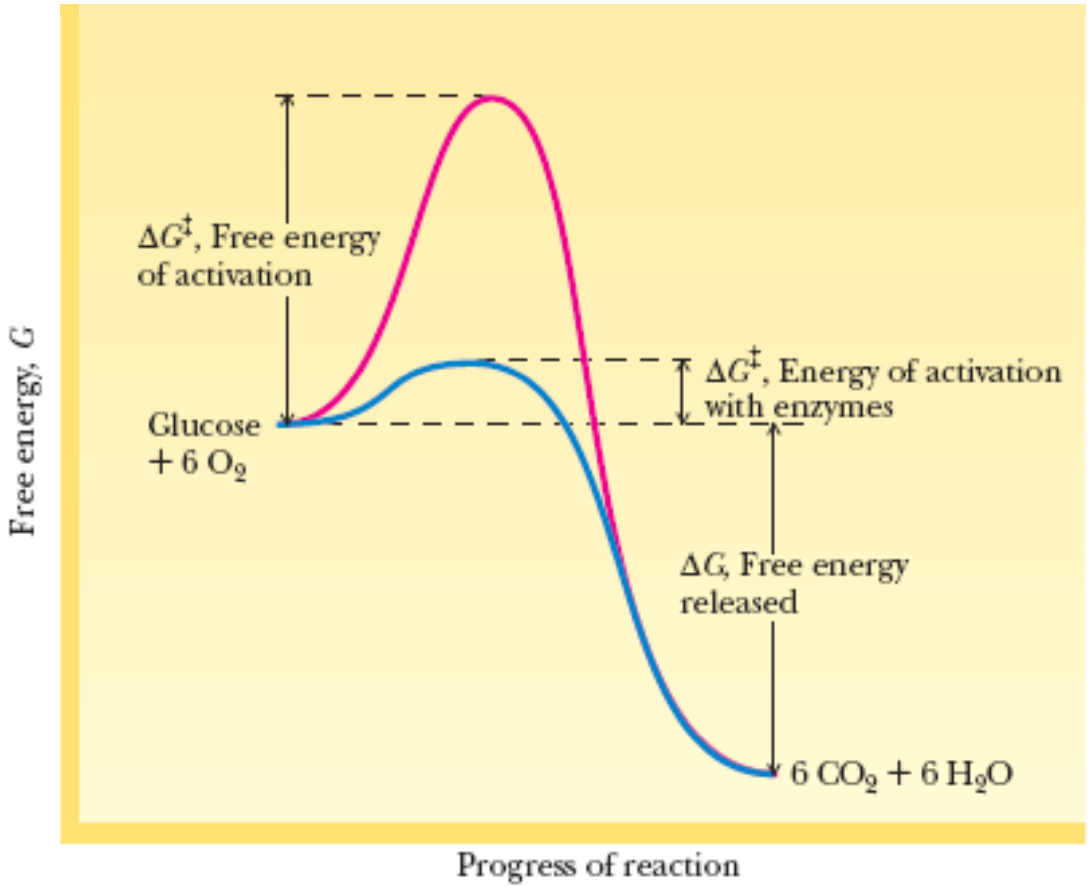
Enzymes

$$C_6H_{12}O_6 + 6\ O_2 \longrightarrow 6\ CO_2 + 6\ H_2O + 2870\ kJ\ of\ energy$$

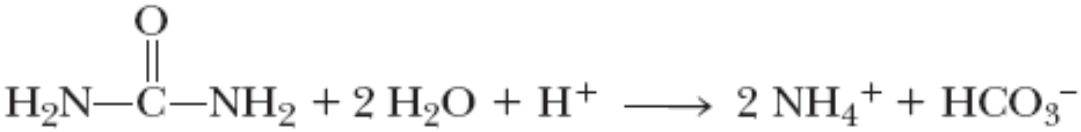


Characteristic Features of Enzymes:

- Catalytic power
- Specificity
- Regulation

Catalytic Power Is Defined as the Ratio of the Enzyme-Catalyzed Rate of a Reaction to the Uncatalyzed Rate

Enzymes display enormous catalytic power, accelerating reaction rates as much as 10^{21} over uncatalyzed levels, which is far greater than any synthetic catalysts can achieve, and enzymes accomplish these astounding feats in dilute aqueous solutions under mild conditions of temperature and pH. For example, the enzyme jack bean *urease* catalyzes the hydrolysis of urea:



$$H_2N{-}\overset{\overset{O}{\|}}{C}{-}NH_2 + 2\,H_2O + H^+ \longrightarrow 2\,NH_4^+ + HCO_3^-$$

At 20°C, the rate constant for the enzyme-catalyzed reaction is 3×10^4/sec; the rate constant for the uncatalyzed hydrolysis of urea is 3×10^{-10}/sec. Thus, 10^{14} is the ratio of the catalyzed rate to the uncatalyzed rate of reaction. Such a ratio is defined as the relative **catalytic power** of an enzyme, so the catalytic power of urease is 10^{14}.

Specificity Is the Term Used to Define the Selectivity of Enzymes for Their Substrates

A given enzyme is very selective, both in the substances with which it interacts and in the reaction that it catalyzes. The substances upon which an enzyme acts are traditionally called **substrates.** In an enzyme-catalyzed reaction, none of the substrate is diverted into nonproductive side reactions, so no wasteful by-products are produced. It follows then that the products formed by a given enzyme are also very specific. This situation can be contrasted with your own experiences in the organic chemistry laboratory, where yields of 50% or even 30% are viewed as substantial accomplishments (Figure 13.3). The selective qualities of an enzyme are collectively recognized as its **specificity.** Intimate interaction between an enzyme and its substrates occurs through molecular recognition based on structural complementarity; such mutual recognition is the basis of specificity. The specific site on the enzyme where substrate binds and catalysis occurs is called the **active site.**

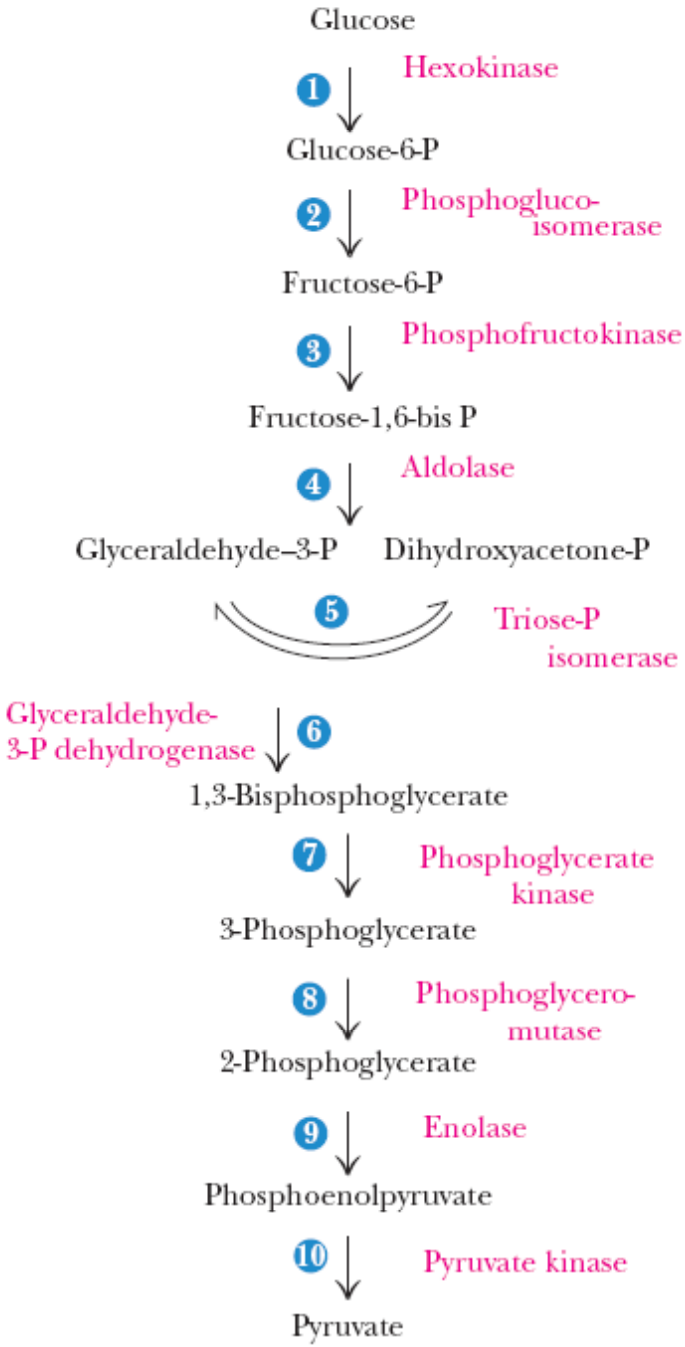


FIGURE 13.2 The breakdown of glucose by *glycolysis* provides a prime example of a metabolic pathway.

