

BCMB/BIOL/CHEM 3100
Chapter 6 Mechanisms of Enzymes

- **Energy diagrams**
- **Chemical modes of enzyme catalysis**
 - Acid-Base catalysis**
 - Covalent catalysis**
- **Binding modes of enzyme catalysis**
 - Proximity effect**
 - Transition state stabilization**
- **Transition state analogs**
- **Induced fit**
- **Serine Proteases**

Energy diagrams show the progress of a reaction

Transition state: high energy, unstable state in which a molecule is best suited to undergo a chemical reaction; state in which chemical bonds are being broken and formed. Lifetime $\sim 10^{-14}$ to 10^{-13} sec

Fig 6.1 Energy diagram for a single-step reaction

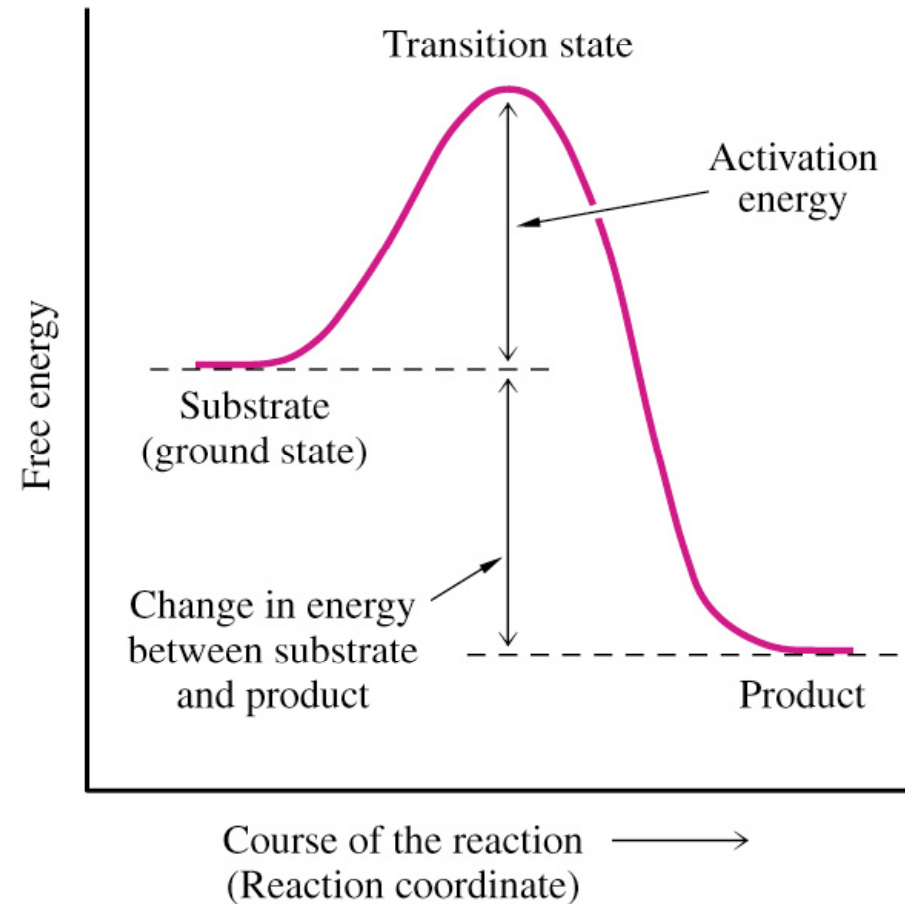


Fig 6.2 Energy diagram for reaction with intermediate

- _____ occurs in the trough between the two transition states
- Lifetime $> \sim 10^{-14}$ to 10^{-13} sec
- In this case, the rate determining step in the forward direction is formation of the first transition state

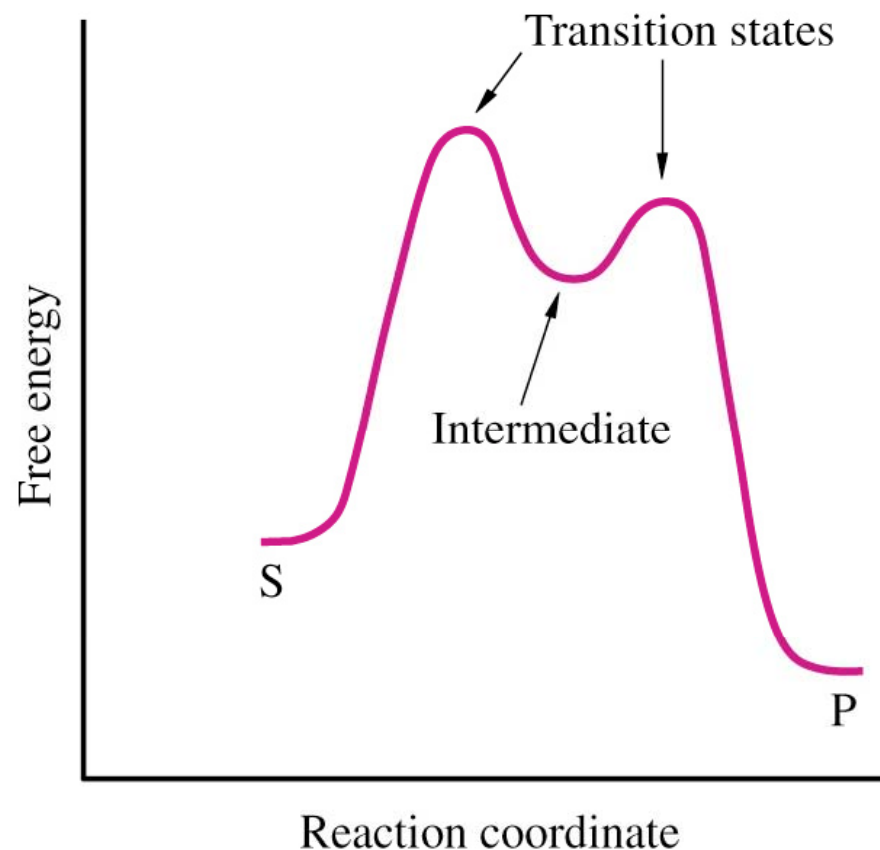
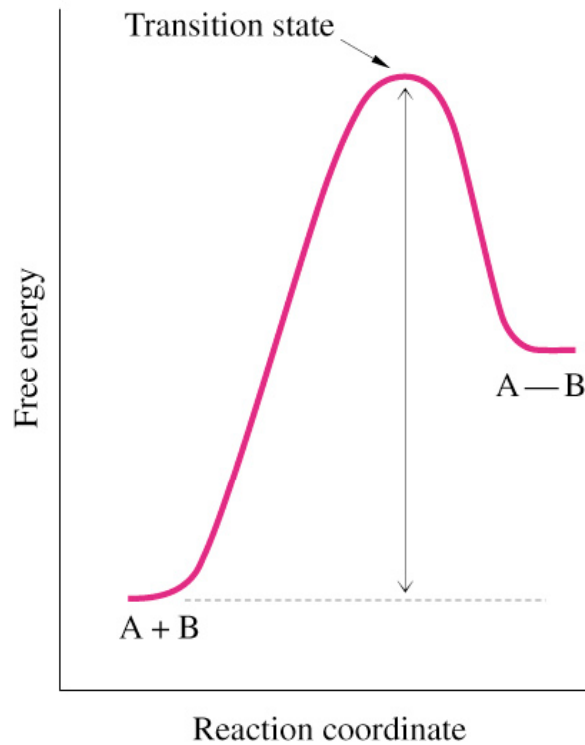


Fig 6.3 Enzymatic catalysis of the reaction $A+B \rightarrow A-B$

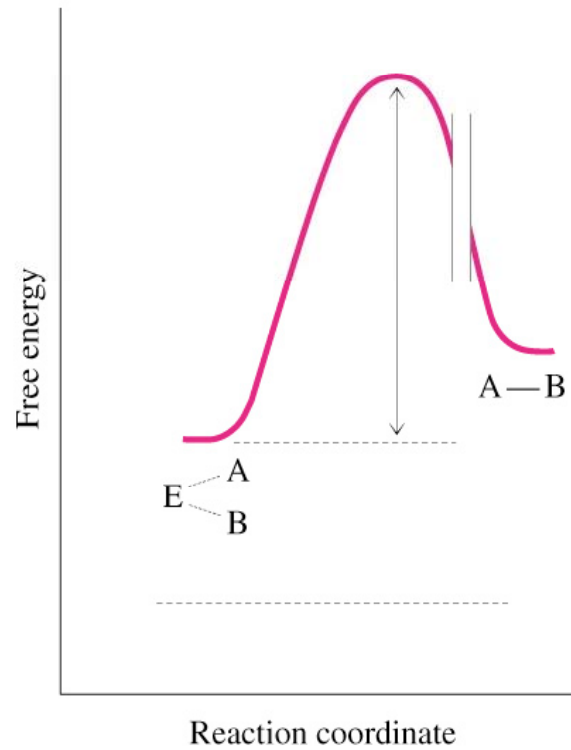
Proximity effect:
“proper” positioning of
substrates

**Transition state
stabilization: lowers
activation energy**

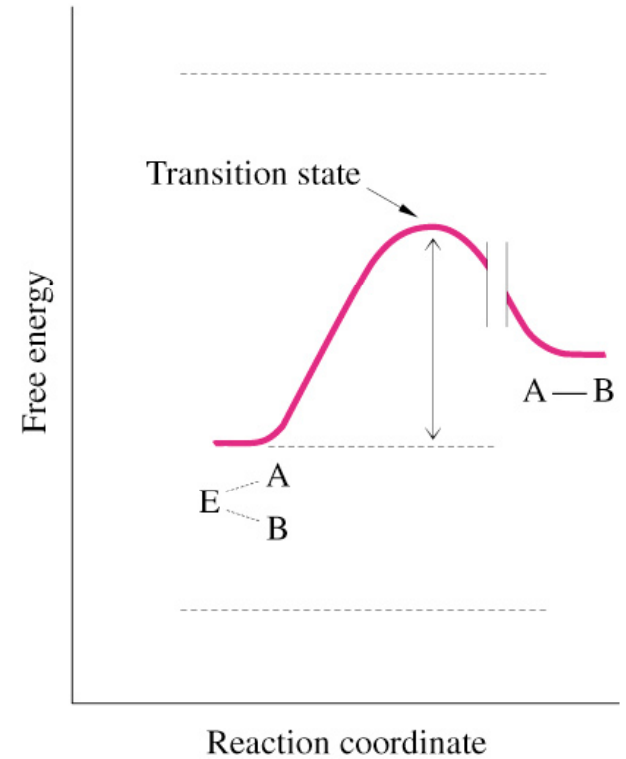
(a) Uncatalyzed reaction



(b) Effect of reactants being bound by enzyme



(c) Effect of reactants and transition state being bound by enzyme



What is the enzyme active site?

A few polar residues and H₂O molecules are found at the otherwise hydrophobic active site of an enzyme

_____ : polar amino acids that undergo changes during enzymatic catalysis

Ionic side chains are involved in two types of chemical catalysis:

- 1. _____**
- 2. _____**

Table 6.1

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 [⊖]	−1	Cation binding; proton transfer
Glutamate	—COO [⊖]	−1	Cation binding; proton transfer
Histidine	Imidazole	Near 0	Proton transfer
Cysteine	—CH ₂ SH	Near 0	Covalent binding of acyl groups
Tyrosine	Phenol	0	Hydrogen bonding to ligands
Lysine	—NH ₃ [⊕]	+1	Anion binding; proton transfer
Arginine	Guanidinium	+1	Anion binding
Serine	—CH ₂ OH	0	Covalent binding of acyl groups

Note: pKa of ionizable groups of amino acids in proteins vary from pKa of free amino acids (compare Table 3.2 to Table 6.2)

Table 6.2 pKa Values of amino acid ionizable groups in proteins

<u>Group</u>	<u>pK_a</u>
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

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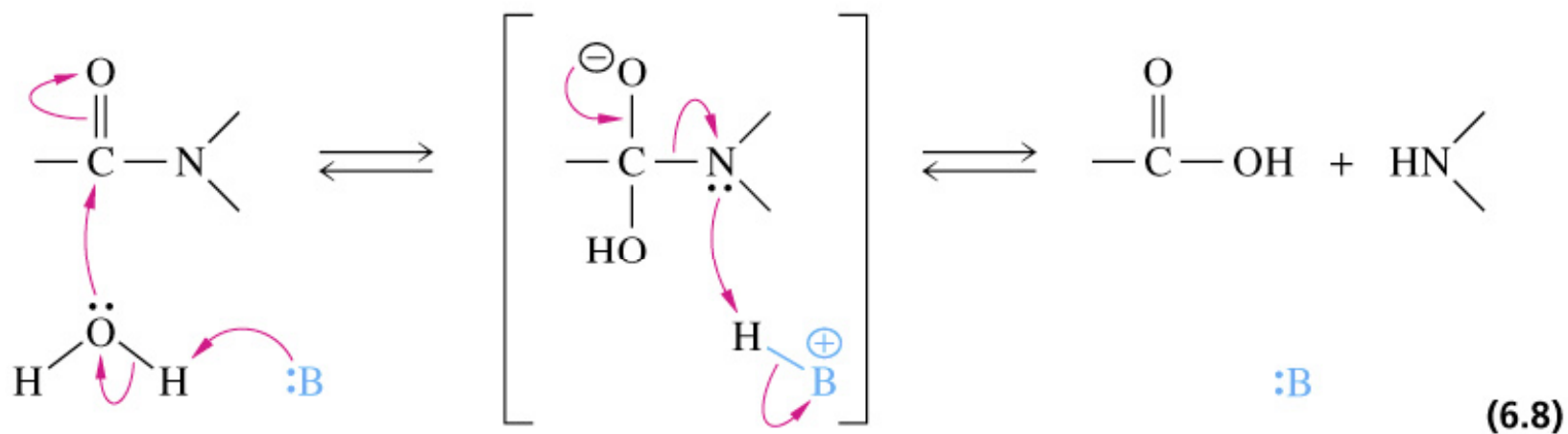
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Chemical modes of enzyme catalysis

