

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

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Energy diagrams show the progress of a reaction

_____ : 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

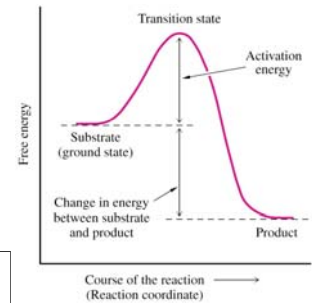
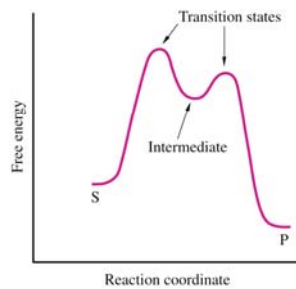


Fig 6.1 Energy diagram for a single-step reaction

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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

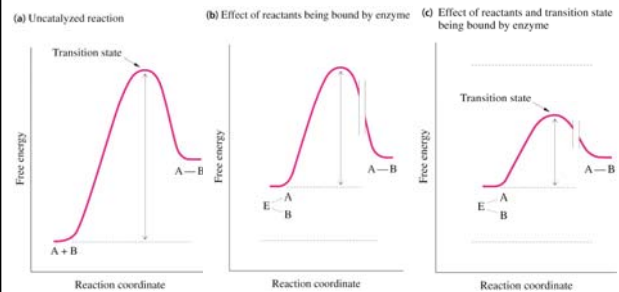


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Fig 6.3 Enzymatic catalysis of the reaction $A+B \rightarrow A-B$

Proximity effect: "proper" positioning of substrates

Transition state stabilization: lowers activation energy



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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. _____

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

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Table 6.2 pK_a 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

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Chemical modes of enzyme catalysis

- Acid-Base catalysis
- Covalent catalysis

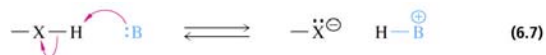
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_____ : acceleration of a reaction by transfer of a proton

B: = base (proton acceptor)

BH⁺ = conjugate acid (proton donor)

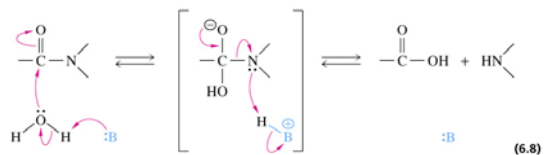
- A **general base (B:)** can act as a proton acceptor to remove protons from OH, NH, CH or other XH
- This produces a stronger nucleophilic reactant (X⁻)



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General base catalysis reactions (continued)

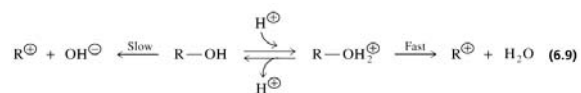
- A _____ (**B:**) can remove a proton from water and thereby generate the equivalent of OH⁻ in neutral solution



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Proton donors can also catalyze reactions

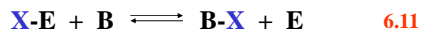
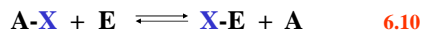
- A _____ (**BH⁺**) can donate protons
- A covalent bond may break more easily if one of its atoms is protonated (below)



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_____ : part or entirety of S forms covalent bond with E and then with second S

- All or part of a substrate is bound _____ to the enzyme to form a _____
- Group X can be transferred from A-X to B in two steps via the covalent ES complex X-E



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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)

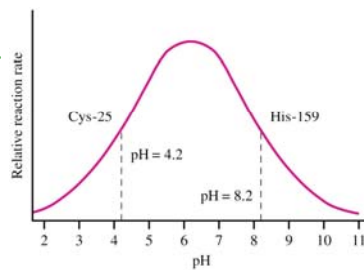
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Fig 6.4 pH-rate profile for papain

_____ 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



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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)

