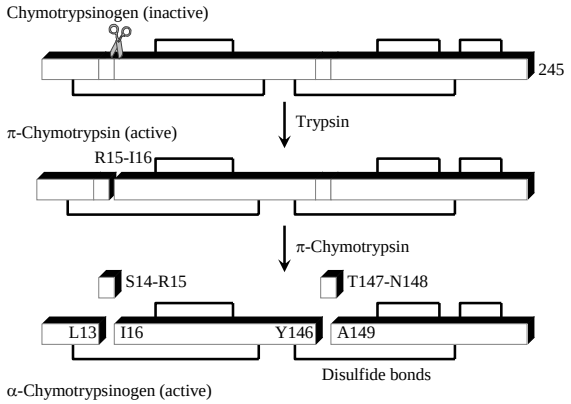
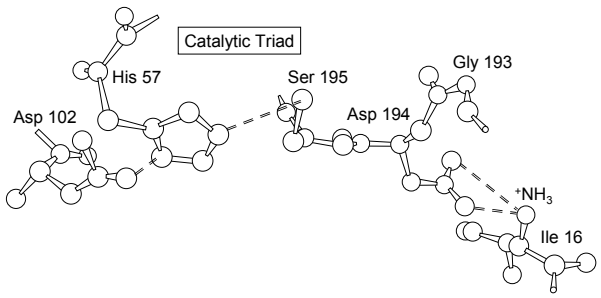
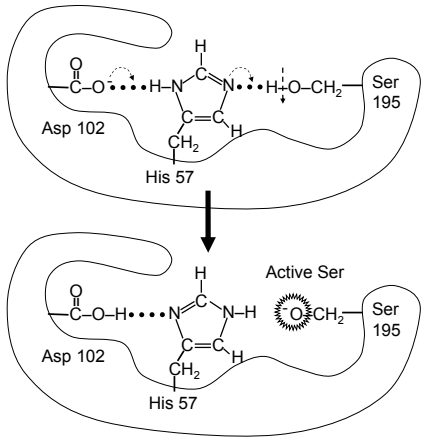
Chymotrypsin Is Activated by Proteolysis