- **Acid-Base catalysis**
- **Covalent catalysis**

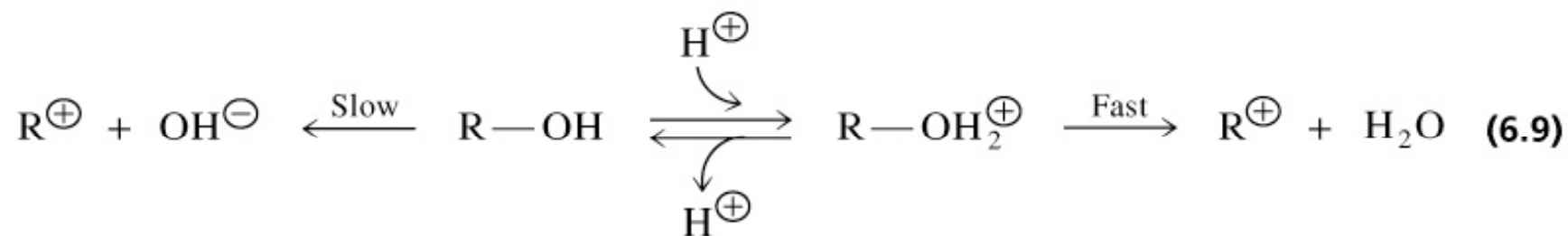
General base catalysis reactions (continued)

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



Proton donors can also catalyze reactions

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



Sucrose phosphorylase exhibits covalent catalysis (6.11-6.13)

Step one: a glucosyl residue is transferred to enzyme



Step two: Glucose is donated to phosphate



*(Sucrose is composed of a glucose and a fructose)

Fig 6.4 pH-rate profile for papain

_____ of an enzyme can give information about ionic residues at the active site.

A simple bell-shaped curve can result from two overlapping titrations of active site amino acids.

- The two inflection points approximate the pK_a values of the two ionizable residues

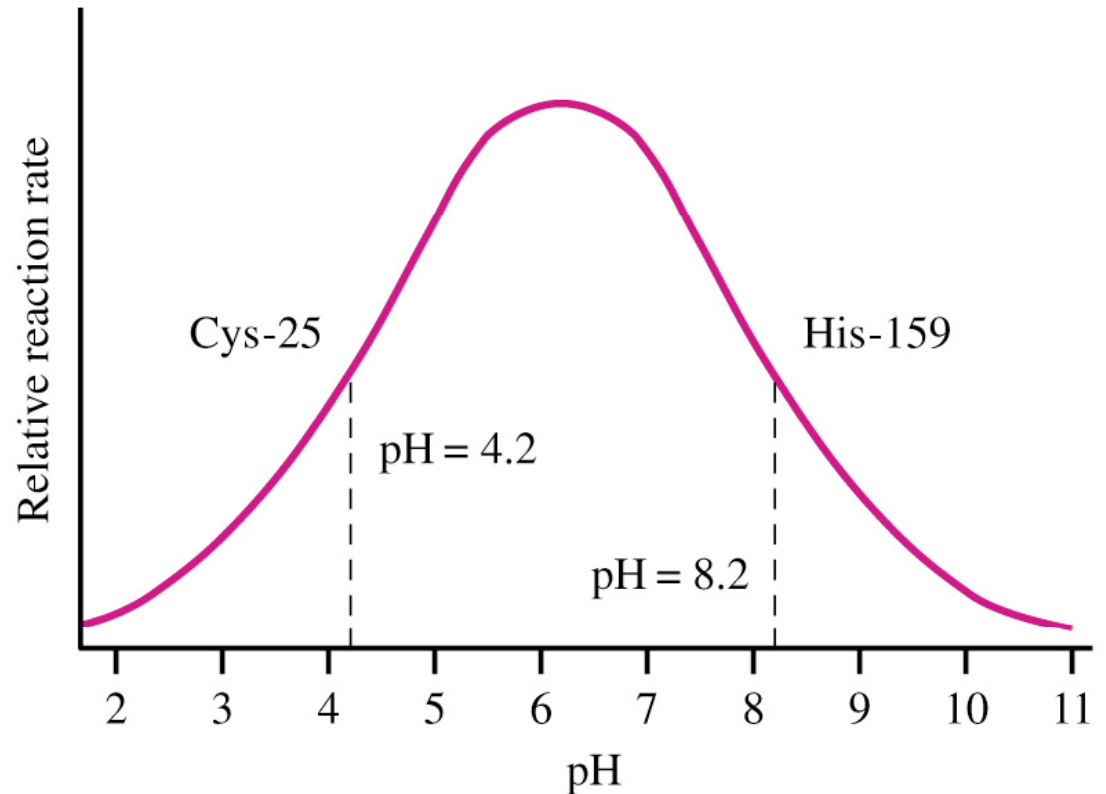


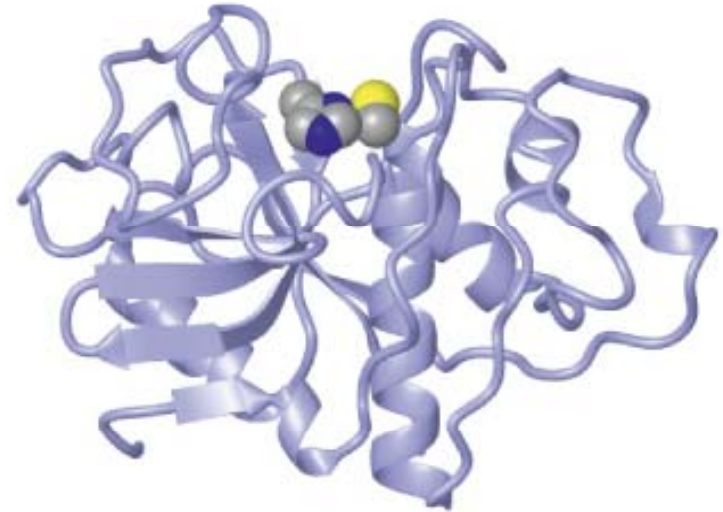
Fig 6.5

- Papain's activity depends upon ionizable residues:
His-159 and **Cys-25**

(a) Ribbon model

(b) Active site residues
(N blue, S yellow)

(a)



(b)

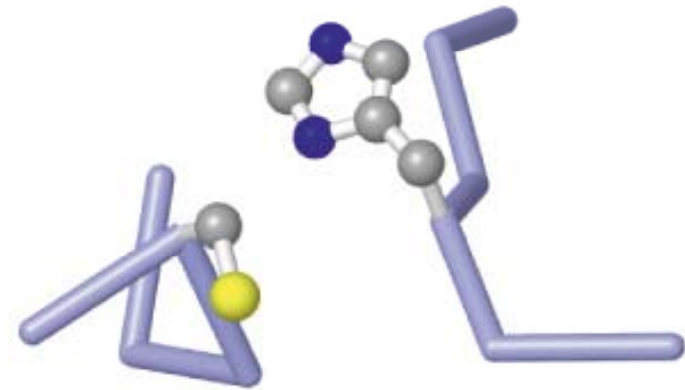
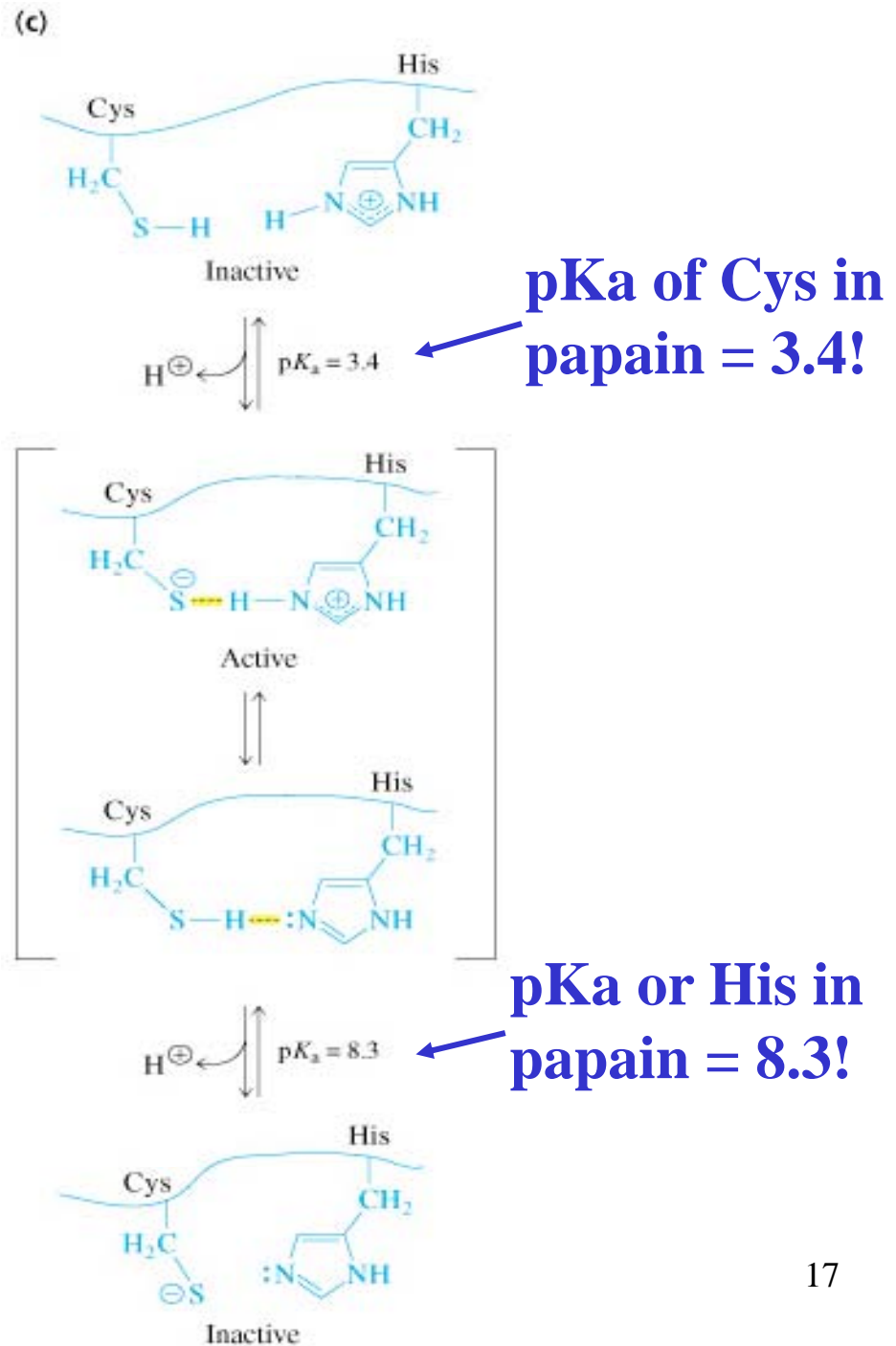


Fig. 6.5 continued

Three ionic forms of papain. Only the upper tautomer of the middle pair is active



Fastest Reactions are Diffusion-Controlled Reactions:

rates approach rate of diffusion: 10^8 to $10^9 \text{ M}^{-1}\text{s}^{-1}$; speed of binding of substrates to the enzyme

Table 6.4

TABLE 6.4 Enzymes with second-order rate constants near the upper limit

Enzyme	Substrate	$k_{\text{cat}}/K_{\text{m}}$ ($\text{M}^{-1} \text{s}^{-1}$)*
Catalase	H_2O_2	4×10^7
Carbonic anhydrase	CO_2	1.2×10^8
Acetylcholinesterase	Acetylcholine	1.6×10^8
Fumarase	Fumarate	1.6×10^8
Triose phosphate isomerase	D-Glyceraldehyde 3-phosphate	4×10^8
Superoxide dismutase	$\cdot\text{O}_2^\ominus$	2×10^9

*The ratio $k_{\text{cat}}/K_{\text{m}}$ is the apparent second-order rate constant for the enzyme-catalyzed reaction $\text{E} + \text{S} \rightarrow \text{E} + \text{P}$. For these enzymes, the formation of the ES complex can be the slowest step.

