

CHAPTER 6

ENZYMES

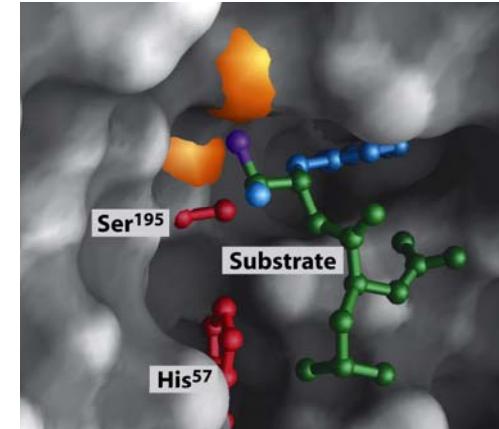
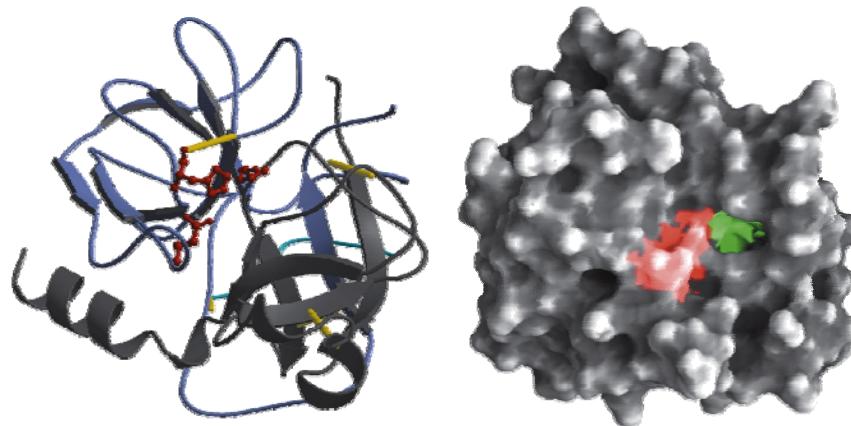
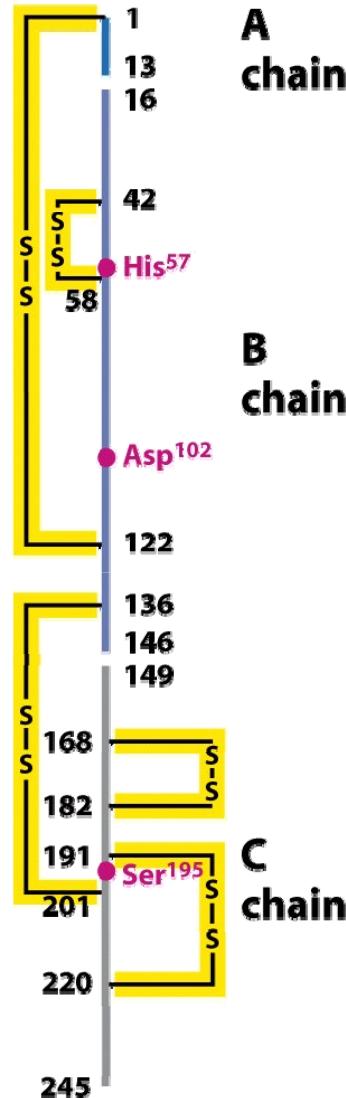
- 6.1 An introduction to enzymes
- 6.2 How enzymes work
- 6.3 Enzyme kinetics as an approach to understanding mechanism
- 6.4 Examples of enzymatic reactions
- 6.5 Regulatory enzymes

Examples of Enzymatic Reactions

- An understanding of the complete mechanism of action of a purified enzyme requires identification of all substrates, cofactors, products, and regulators. Moreover, it requires a knowledge of:
 - 1) the temporal sequence in which enzyme-bound reaction intermediates form,
 - 2) the structure of each intermediate and each transition state,
 - 3) the rates of interconversion between intermediates,
 - 4) the structural relationship of the enzyme to each intermediate,
 - 5) the energy contributed by all reacting and interacting groups to intermediate complexes and transition states.

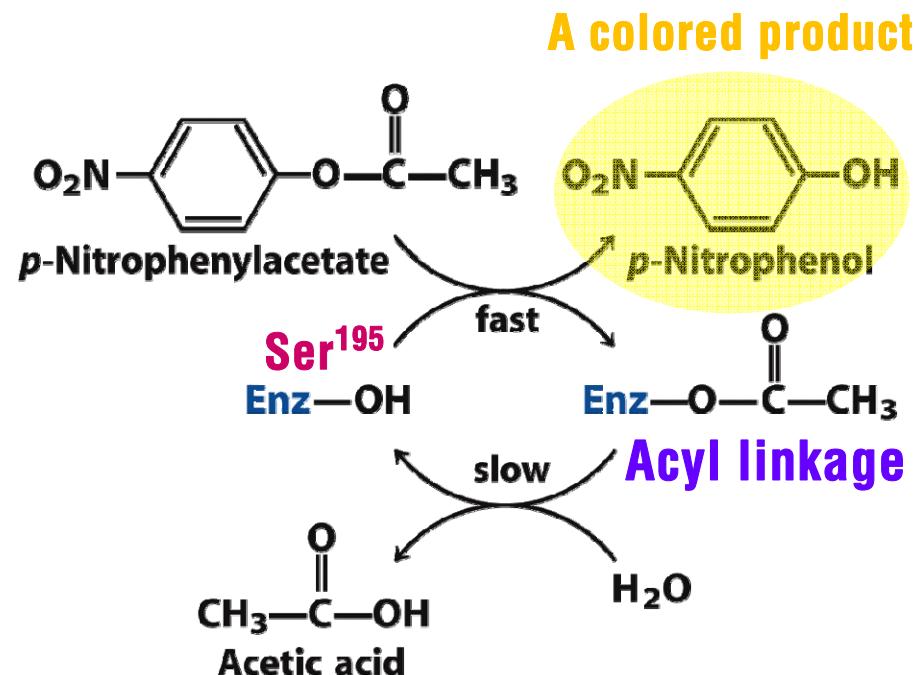
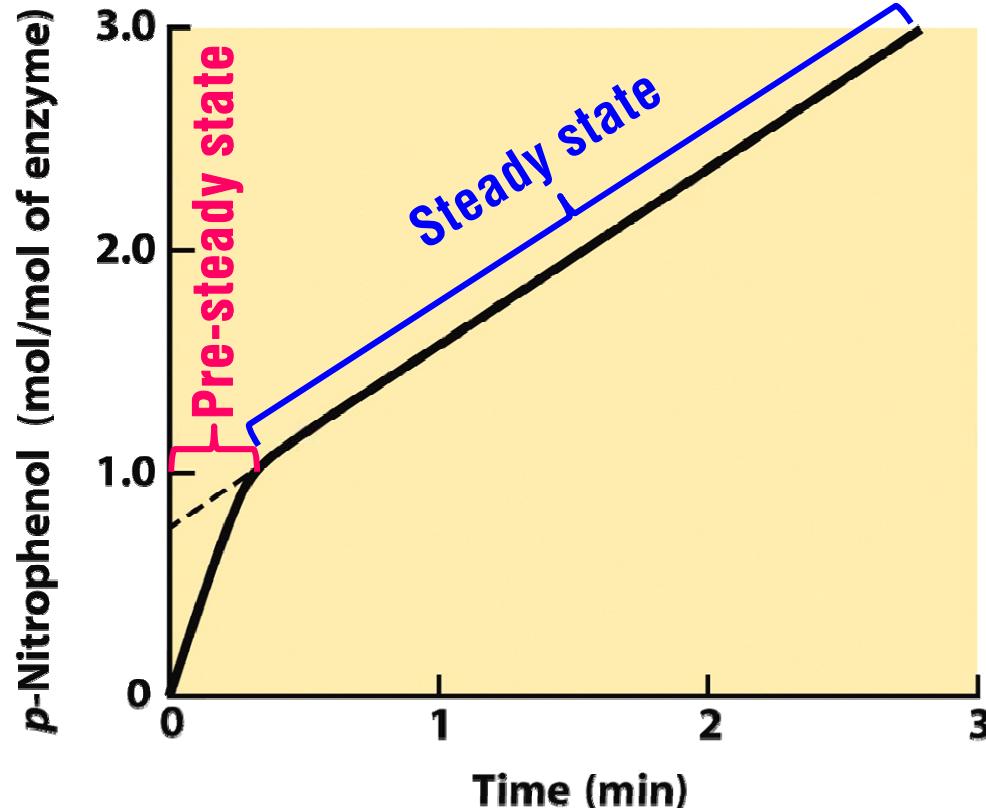
The chymotrypsin mechanism involves acylation and deacylation of a Ser residue

The reaction catalyzed by chymotrypsin illustrates the principle of transition-state stabilization and also provides a classic example of general acid-base catalysis and covalent catalysis.



- The active chymotrypsin consists of three polypeptide chains linked by disulfide bonds.
- In chymotrypsin, Ser¹⁹⁵ is linked to His⁵⁷ and Asp¹⁰² in a hydrogen-bonding network referred to as the **catalytic triad (D-H-S)**.
- The active-site amino acid residues are grouped together in the three-dimensional structure.
- Residues 14, 15, 147, and 148 in **active chymotrypsin are removed (missing)**.
- The pocket in which the **aromatic amino acid side chain of the substrate (Trp, Phe or Tyr)** is bound.

