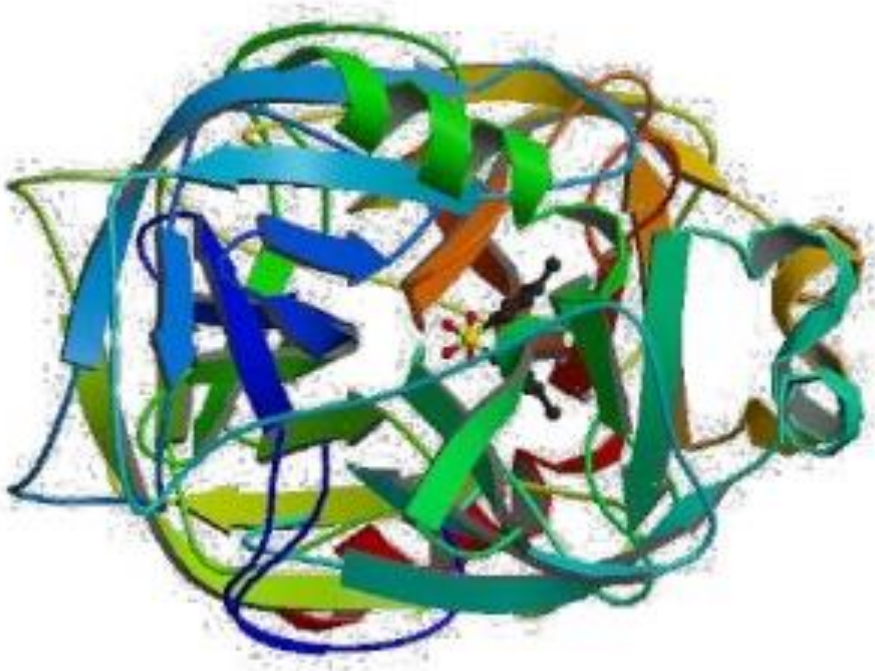
Chymotrypsin



Mechanism of action of Chymotrypsin

- Introduction
- Structure of chymotrypsin
- Mechanism of action

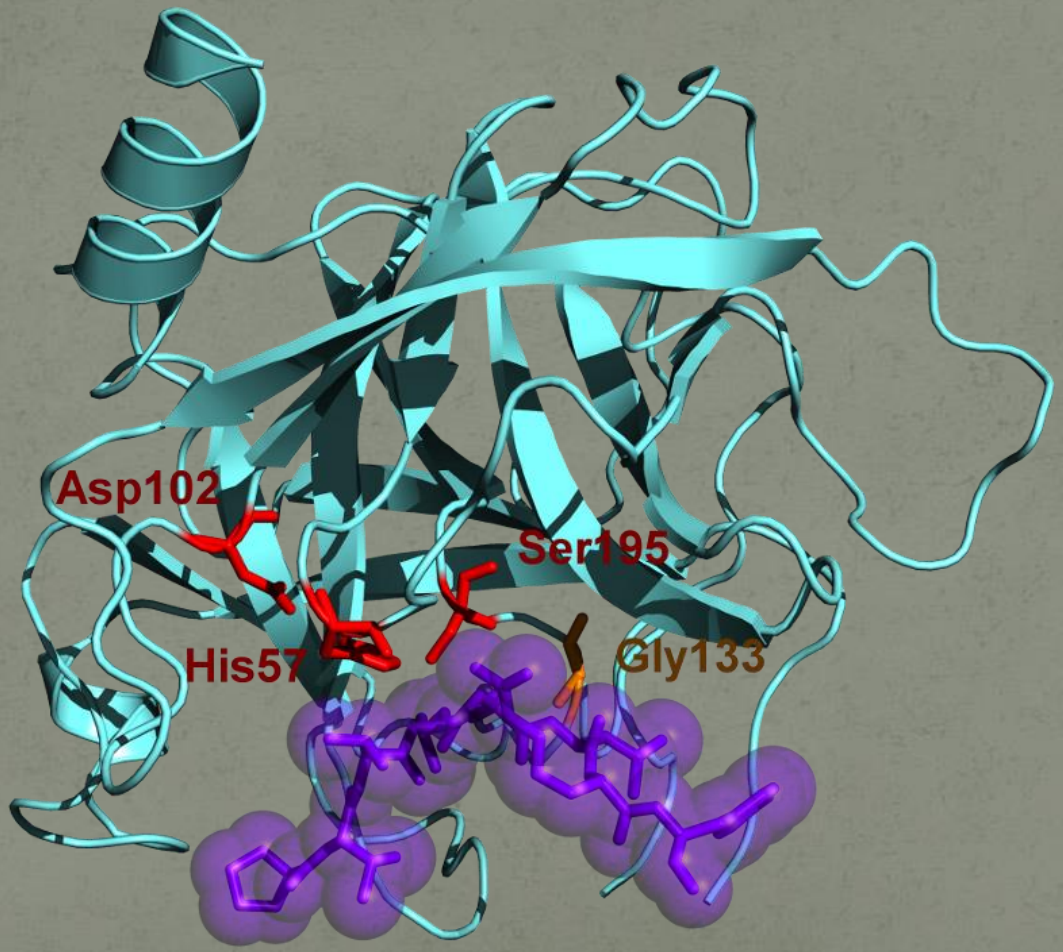
INTRODUCTION

- Chymotrypsin is an enzyme used for digesting proteins.
- Chymotrypsin is found in the duodenum that selectively cleaves (cuts) off pieces of amino acids from the protein chain.
- Specifically chymotrypsin cleaves phenylalanine, tyrosine, and tryptophan bonds, or in other words the aromatic amino acids. It cleaves these amino acids starting from the C-terminus of the protein.

Chymotrypsin Structure

- The primary structure shows that disulfide bonds are the crucial role to the protein folding. The protein is spherical and itself consists of three polypeptide chains. There is also a pocket in the enzyme other than the active site and known as S1 pocket which, in the case of chymotrypsin, is lined with relatively hydrophobic residues such as Ser-189, Ser-214, Trp-215, Gly-216, and Gly-226.
- The active site includes Ser-195, His-57, and Asp-102 (the catalytic triad). Ser-195 is hydrogen bonded to the His-57 and it in turn is hydrogen bonded to the Asp-102 residue. The His-57 role is to position the serine residue and polarize the hydroxyl group so it can be deprotonated to the alkoxide ion. In the presence of the substrate, this accepts a proton by acting as a base. Asp-102 orients the His-57 and stabilizes it through hydrogen bonding and electrostatics

Chymotrypsin Structure



Mechanism of action