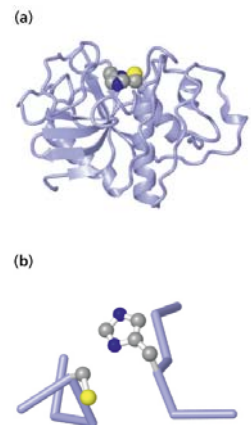
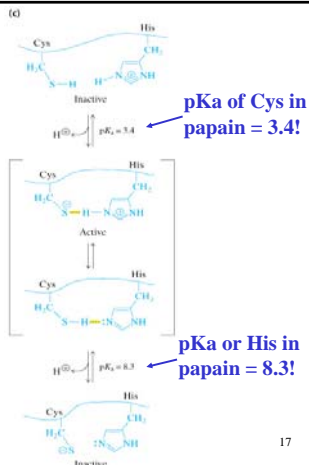


Fig. 6.5 continued

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



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Fastest Reactions are Diffusion-Controlled Reactions: rates approach rate of diffusion: 10^8 to 10^9 $M^{-1}s^{-1}$; speed of binding of substrates to the enzyme

Table 6.4

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

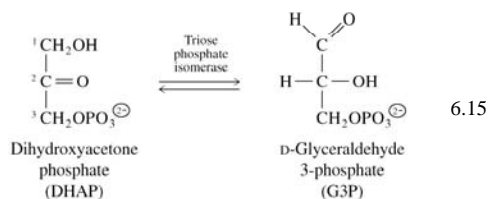
Enzyme	Substrate	k_{cat}/K_m ($M^{-1}s^{-1}$) ^a
Catalase	H_2O_2	4×10^7
Carbonic anhydrase	CO_2	1.2×10^8
Acetylcholinesterase	Acetylcholine	1.6×10^8
Fumarase	Fumarate	1.6×10^8
Triose phosphate isomerase	D-Glyceraldehyde 3-phosphate	4×10^8
Superoxide dismutase	$\cdot O_2^-$	2×10^9

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

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A. Triose Phosphate Isomerase (TPI)

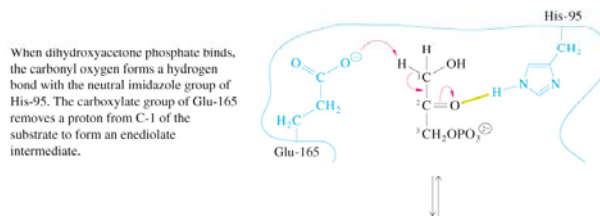
- TPI catalyzes a rapid aldehyde-ketone interconversion



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Fig 6.7 Proposed mechanism for TPI

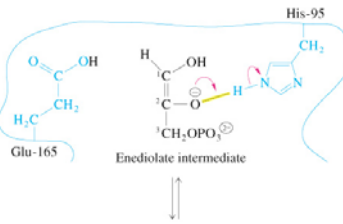
- General acid-base catalysis mechanism (4 slides)



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Fig 6.7 TPI mechanism (continued)

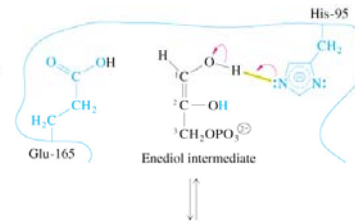
His-95 forms a strong hydrogen bond to the C-2 oxygen atom of the enediolate, and protonates this oxygen atom.



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Fig 6.7 TPI mechanism (continued)

Next, the imidazole 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.



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Fig 6.7 TPI mechanism (continued)

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

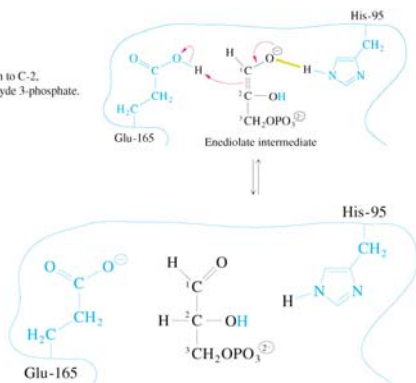
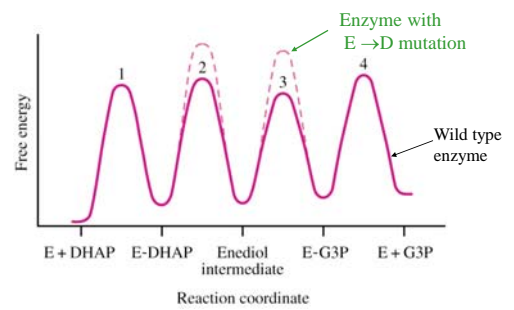