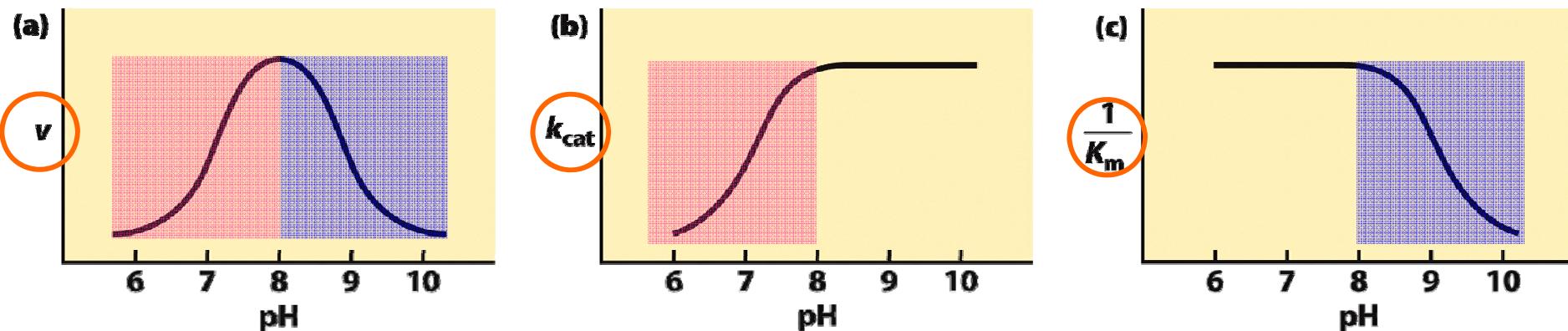
Pre-steady state kinetic evidence for an acyl-enzyme intermediate



Acylation at Ser¹⁹⁵

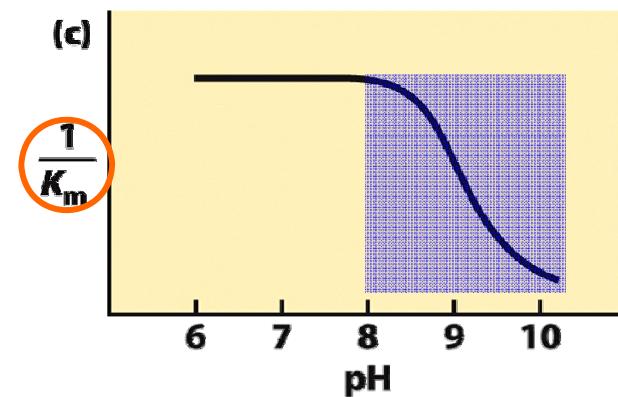
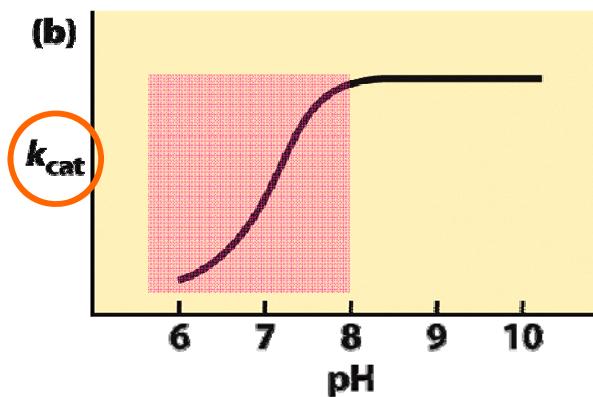
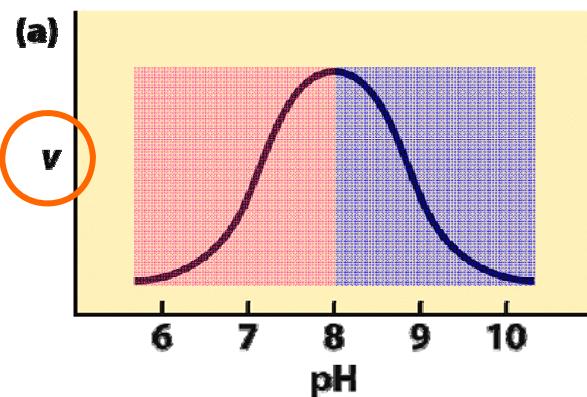
- The reaction releases a rapid burst of *p*-nitrophenol nearly stoichiometric with the amount of enzyme present. This reflects the fast acylation phase of the reaction. The subsequent rate is slower, because enzyme turnover is limited by the rate of the slower deacylation phase.

The pH dependence of chymotrypsin-catalyzed reactions



- The rates of chymotrypsin-mediated cleavage produce a bell-shaped pH-rate profile with an optimum at pH 8.0.
- The plot can be broken down to its components by using kinetic methods to determine the terms k_{cat} and K_m separately at each pH.
- The transition just above pH 7 is due to changes in k_{cat} , whereas the transition above pH 8.5 is due to changes in $1/K_m$.
- Kinetic and structural studies have shown that the transitions illustrated in (b) and (c) reflect the ionization states of the His⁵⁷ side chain (when substrate is not bound) and the α -amino group of Ile¹⁶ (at the amino terminus of the B chain), respectively. For optimal activity, His⁵⁷ must be unprotonated and Ile¹⁶ must be protonated. Protonated Ile¹⁶ formed salt-bridge with Asp¹⁹⁴.

The pH dependence of chymotrypsin-catalyzed reactions



- Additional features of the chymotrypsin mechanism have been discovered by analyzing the dependence of the reaction on pH.
- The decline in k_{cat} at low pH results from protonation of His⁵⁷ (so that it cannot extract a proton from Ser¹⁹⁵ in step ① of the reaction. This rate reduction illustrates the importance of general acid and general base catalysis in the mechanism for chymotrypsin.
- For optimal activity, His⁵⁷ must be unprotonated and Ile¹⁶ must be protonated.

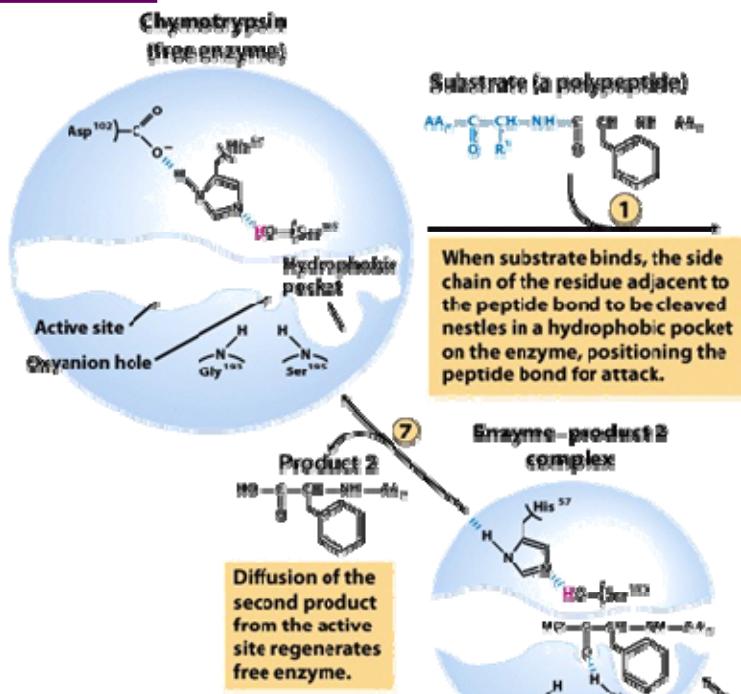
Unprotonation of His⁵⁷

207

- When a peptide substrate binds to chymotrypsin, a subtle change in conformation compresses the hydrogen bond between His⁵⁷ and Asp¹⁰², resulting in a stronger interaction, called a low-barrier hydrogen bond.

Hydrolytic cleavage of a peptide bond by chymotrypsin

209



Interaction of Ser¹⁹⁵ and His⁵⁷ generates a strongly nucleophilic alkoxide ion on Ser¹⁹⁵; the ion attacks the peptide carbonyl group, forming a tetrahedral acyl-enzyme. This is accompanied by formation of a short-lived negative charge on the carbonyl oxygen of the substrate, which is stabilized by hydrogen bonding in the oxyanion hole.

E2 complex

Short-lived intermediate* (acylation)

Instability of the negative charge on the substrate carbonyl oxygen leads to collapse of the tetrahedral intermediate; re-formation of a double bond with carbon displaces the bond between carbon and the amino group of the peptide linkage, breaking the peptide bond. The amino leaving group is protonated by His⁵⁷, facilitating its displacement.

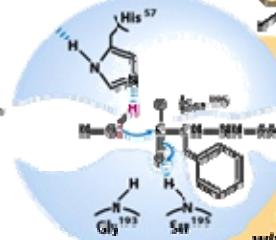
Acylation phase (steps ① to ③)

Deacylation phase (steps ④ to ⑦)

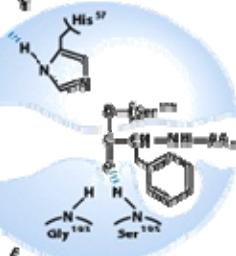
Short-lived intermediate* (deacylation)

Collapse of the tetrahedral intermediate forms the second product, a carboxylate anion, and displaces Ser¹⁹⁵.