Step 1: When substrate (polypeptide) binds, the side of chain of the residue next to the peptide bond to be cleaved nestles in a hydrophobic pocket on the enzyme, positioning the peptide bond for attack. Histidine extracts one proton from serine to form an alkoxide ion. This serine ion reacts with the substrate.

Step 2: In chymotrypsin, the carboxylate R-group of Asp102 forms a hydrogen bond with R group of His 57. When this happens, it compresses this hydrogen bond and shifts electron density to the other nitrogen atom (not involved in the H-bond) in the R-group of His57 becomes a very strong base. This allows His 57 to deprotonate Ser195 and turn it into a strong nucleophile that can attack the substrate.

Oxygen develops a partially negative charge in the oxyanion hole

Step 3: Instability of the negative charge on the substrate carbonyl oxygen when will leads to collapse of the tetrahedral intermediate, re-formation of a double bond with carbon which breaks the peptide bond between the carbon and amino acid group. The amino leaving group is protonated by His57, facilitating its displacement. Once the oxyanion hole stabilizes the negative charge, the bond breaks because the proton from Histidine is binding to nitrogen to make it less likely to carbon. The leaving group is stabilized and the acyl-enzyme is formed

Mechanism of action

Step 4: The amine component is departed from the enzyme (metabolized by the body) and binds to serine. This completes the first stage (acylation of enzyme). The first product has been made.

