Chapter 14

Introduction to Mechanisms of Enzymes

Mechanism of Action of Enzymes

To Understand the Major Mechanism Action of Enzyme, we are going to study in depth the mechanism of chymotrypsin

Do you remember what that enzyme does?

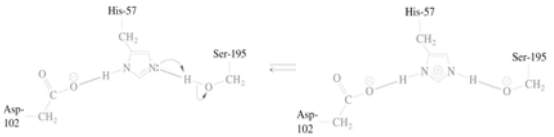
Chymotrypsin is a Serine Protease

- Serine protease is an enzyme that catalyzed the hydrolysis of peptide bonds with an active site serine residue that acts as a nucleophile during catalysis.

Chymotrypsin has 28 serine residues, but only one is very reactive

Catalytic triad of chymotrypsin

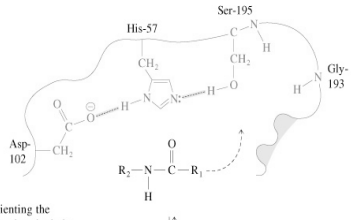
- **Imidazole ring (His-57) removes H from Ser-195 hydroxyl to make it a strong nucleophile (-CH_2O^-)**
- **Buried carboxylate (Asp-102) stabilizes the positively-charged His-57 to facilitate serine ionization**



a-Chymotrypsin mechanism (8 slides)

Step (1): E + S

E + S



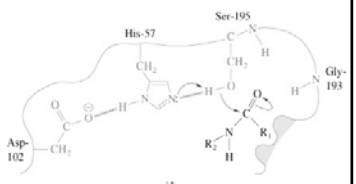
The noncovalent enzyme-substrate complex is formed, orienting the substrate for reaction. Interactions holding the substrate in place include binding of the R_1 group in the specificity pocket (shaded). The binding interactions position the carbonyl carbon of the scissile peptide bond (the bond susceptible to cleavage) next to the oxygen of Ser-195.

(1)

(E-S)

Binding of the substrate compresses Asp-102 and His-57. This strain is relieved by formation of a low-barrier hydrogen bond. The raised pK_a of His-57 enables the imidazole ring to remove a proton from the hydroxyl group of Ser-195. The nucleophilic oxygen of Ser-195 attacks the carbonyl carbon of the peptide bond to form a tetrahedral intermediate (E-TI_1), which is believed to resemble the transition state.

E-S

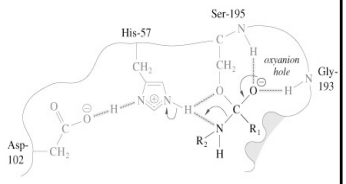


(2)

(E-TI_1)

When the tetrahedral intermediate is formed, the substrate C—O bond changes from a double bond to a longer single bond. This allows the negatively charged oxygen (the oxyanion) of the tetrahedral intermediate to move to a previously vacant position, called the oxyanion hole, where it can form hydrogen bonds with the peptide-chain —NH groups of Gly-193 and Ser-195.

E-TI_1



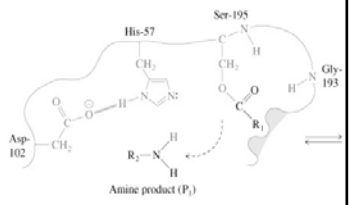
The imidazolium ring of His-57 acts as an acid catalyst, donating a proton to the nitrogen of the scissile peptide bond, thus facilitating its cleavage.

(3)

(Acyl E + P_1)

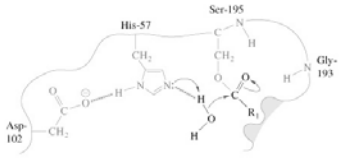
The carbonyl group from the peptide forms a covalent bond with the enzyme, producing an acyl-enzyme intermediate. After the peptide product (P_1) with the new amino terminus leaves the active site, water enters.

Acyl E + P_1



(Acyl E + H_2O)

(4)

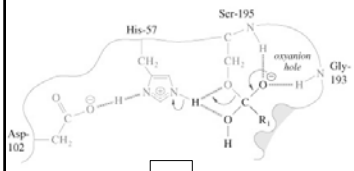


Acyl E + H_2O

Hydrolysis (deacylation) of the acyl-enzyme intermediate starts when Asp-102 and His-57 again form a low-barrier hydrogen bond and His-57 removes a proton from the water molecule to provide an $OH^{\ominus}$ group to attack the carbonyl group of the ester.

($E\text{-}TI_2$)

(5)



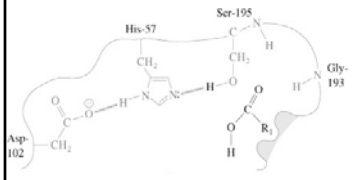
$E\text{-}TI_2$

His-57, once again an imidazolium ion, donates a proton, leading to the collapse of the second tetrahedral intermediate.

