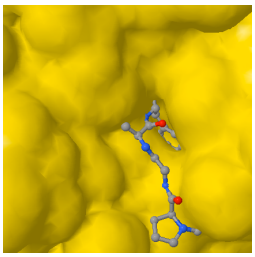
(Click to start animation)

Chymotrypsin

- Covalent Catalysis
  - Ser 195 is converted into powerful nucleophile and leads to a covalent, enzyme-bound intermediate.
- General Acid/Base Catalysis
  - His 57 does both
- Catalysis by Approximation
  - Binds the substrate with specificity and arranges the various players next to one another.
- Metal Ion Catalysis
  - Nothing here
- Transition State Stabilization
  - The oxyanion hole stabilizes the negatively charged, tetrahedral transition state.

Chymotrypsin

- Substrate Specificity



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Chymotrypsin

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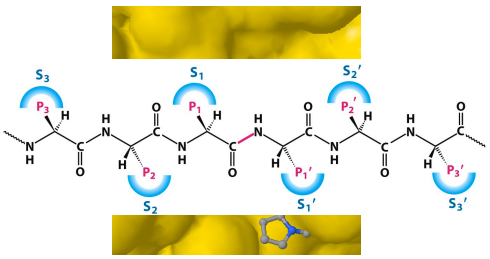

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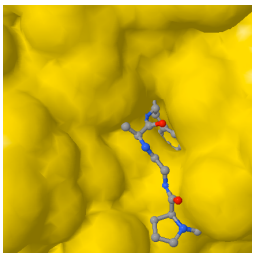

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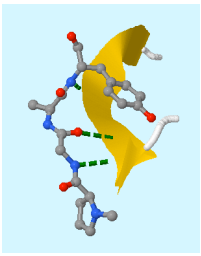
Chymotrypsin

Also illustrates substrate specificity



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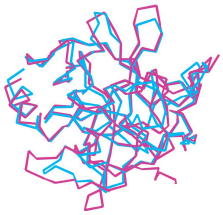
Other Serine Proteases

- Other Homologous Serine Proteases include **trypsin** and **elastase**



Trypsin's structure overlaid on Chymotrypsin's

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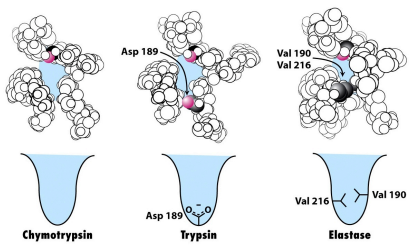
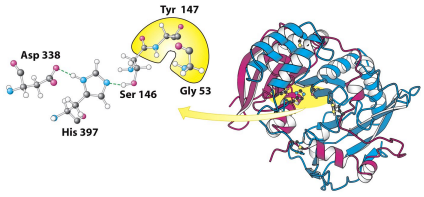


Other Serine Proteases

- Some serine proteases are not homologues of chymotrypsin
  - **carboxypeptidase II**



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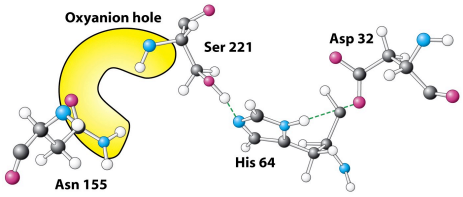
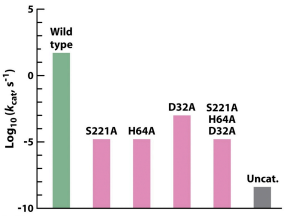
  - **subtilisin**



Other Serine Proteases

- Investigating the catalytic triad by site-directed mutagenesis



Subtilisin

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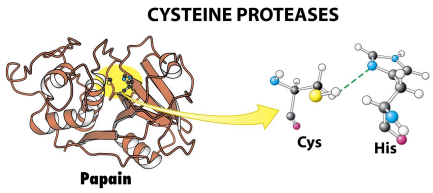

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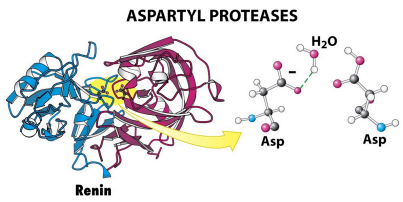

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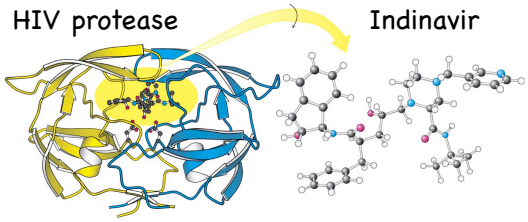

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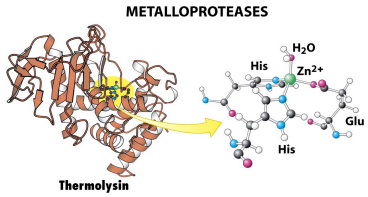

Introduction

- Some Basic Catalytic Principles
  - Covalent Catalysis
  - General Acid/Base Catalysis
  - Catalysis by Approximation (Juxtaposition, or the proximity effect)
  - Metal Ion Catalysis
  - Transition state stabilization

Next up

- Catalytic Strategies, con'd (Chapter 9)
  - Carbonic Anhydrase
  - EcoRV
  - Myosin II ATPase