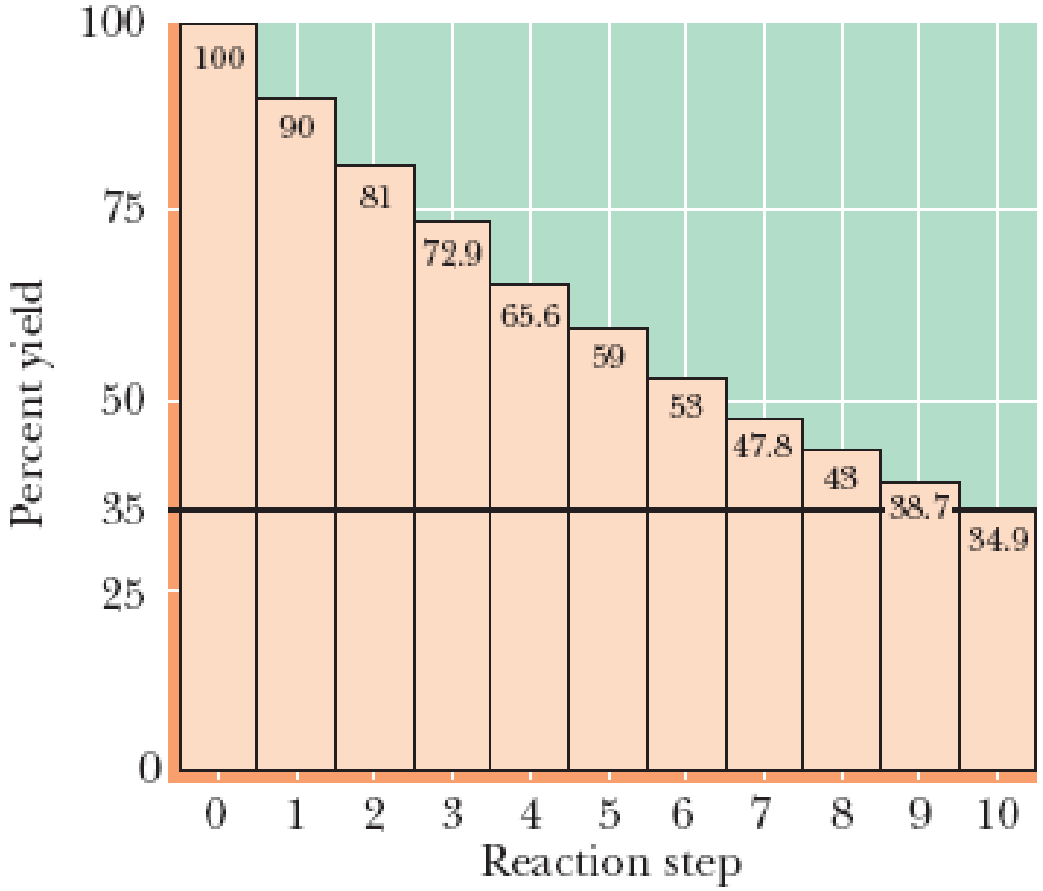


FIGURE 13.3 A 90% yield over 10 steps, for example, in a metabolic pathway, gives an overall yield of 35%. Therefore, yields in biological reactions *must be substantially greater;* otherwise, unwanted by-products would accumulate to unacceptable levels.

Regulation of Enzyme Activity Ensures That the Rate of Metabolic Reactions Is Appropriate to Cellular Requirements

Regulation of enzyme activity is essential to the integration and regulation of metabolism. Enzyme regulation is achieved in a variety of ways, ranging from controls over the amount of enzyme protein produced by the cell to more rapid, reversible interactions of the enzyme with metabolic inhibitors and activators.

TABLE 13.2 Enzyme Cofactors: Some Metal Ions and Coenzymes and the Enzymes with Which They Are Associated

Metal Ions and Some Enzymes That Require Them		Coenzymes Serving as Transient Carriers of Specific Atoms or Functional Groups		
Metal Ion	Enzyme	Coenzyme	Entity Transferred	Representative Enzymes Using Coenzymes
Fe^{2+} or Fe^{3+}	Cytochrome oxidase	Thiamine pyrophosphate (TPP)	Aldehydes	Pyruvate dehydrogenase
	Catalase	Flavin adenine dinucleotide (FAD)	Hydrogen atoms	Succinate dehydrogenase
	Peroxidase	Nicotinamide adenine dinucleotide (NAD)	Hydride ion ($:H^-$)	Alcohol dehydrogenase
Cu^{2+}	Cytochrome oxidase			
Zn^{2+}	DNA polymerase	Coenzyme A (CoA)	Acyl groups	Acetyl-CoA carboxylase
	Carbonic anhydrase	Pyridoxal phosphate (PLP)	Amino groups	Aspartate aminotransferase
	Alcohol dehydrogenase			
Mg^{2+}	Hexokinase	5′-Deoxyadenosylcobalamin (vitamin B_{12})	H atoms and alkyl groups	Methylmalonyl-CoA mutase
	Glucose-6-phosphatase			
Mn^{2+}	Arginase	Biotin (biocytin)	CO_2	Propionyl-CoA carboxylase
K^+	Pyruvate kinase (also requires Mg^{2+})	Tetrahydrofolate (THF)	Other one-carbon groups, such as formyl and methyl groups	Thymidylate synthase
Ni^{2+}	Urease			
Mo	Nitrate reductase			
Se	Glutathione peroxidase			

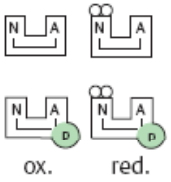
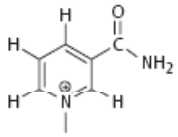
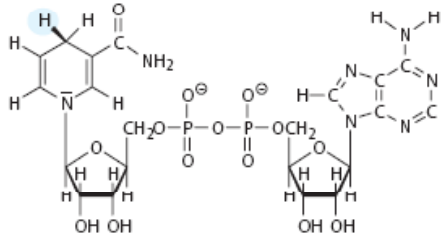
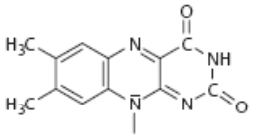
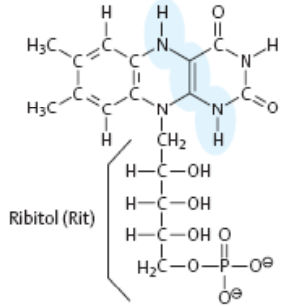
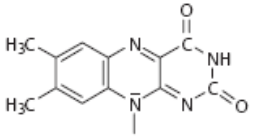
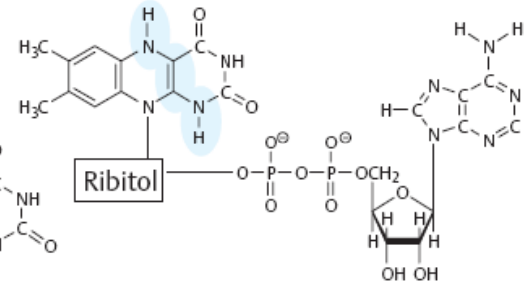
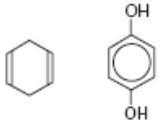
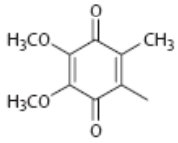
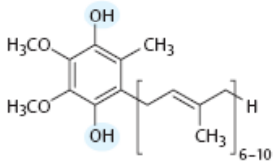
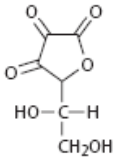
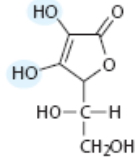
TABLE 6–1 Some Inorganic Elements That Serve as Cofactors for Enzymes

Cu^{2+}	Cytochrome oxidase
Fe^{2+} or Fe^{3+}	Cytochrome oxidase, catalase, peroxidase
K^{+}	Pyruvate kinase
Mg^{2+}	Hexokinase, glucose 6-phosphatase, pyruvate kinase
Mn^{2+}	Arginase, ribonucleotide reductase
Mo	Dinitrogenase
Ni^{2+}	Urease
Se	Glutathione peroxidase
Zn^{2+}	Carbonic anhydrase, alcohol dehydrogenase, carboxypeptidases A and B

TABLE 6–2 Some Coenzymes That Serve as Transient Carriers of Specific Atoms or Functional Groups

Coenzyme	*Examples of chemical groups transferred*	*Dietary precursor in mammals*
Biocytin	CO_2	Biotin
Coenzyme A	Acyl groups	Pantothenic acid and other compounds
5′-Deoxyadenosylcobalamin (coenzyme B_{12})	H atoms and alkyl groups	Vitamin B_{12}
Flavin adenine dinucleotide	Electrons	Riboflavin (vitamin B_2)
Lipoate	Electrons and acyl groups	Not required in diet
Nicotinamide adenine dinucleotide	Hydride ion ($:H^-$)	Nicotinic acid (niacin)
Pyridoxal phosphate	Amino groups	Pyridoxine (vitamin B_6)
Tetrahydrofolate	One-carbon groups	Folate
Thiamine pyrophosphate	Aldehydes	Thiamine (vitamin B_1)

B. Redox coenzymes

Coenzyme	Oxidized form	Reduced form	Type	Trans-ferred
1. NAD(P)$^{\oplus}$ 			L	$H^{\ominus}$
2. Flavin mononucleotide (FMN) 			P	2[H]
3. Flavin adenine dinucleotide (FAD) 			P	2[H]
4. Ubiquinone (coenzym Q) 			L	2[H]
5. Ascorbic acid			L	2[H]

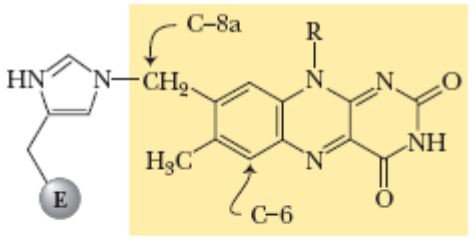
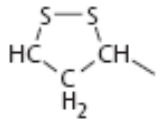
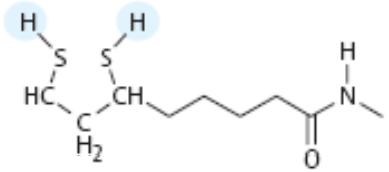
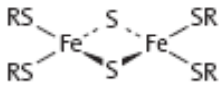
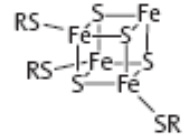
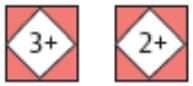
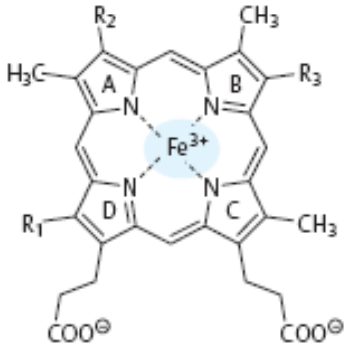
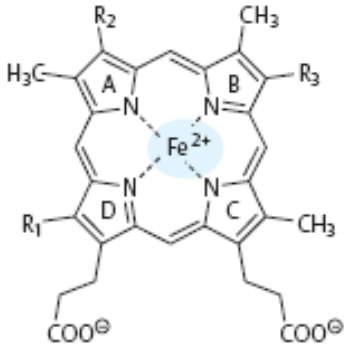
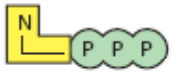
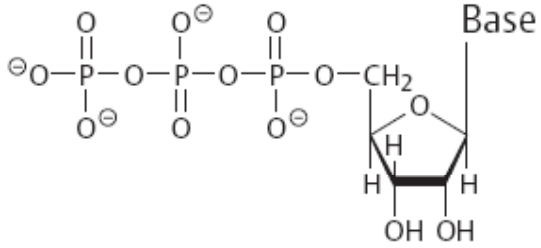
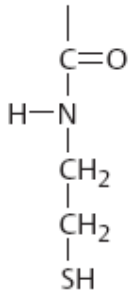
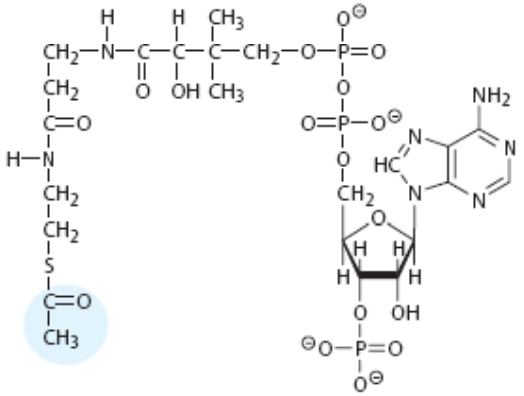
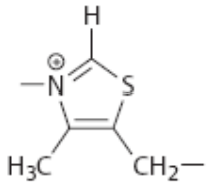
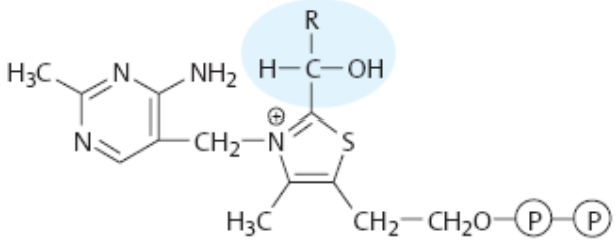


FIGURE 19.12 The covalent bond between FAD and succinate dehydrogenase involves the C-8a methylene group of FAD and the N-3 of a histidine residue on the enzyme.