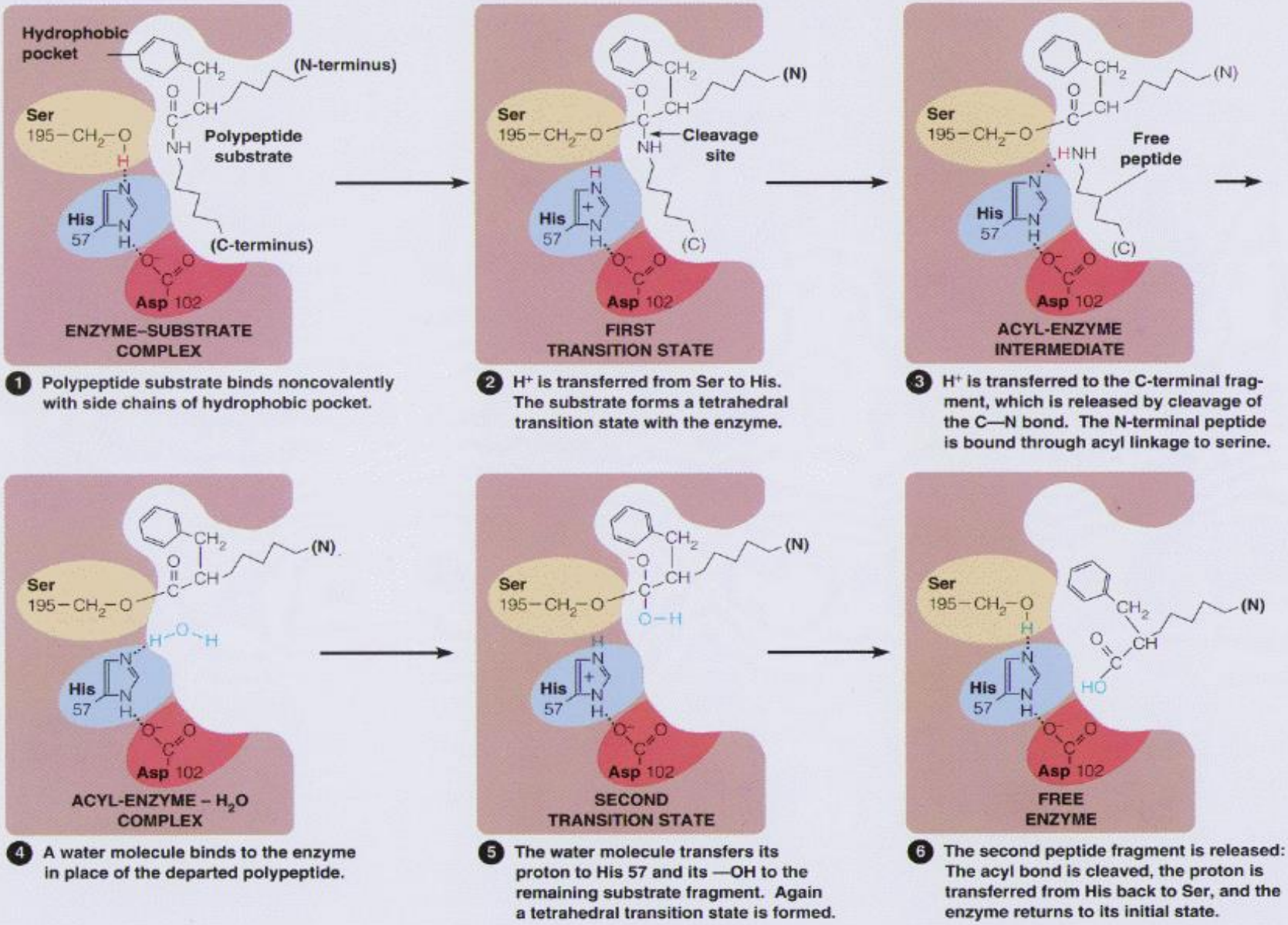
Step 5: A water molecule is added where the N terminus was. Histidine deprotonates the water to form a hydroxyl group. This hydroxyl group attaches to carbon from the carboxyl side and destabilizes the acyl intermediate. The bond is broken.

