Regulatory Enzymes

Regulatory Enzymes

The kinds of enzyme activity regulation are

1. **Allosteric regulation**
2. **Reversible covalent modification**
3. **Proteolytic activation or irreversible covalent modification**

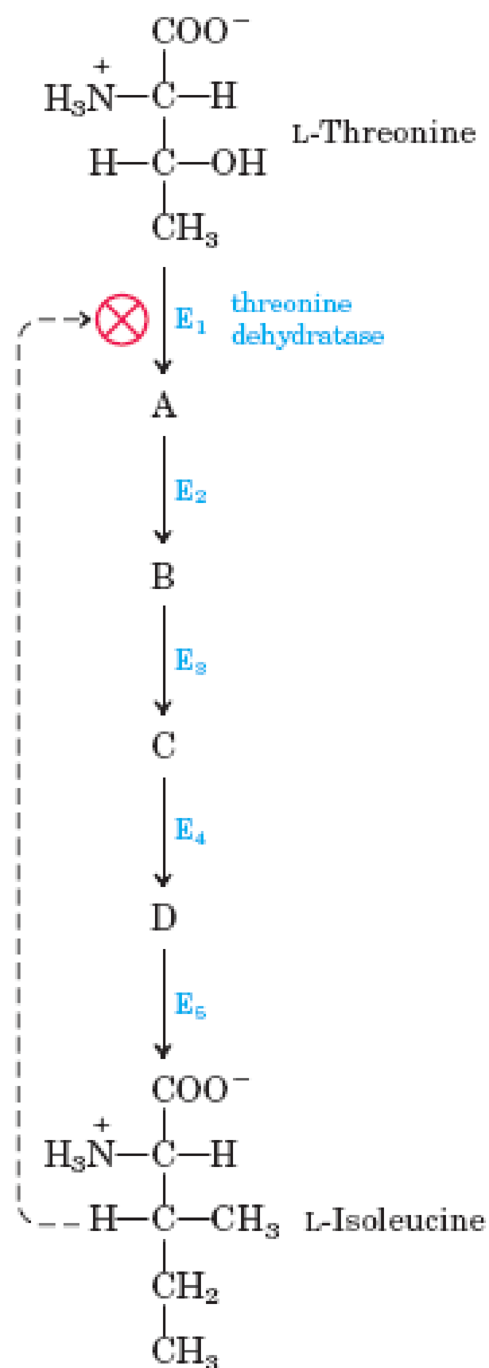


FIGURE 6–28 Feedback inhibition. The conversion of L-threonine to L-isoleucine is catalyzed by a sequence of five enzymes (E_1 to E_5). Threonine dehydratase (E_1) is specifically inhibited allosterically by L-isoleucine, the end product of the sequence, but not by any of the four intermediates (A to D). Feedback inhibition is indicated by the dashed feedback line and the ⊗ symbol at the threonine dehydratase reaction arrow, a device used throughout this book.

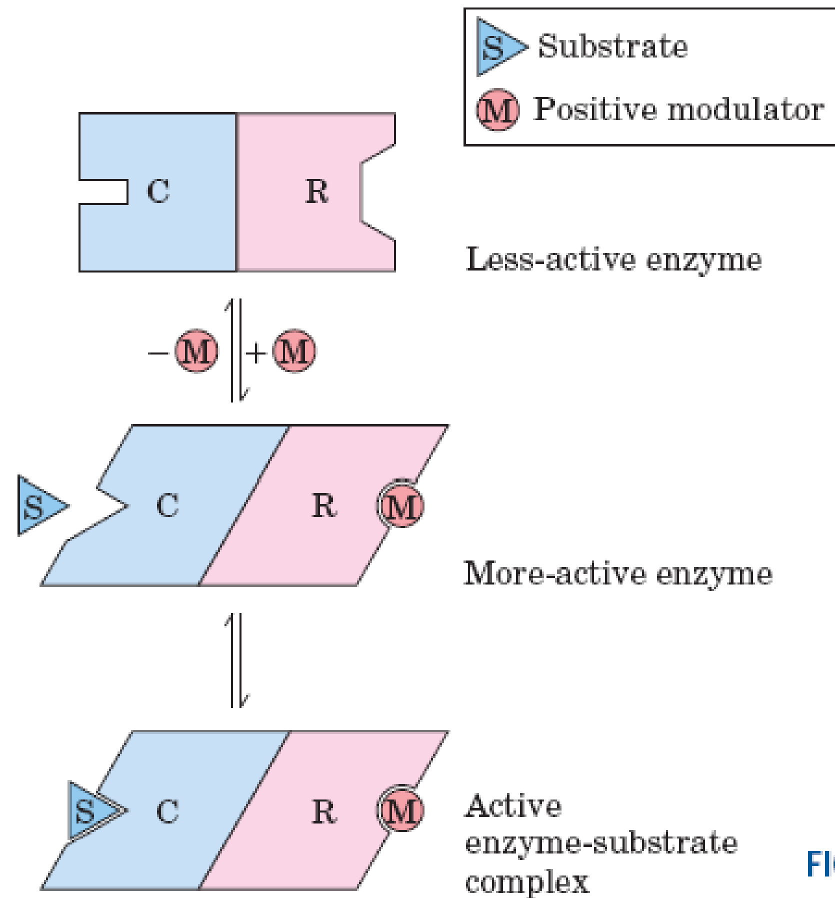
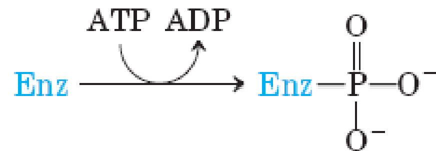