Acyl-enzyme intermediate



Product 1
 $\text{AA}_1-\text{C}(=\text{O})-\text{NH}_2$



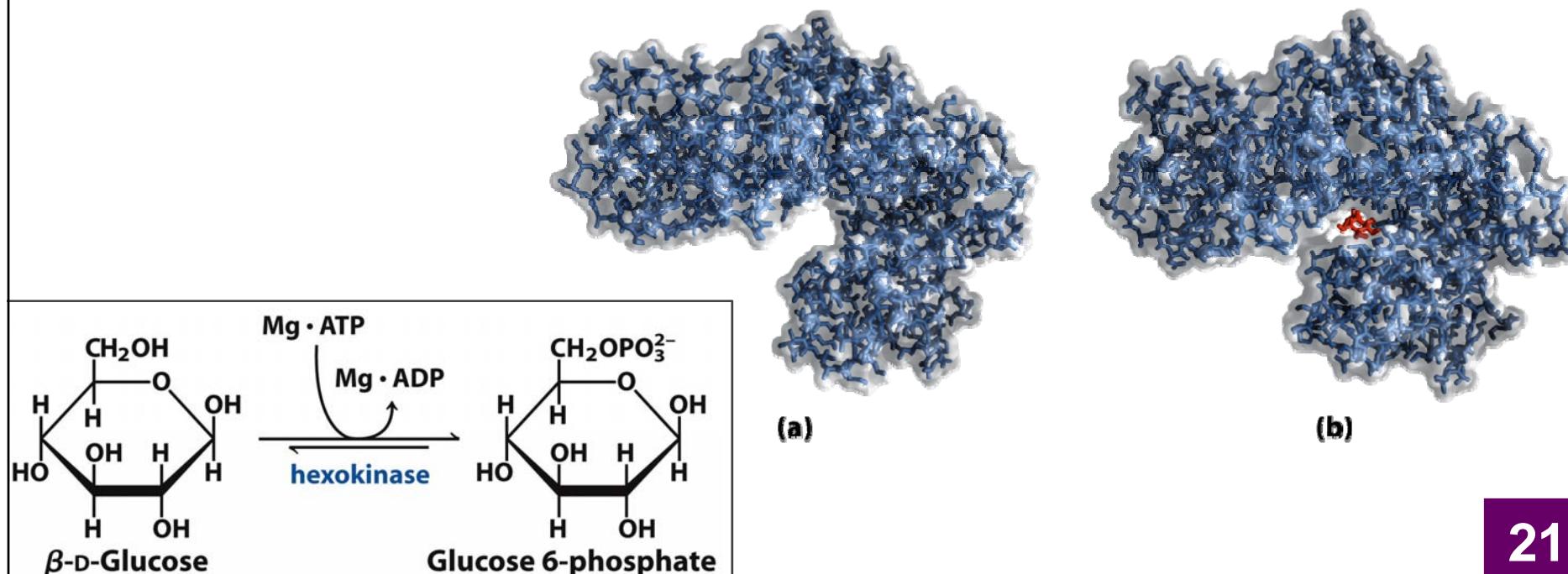
Acyl-enzyme intermediate

An incoming water molecule is deprotonated by general base catalysis, generating a strongly nucleophilic hydroxide ion. Attack of hydroxide on the ester linkage of the acyl-enzyme generates a second tetrahedral intermediate, with oxygen in the oxyanion hole again taking on a negative charge.

The reaction catalyzed by this enzyme illustrates the principle of **transition-state stabilization** and also provides a classic example of **general acid-base catalysis** and **covalent catalysis**.

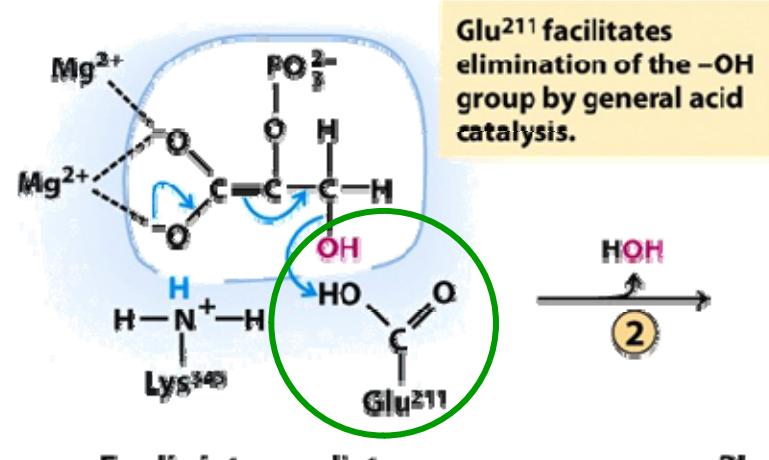
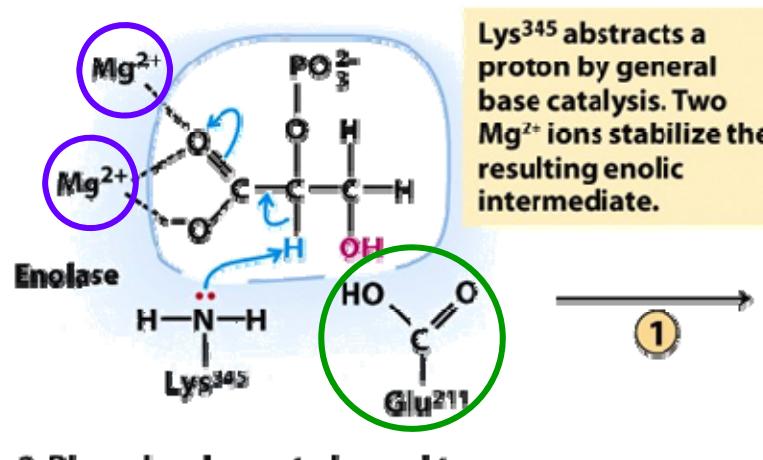
Hexokinase Undergoes Induced Fit on Substrate Binding

- Hexokinase provides a good example of induced fit.
- Induced fit is only one aspect of the catalytic mechanism of hexokinase—like chymotrypsin, hexokinase uses several catalytic strategies.
- Hexokinase has a U-shaped structure. Two ends pinch toward each other in a conformational change induced by binding of D-glucose (red)

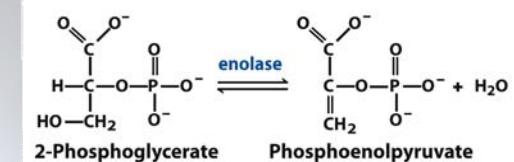
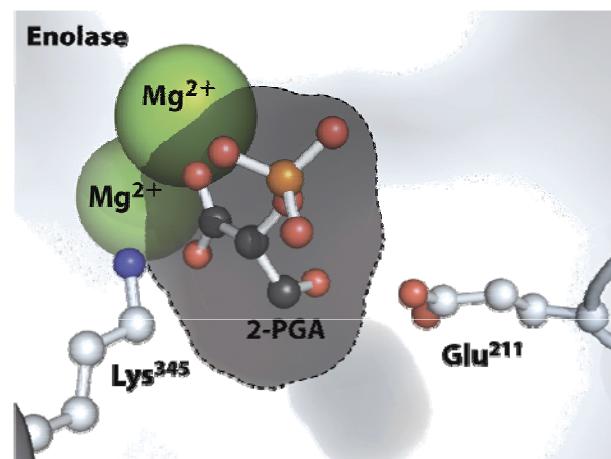


The enolase reaction mechanism requires metal ions

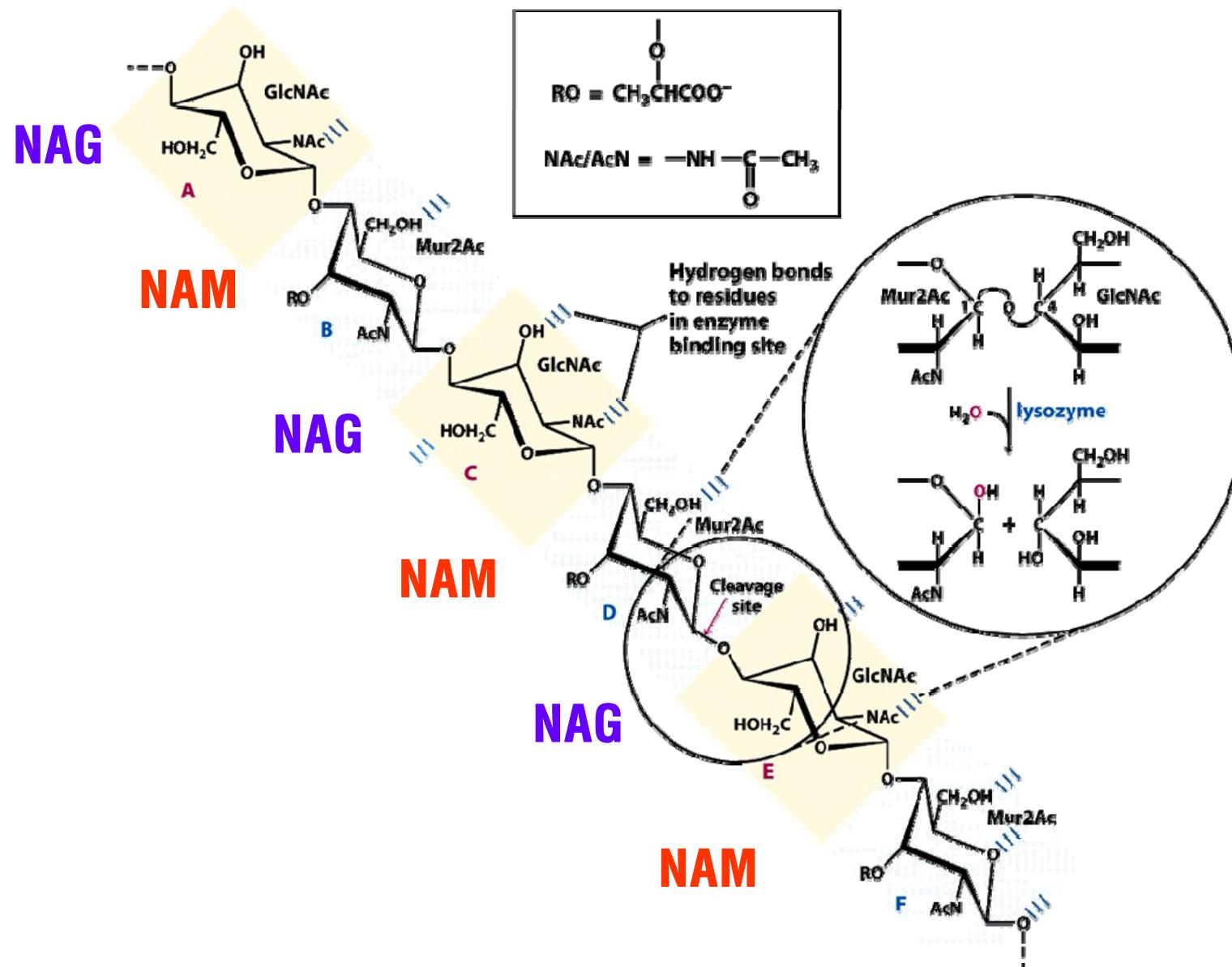
- The enolase reaction illustrates one type of metal ion catalysis and provides an additional example of general acid-base catalysis and transition-state stabilization.