Fig 6.9 Energy diagram for the TPI reaction



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Proximity effect:

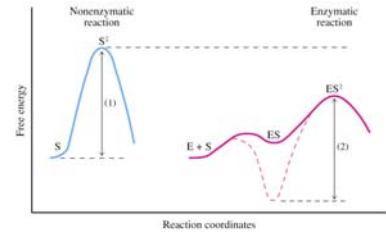
_____ in rate of a reaction due to binding of substrates at binding sites in the enzyme,
results in an _____ effective concentration of reactants,
results in more frequent formation of the _____

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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

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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

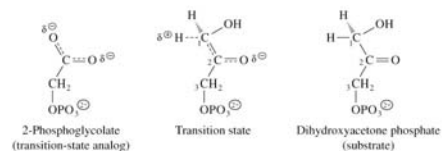
In Emil Fisher's lock-and-key model for SE binding, the _____. Binding of S to E distorts S to → transition state. The transition state must be stabilized for catalysis to occur.

Transition state analogs can → catalytic antibodies

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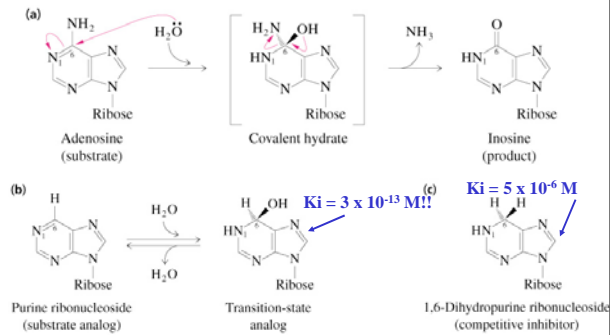
Transition-state (TS) analogs

- **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



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Fig 6.15 Inhibition of adenosine deaminase by a TS analog



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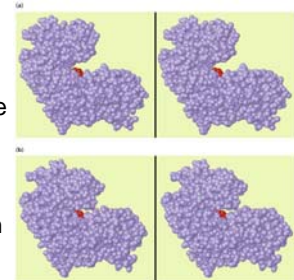
Induced Fit: substrate induced cleft closing (Daniel Koshland, 1950s)

- _____ 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

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~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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Chapter 6 Mechanisms of Enzymes

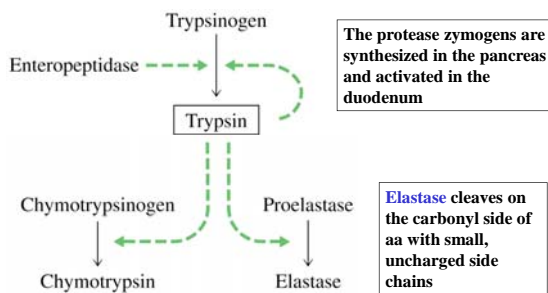
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Properties of Serine Proteases

- 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, $K_d = 10^{-13} \text{ M} !!$) ³⁸

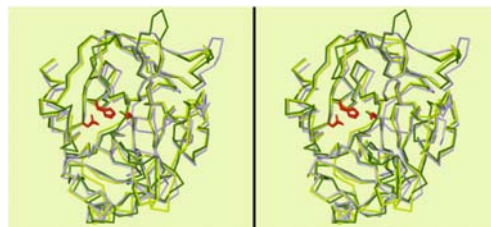
Fig 6.21 Activation of some pancreatic zymogens
Enzyme cascades → rapid signal amplification



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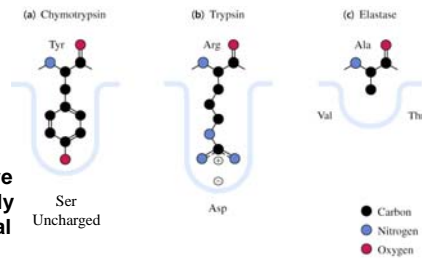
Fig 6.22 The backbones of chymotrypsin (blue), trypsin (yellow), and elastase (green)