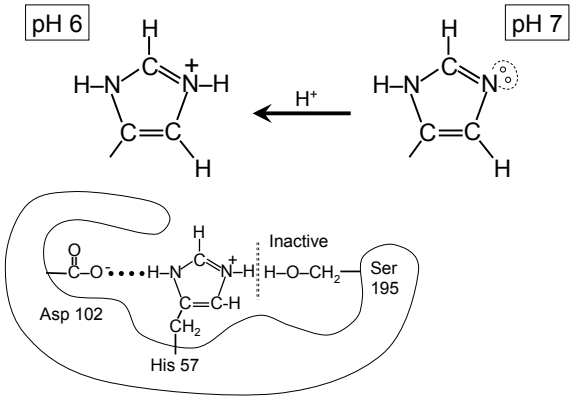
New Ile16 N-Terminal Stabilizes Asp194



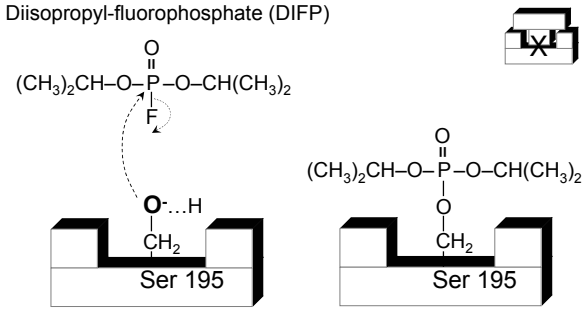
Charge Relay in Active Site



Imidazole on Histidine Is Affected by pH

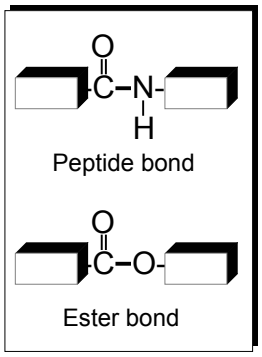


Chymotrypsin Ser195 Inhibited by DIFP

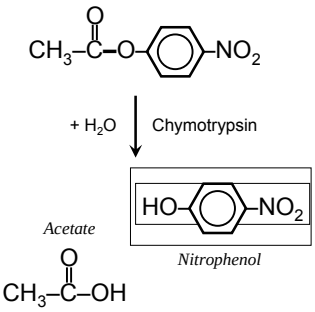


Chymotrypsin Also Catalyzes Acetate

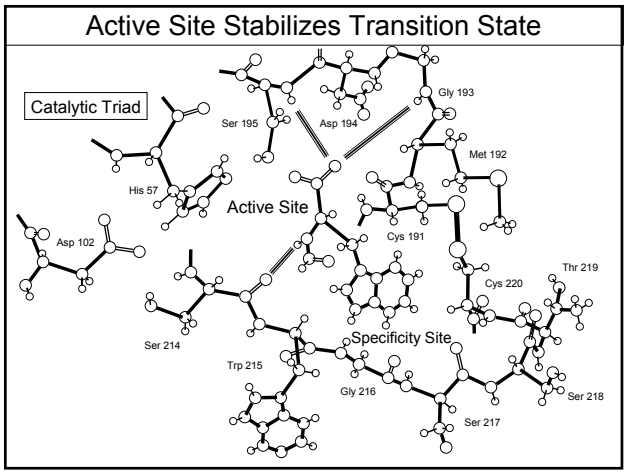
Hartley & Kilby

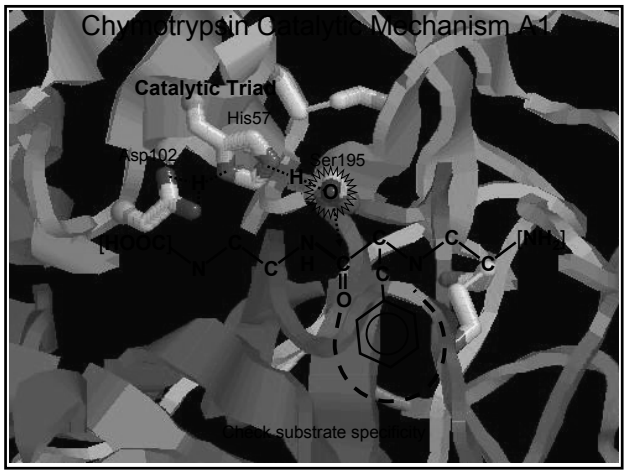


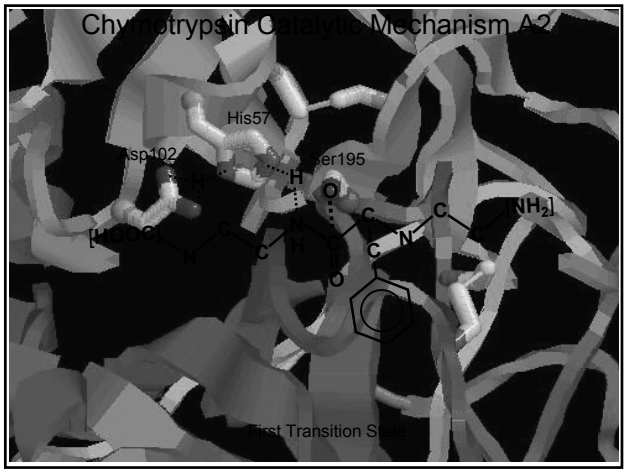
Nitrophenol acetate

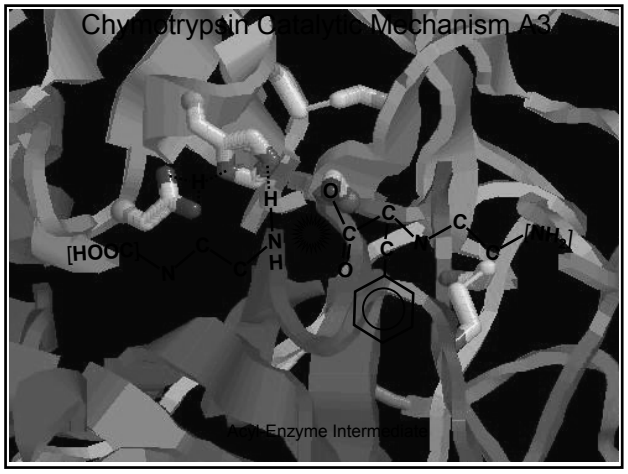


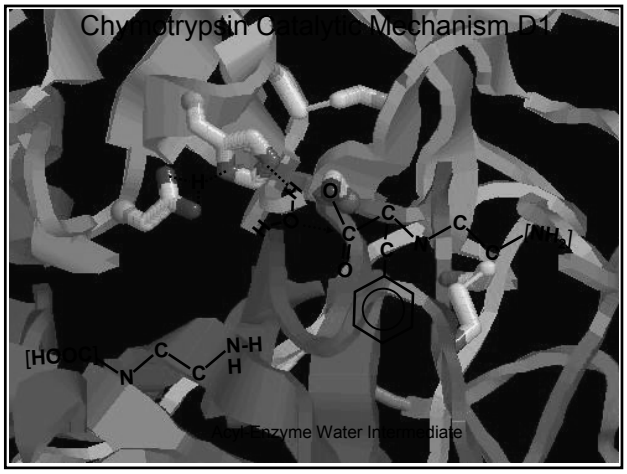
No acetate was detected at early stage

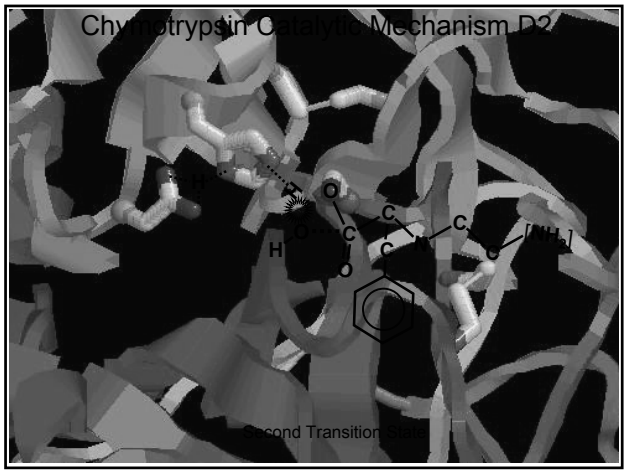

Active Site Stabilizes Transition State
Catalytic Triad
Ser 195
Asp 194
Gly 193
Met 192
His 57
Active Site
Asp 102
Cys 191
Thr 219