- The mechanism by which enolase converts 2-phosphoglycerate (2-PGA) to phosphoenolpyruvate.
- The carboxyl group of 2-PGA is coordinated by two magnesium ions at the active site.

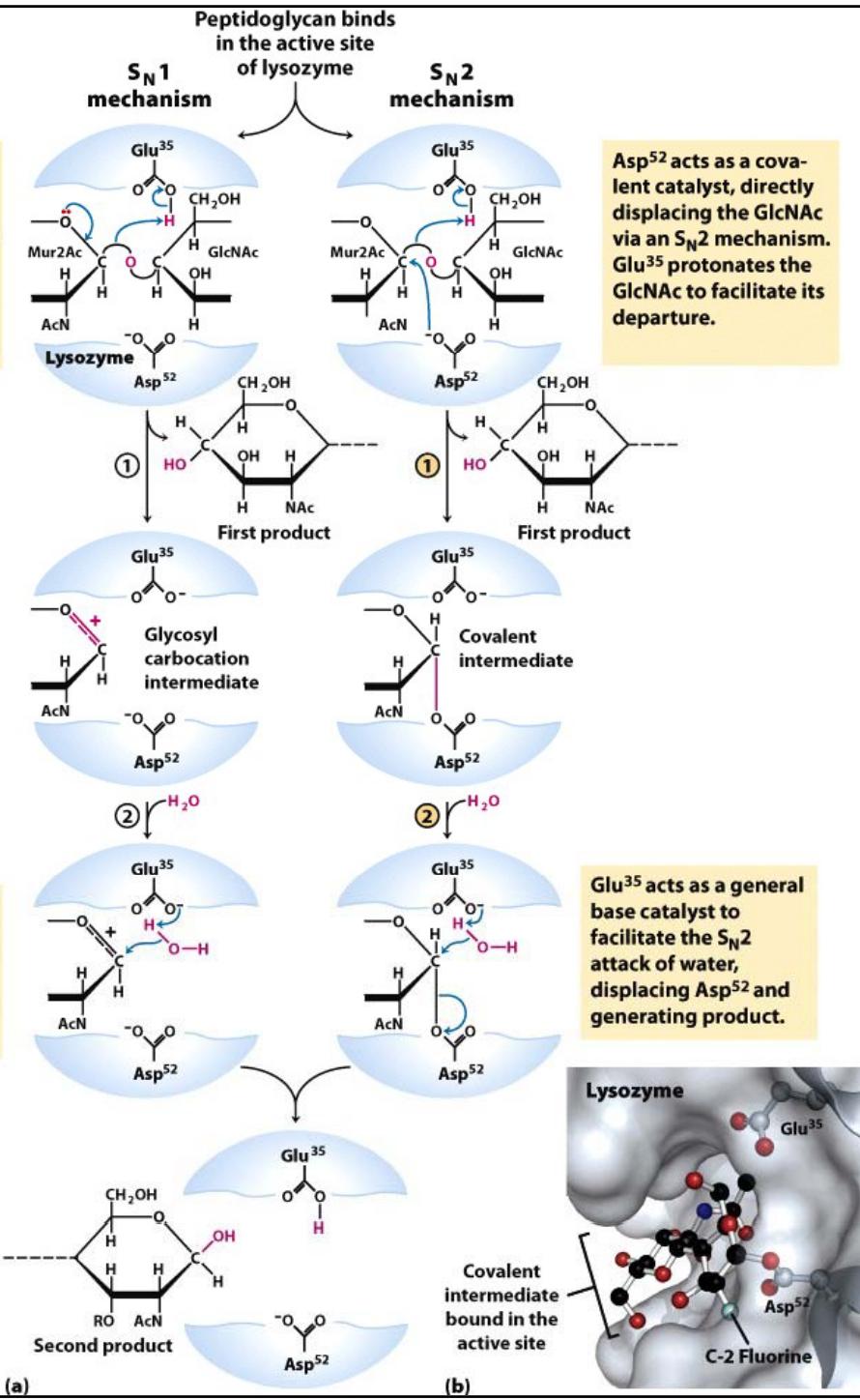


The reaction lysozyme catalyzes



Cleavage of peptidoglycan by lysozyme: two successive S_N2 steps model

A rearrangement produces a glycosyl carbocation. General acid catalysis by Glu⁵² protonates the displaced GlcNAc oxygen and facilitates its departure.

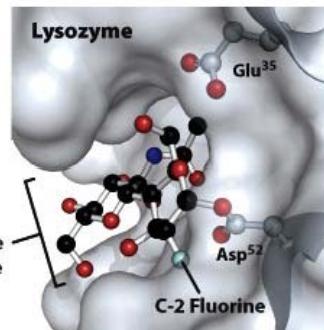


STEP1

- Asp⁵² acts as a nucleophile to attack the anomeric carbon in the first S_N2 step
 - Glu³⁵ acts as a general acid and protonates the leaving group in the transition state.

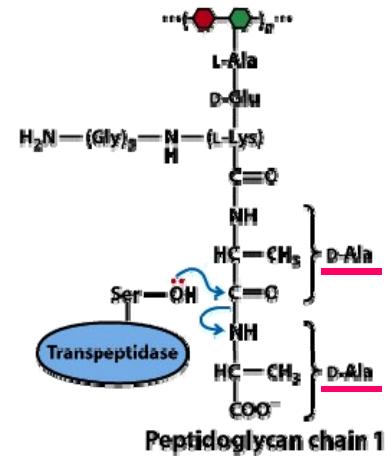
STEP 2

- Water hydrolyzes the covalent **glycosyl-enzyme intermediate**
 - Glu³⁵ acts as a **general base** to deprotonate water in the second S_N2 step

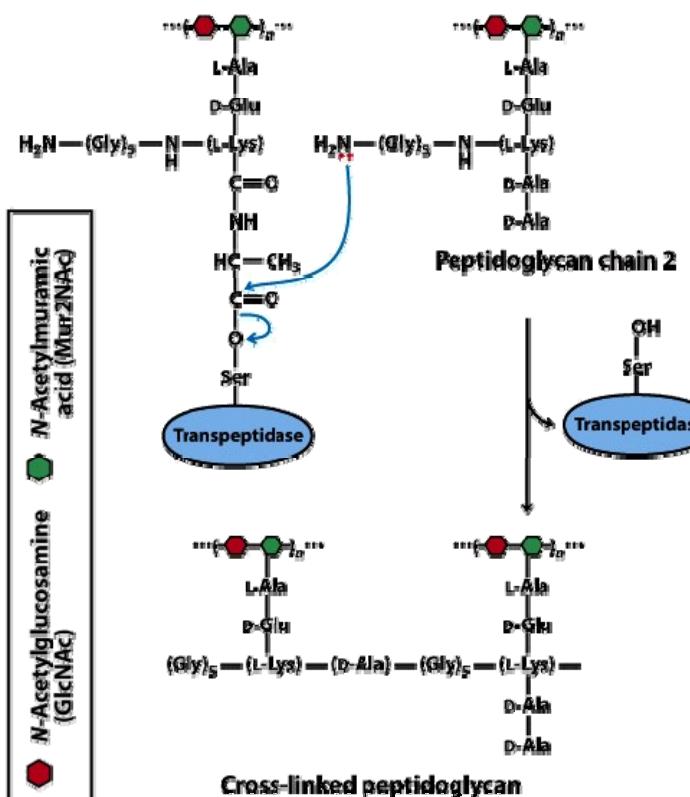


An understanding of enzyme mechanism drives important advances in medicine

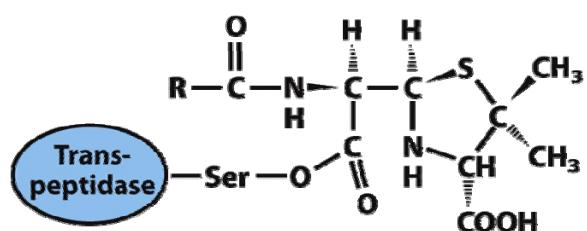
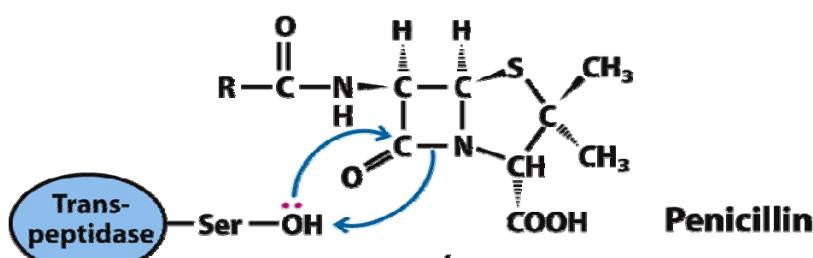
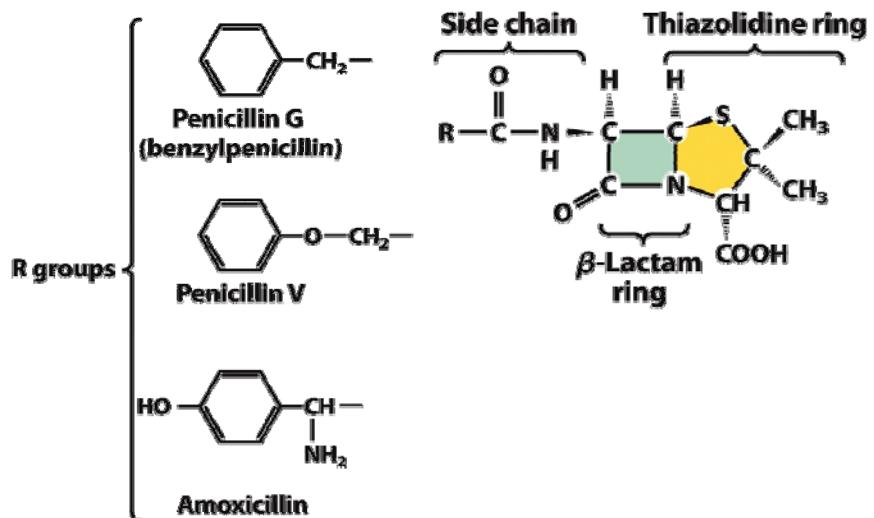
- The drugs used to treat maladies ranging from headache to HIV infection are almost always inhibitors of an enzyme.