Step 6: An incoming water molecule is deprotonated by acid-base catalysis, generating a strongly nucleophilic hydroxide ion. Attack of hydroxide on the ester linkage of the acylenzyme generates a second tetrahedral intermediate.

Step 7: collapse of the tetrahedral intermediate form the second product, a carboxylate anion, and displace Ser195. The proton from Histidine goes back to Serine.

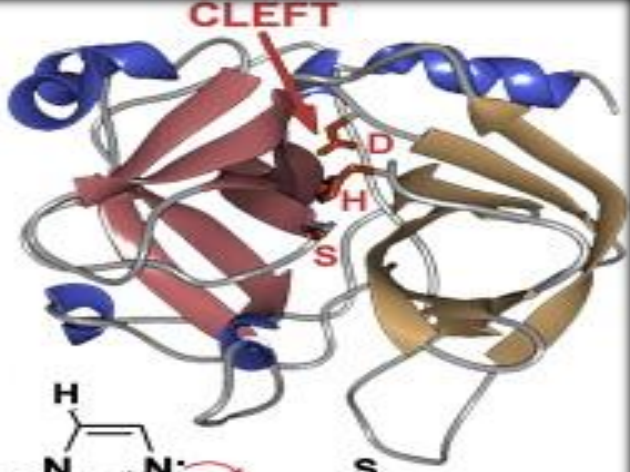
Step 8: The carboxylic acid is released and the enzyme is reformed to catalyze the next reaction with the original active site.

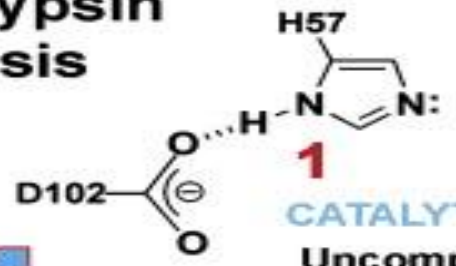
Figure 11.13 Catalysis of peptide bond hydrolysis by chymotrypsin



1. Polypeptide substrate binds noncovalently with side chains of hydrophobic pocket.
2. H^+ is transferred from Ser to His. The substrate forms a tetrahedral transition state with the enzyme.
3. H^+ is transferred to the C-terminal fragment, which is released by cleavage of the C—N bond. The N-terminal peptide is bound through acyl linkage to serine.
4. A water molecule binds to the enzyme in place of the departed polypeptide.
5. The water molecule transfers its proton to His 57 and its —OH to the remaining substrate fragment. Again a tetrahedral transition state is formed.
6. The second peptide fragment is released: The acyl bond is cleaved, the proton is transferred from His back to Ser, and the enzyme returns to its initial state.

Chymotrypsin Catalysis





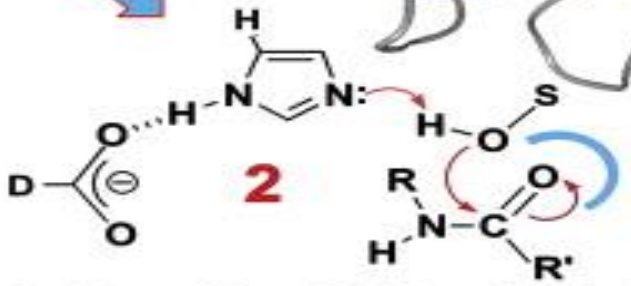
oxy-anion "hole"