A. Triose Phosphate Isomerase (TPI)

- TPI catalyzes a rapid aldehyde-ketone interconversion

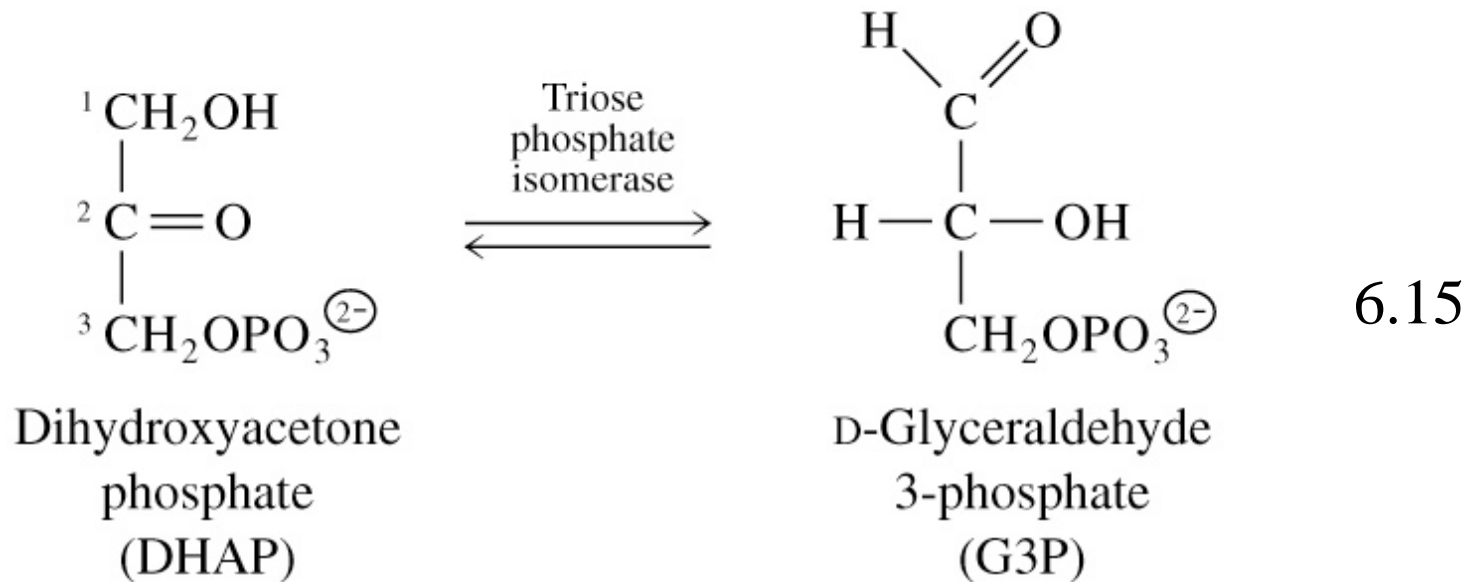


Fig 6.7 Proposed mechanism for TPI

- General acid-base catalysis mechanism (4 slides)

When dihydroxyacetone phosphate binds, the carbonyl oxygen forms a hydrogen bond with the neutral imidazole group of His-95. The carboxylate group of Glu-165 removes a proton from C-1 of the substrate to form an enediolate intermediate.

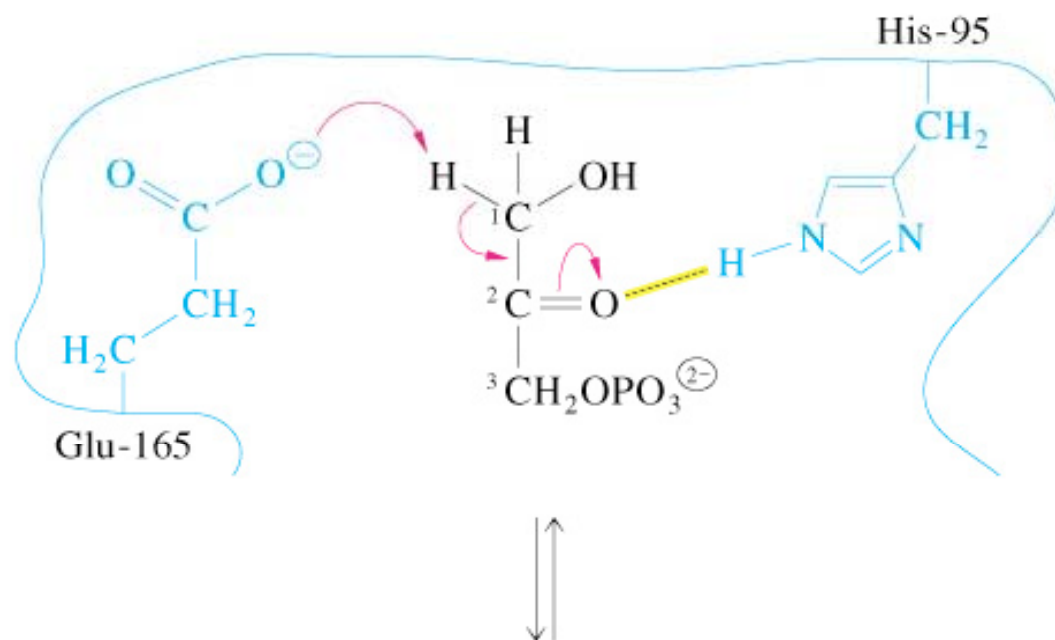


Fig 6.7 TPI mechanism (continued)

His-95 forms a strong hydrogen bond to the C-2 oxygen atom of the enediolate, and protonates this oxygen atom.

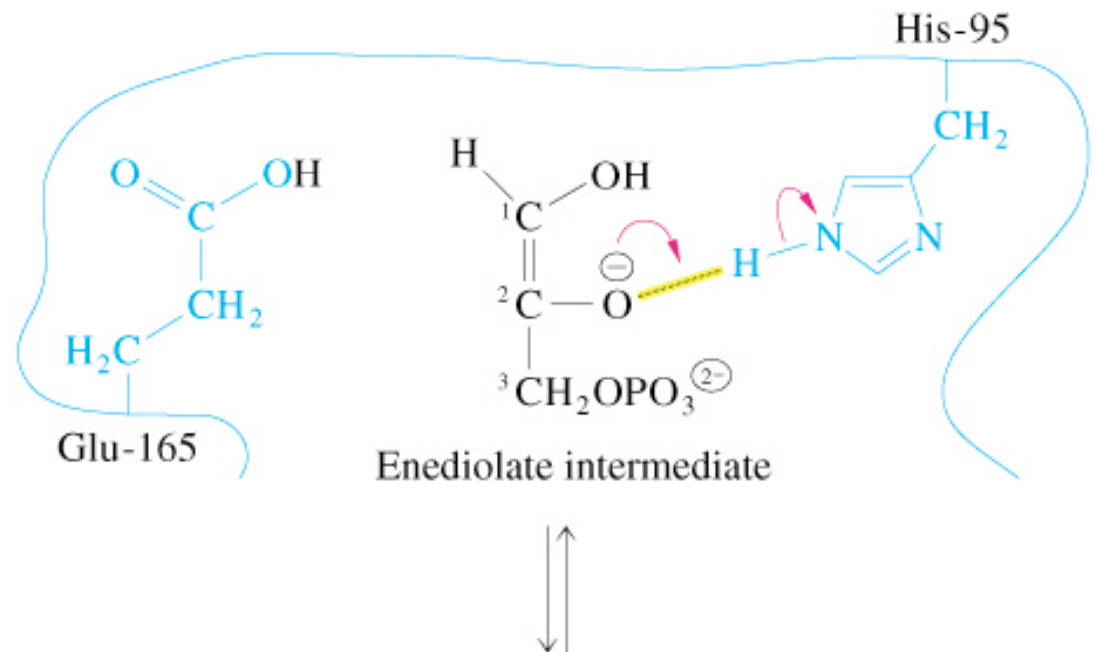


Fig 6.7 TPI mechanism (continued)

Next, the imidazolate form of His-95 abstracts a proton from the hydroxyl group at C-1 and shuttles the proton between oxygen atoms, producing another unstable enediolate intermediate.

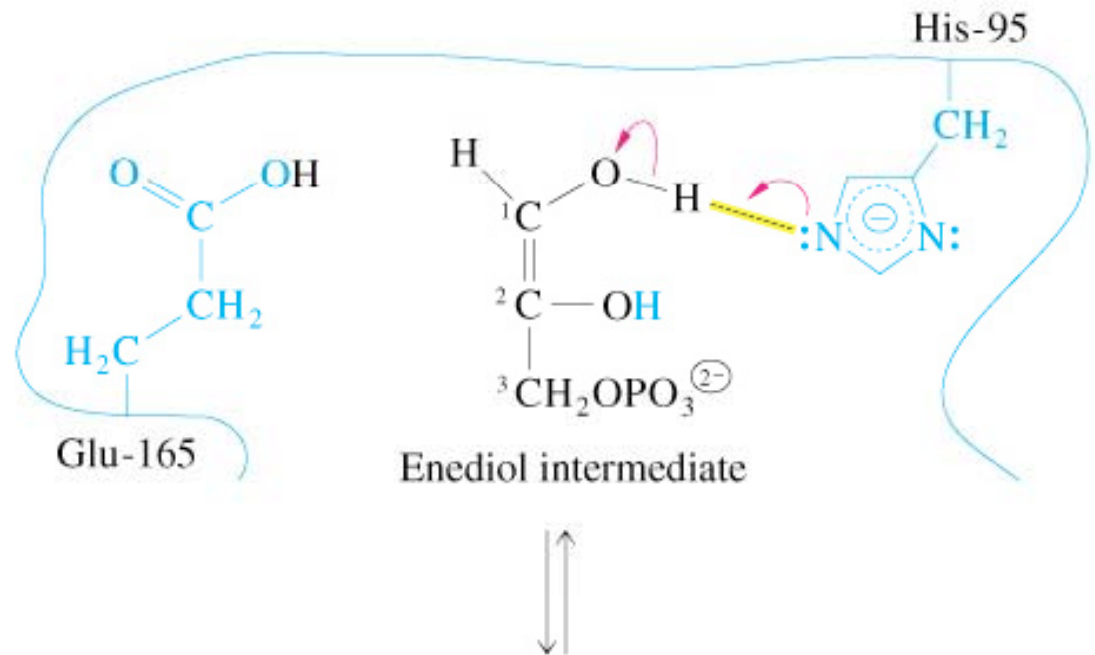


Fig 6.7 TPI mechanism (continued)

Glu-165 donates a proton to C-2,
producing D-glyceraldehyde 3-phosphate.