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



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Fig 6.24 Binding sites of chymotrypsin, trypsin, and elastase

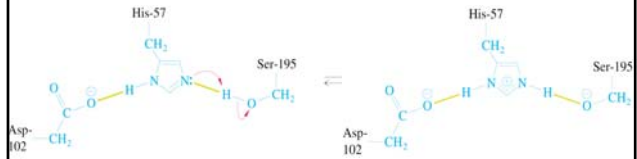


- Substrate specificities are due to relatively small structural differences in active-site binding cavities

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Fig 6.26 _____ of chymotrypsin

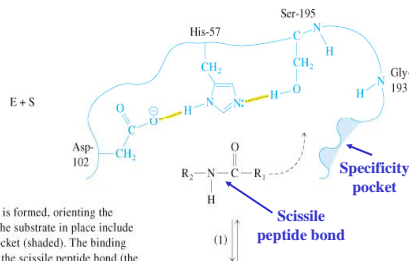
- Imidazole ring (His-57) removes H from Ser-195 hydroxyl to make it a strong nucleophile ($-\text{CH}_2\text{O}^-$)
- Buried carboxylate (Asp-102) stabilizes the positively charged His-57 to facilitate serine ionization



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

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

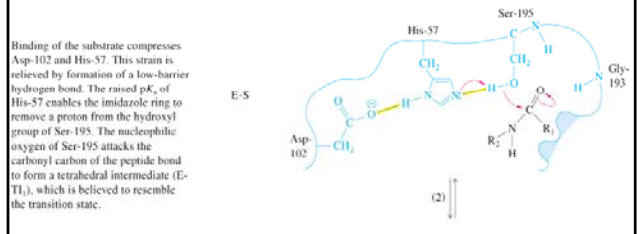
Step (1): E + S



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

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Fig 6.27 (E-S) (2)



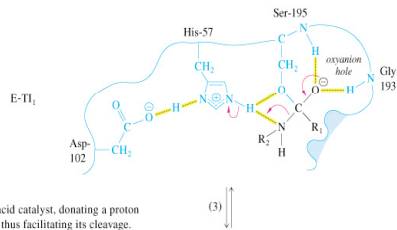
Binding of the substrate compresses Asp-102 and His-57. This strain is relieved by formation of a low-barrier hydrogen bond. The raised pK_a of His-57 enables the imidazole ring to remove a proton from the hydroxyl group of Ser-195. The nucleophilic oxygen of Ser-195 attacks the carbonyl carbon of the peptide bond to form a tetrahedral intermediate (E-TI₁), which is believed to resemble the transition state.

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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.



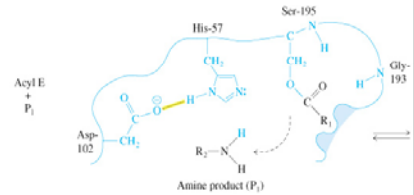
The imidazolium ring of His-57 acts as an acid catalyst, donating a proton to the nitrogen of the scissile peptide bond, thus facilitating its cleavage.

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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.

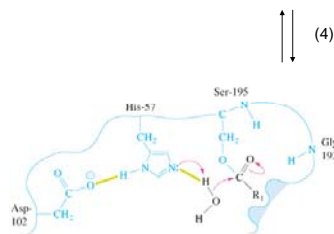


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Fig 6.27 (Acyl E + H₂O) (5)

Hydrolysis

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.

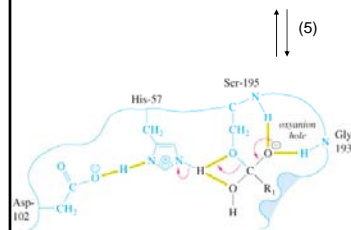


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Fig 6.27 (E-TI₂) (6)