S195

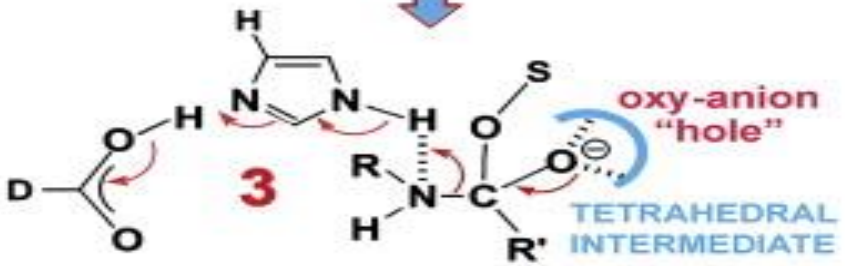
HO

CATALYTIC TRIAD

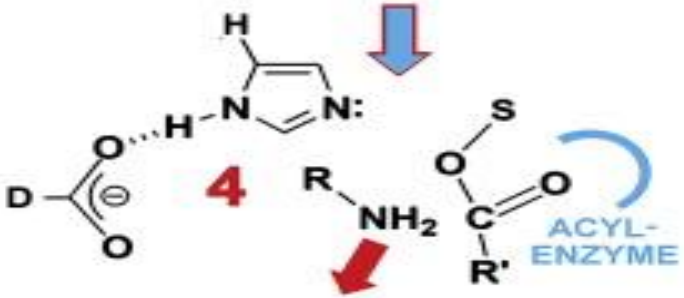
Uncomplexed Enzyme



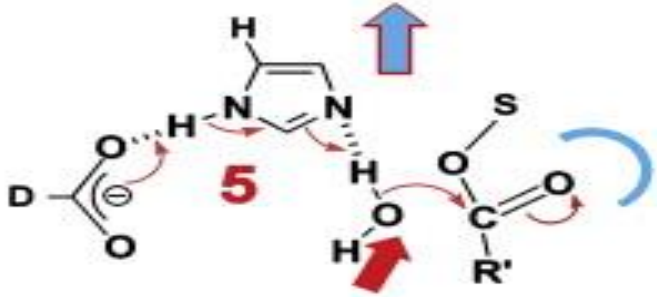
Activated Ser–OH Attacks C=O



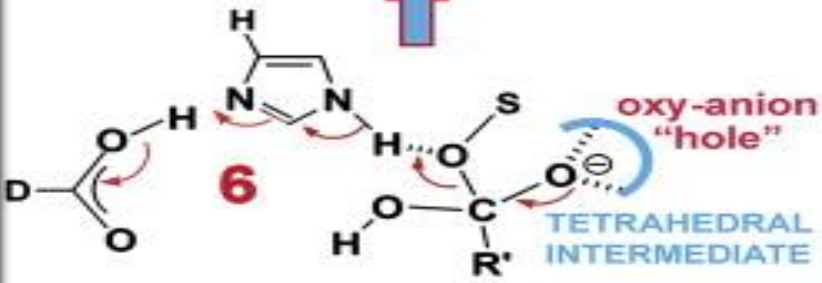
Intermediate Rearranges



First Product Departs



Water Binds & Attacks C=O



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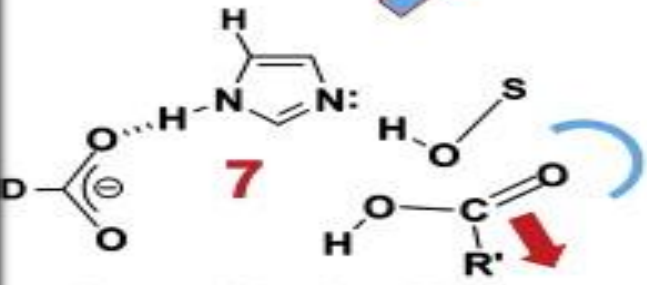

Second Product Departs

THANKYOU

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