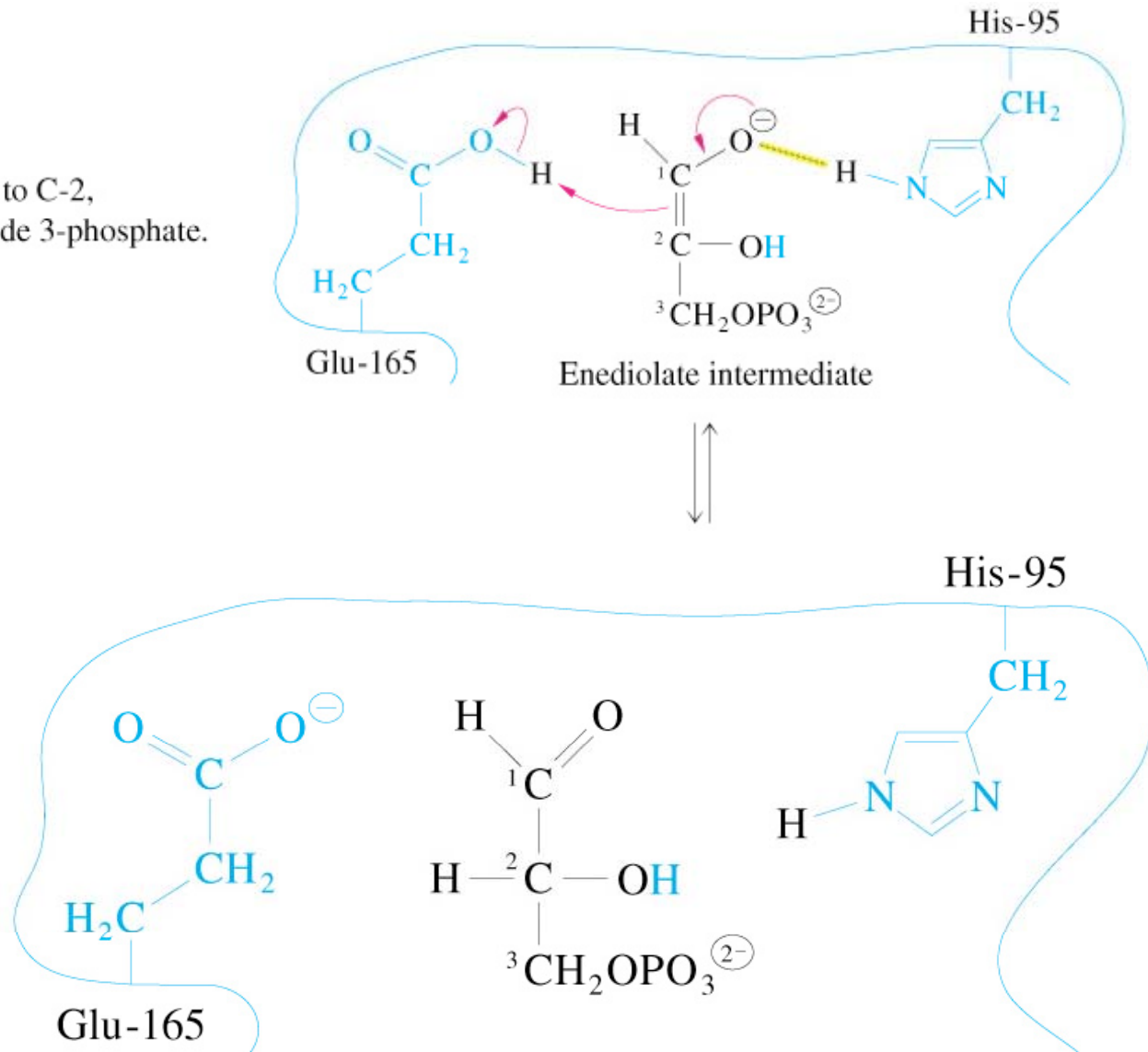
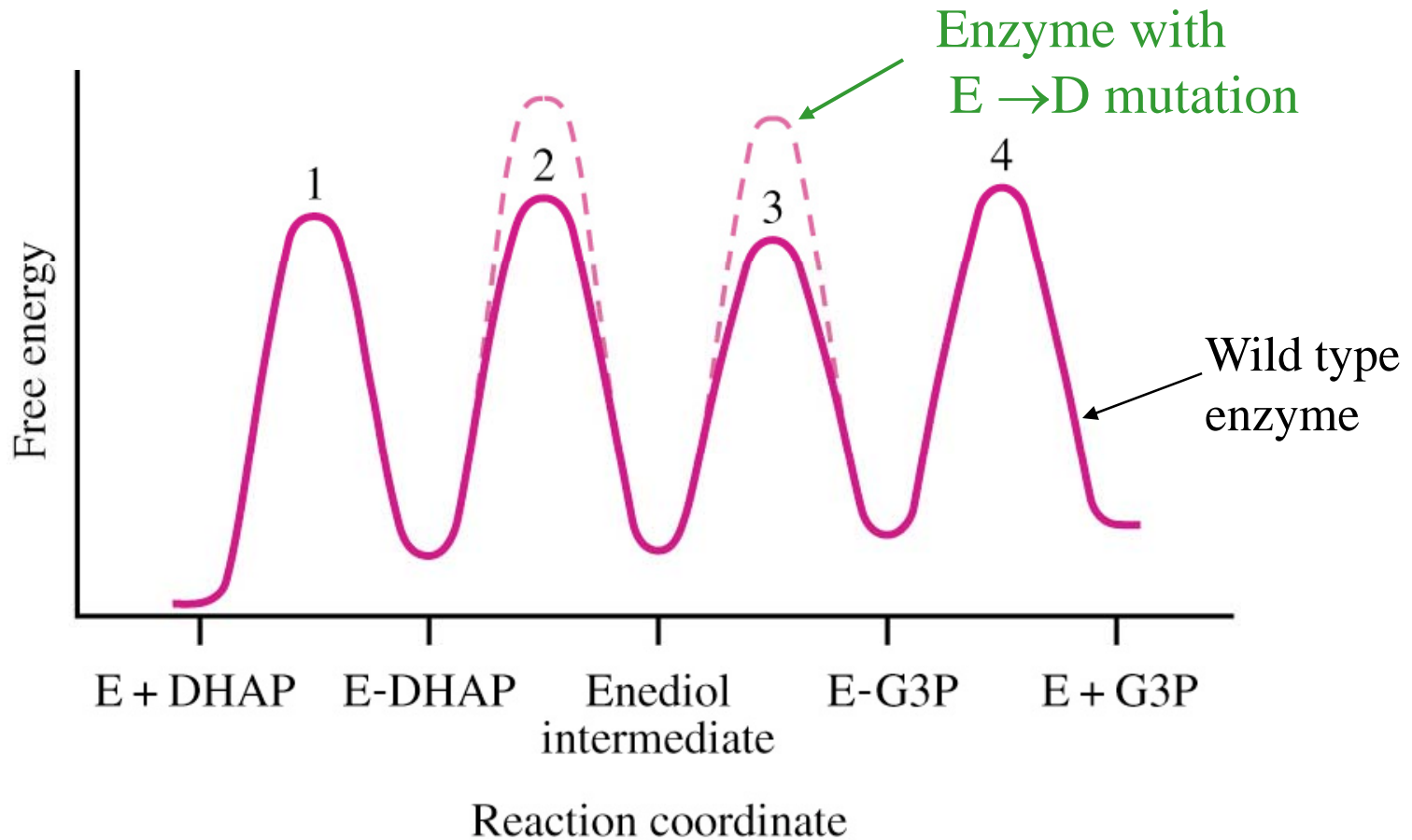


Fig 6.9 Energy diagram for the TPI reaction



Proximity effect:

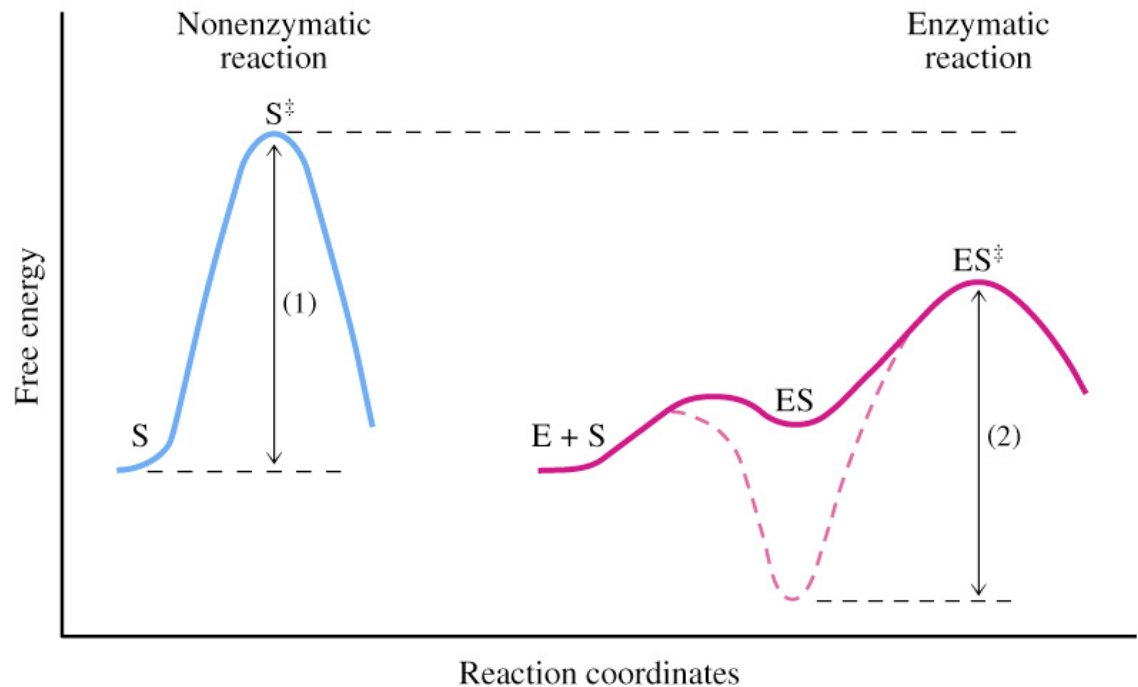
_____ in rate of a reaction due to binding of substrates at binding sites in the enzyme,

results in an _____ effective concentration of reactants,

results in more frequent formation of the

Fig 6.12 Excessive ES stabilization would create a “thermodynamic pit” and give little or no catalysis

- if E binds S too tightly (dashed profile), the activation barrier (2) could be similar to that of the uncatalyzed reaction (1)
- most K_m values (substrate dissociation constants) indicate weak binding to enzymes



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Transition-State (TS) Stabilization

- increased interaction of E with S in transition-state (ES^\ddagger)
- E distorts S, forcing it toward the transition state
- E must be complementary to transition-state in shape and chemical character
- E binds transition states 10^{10} to 10^{15} times more tightly than S

Basis for enzymatic catalysis

1. _____ (e.g. **acid-base & covalent catalysis**) →
10-100 ↑

2. _____

_____ : "weak" binding (~0.1 M) of S to active site raises the effective concentration of S and favors more frequent transition states → 10^4 - 10^5 ↑

effective molarity: enhanced relative concentration of reactants due to binding to E

_____ : greater binding of transition states than S or P to E → lower activation energy → 10^4 - 10^5 ↑

Enzyme rate accelerations ~ 10^8 - 10^{12}

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Wolfenden & Lienhard (1970s): showed that chemical analogs of _____ are enzyme inhibitors

In Emil Fisher's lock-and-key model for SE binding, the _____. Binding of S to E distorts S to → transition state. The transition state must be stabilized for catalysis to occur.

Transition state analogs can → catalytic antibodies

Transition-state (TS) analogs

- **Transition-state analogs** are stable compounds whose structures resemble unstable transition states
- **Fig. 6.14** 2-Phosphoglycolate, a TS analog for the enzyme triose phosphate isomerase

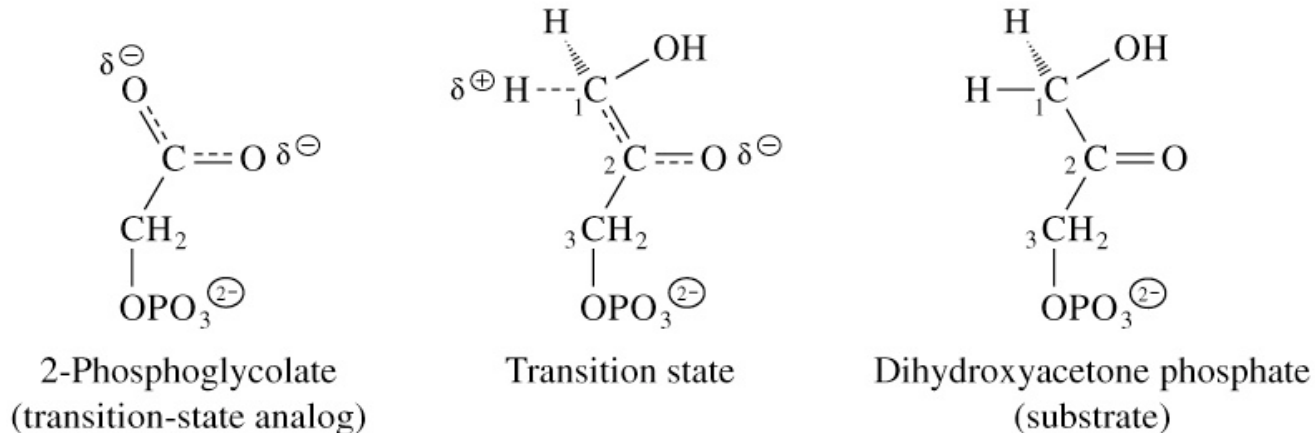
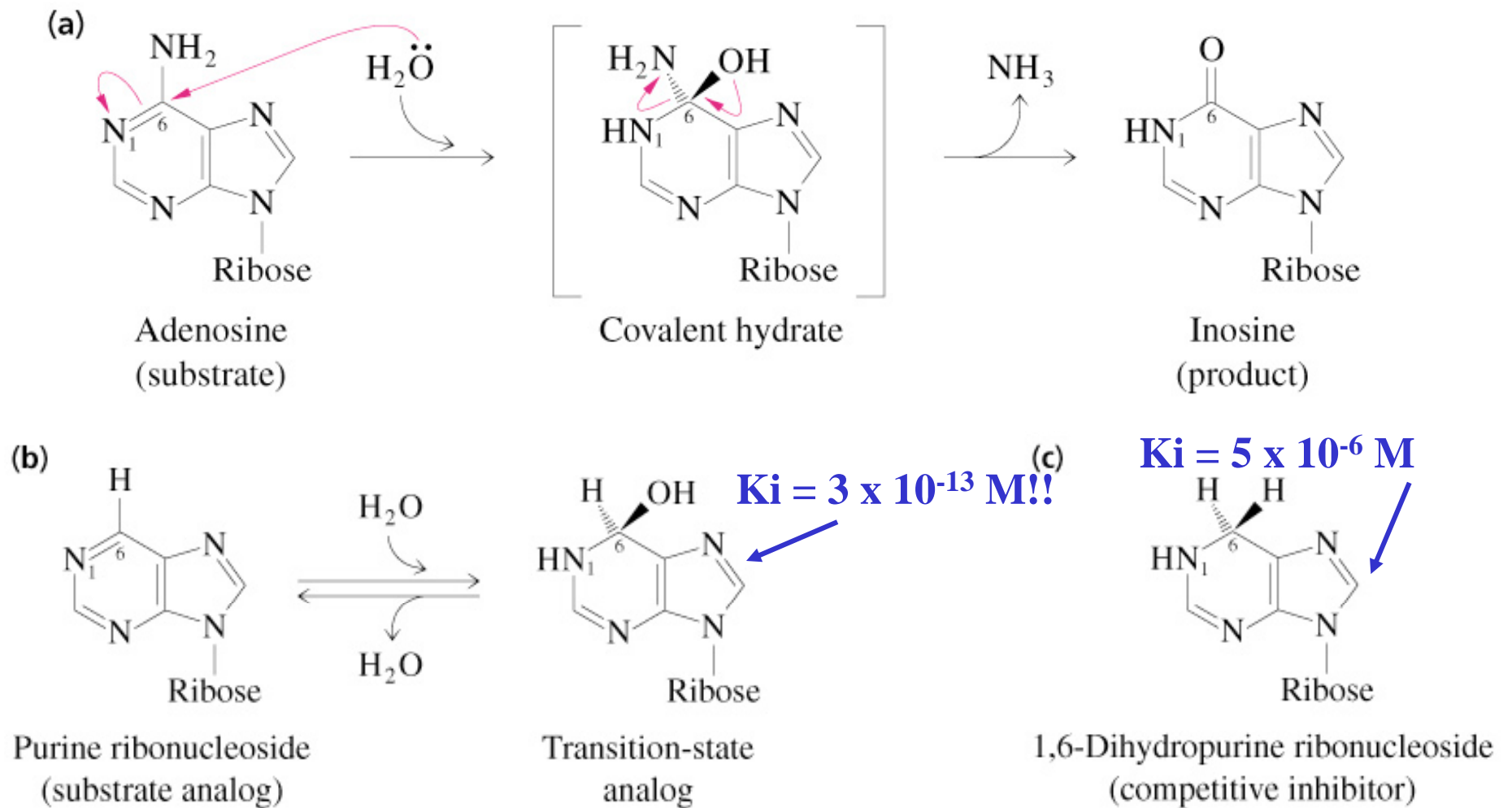