D-Ala

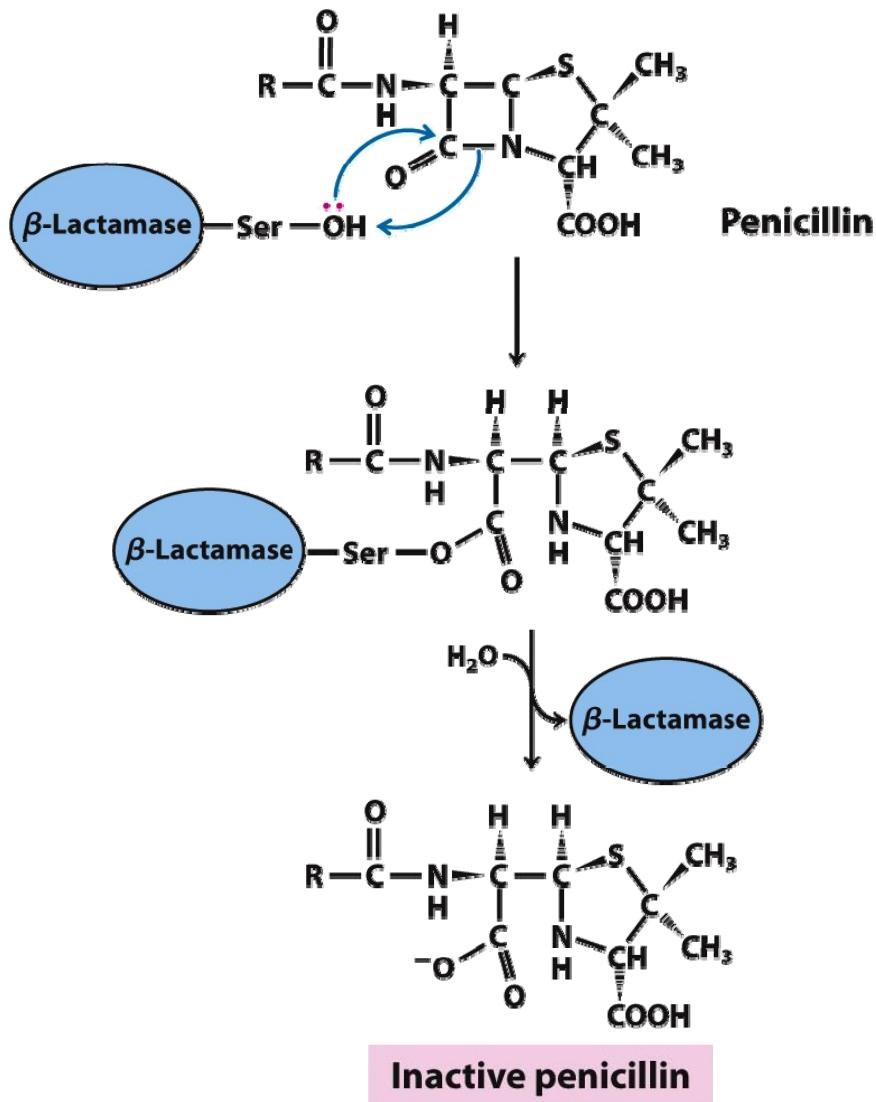


Transpeptidase inhibition by β -lactam antibiotics



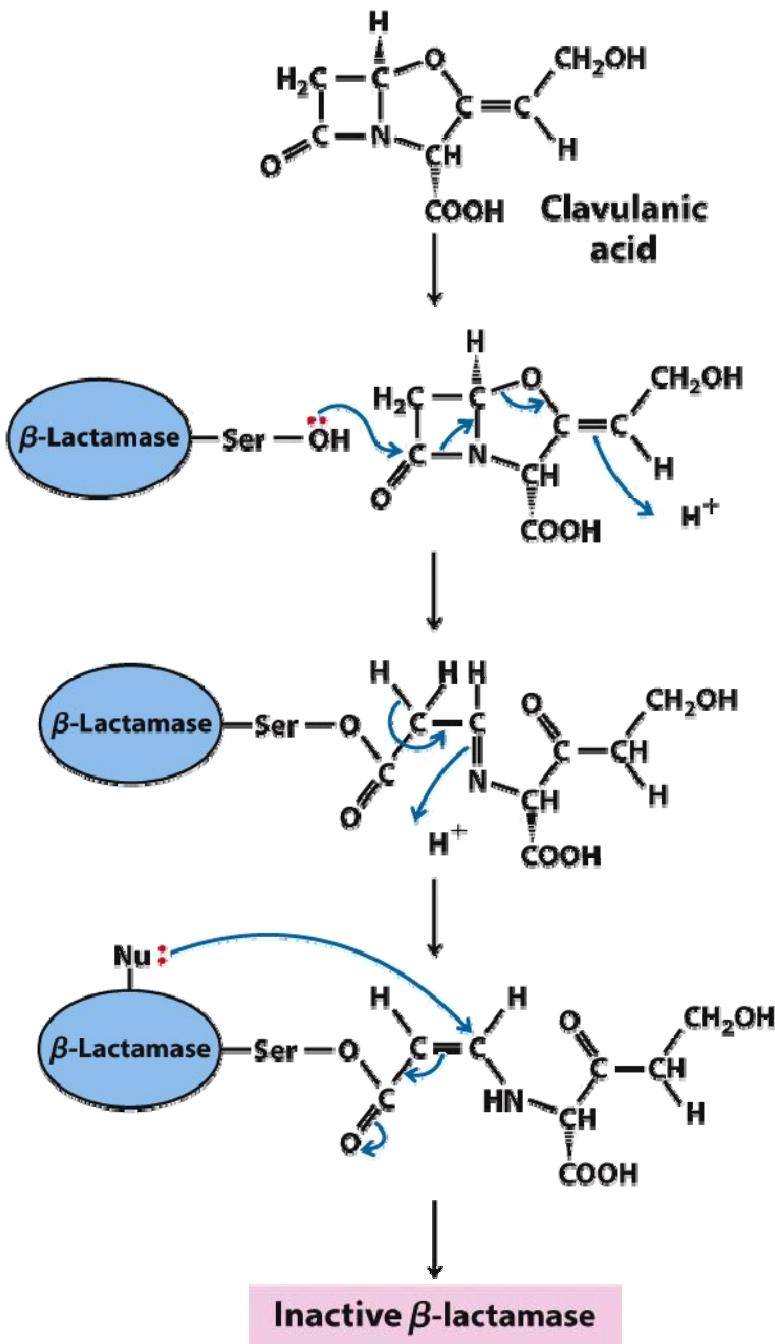
Stably derivatized,
inactive transpeptidase

- Human use of penicillin and its derivatives has led to the evolution of strains of pathogenic bacteria that express **β -lactamases**, enzymes that cleave β -lactam antibiotics, rendering them inactive. The bacteria thereby become resistant to the antibiotics.