A. Redox coenzymes 2

Coenzyme	Oxidized form	Reduced form	Type	Transferred
6. Lipoamide 			P	2[H]
7. Iron–sulfur cluster	$[Fe_2S_2]^{n+}$ 	$[Fe_4S_4]^{m+}$ 	P	$1e^{\ominus}$
8. Heme 			P	$1e^{\ominus}$

B. Group-transferring coenzymes 1

Coenzyme (symbol)	Free form	Charged form	Group(s) transferred	Important enzymes
1. Nucleoside phosphates 			Ⓟ B-Rib B-Rib-Ⓟ B-Rib-ⓅⓅ	Phospho-transferases Nucleotidyl-transferases (2.7.n.n) Ligases (6.n.n.n)
2. Coenzyme A 			Acyl residues	Acyltrans-ferases (2.3.n.n) CoA trans-ferases (2.8.3.n)
3. Thiamine diphosphate 			Hydroxy-alkyl residues	Decarboxy-lases (4.1.1.n) Oxoacid de-hydrogenases (1.2.4. n) Transketolase (2.2.1.1)

A. Group-transferring coenzymes 2

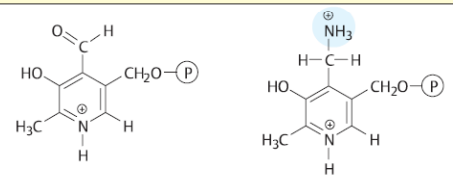
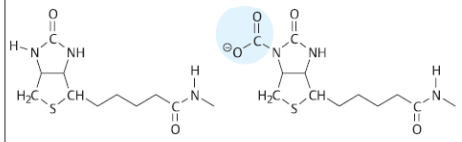
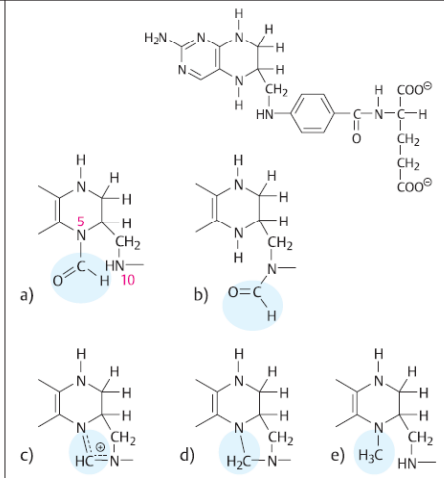
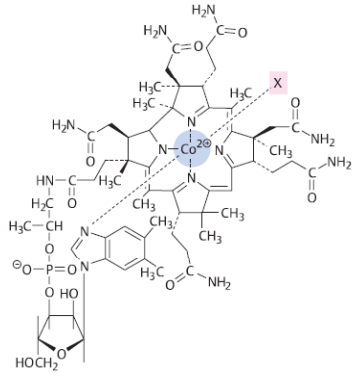
Coenzyme	Free form / Charged form	Group(s) transferred	Important enzymes
4. Pyridoxal phosphate PLP		Amino group Amino acid residues	Transaminases (2.6.1.n) Many lyases (4.n.n.n)
5. Biotin B		$[CO_2]$	Carboxylases (6.4.1.n)
4. Pyridoxal phosphate THF		C_1 groups a) N^5-Formyl b) N^{10}-Formyl c) N^5N^{10}-Methenyl d) N^5N^{10}-Methylene e) N^5N^{10}-Methyl	C_1 transferases (2.1.n.n)
7. Cobalamin coenzymes		X = Adenosyl- X = Methyl-	Mutases *(5.4.n.n)* Methyltransferases *(2.1.1.n.)*

TABLE 6–3 International Classification of Enzymes

No.	*Class*	*Type of reaction catalyzed*
1	Oxidoreductases	Transfer of electrons (hydride ions or H atoms)
2	Transferases	Group transfer reactions
3	Hydrolases	Hydrolysis reactions (transfer of functional groups to water)
4	Lyases	Addition of groups to double bonds, or formation of double bonds by removal of groups
5	Isomerases	Transfer of groups within molecules to yield isomeric forms
6	Ligases	Formation of C—C, C—S, C—O, and C—N bonds by condensation reactions coupled to ATP cleavage

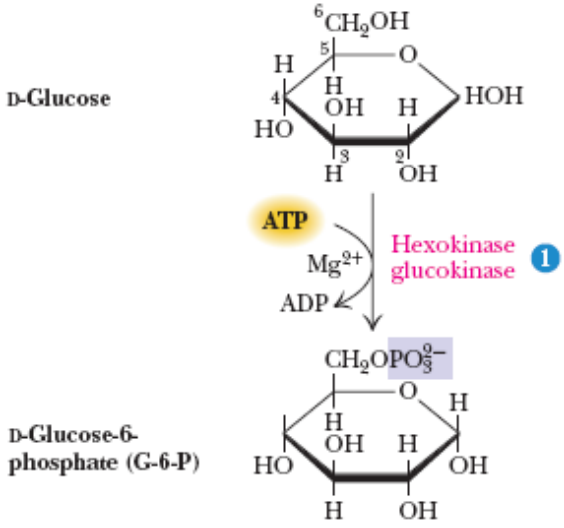
TABLE 13.1 Systematic Classification of Enzymes According to the Enzyme Commission

E.C. Number	Systematic Name and Subclasses
1	*Oxidoreductases* (oxidation–reduction reactions)
1.1	Acting on CH—OH group of donors
1.1.1	With NAD or NADP as acceptor
1.1.3	With O_2 as acceptor
1.2	Acting on the $\rangle$C=O group of donors
1.2.3	With O_2 as acceptor
1.3	Acting on the CH—CH group of donors
1.3.1	With NAD or NADP as acceptor
2	*Transferases* (transfer of functional groups)
2.1	Transferring C-1 groups
2.1.1	Methyltransferases
2.1.2	Hydroxymethyltransferases and formyltransferases
2.1.3	Carboxyltransferases and carbamoyltransferases
2.2	Transferring aldehydic or ketonic residues
2.3	Acyltransferases
2.4	Glycosyltransferases
2.6	Transferring N-containing groups
2.6.1	Aminotransferases
2.7	Transferring P-containing groups
2.7.1	With an alcohol group as acceptor
3	*Hydrolases* (hydrolysis reactions)
3.1	Cleaving ester linkage
3.1.1	Carboxylic ester hydrolases
3.1.3	Phosphoric monoester hydrolases
3.1.4	Phosphoric diester hydrolases
4	*Lyases* (bond cleavage by means other than hydrolysis or oxidation)
4.1	C—C lyases
4.1.1	Carboxy lyases
4.1.2	Aldehyde lyases
4.2	C—O lyases
4.2.1	Hydrolases
4.3	C—N lyases
4.3.1	Ammonia lyases
5	*Isomerases* (isomerization reactions)
5.1	Racemases and epimerases
5.1.3	Acting on carbohydrates
5.2	*Cis-trans* isomerases
6	*Ligases* (formation of bonds with ATP cleavage)
6.1	Forming C—O bonds
6.1.1	Amino acid–RNA ligases
6.2	Forming C—S bonds
6.3	Forming C—N bonds
6.4	Forming C—C bonds
6.4.1	Carboxylases

ATP + D-glucose ⟶ ADP + D-glucose-6-phosphate

A phosphate group is transferred from ATP to the C-6-OH group of glucose, so the enzyme is a *transferase* (class 2, Table 13.1). Subclass 7 of transferases is *enzymes transferring phosphorus-containing groups,* and sub-subclass 1 covers those *phosphotransferases with an alcohol group as an acceptor.* Entry 2 in this sub-subclass is **ATP:D-glucose-6-phosphotransferase,** and its classification number is **2.7.1.2.** In use, this number is written preceded by the letters **E.C.,** denoting the Enzyme Commission. For example, entry 1 in the same sub-subclass is E.C.2.7.1.1, ATP:D-hexose-6-phosphotransferase, an ATP-dependent enzyme that transfers a phosphate to the 6-OH of hexoses (that is, it is nonspecific regarding its hexose acceptor). These designations can be cumbersome, so in everyday usage, trivial names are commonly used. The glucose-specific enzyme E.C.2.7.1.2 is called *glucokinase,* and the nonspecific E.C.2.7.1.1 is known as *hexokinase. Kinase* is a trivial term for enzymes that are ATP-dependent phosphotransferases.

E.C.2.7.1.1 (Hexokinase)
E.C.2.7.1.2 (Glucokinase)



Oxidoreductases

What are oxidoreductases?