Fig 6.15 Inhibition of adenosine deaminase by a TS analog



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Induced Fit: substrate induced cleft closing
(Daniel Koshland, 1950s)

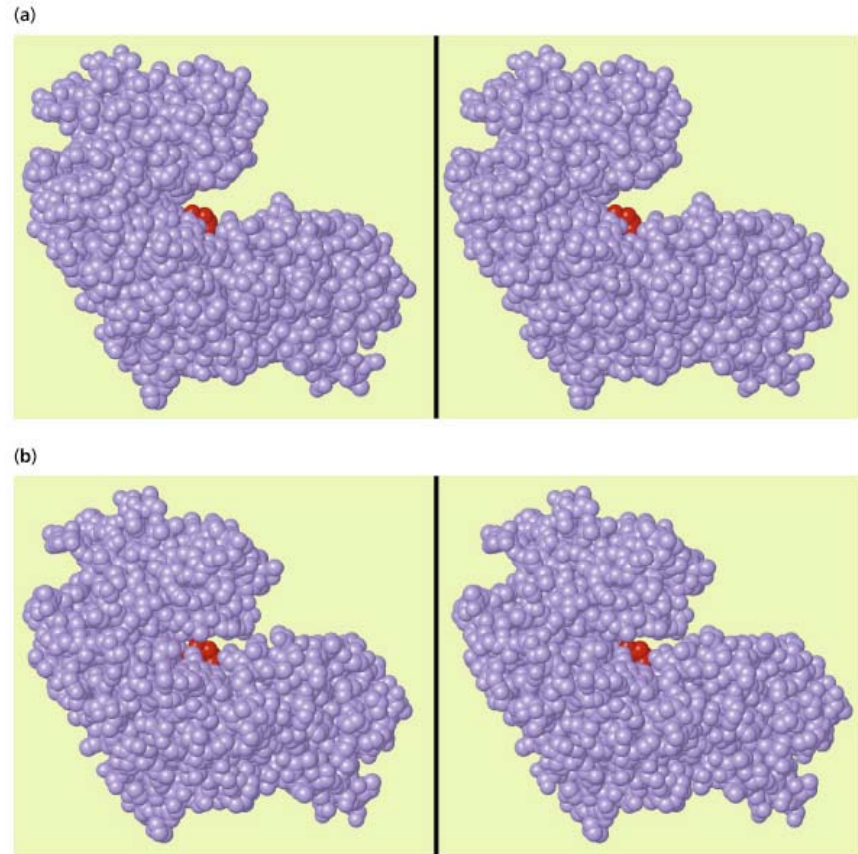
- _____ activates an enzyme by substrate-initiated conformation effect
- Induced fit is a substrate specificity effect, not a catalytic mode
- **Hexokinase** mechanism requires sugar-induced closure of the active site
- Other examples: **pyruvate kinase, phosphoglycerate kinase, phosphofructokinase**

~Fig 6.13 Stereo views of yeast hexokinase

- Yeast hexokinase contains 2 domains connected by a hinge region. Domains close on glucose binding.

(a) Open conformation

(b) Closed conformation



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Properties of Serine Proteases

- Digestive serine proteases including _____, _____, and _____ are synthesized and stored in the pancreas as zymogens
- _____ are inactive enzyme precursors that must be covalently modified to become active
- Storage of hydrolytic enzymes as _____ prevents damage to cell proteins
- Pancreatic zymogens are activated by _____
- The pancreatic zymogens are also regulated by enzyme inhibitors (e.g. trypsin inhibitor, $K_d = 10^{-13}$ M !!)

Fig 6.21 Activation of some pancreatic zymogens
Enzyme cascades → rapid signal amplification

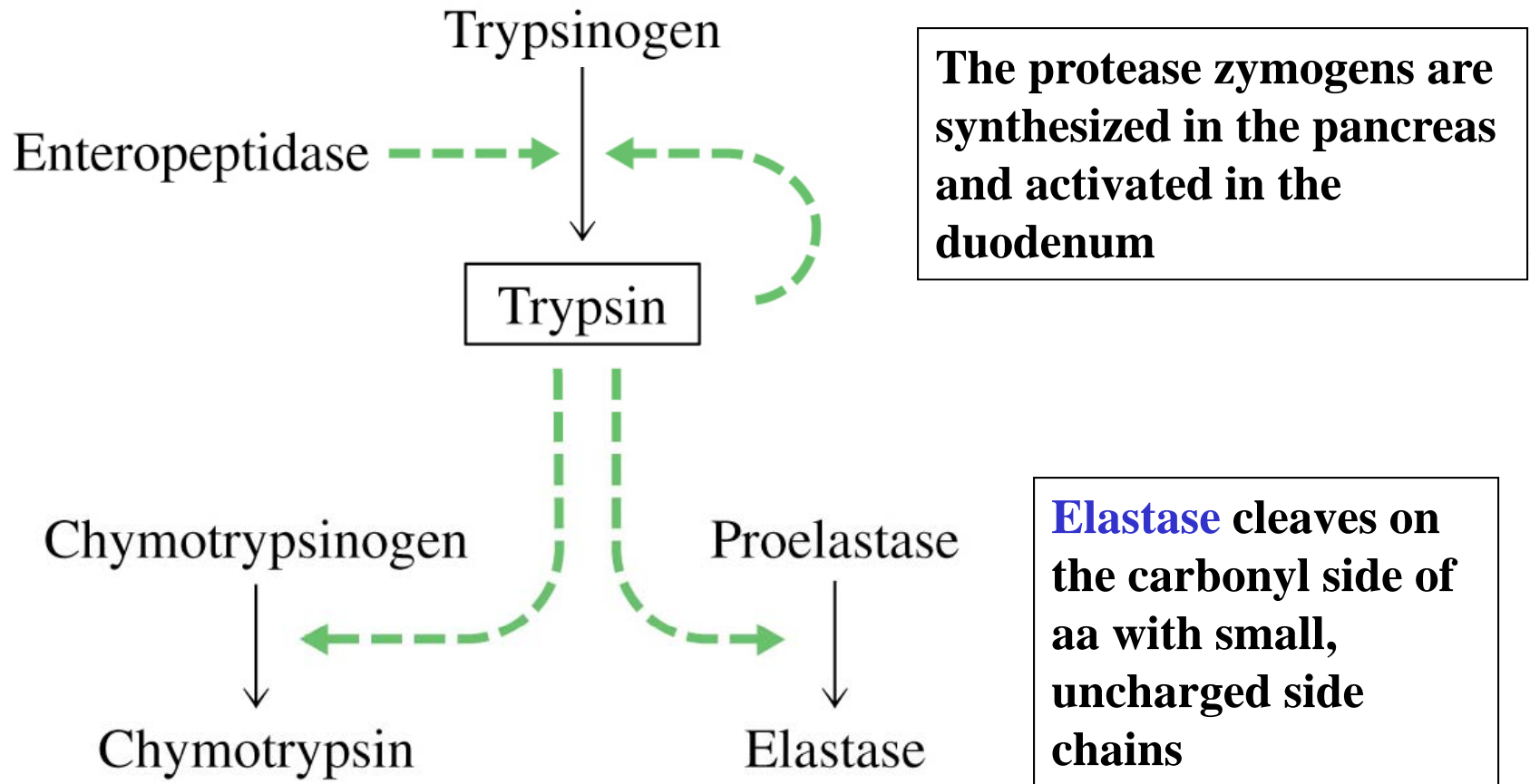


Fig 6.22 The backbones of **chymotrypsin** (blue),
trypsin (yellow), and **elastase** (green)

- Backbone conformations and active-site residues (red) are similar in these three enzymes

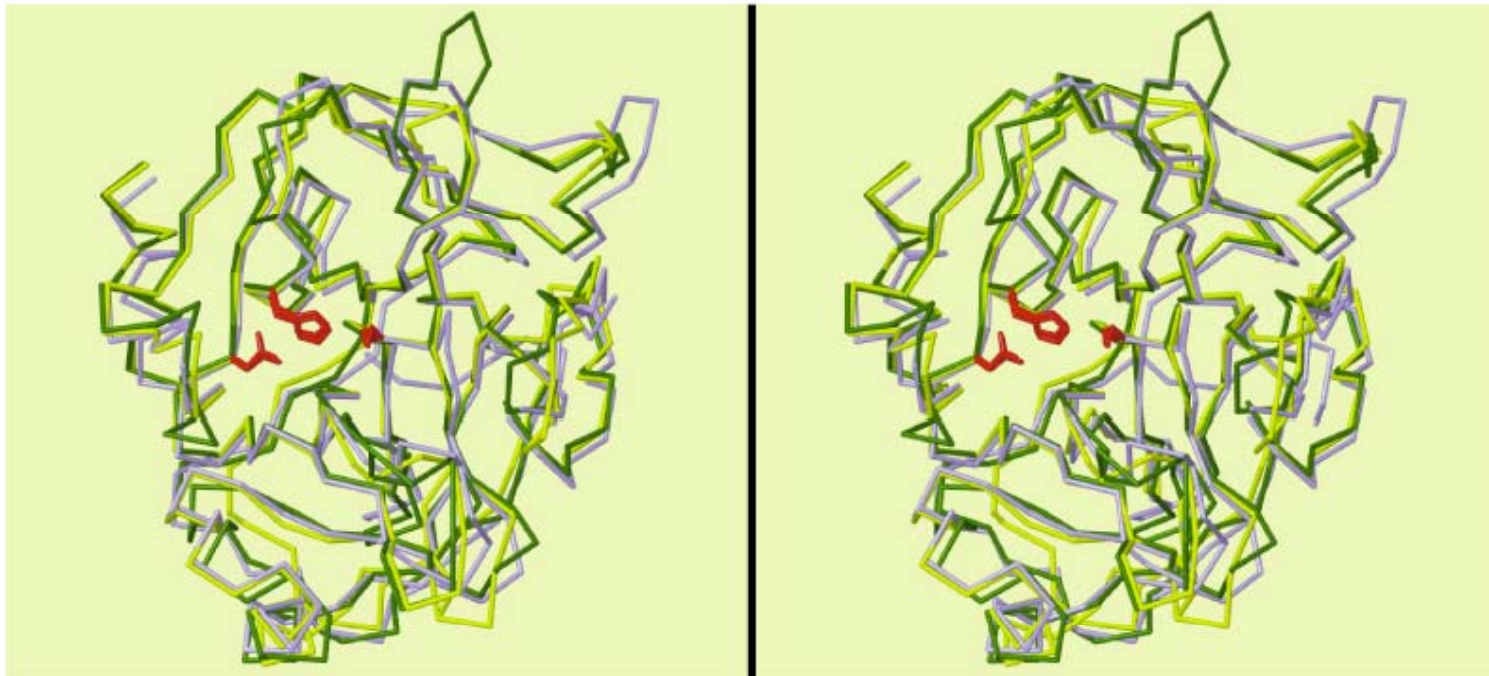
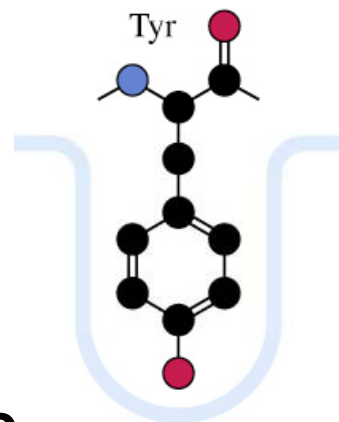