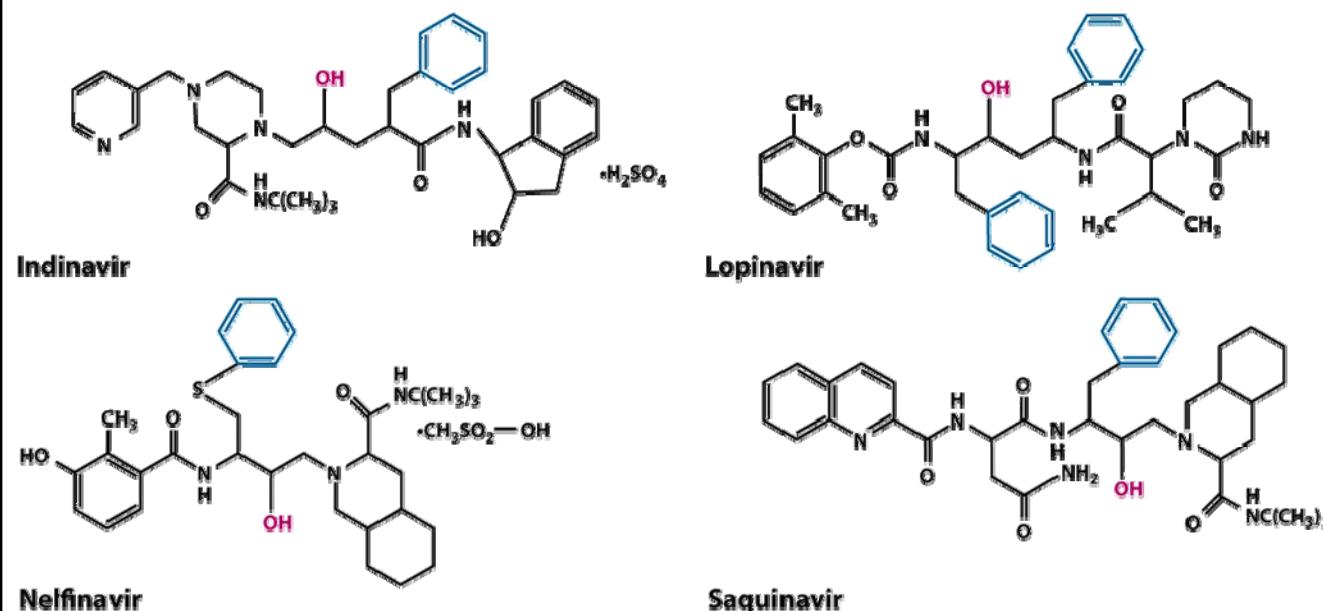
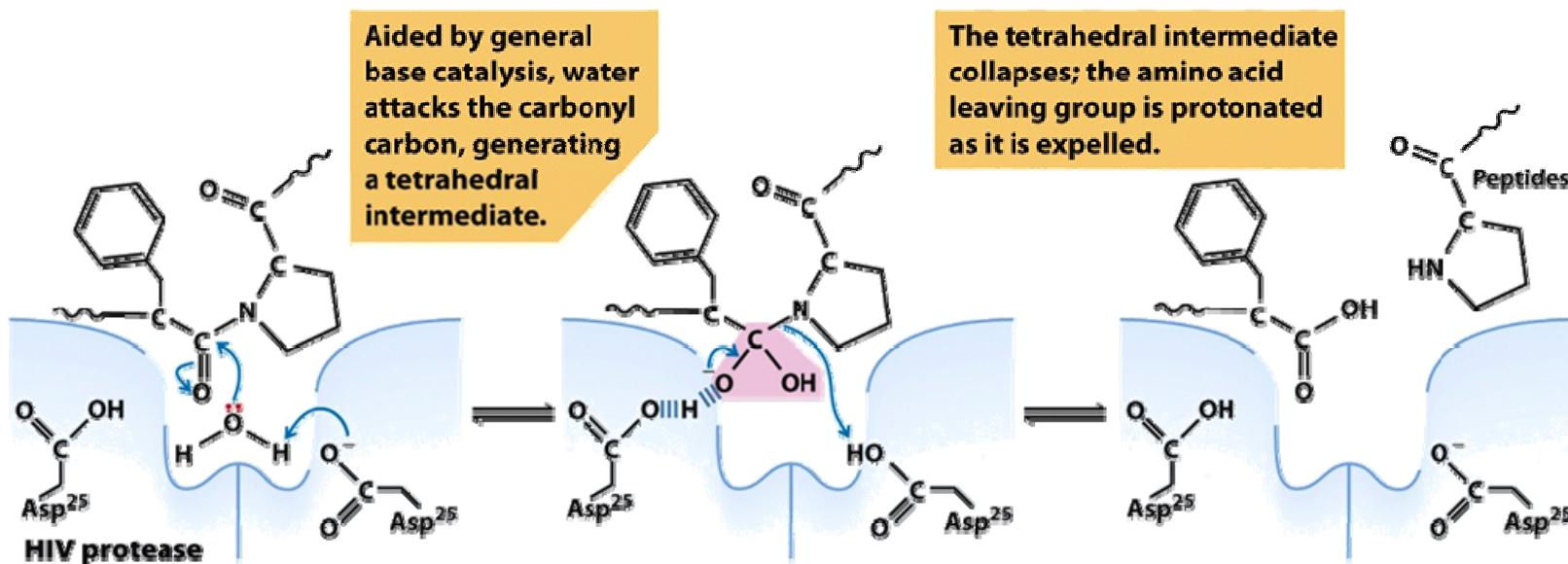
β -Lactamases promote cleavage of the β -lactam ring in β -lactam antibiotics, inactivating them.

β -lactamase inhibition



■ Clavulanic acid is a suicide inhibitor, making use of the normal chemical mechanism of β -lactamases to create a reactive species at the active site. This reactive species is attacked by groups in the active site to irreversibly acylate the enzyme.

Mechanism of action of HIV protease and HIV protease inhibitors

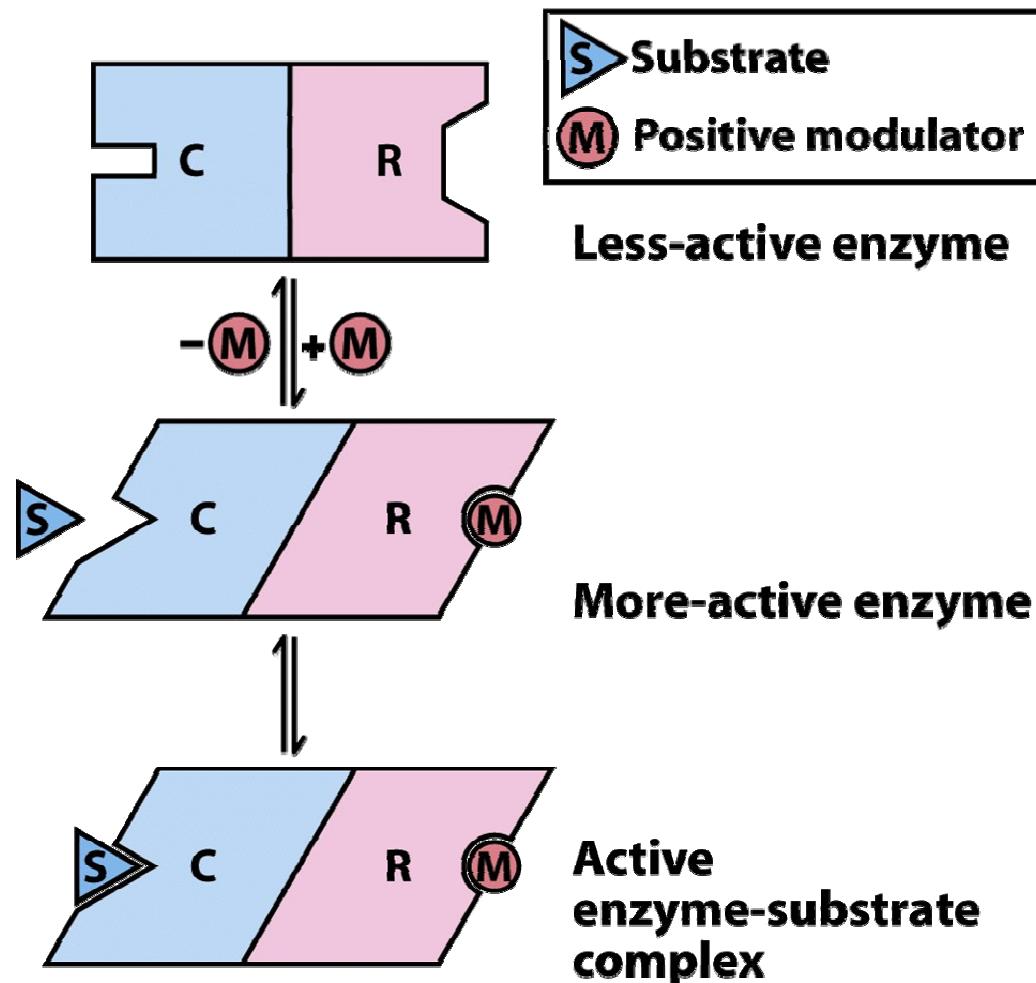


Hydroxyl group (red) acts as a transition-state analog, mimicking the oxygen of the tetrahedral intermediate. The adjacent **benzyl group (blue)** helps to properly position the drug in the active site.

Regulatory Enzymes

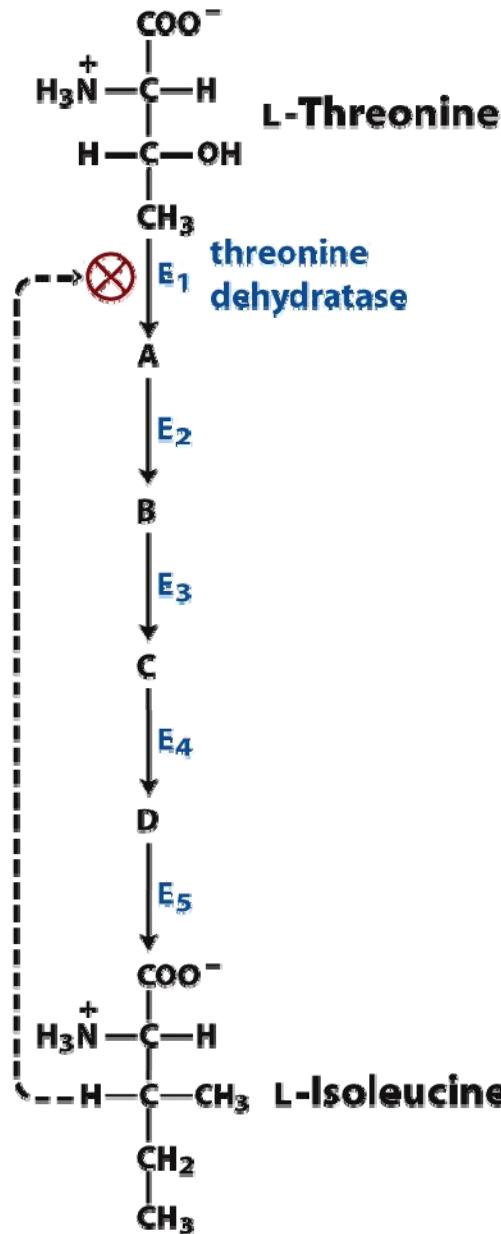
- **Regulatory enzymes** exhibit increased or decreased catalytic activity in response to certain signals.
- The activities of regulatory enzymes are modulated in a variety of ways.
- **Allosteric enzymes** function through reversible, noncovalent binding of regulatory compounds called **allosteric modulators** or **allosteric effectors**, which are generally small metabolites or cofactors.
- Other enzymes are regulated by reversible **covalent modification**.