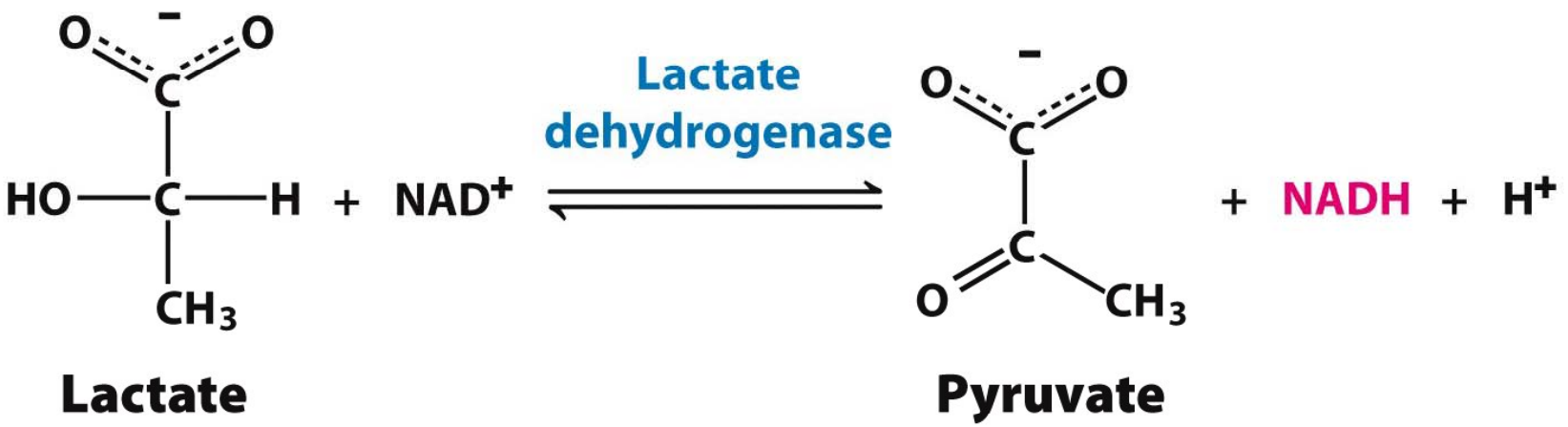
Oxidoreductases catalyse oxidation or reduction reactions, where electrons are transferred from one molecule (the reductant) to another molecule (the oxidant).

This can be shown as:

A- + B --> A + B-

Where A= the reductant and B= the oxidant and an electron has transferred from A to B.

This process often requires co-factors such as NAD(P)H.



TRANSFERASES

What are transferases?

Transferases are enzymes that catalyse the movement of a functional group from one molecule to another. These functional groups are very diverse can include phosphate, methyl and glycosyl groups.

The basic reaction can be shown as:

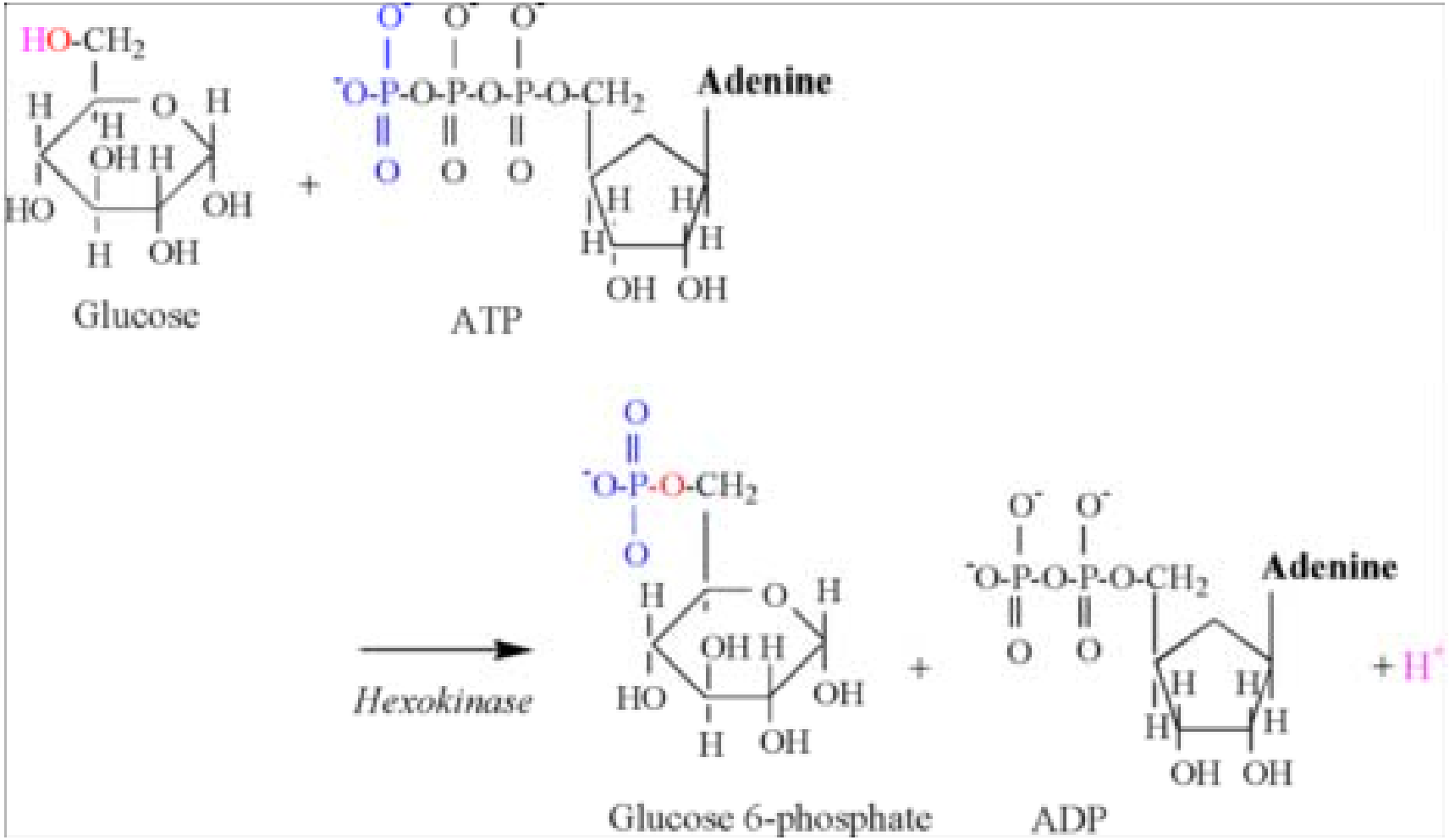
AX + B --> A + BX

Where **A**= the donor, **B**= the acceptor and **X**= the functional group

There are many transferase enzymes, here two sub-groups will be focused on.

Kinases

Kinases enzymes are involved in catalysing the transfer of phosphate groups in a process called phosphorylation. They can act on a range of different molecules, for example lipids, carbohydrates and nucleotides. This is often occurs to prime the molecule ready for different metabolic pathways. Protein kinases are extremely important, as they are used extensively in signal transduction and in controlling complex processes within the cell. They are very diverse, with more than 500 different kinases being identified in the human body alone!


Glucose
ATP
Adenine
Hexokinase
Glucose 6-phosphate
ADP
Adenine
+ H+

HYDROLASES

What are hydrolases?

Hydrolase enzymes simply catalyse hydrolysis; the breaking of single bonds through the addition of water.

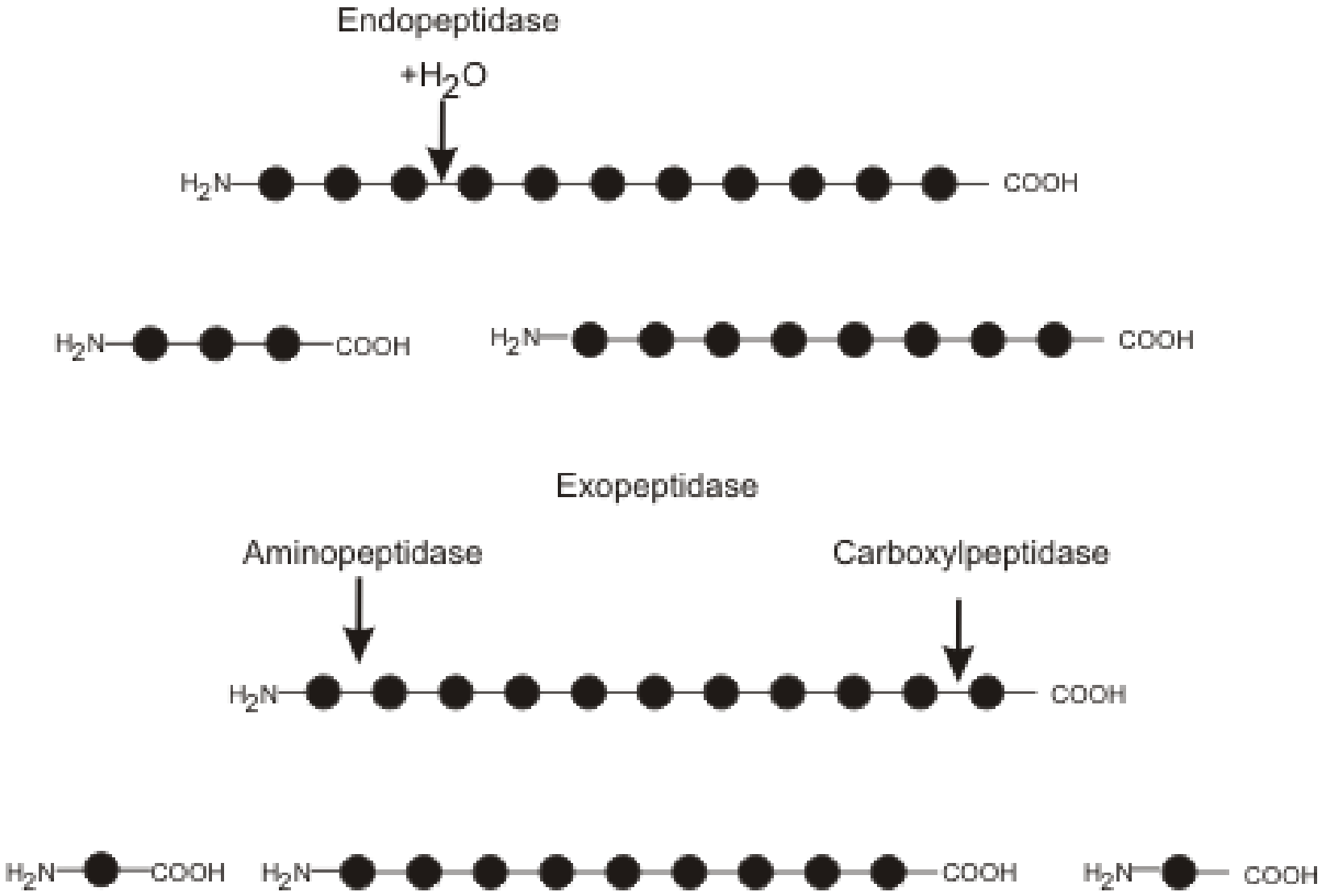
There are a huge variety of hydrolase enzymes. For example, the digestive enzymes that are classified based on their target:

*proteases/ peptidases cleave peptide bonds between amino acids in order to breakdown proteins

*lipases break down lipids into fatty acids and glycerol by cleaving ester bonds

*nucleases cleave phosphodiester bonds between nucleotide subunits in nucleic acids

They are termed exo or endo depending on where they cut. Endo enzymes cut in the middle of the chain, whereas exo enzymes cut at the end of the chain to release an individual monomer.