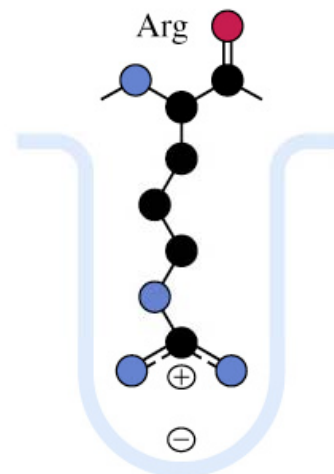
Fig 6.24 Binding sites of chymotrypsin, trypsin, and elastase

(a) Chymotrypsin



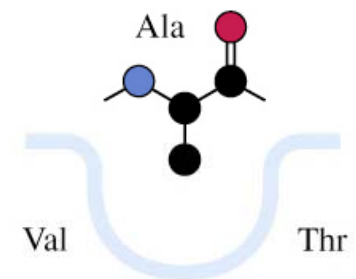
Ser
Uncharged

(b) Trypsin



Asp

(c) Elastase

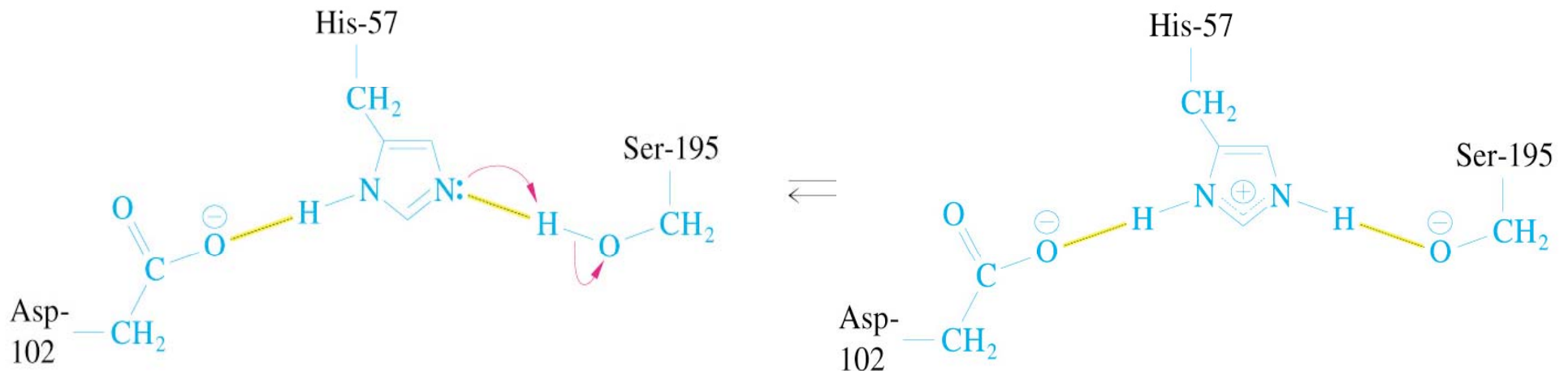


● Carbon
● Nitrogen
● Oxygen

- **Substrate specificities are due to relatively small structural differences in active-site binding cavities**

Fig 6.26 _____ of chymotrypsin

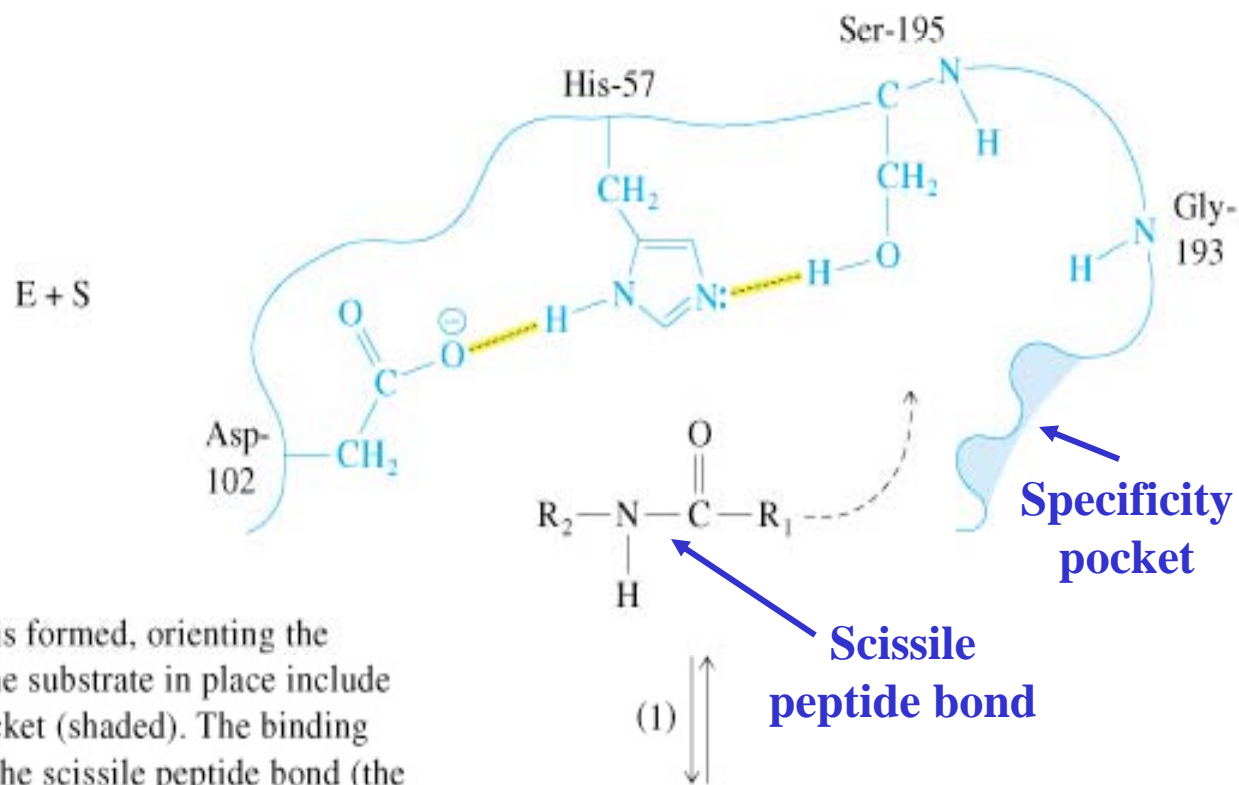
- Imidazole ring (His-57) removes H from Ser-195 hydroxyl to make it a strong nucleophile ($-\text{CH}_2\text{O}^-$)
- Buried carboxylate (Asp-102) stabilizes the positively-charged His-57 to facilitate serine ionization



Catalytic triad of serine proteases = **Asp, His, Ser**₄₂

Fig 6.27 α -Chymotrypsin mechanism (8 slides; 1)

Step (1): E + S



The noncovalent enzyme-substrate complex is formed, orienting the substrate for reaction. Interactions holding the substrate in place include binding of the R₁ 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.

Fig 6.27 (E-S) (2)

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-TI₁), which is believed to resemble the transition state.

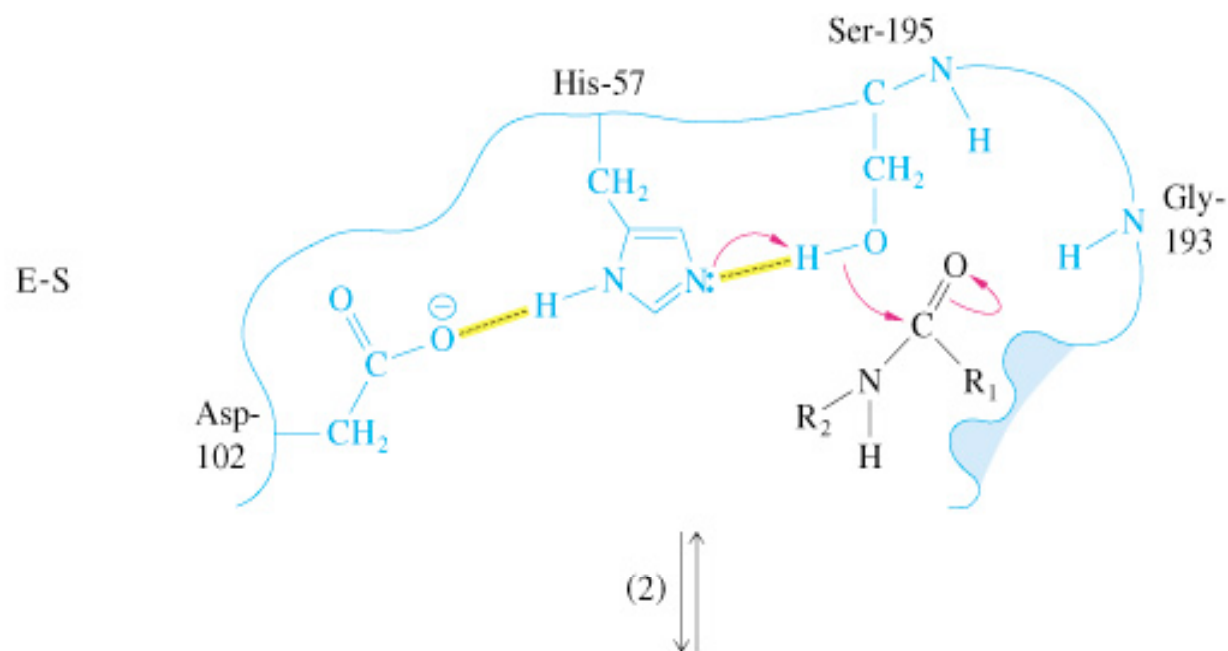
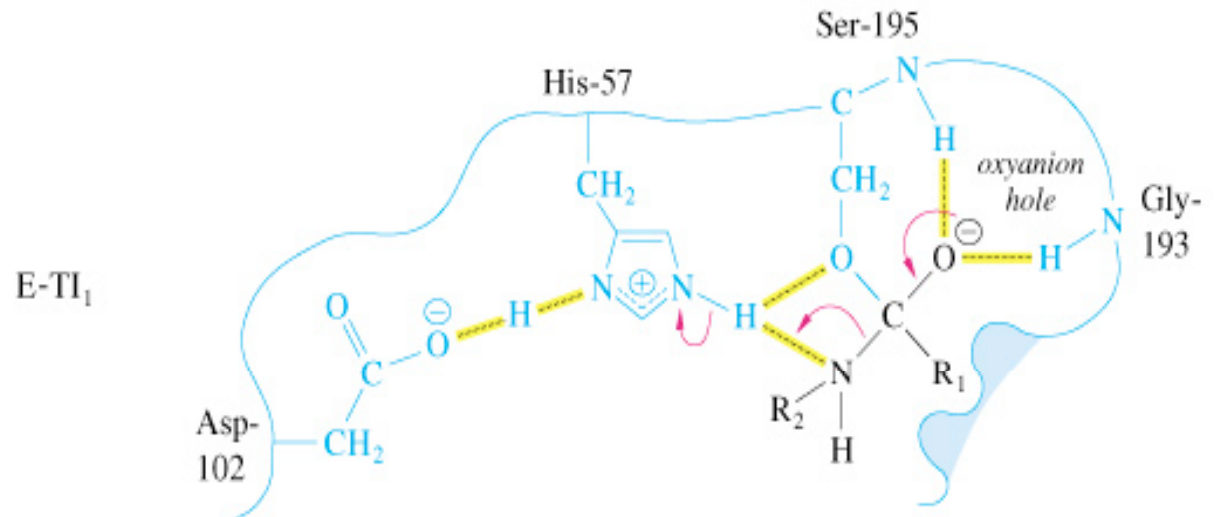


Fig 6.27 (E-TI₁) (3)

Tetrahedral intermediate

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.



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) ⇌

Fig 6.27 (Acyl E + P₁) (4)

Acid-base & covalent catalysis

The carbonyl group from the peptide forms a covalent bond with the enzyme, producing an acyl-enzyme intermediate. After the peptide product (P₁) with the new amino terminus leaves the active site, water enters.