Cys 220
Ser 214
Specificity Site
Trp 215
Gly 216
Ser 218
Ser 217

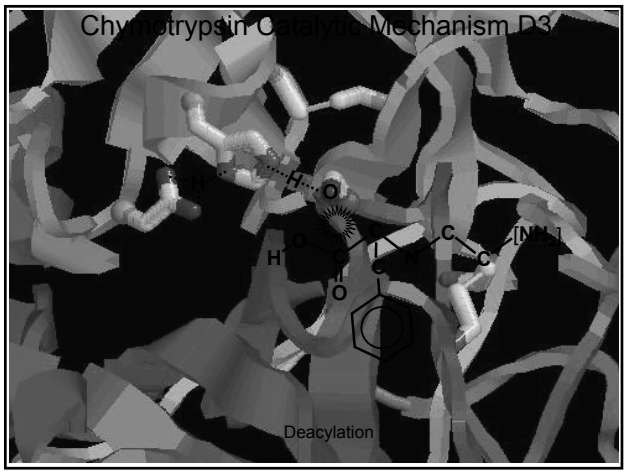

Chymotrypsin Catalytic Mechanism A1
Catalytic Triad
His57
Ser195
Check substrate specificity


Chymotrypsin Catalytic Mechanism A2
His57
Ser195
First Transition State


Chymotrypsin Catalytic Mechanism A3
Acyl-Enzyme Intermediate


Chymotrypsin Catalytic Mechanism D1
Acyl-Enzyme Water Intermediate

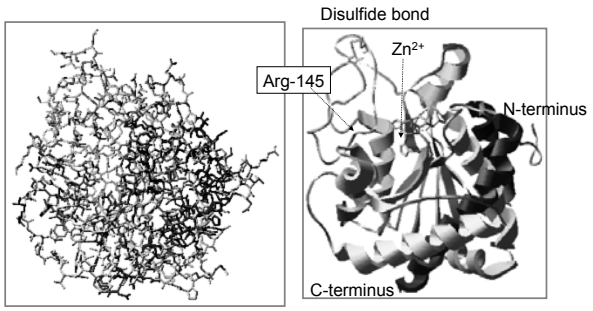

Chymotrypsin Catalytic Mechanism D2
Second Transition State



Carboxypeptidase

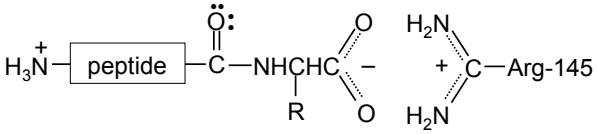
- Carboxypeptidase is an enzyme that catalyzes the hydrolysis of proteins at their C-terminus.
- It is a metalloenzyme containing Zn^{2+} at its active site.
- An amino acid with a positively charged side chain (Arg-145) is near the active site.