Endopeptidase
+H2O
H2N
COOH
H2N
COOH
H2N
COOH
Exopeptidase
Aminopeptidase
Carboxylpeptidase
H2N
COOH
H2N
COOH
H2N
COOH
H2N
COOH

LYASES

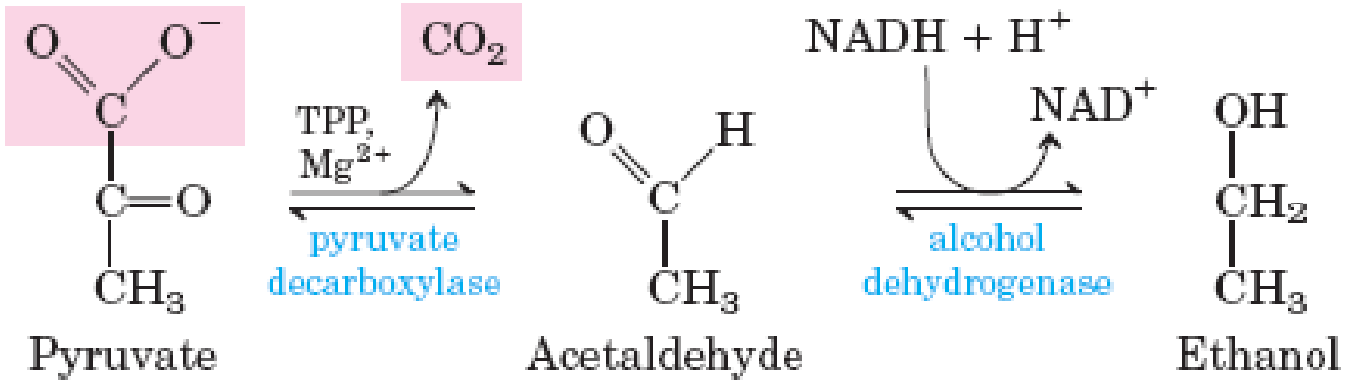
What are lyases?

Lyases catalyse lysis reactions that generate a double bond. These are a type of elimination reaction but are not hydrolytic or oxidative. The reverse reaction catalyses an addition reaction, where a substrate is added to a double bond. These are often referred to as synthase enzymes.

An example lyase reaction would be:

ATP <--> cAMP + PPi

Generally one substrate is required in the forward direction, whereas two are needed for the backward reaction.

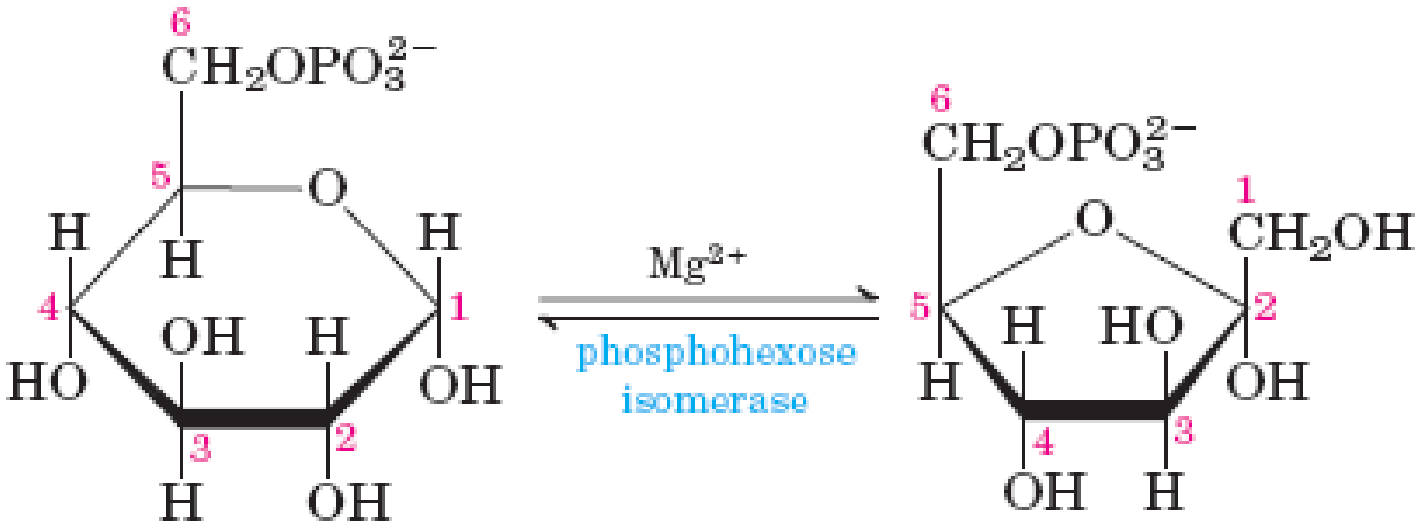


ISOMERASES

What are isomerases?

Isomerases are enzymes that can catalyse structural changes within a molecule. There is only one substrate and one product with nothing gained or lost, so they represent only a change in shape. The diagram shows a simple example of this sort of reaction.

Isomers have the same molecular formula but differ in their structural formula. These differences can change the chemical properties of the molecule. There are multiple classes of isomerases, for example geometric, structural, enantiomers and stereoisomers.



Glucose 6-phosphate

Fructose 6-phosphate

LIGASES

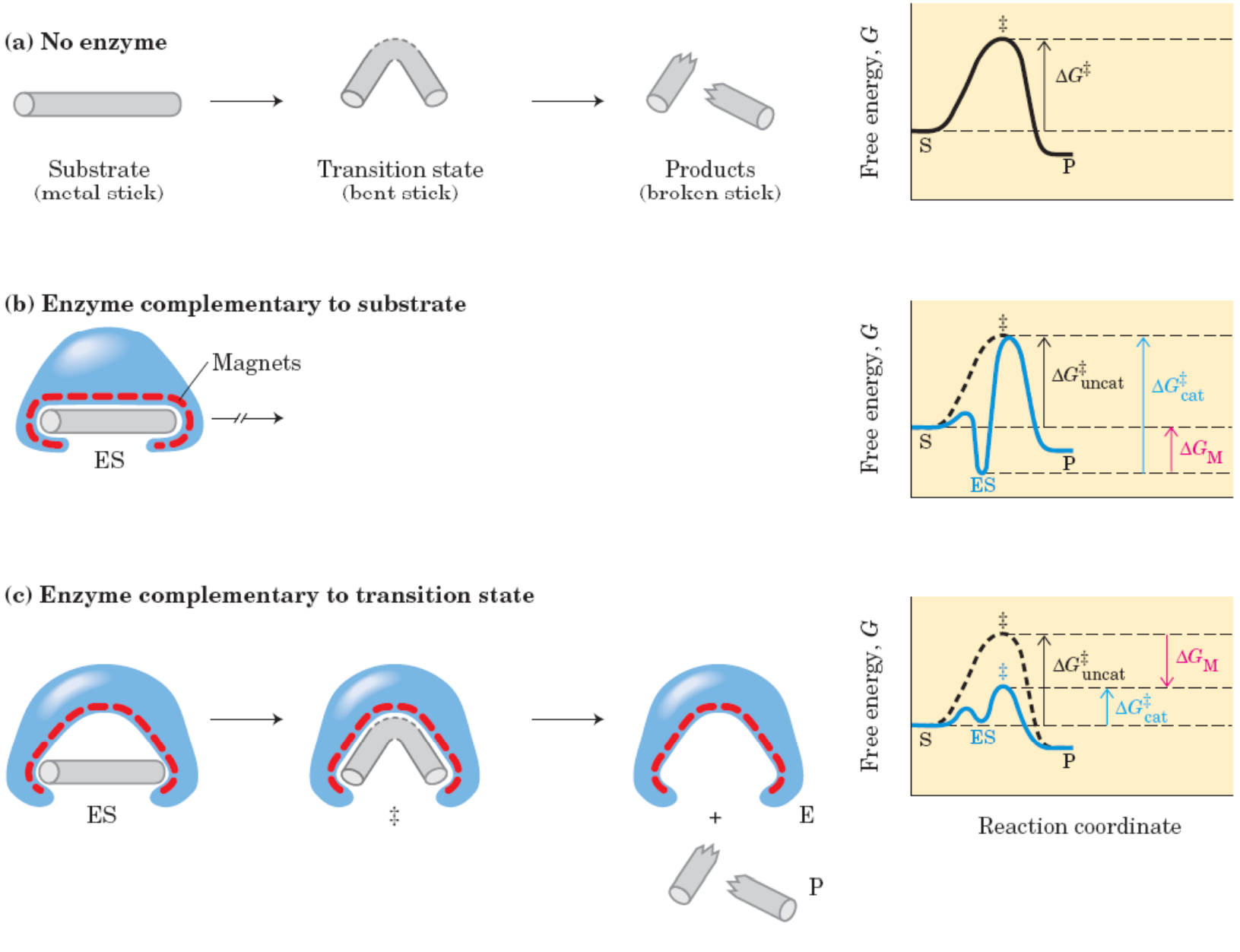
What are ligases?

Ligases are responsible for the catalysis of ligation; the joining of two substrates. Usually chemical potential energy is required, so the reaction is coupled to the hydrolysis of a disphosphate bond in a nucleotide triphosphate such as ATP.

DNA ligase

A very important ligase enzyme which will be focused on here is DNA ligase. It catalyses the ligation between breaks in DNA by forming a phosphodiester bond. There are different forms of the enzyme, and they catalyse different breaks. (In mammals there are 4 different types.) For example, double strand breaks are repaired by DNA ligase IV. Whereas DNA ligase I repairs single stranded breaks using the complementary strand as a template, like in DNA replication of the lagging strand. The reaction requires ATP, yet in some bacterial species the co-factor NAD has been shown to be a requirement.