A second tetrahedral intermediate ($E\text{-}TI_2$) is formed and stablized by the oxyanion hole.

($E\text{-}P_2$)

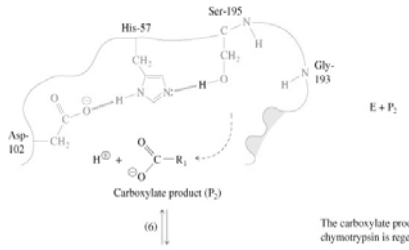
(6)



$E\text{-}P_2$

The second product (P_2)—a polypeptide with a new carboxy terminus—is formed.

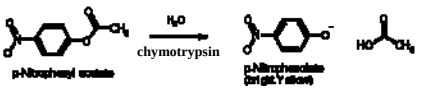
$(E + P_2)$



The carboxylate product is released from the active site, and free chymotrypsin is regenerated.

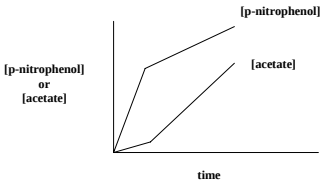
Can One Get Experimental Evidence for an Intermediate in Chymotrypsin Reaction?

Chymotrypsin catalyzes the hydrolysis of some esters



Experiment: Incubate chymotrypsin with p-nitrophenylacetate.
Drop pH to 3
Isolate chymotrypsin-CH_2-O-C-CH_3
O

Kinetic Data



Acetate lags p-nitrophenol initially- burst of p-nitrophenol while little acetate is being produced.

General Statement About How Enzymes Work
-Using Chymotrypsin As An Example-

1. Acid-Base Catalysis

A. Specific Acid-Base Catalysis

<u>Rate enhancement is proportional to pH or pOH</u>

General Statement About How Enzymes Work
-Using Chymotrypsin As An Example-

1. Acid-Base Catalysis

B. General Acid-Base Catalysis

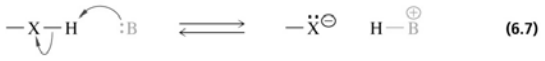
Rate enhancement is proportional to weak acid or weak base

Whether something is protonated or not.

Examples of general acid-base catalysis- His-57 and Asp-102

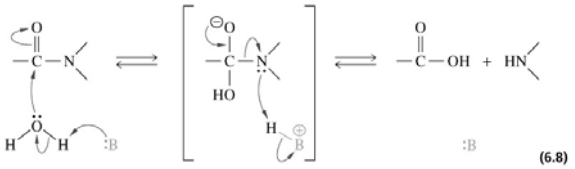
Acid-Base Catalysis

- **Reaction acceleration is achieved by catalytic transfer of a proton**
- **A general base (B:) can act as a proton acceptor to remove protons from OH, NH, CH or other XH**
- **This produces a stronger nucleophilic reactant ($X:^-$)**



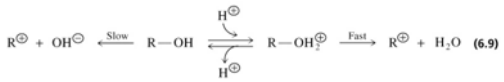
General base catalysis reactions (continued)

- A **general base** (**B:**) can remove a proton from water and thereby generate the equivalent of OH^- in neutral solution



Proton donors can also catalyze reactions

- **A general acid (BH^+) can donate protons**
- **A covalent bond may break more easily if one of its atoms is protonated (below)**



Polar Amino Acid Residues in Active Sites

- Active-site cavity of an enzyme is lined with hydrophobic amino acids
- Polar, ionizable residues at the active site participate in the mechanism
- Anions and cations of certain amino acids are commonly involved in catalysis

TABLE 6.1 Catalytic functions of reactive groups of ionizable amino acids

Amino acid	Reactive group	Net charge at pH 7	Principal functions
Aspartate	$-COO^{\ominus}$	−1	Cation binding; proton transfer
Glutamate	$-COO^{\ominus}$	−1	Cation binding; proton transfer
Histidine	Imidazole	Near 0	Proton transfer
Cysteine	$-CH_2SH$	Near 0	Covalent binding of acyl groups
Tyrosine	Phenol	0	Hydrogen bonding to ligands
Lysine	$-NH_3^{\oplus}$	+1	Anion binding; proton transfer
Arginine	Guanidinium	+1	Anion binding
Serine	$-CH_2OH$	0	Covalent binding of acyl groups

Table 6.2 pKa Values of amino acid ionizable groups in proteins

Group	pK_a
Terminal α-carboxyl	3-4
Side-chain carboxyl	4-5
Imidazole	6-7
Terminal α-amino	7.5-9
Thiol	8-9.5
Phenol	9.5-10
ε-Amino	~10
Guanidine	~12
Hydroxymethyl	~16

General Statement About How Enzymes Work
-Using Chymotrypsin As An Example-

2. Covalent Catalysis

Formation of a transient covalent bond between enzyme and substrate

Example: Ser 195

chymotrypsin-CH_2-O-C-CH_3
||
O

What other kinds of bonds-
Thioester
Imine (Schiff base)

General Statement About How Enzymes Work
-Using Chymotrypsin As An Example-

3. Proximity and Orientation Effects

Experiment Designed to Assess the Effect of Holding Reacting Group Near Catalytic Site

$$\frac{k_{cat}}{k_{uncat}}$$

The Proximity Effect

The rate of reaction is faster when the reactants are held more rigidly in proximity.