Allosteric enzymes undergo conformational changes in response to modulator binding



- In addition to active sites, allosteric enzymes generally have one or more regulatory, or allosteric, sites for binding the modulator. Just as an enzyme's active site is specific for its substrate, each regulatory site is specific for its modulator.

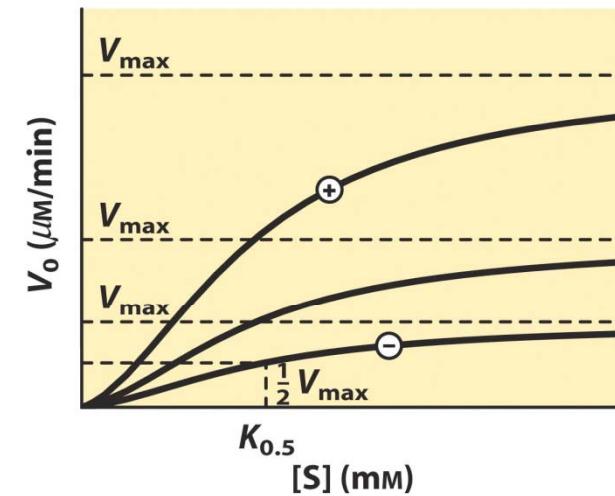
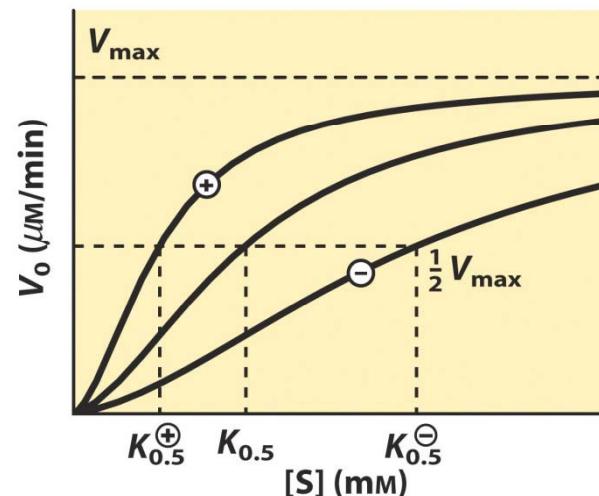
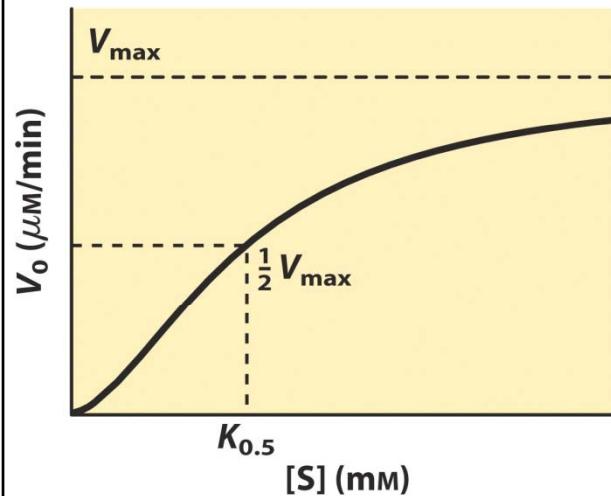
Feedback inhibition: Buildup of the end product ultimately slows the entire pathway



- In addition to active sites, allosteric enzymes generally have one or more regulatory, or allosteric, sites for binding the modulator. Just as an enzyme's active site is specific for its substrate, each regulatory site is specific for its modulator.
- The conversion of L-threonine to L-isoleucine is catalyzed by a sequence of five enzymes (E_1 to E_5). Threonine dehydratase (E_1) is specifically inhibited allosterically by L-isoleucine, the end product of the sequence, but not by any of the four intermediates (A to D).

Allosteric regulation 異位調控

■ **Allosteric regulation** is the regulation of an enzyme or other protein by binding an effector molecule at the protein's **allosteric site** (that is, a site other than the protein's active site). Effectors that enhance the protein's activity are referred to as ***allosteric activators***, whereas those that decrease the protein's activity are called ***allosteric inhibitors***.

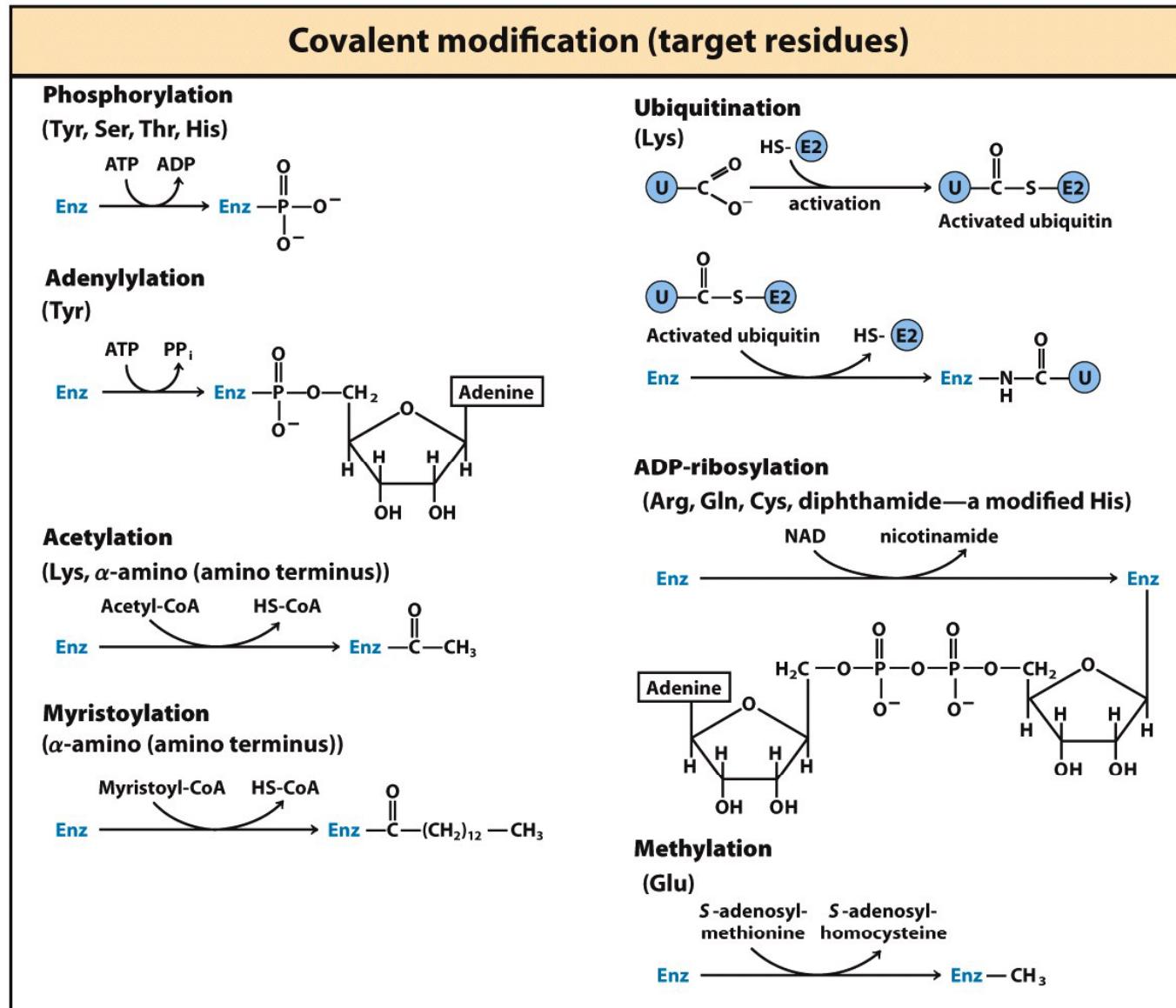


The sigmoid curve of a homotropic enzyme, in which the substrate also serves as a positive (stimulatory) modulator, or activator.