Carboxypeptidase

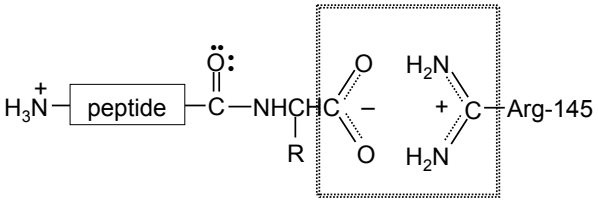


tube model ribbon model

What happens at the active site?

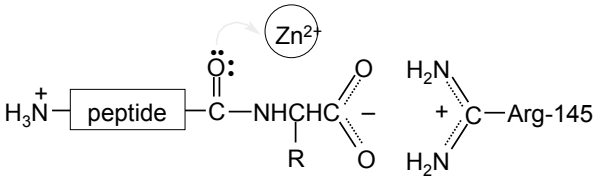


What happens at the active site?



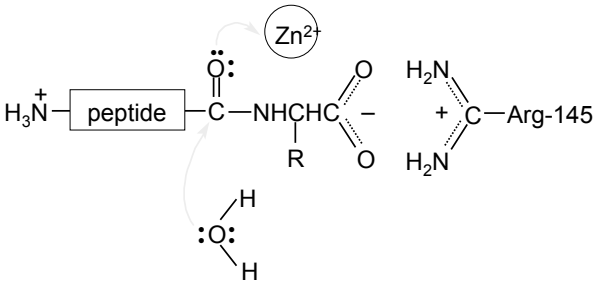
- The peptide or protein is bound at the active site by electrostatic attraction between its negatively charged carboxylate ion and arginine-145.

What happens at the active site?



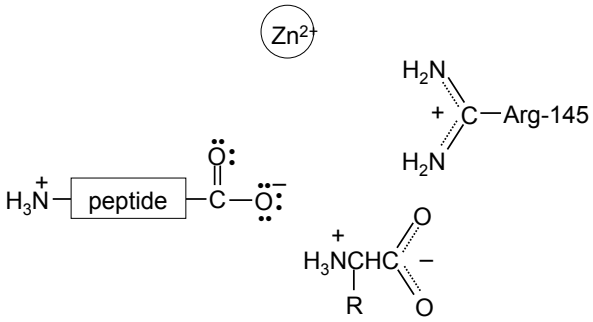
- Zn^{2+} acts as a Lewis acid toward the carbonyl oxygen, increasing the positive character of the carbonyl carbon.

What happens at the active site?



•Water attacks the carbonyl carbon. Nucleophilic acyl substitution occurs.

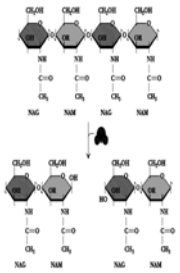
What happens at the active site?



Lysozyme

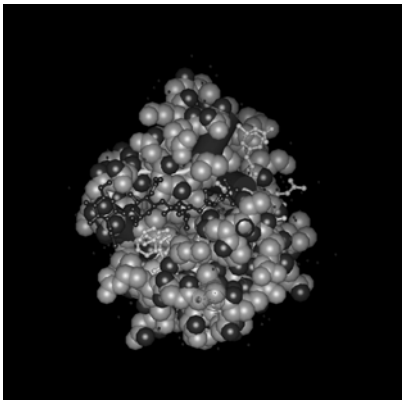
- Lysozyme cleaves the polysaccarides of bacterial cell walls.
- Lysozyme is a 14.6 kd polypeptide stabilized by 4 disulfide bridges.
- The secondary structure of lysozyme has both β conformation and **α helix**.
- It is compactly folded with only non-polar residues on the interior.

Lysozyme



- Lysozyme is a small globular protein composed of 129 amino acids.
- **It is an enzyme which hydrolyzes polysaccharide chains**,
  - particularly those found in the peptidoglycan cell wall of bacteria.
  - In particular, it hydrolyzes the glycosidic bond between C-1 of N-acetyl muramic acid and C-4 of N-acetyl glucosamine.
- It is found in many body fluids, such as tears, and is one of the body's defenses against bacteria.
- Although crystal structures of other proteins had been determined previously, lysozyme was the first enzyme to have its structure determined.