- Correct positioning of two reacting groups (in model reactions or at enzyme active sites):

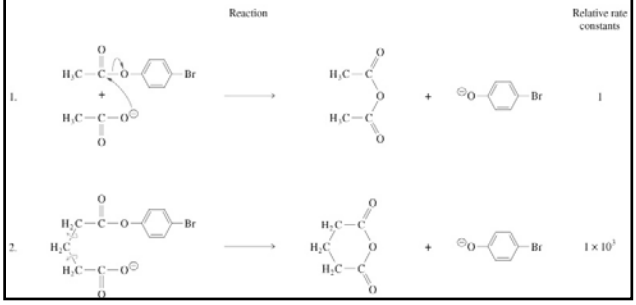
  (1) Reduces their degrees of freedom

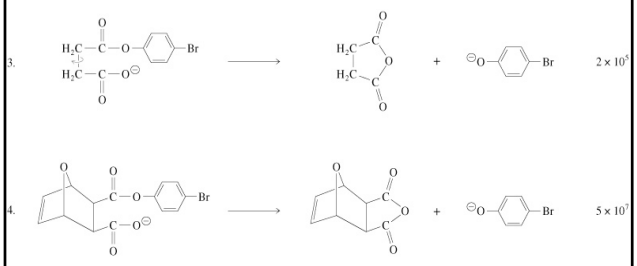
  (2) Results in a large loss of entropy

  (3) The relative enhanced concentration of substrates ("effective molarity") predicts the rate acceleration expected due to this effect

Reactions of carboxylates with phenyl esters

- Increased rates are seen when the reactants are held more rigidly in proximity (continued next slide)



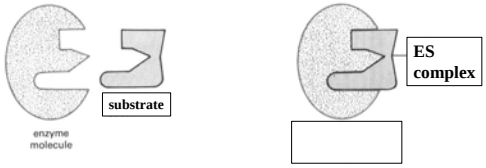


General Statement About How Enzymes Work
-Using Chymotrypsin As An Example-

4. Strain

When enzymes were first discovered, it was thought that the substrate and active site of the enzyme were structurally complementary

Lock and Key Mechanism



General Statement About How Enzymes Work
-Using Chymotrypsin As An Example-

4. Strain

Modification to the Lock and Key Mechanism

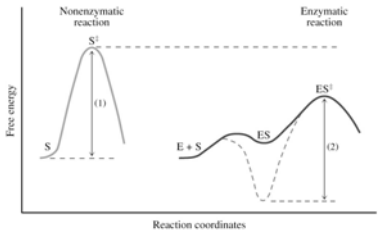
E and transition state fit together like a lock and key (not ES)

Weak Binding of Substrates to Enzymes

- Energy is required to reach the transition state from the ES complex
- Excessive ES stabilization would create a "thermodynamic pit" and mean little or no catalysis
- Most K_m values (substrate dissociation constants) indicate weak binding to enzymes

Energy of substrate binding

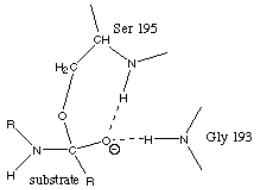
- If an enzyme binds the substrate too tightly (dashed profile), the activation barrier (2) could be similar to that of the uncatalyzed reaction (1)



Transition-State (TS) Stabilization

- An increased interaction of the enzyme and substrate occurs in the transition-state ($ES^{\ddagger}$)
- The enzyme distorts the substrate, forcing it toward the transition state
- An enzyme must be complementary to the transition-state in shape and chemical character
- Enzymes may bind their transition states 10^{10} to 10^{15} times more tightly than their substrates

Specific Binding of Chymotrypsin to the Transition State is Called "oxyanion hole"



In transition state, the H-Bonds are stronger b/c:

1. **Different molecular geometry**
2. **C-O^- bond is longer than C=O bond**
3. **Has negative charge and closer to H atom**

Consequences of Strain

Enzymes should bind transition state analogues more strongly than substrate

Consequences of Strain

Catalytic Antibodies

Antibodies that have catalytic activity can be induced by using transition state analogs bound to carrier proteins as antigens

Antibodies are proteins made by organisms that specifically bind antigens (which are foreign materials)