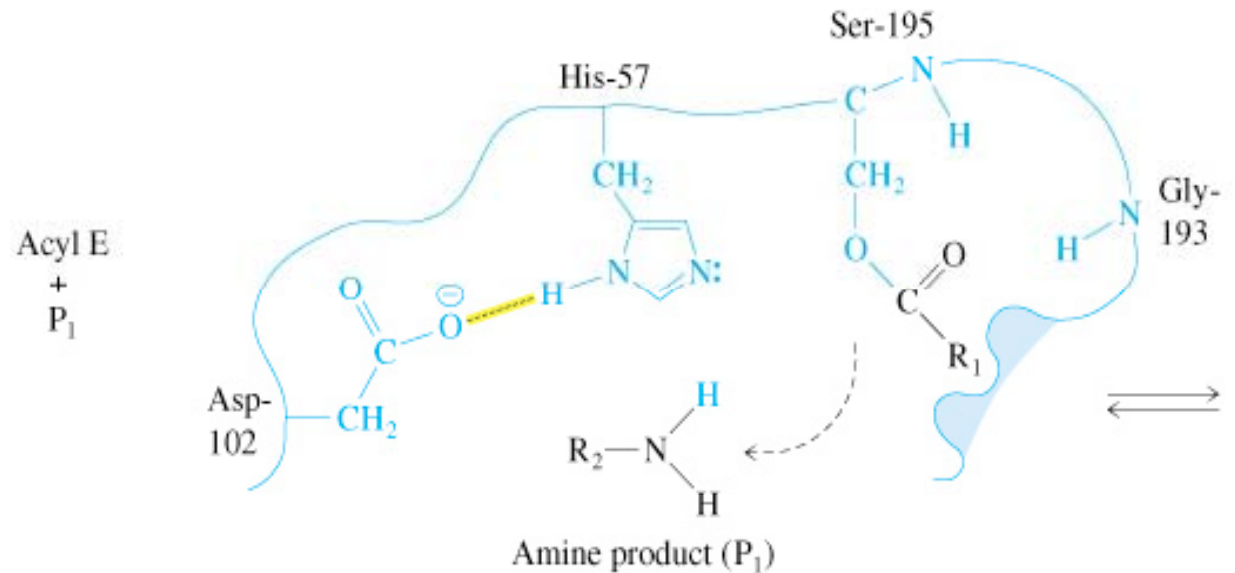
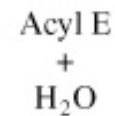
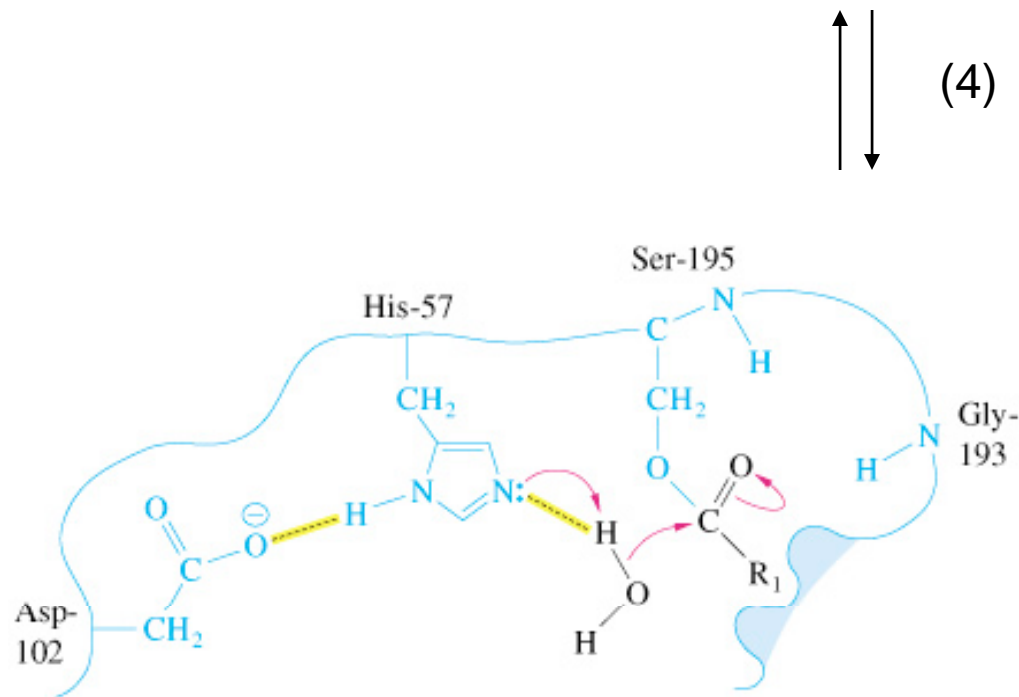


Fig 6.27 (Acyl E + H₂O) (5)

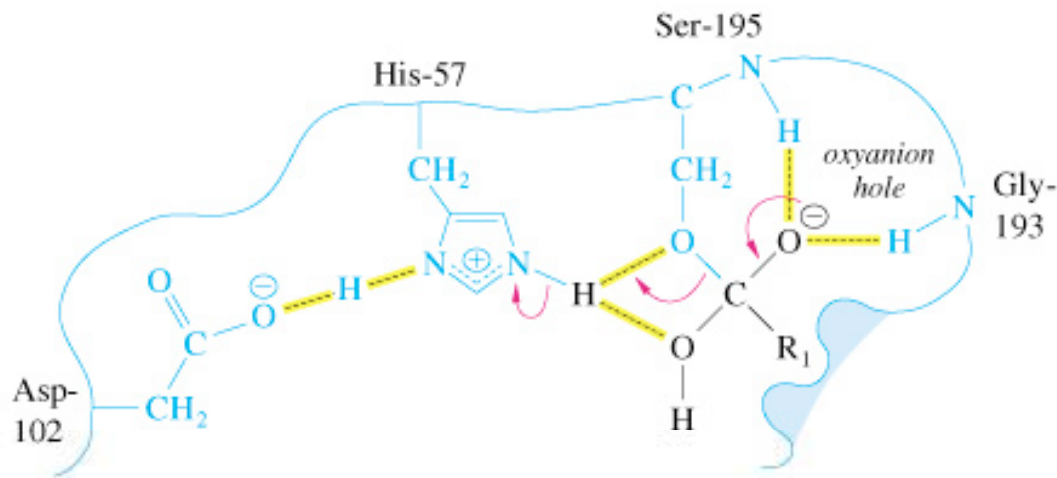


Hydrolysis (deacylation) of the acyl-enzyme intermediate starts when Asp-102 and His-57 again form a low-barrier hydrogen bond and His-57 removes a proton from the water molecule to provide an OH[⊖] group to attack the carbonyl group of the ester.

Hydrolysis

Fig 6.27 (E-TI₂) (6)

↑
↓ (5)



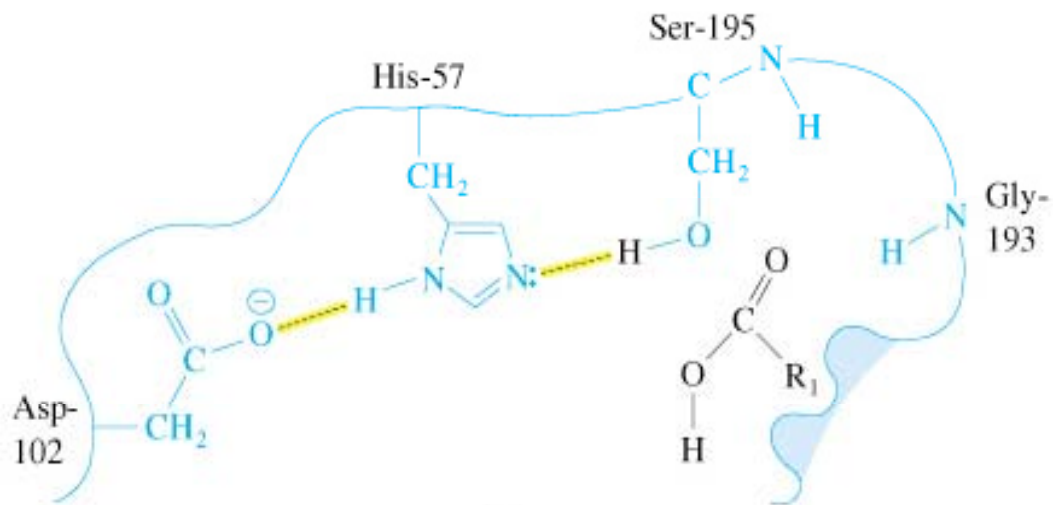
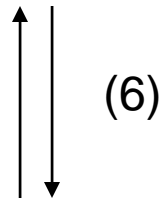
Tetrahedral intermediate

E-TI₂

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

A second tetrahedral intermediate (E-TI₂) is formed and stabilized by the oxyanion hole.

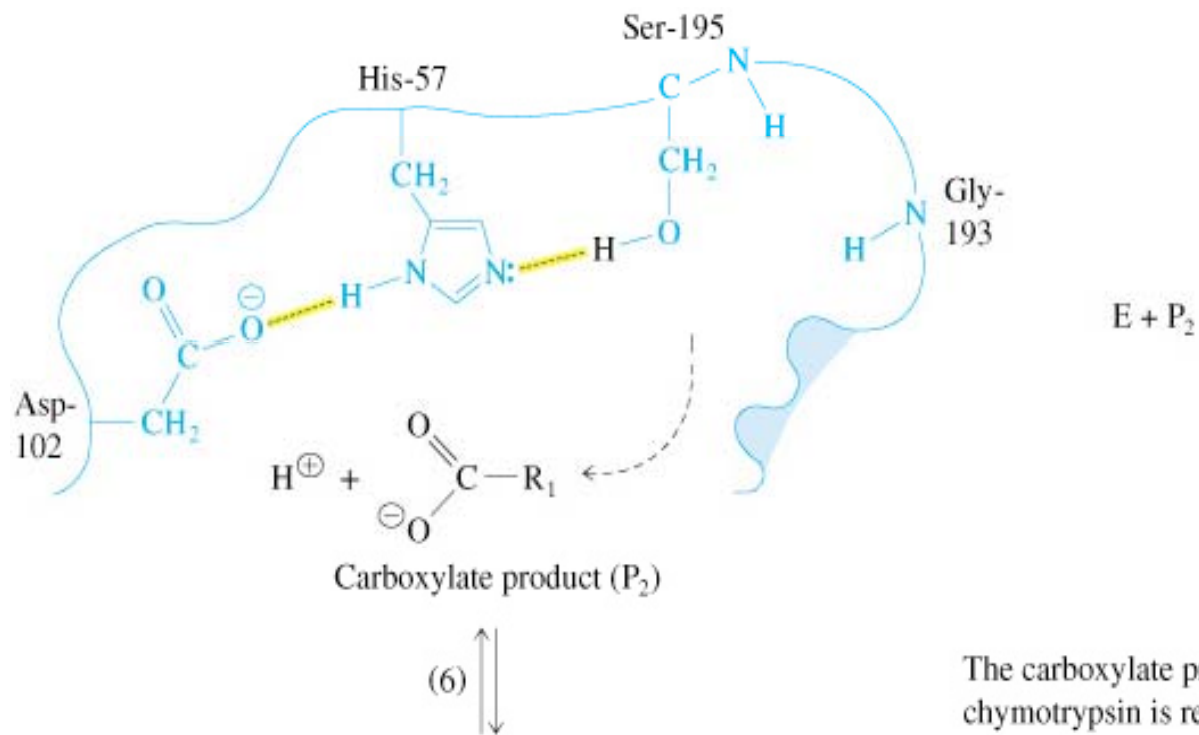
Fig 6.27 (E-P₂) (7)



E-P₂

The second product (P₂)—a polypeptide with a new carboxy terminus—is formed.