Lysozyme

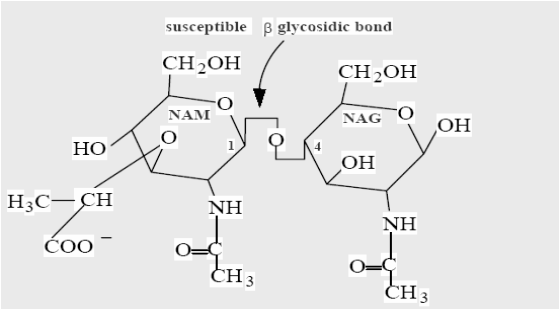


Lysozyme

- Lysozyme binds 3 disaccarides at once.
- It hydrolyzes the **glycosidic bond**between the C-1 of NAM (4thmonosaccaride) and C-4 of NAG (5th monosaccaride).

Lysozyme

Susceptible glycosidic bond



Nuclease

- A **nuclease** is an enzyme capable of cleaving the phosphodiester bonds between the nucleotide subunits of nucleic acids.
- These are of two types
  - **Deoxyribonuclease** (**DNase**) is any enzyme that catalyzes the hydrolytic cleavage of phosphodiester linkages in the DNA backbone.
  - **Ribonuclease (RNase)**, is a nuclease that catalyzes the hydrolysis of RNA into smaller components

Nuclease

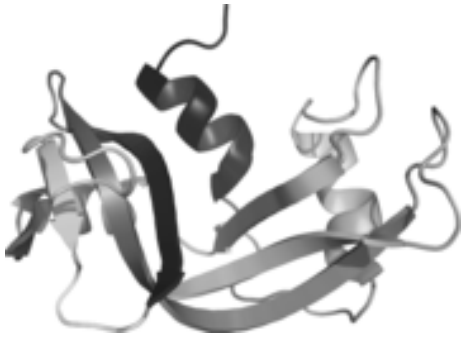
- **Endonucleases** are enzymes that cleave the phosphodiester bond within a polynucleotide chain.
- **Exonucleases** are enzymes (found as individual enzymes, or as parts of larger enzyme complexes) that cleave nucleotides one at a time from an end of a polynucleotide chain.
  - These enzymes hydrolyse phosphodiester bonds from either the 3' or 5' terminus of a polynucleotide molecule.

Ribonuclease

Ribinuclease can be classified as
- Endoribonucleases
- Exoribonucleases

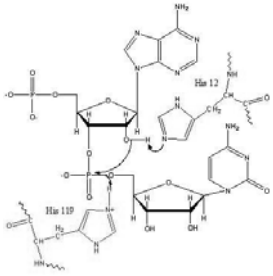
- **An endoribonuclease** is hydrolase enzyme which makes oligonucleotides and polynucleotides by hydrolyzing the interior bonds of ribonucleotides.
- An **exoribonuclease** is an enzymes which degrades RNA by removing terminal nucleotides from either the 5' end or 3' end of the RNA molecule.
  - Enzymes that remove nucleotides from the 5' end are called ***5'-3' exoribonucleases***
  - Enzymes that remove nucleotides from the 3' end are called ***3'-5' exoribonucleases*****.**

Ribonuclease



Ribonuclease

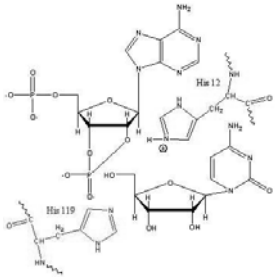
- **His 12**
  - General Base
  - Abstracts a proton from 2' hydroxyl of 3' nucleotide.
- **His 119**
  - General acid
  - Donates a proton to 5' hydroxyl of nucleoside.



Ribonuclease

- **His 12**
  - General Base
  - Abstracts a proton from 2' hydroxyl of 3' nucleotide.
- **His 119**
  - General acid
  - Donates a proton to 5' hydroxyl of nucleoside.

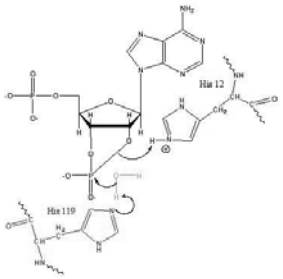
2'-3' cyclic phosphate intermediate
Net Proton Transfer from His119 to His12



Ribonuclease

- **His 12**
  - General Base
  - Abstracts a proton from 2' hydroxyl of 3' nucleotide.
- **His 119**
  - General acid
  - Donates a proton to 5' hydroxyl of nucleoside.

Water replaces the released nucleoside
Acid and base roles are reversed for H12 and H119



Ribonuclease

- **His 12**
  - General Base
  - Abstracts a proton from 2' hydroxyl of 3' nucleotide.
- **His 119**
  - General acid
  - Donates a proton to 5' hydroxyl of nucleoside.

Original Histidine protonation states are restored