ATP + Acetate + CoA <=> AMP + Pyrophosphate + Acetyl-CoA


(a) No enzyme
Substrate
(metal stick)
Transition state
(bent stick)
Products
(broken stick)
Free energy, G
‡
ΔG‡
S
P
(b) Enzyme complementary to substrate
Magnets
ES
Free energy, G
ΔG‡uncat
ΔG‡cat
ΔGM
S
P
ES
(c) Enzyme complementary to transition state
ES
‡
+
E
P
Free energy, G
ΔG‡uncat
ΔGM
ΔG‡cat
S
ES
P
Reaction coordinate

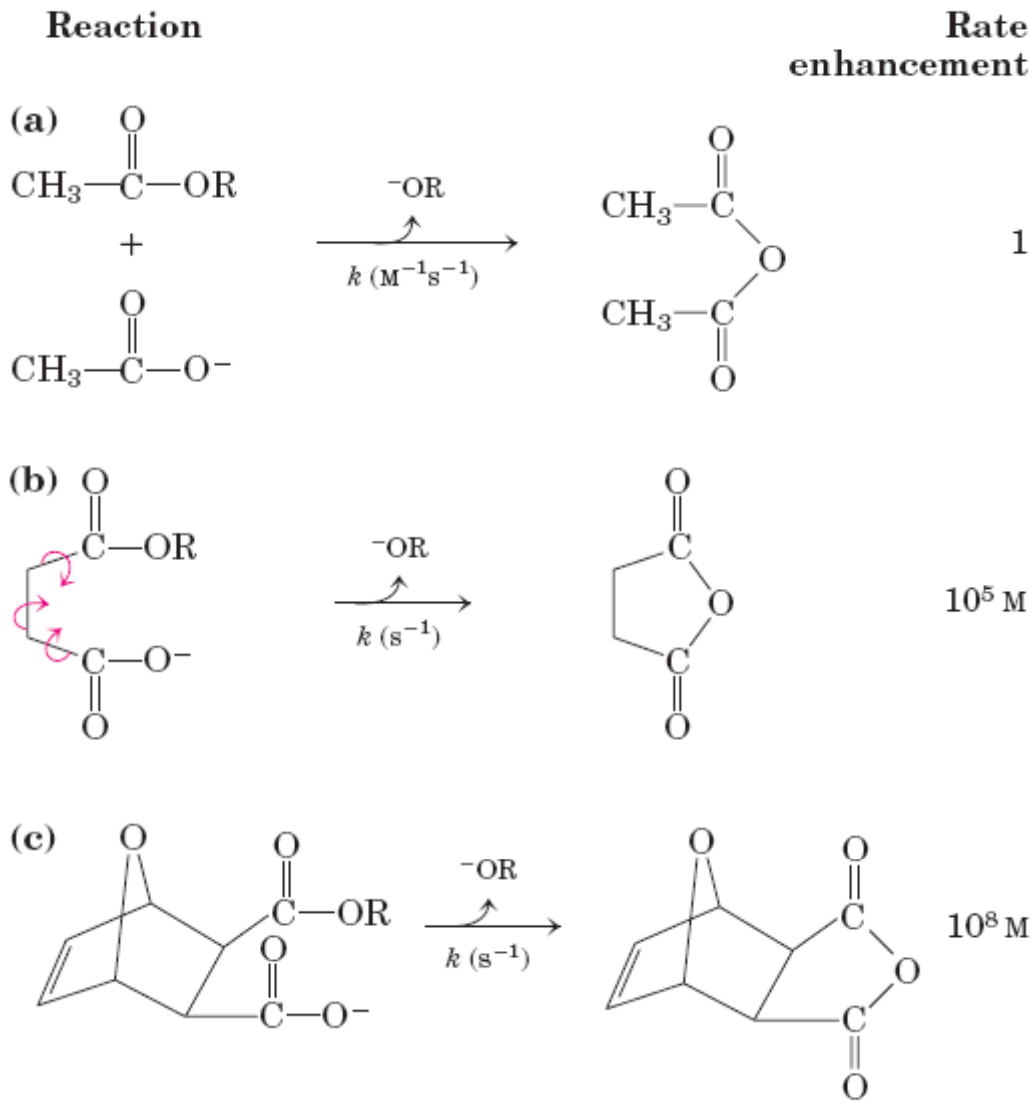


FIGURE 6–7 Rate enhancement by entropy reduction. Shown here are reactions of an ester with a carboxylate group to form an anhydride. The R group is the same in each case. **(a)** For this bimolecular reaction, the rate constant k is second order, with units of $M^{-1}s^{-1}$. **(b)** When the two reacting groups are in a single molecule, the reaction is much faster. For this unimolecular reaction, k has units of s^{-1}. Dividing the rate constant for **(b)** by the rate constant for **(a)** gives a rate enhancement of about 10^5 M. (The enhancement has units of molarity because we are comparing a unimolecular and a bimolecular reaction.) Put another way, if the reactant in **(b)** were present at a concentration of 1 M, the reacting groups would *behave* as though they were present at a concentration of 10^5 M. Note that the reactant in **(b)** has freedom of rotation about three bonds (shown with curved arrows), but this still represents a substantial reduction of entropy over **(a)**. If the bonds that rotate in **(b)** are constrained as in **(c)**, the entropy is reduced further and the reaction exhibits a rate enhancement of 10^8 M relative to **(a)**.

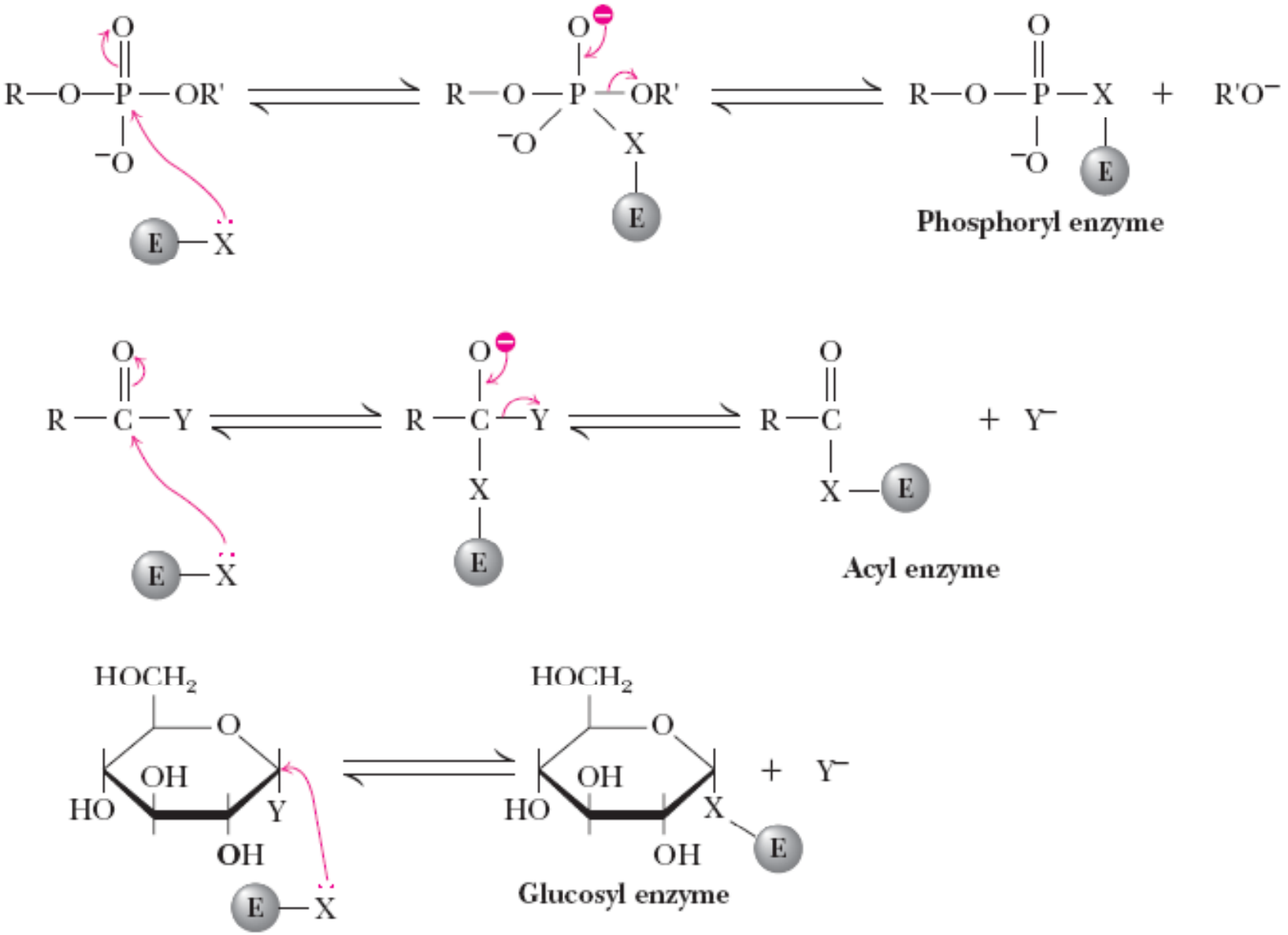


FIGURE 14.11 Examples of covalent bond formation between enzyme and substrate. In each case, a nucleophilic center (X:) on an enzyme attacks an electrophilic center on a substrate.