常見

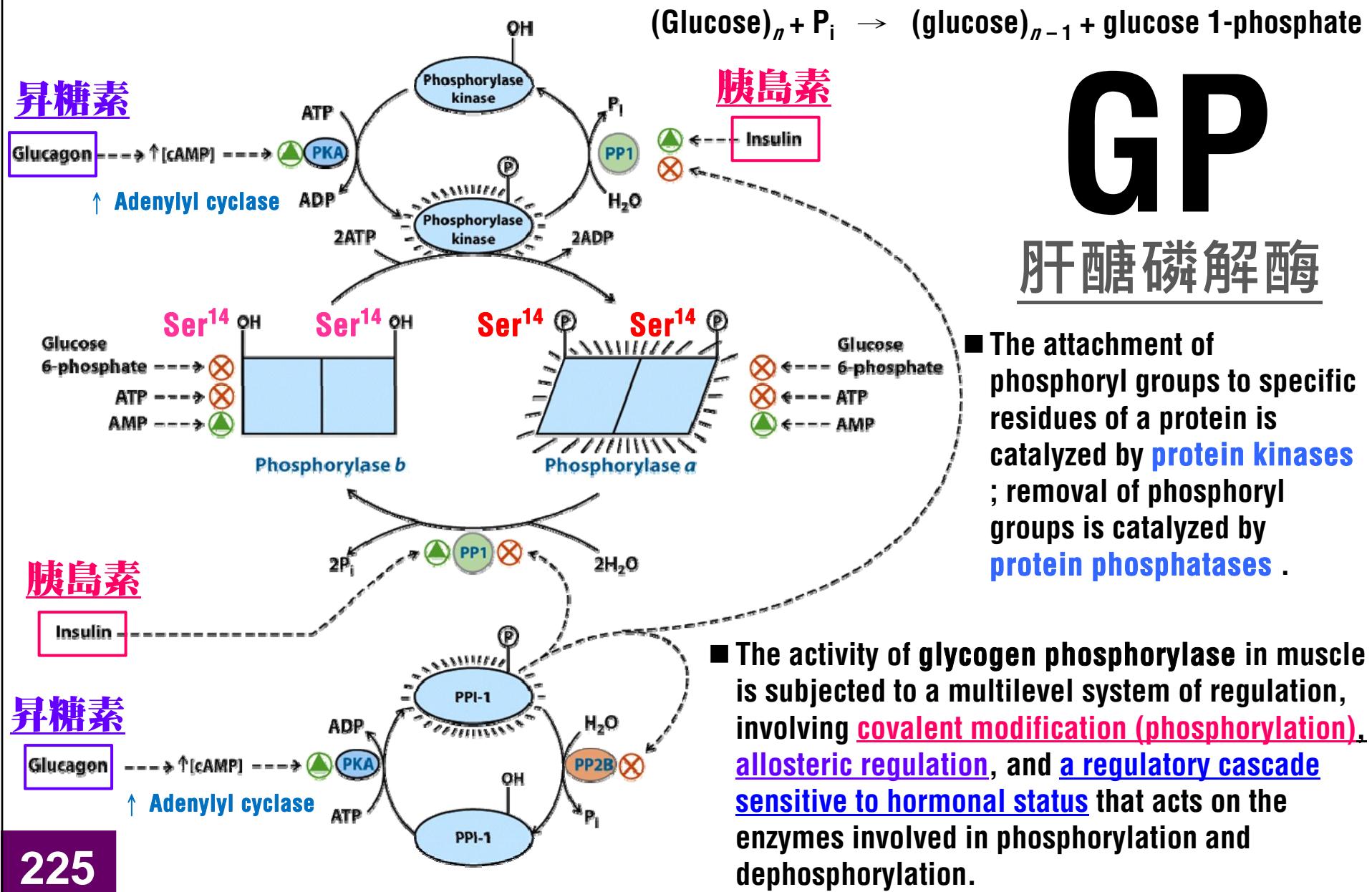
少見

Some enzyme modification reactions



Some enzymes are regulated by reversible covalent modification.

Regulation of muscle glycogen phosphorylase activity by multiple mechanisms

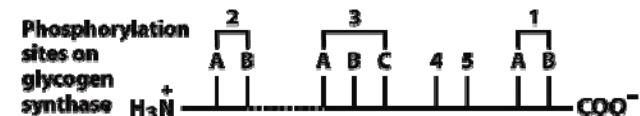


Multiple Phosphorylations Allow Exquisite Regulatory Control

- The Ser, Thr, or Tyr residues that are phosphorylated in regulated proteins occur within common structural motifs, called consensus sequences, that are recognized by specific protein kinases.
- To serve as an effective regulatory mechanism, phosphorylation must be reversible. In general, phosphoryl groups are added and removed by different enzymes, and the processes can therefore be separately regulated.

TABLE 6-10 Consensus Sequences for Protein Kinases

Protein kinase	Consensus sequence and phosphorylated residue*
Protein kinase A	-x-R-[RK]-x-[ST]-B-
Protein kinase G	-x-R-[RK]-x-[ST]-x-
Protein kinase C	-[RK](2)-x-[ST]-B-[RK](2)-
Protein kinase B	-x-R-x-[ST]-x-K-
Ca ²⁺ /calmodulin kinase I	-B-x-R-x(2)-[ST]-x(3)-B-
Ca ²⁺ /calmodulin kinase II	-B-x-[RK]-x(2)-[ST]-x(2)-
Myosin light chain kinase (smooth muscle)	-K(2)-R-x(2)-S-x-B(2)-
Phosphorylase b kinase	-K-R-K-Q-I-S-V-R-
Extracellular signal-regulated kinase (ERK)	-P-x-[ST]-P(2)-
Cyclin-dependent protein kinase (cdc2)	-x-[ST]-P-x-[KR]-
Casein kinase I	-[SpTp]-x(2)-[ST]-B [†]
Casein kinase II	-x-[ST]-x(2)-[ED]-x-
β-Adrenergic receptor kinase	-[DE](n)-[ST]-x(3)
Rhodopsin kinase	-x(2)-[ST]-E(n)-
Insulin receptor kinase	-x-E(3)-Y-M(4)-K(2)-S-R-G-D-Y-M-T-M-Q-I-G-K(3)-L-P-A-T-G-D-Y-M-N-M-S-P-V-G-D-
Epidermal growth factor (EGF) receptor kinase	-E(4)-Y-F-E-L-V-



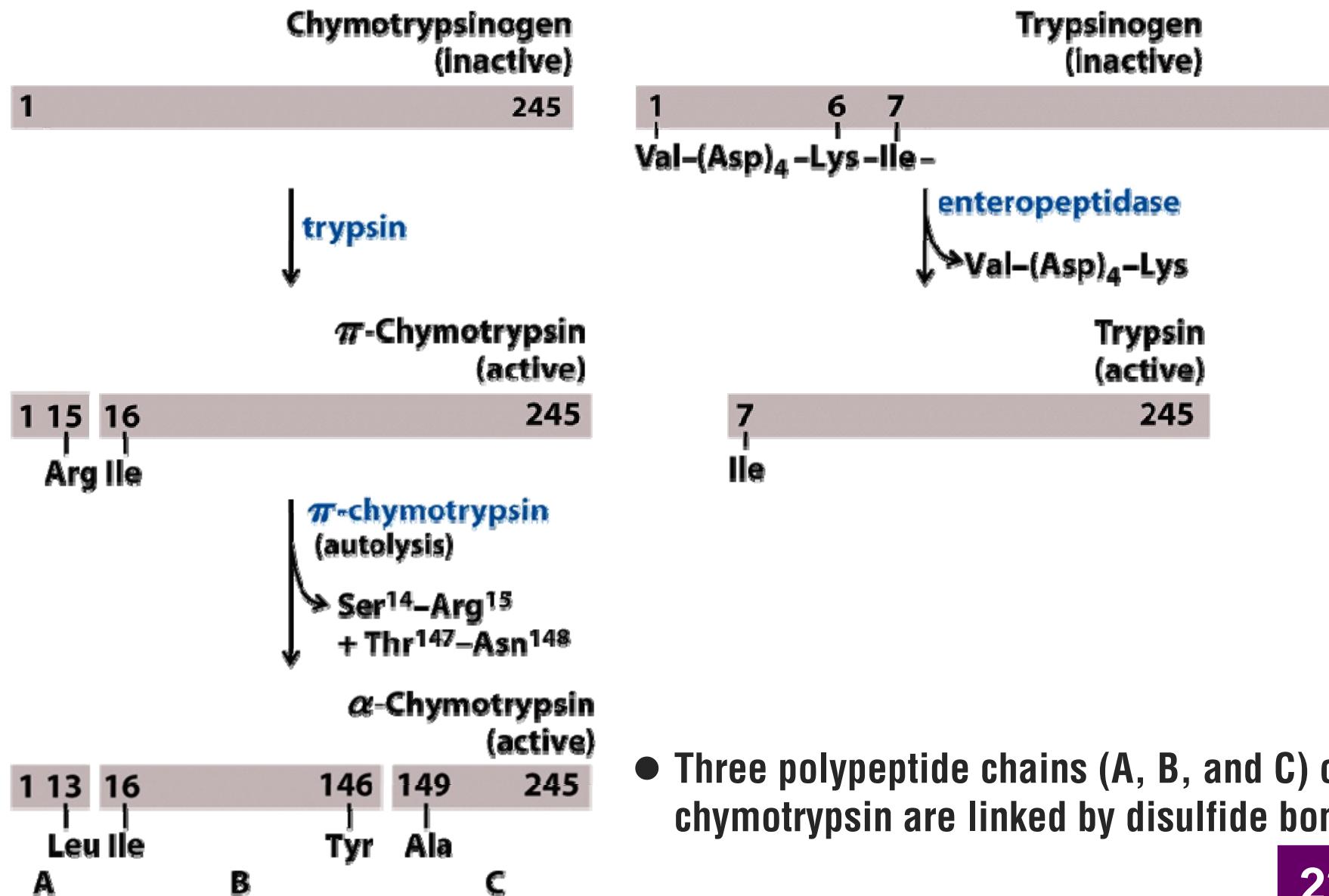
Kinase	Phosphorylation sites	Degree of synthase inactivation
Protein kinase A	1A, 1B, 2, 4	+
Protein kinase G	1A, 1B, 2	+
Protein kinase C	1A	+
Ca ²⁺ /calmodulin kinase	1B, 2	+
Phosphorylase b kinase	2	+
Casein kinase I	At least nine	+++ +
Casein kinase II	5	0
Glycogen synthase kinase 3	3A, 3B, 3C	+++
Glycogen synthase kinase 4	2	+

- Glycogen synthase has at least nine separate sites in five designated regions susceptible to phosphorylation by one of the cellular protein kinases. Regulation of this enzyme is a matter not of binary (on/off) switching but of finely tuned modulation of activity over a wide range in response to a variety of signals.

Some enzymes and other proteins are regulated by proteolytic cleavage of an enzyme precursor

- For some enzymes, an inactive precursor called a **zymogen** is cleaved to form the active enzyme.
- Proteases are not the only proteins activated by proteolysis. In other cases, however, the precursors are called not zymogens but, more generally, **proproteins** or **proenzymes**, as appropriate.
- Specific cleavage causes conformation changes that expose the enzyme active site.
- Because this type of activation is irreversible, other mechanisms are needed to inactivate these enzymes.
- For example, proteases are inactivated by inhibitor proteins that bind very tightly to the enzyme active site.

Activation of zymogens by proteolytic cleavage



- Three polypeptide chains (A, B, and C) of chymotrypsin are linked by disulfide bonds