Fig 6.27 (E + P₂) (8)



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

**Additional material to aid in learning the
material covered in the chapter**

Review of Chemical Mechanisms

1. **Nucleophilic Substitution Reactions:** ionic reaction where both electrons stay with one atom → ionic intermediate + leaving group

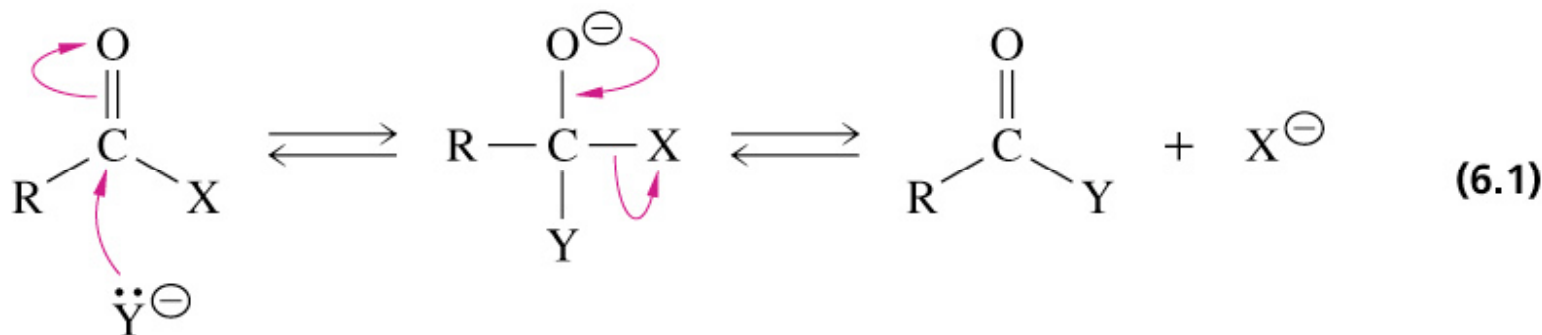
ionic reactions have **nucleophile** + **electrophile**

Formation of tetrahedral intermediate

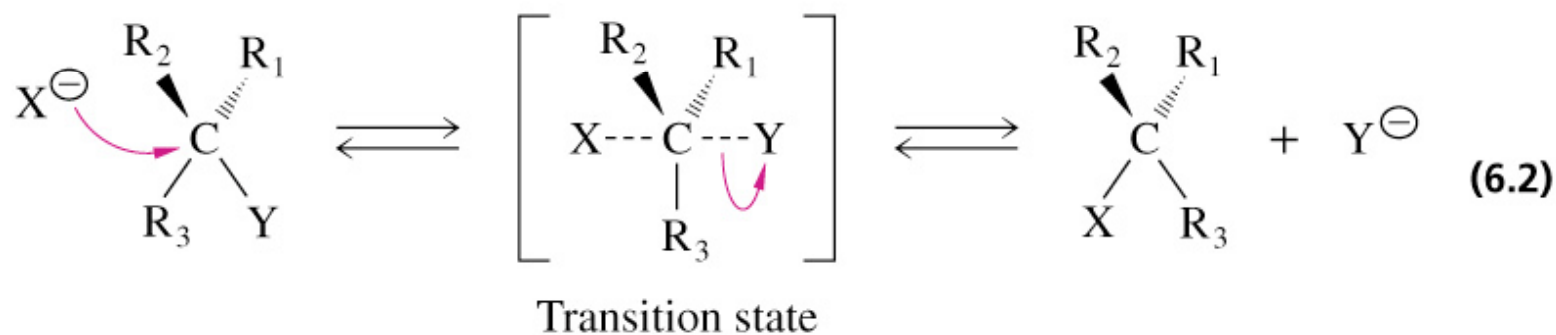
Direct displacement: two molecules react to form a five group transition state

Two types of nucleophilic substitution reactions

- Formation of a tetrahedral intermediate



- Direct displacement

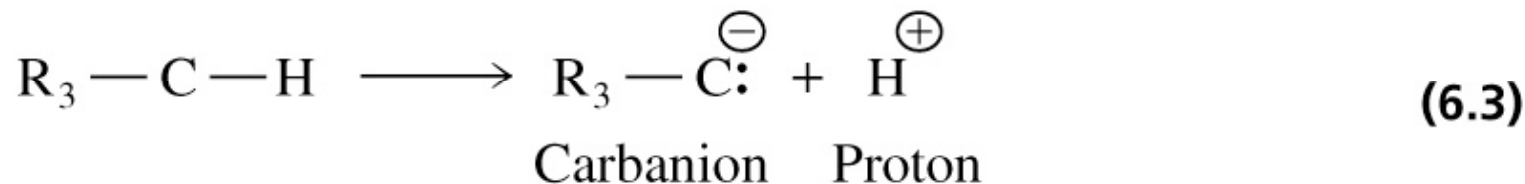


2. Cleavage reactions : most common when both electrons stay with one atom

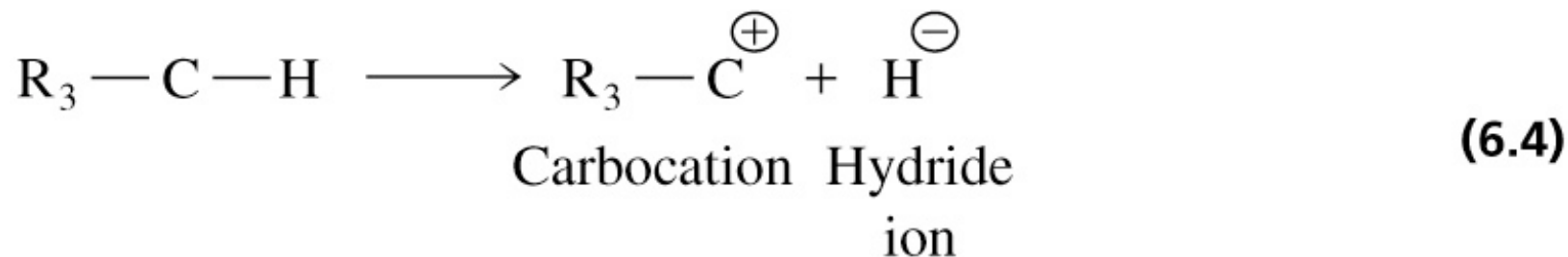
* formation of a carbanion (C retains both e-)

* formation of carbocation ion (C loses both e-)

• Carbanion formation



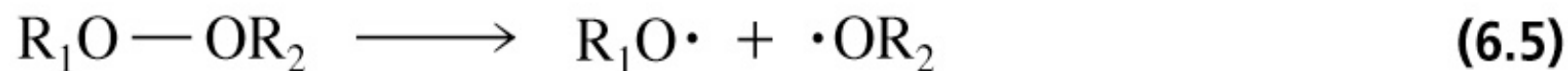
• Carbocation formation



3. Cleavage reactions : less common
when one electron remains with each
product

→ two free radicals

•Free radical formation



4. Oxidation-reduction reactions

Oxidation

- * **addition of oxygen**
- * **removal of hydrogen**
- * **removal of electrons**

- **Electrons are transferred between two species**
- **Oxidizing agent** gains electrons (is reduced)
- **Reducing agent** donates electrons (is oxidized)

Enzymes lower the activation energy of a reaction

(1) Substrate binding

- Enzymes properly position substrates for reaction (makes the formation of the transition state more frequent and lowers the energy of activation)

(2) Transition state binding

- Transition states are bound more tightly than substrates (this also lowers the activation energy)

Binding Modes of Enzymatic Catalysis

- Proper binding of reactants in enzyme active sites provides substrate specificity and catalytic power
- Two catalytic modes based on binding properties can each increase reaction rates over 10,000-fold :
 - (1) **Proximity effect** - collecting and positioning substrate molecules in the active site
 - (2) **Transition-state (TS) stabilization** - transition states bind more tightly than substrates

Binding forces utilized for catalysis

- 1. Charge-charge interactions**
- 2. Hydrogen bonds**
- 3. Hydrophobic interactions**
- 4. Van der Waals forces**

A. The Proximity Effect

- 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

Fig 6.11 Reactions of carboxylates with phenyl esters with phenyl esters

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

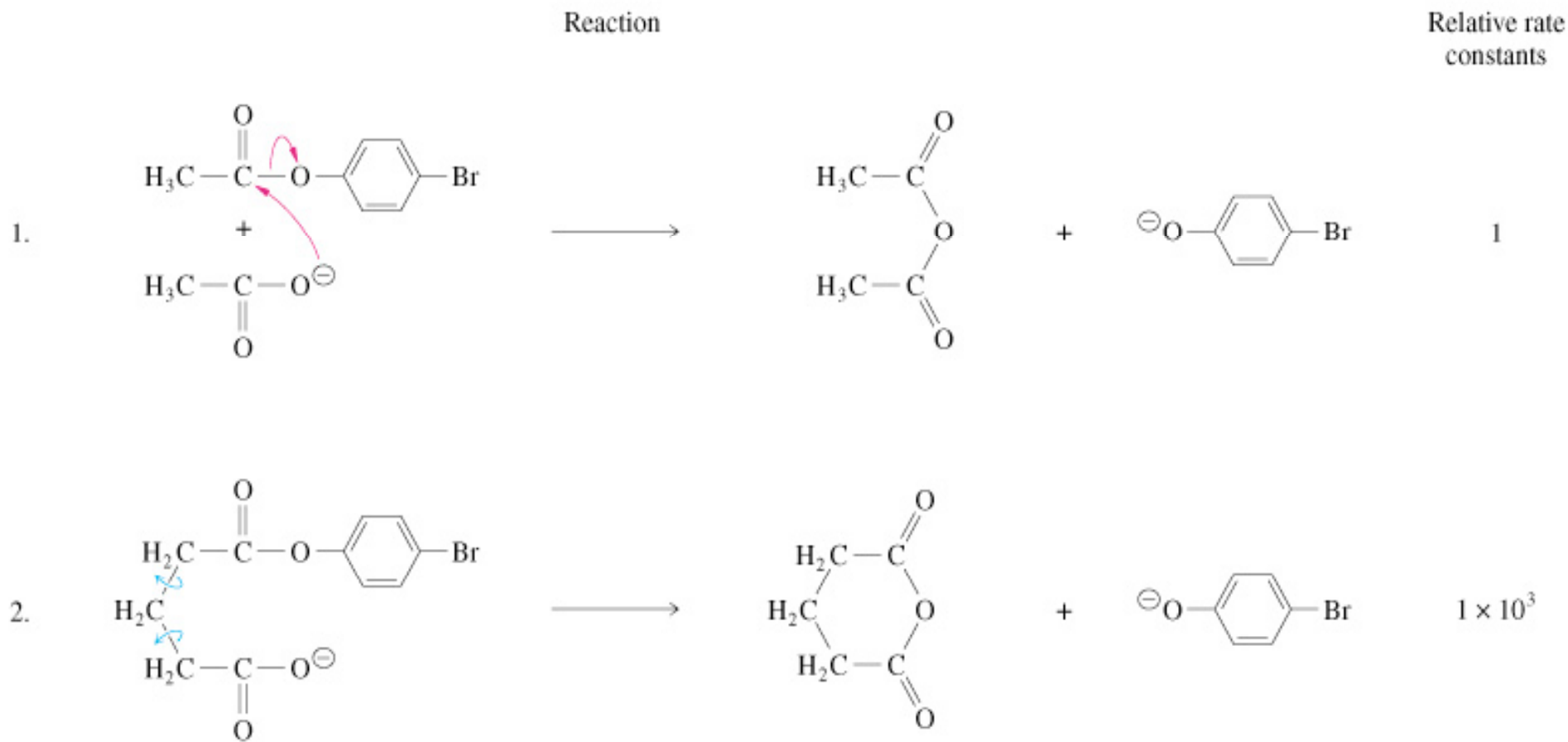
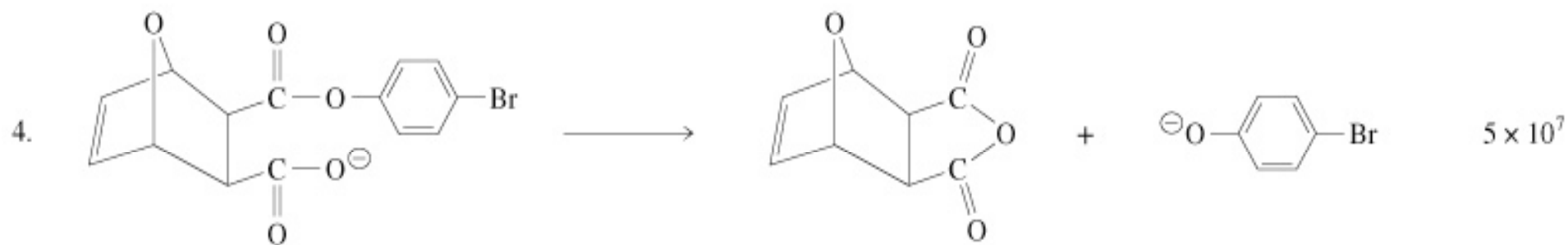
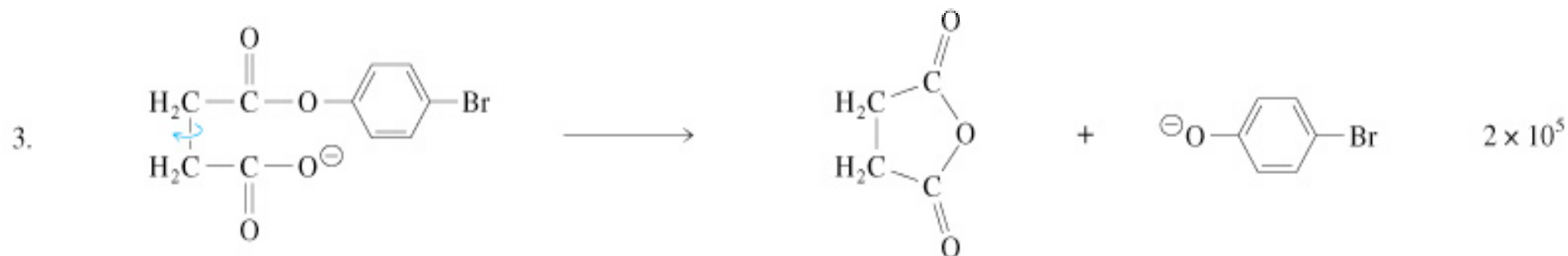


Fig. 6.11 (continued)



B. 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

Catalytic triad of serine proteases = Asp, His, Ser

- Active-site Asp-102, His-57, Ser-195 are arrayed in a hydrogen-bonded network (O red, N blue)

Fig 6.23
Stereo
view of
the
catalytic
site of
chymo-
trypsin