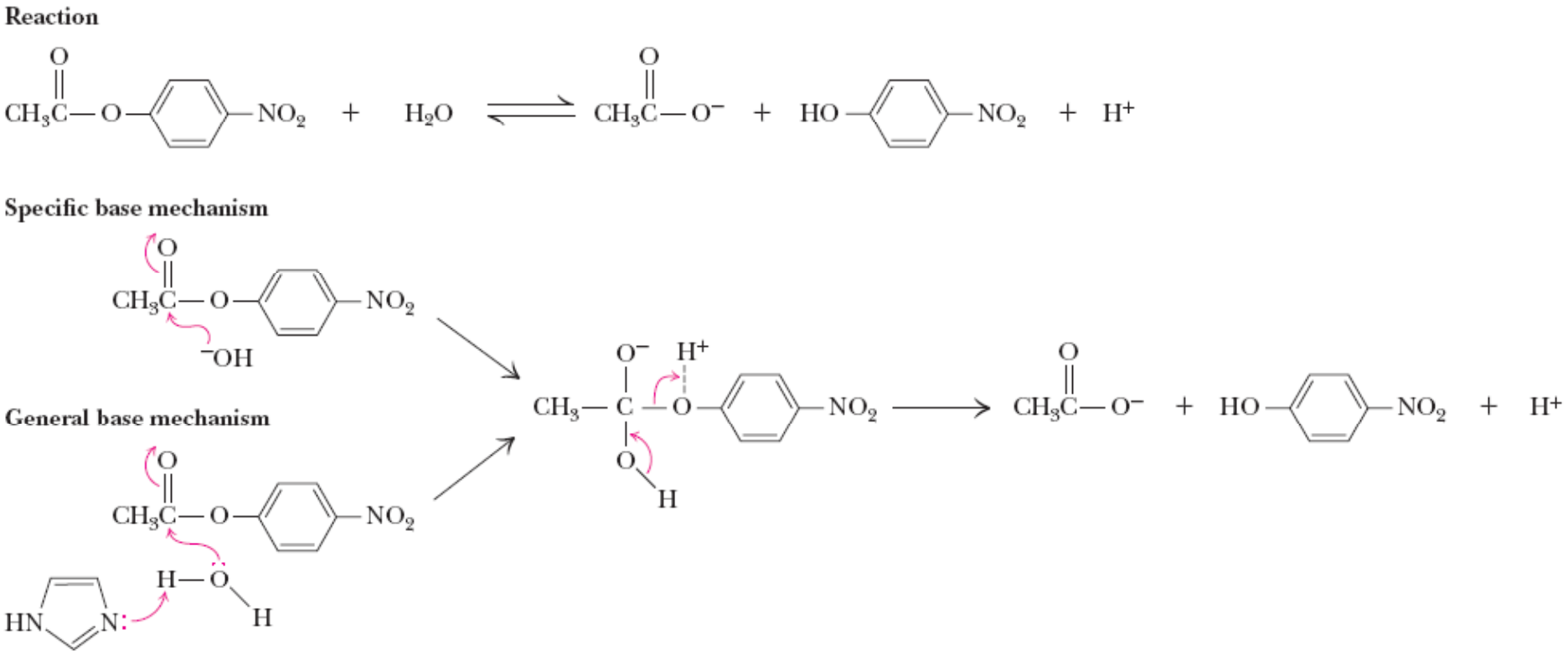
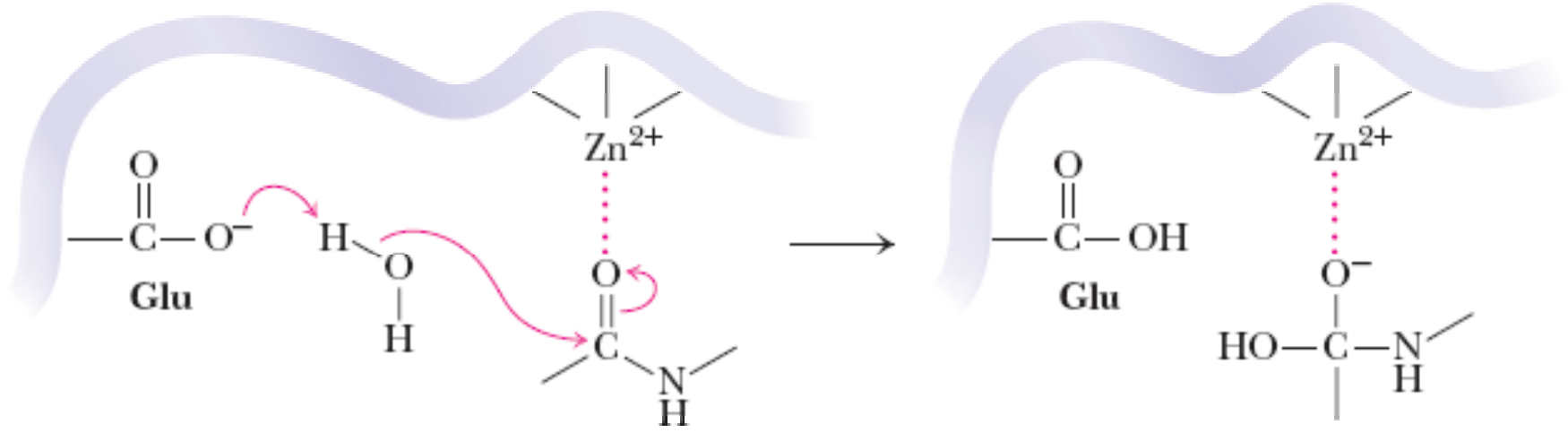


FIGURE 14.12 Catalysis of *p*-nitrophenylacetate hydrolysis can occur either by specific base hydrolysis (where hydroxide from the solution is the attacking nucleophile) or by general base catalysis (in which a base like imidazole can promote hydroxide attack on the substrate carbonyl carbon by removing a proton from a nearby water molecule).



Initial step of thermolysin reaction

FIGURE 14.14 Thermolysin is an endoprotease (that is, it cleaves polypeptides in the middle of the chain) with a catalytic Zn^{2+} ion in the active site. The Zn^{2+} ion stabilizes the buildup of negative charge on the peptide carbonyl oxygen, as a glutamate residue deprotonates water, promoting hydroxide attack on the carbonyl carbon. Thermolysin is found in certain laundry detergents, where it is used to remove protein stains from fabrics.