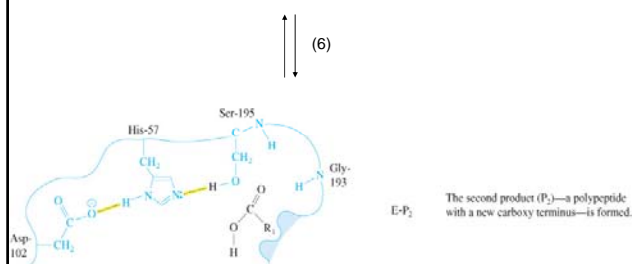
Tetrahedral intermediate

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



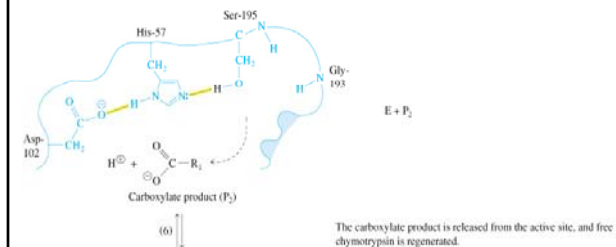
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Fig 6.27 (E-P₂) (7)



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Fig 6.27 (E + P₂) (8)



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

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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**

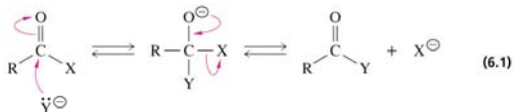
Formation of tetrahedral intermediate

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

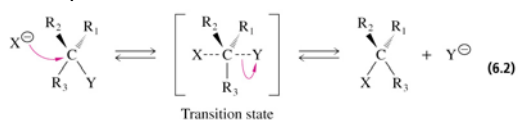
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Two types of nucleophilic substitution reactions

- Formation of a tetrahedral intermediate



- Direct displacement

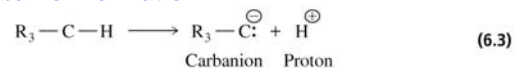


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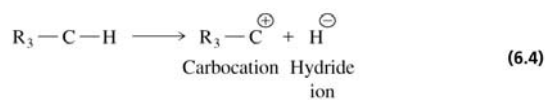
2. Cleavage reactions: most common when both electrons stay with one atom

- * 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



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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)

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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)

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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

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Binding forces utilized for catalysis

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

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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

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Fig 6.11 Reactions of carboxylates with phenyl esters

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

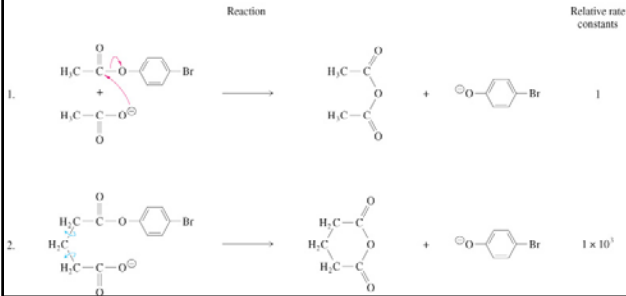
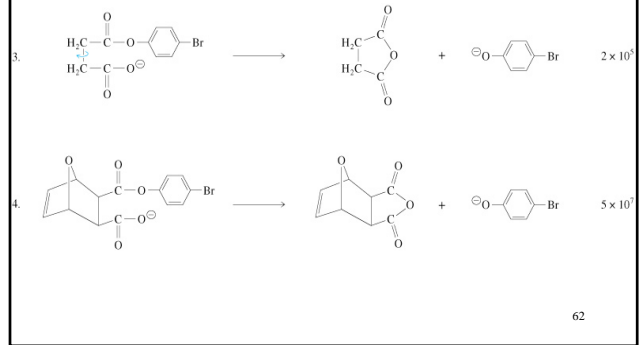


Fig. 6.11 (continued)



B. Weak Binding of Substrates to Enzymes

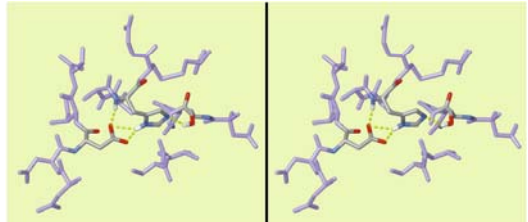
- Energy is required to reach the transition state from the ES complex
- Excessive ES stabilization would create a "thermodynamic pit" and mean little or no catalysis
- Most K_m values (substrate dissociation constants) indicate weak binding to enzymes

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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 chymotrypsin



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