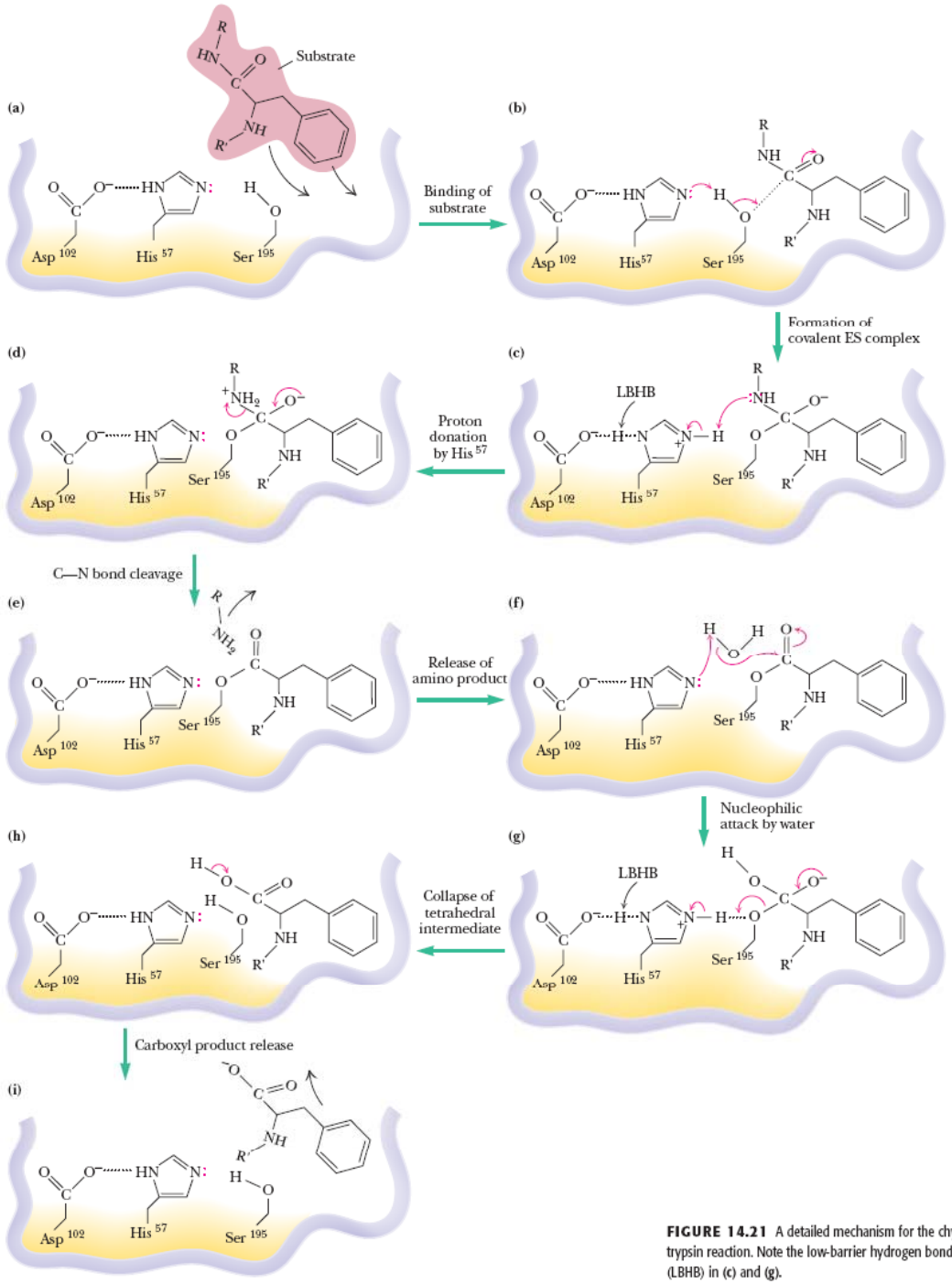
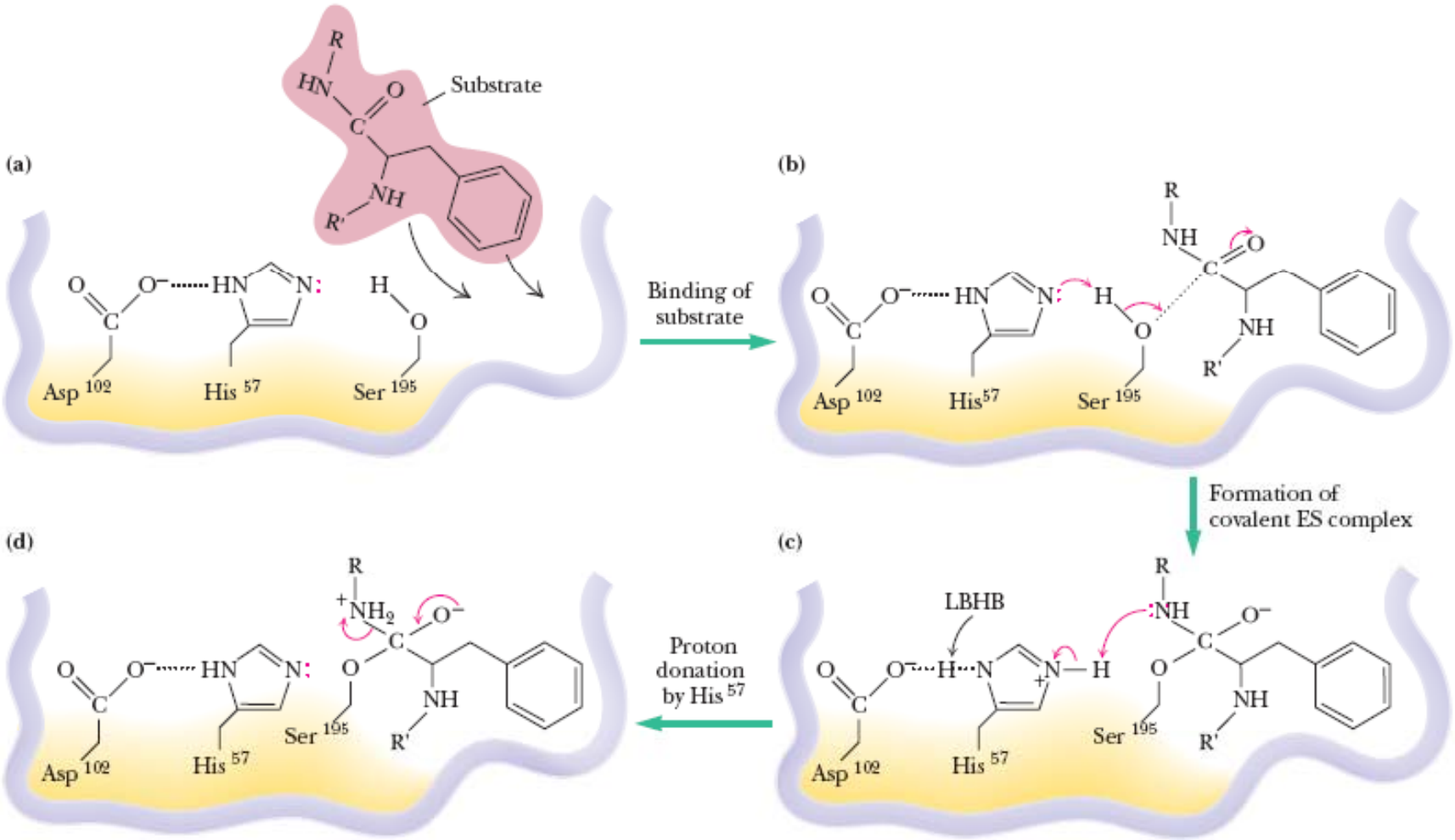
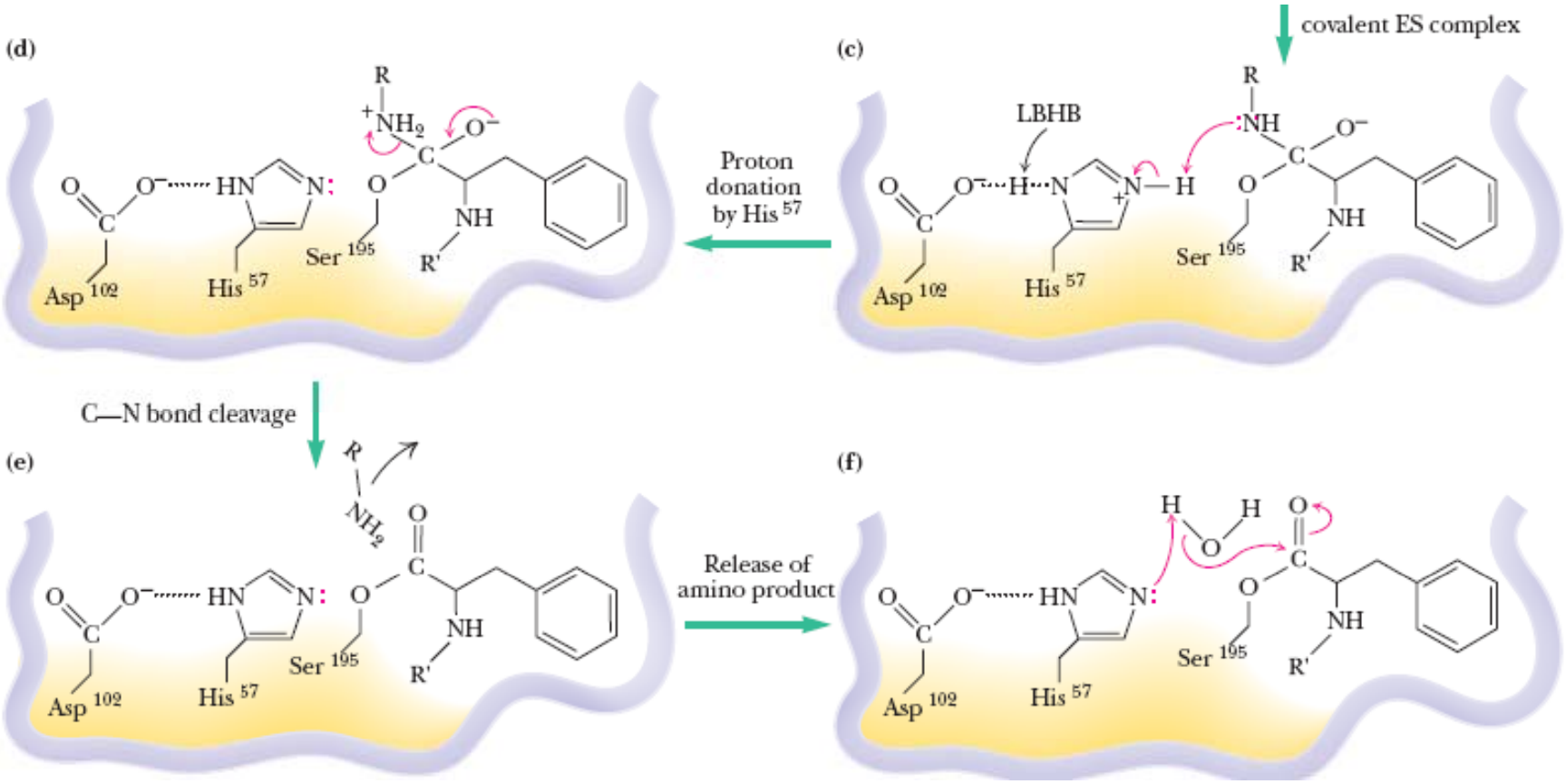
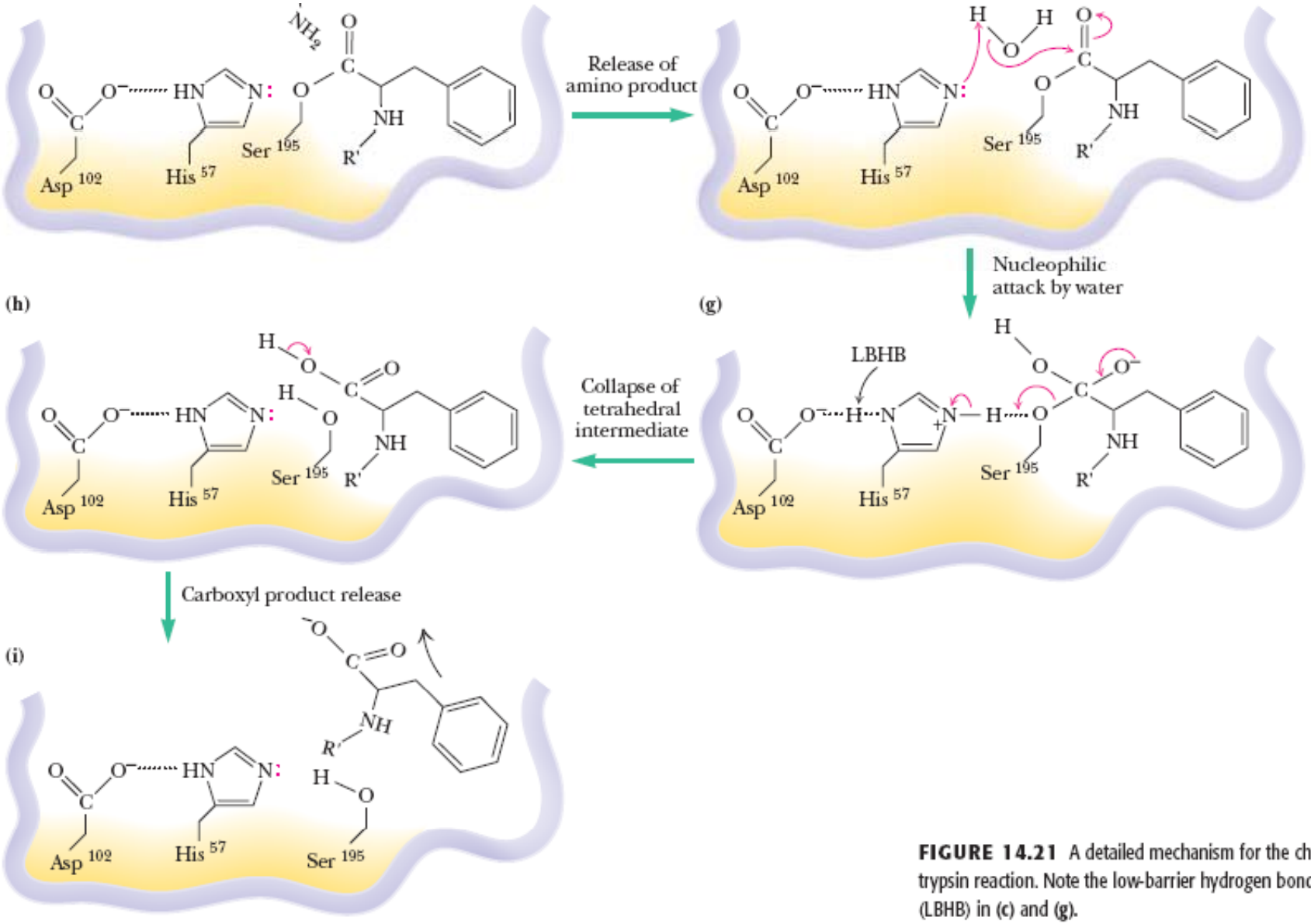


FIGURE 14.21 A detailed mechanism for the chymotrypsin reaction. Note the low-barrier hydrogen bond (LBHB) in (c) and (g).


(a)
Substrate
Asp 102
His 57
Ser 195
Binding of substrate
(b)
Asp 102
His 57
Ser 195
Formation of covalent ES complex
(c)
LBHB
Asp 102
His 57
Ser 195
Proton donation by His 57
(d)
Asp 102
His 57
Ser 195


(d)
(c)
covalent ES complex
LBHB
Proton donation by His 57
C—N bond cleavage
(e)
(f)
Release of amino product
Asp 102
His 57
Ser 195



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