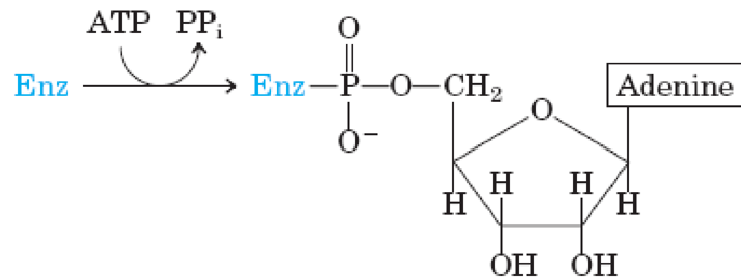
FIGURE 6–26 Subunit interactions in an allosteric enzyme, and interactions with inhibitors and activators. In many allosteric enzymes the substrate binding site and the modulator binding site(s) are on different subunits, the catalytic (C) and regulatory (R) subunits, respectively. Binding of the positive (stimulatory) modulator (M) to its specific site on the regulatory subunit is communicated to the catalytic subunit through a conformational change. This change renders the catalytic subunit active and capable of binding the substrate (S) with higher affinity. On dissociation of the modulator from the regulatory subunit, the enzyme reverts to its inactive or less active form.

Covalent modification (target residues)

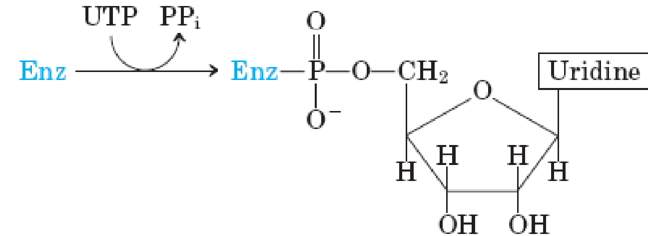
Phosphorylation
(Tyr, Ser, Thr, His)



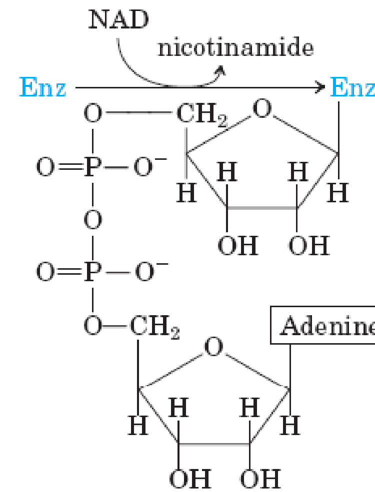
Adenylylation
(Tyr)



Uridylylation
(Tyr)



ADP-ribosylation
(Arg, Gln, Cys, diphthamide—a modified His)



Methylation
(Glu)

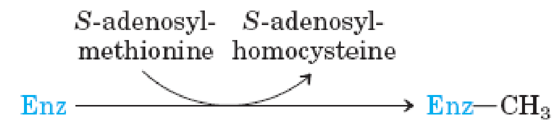
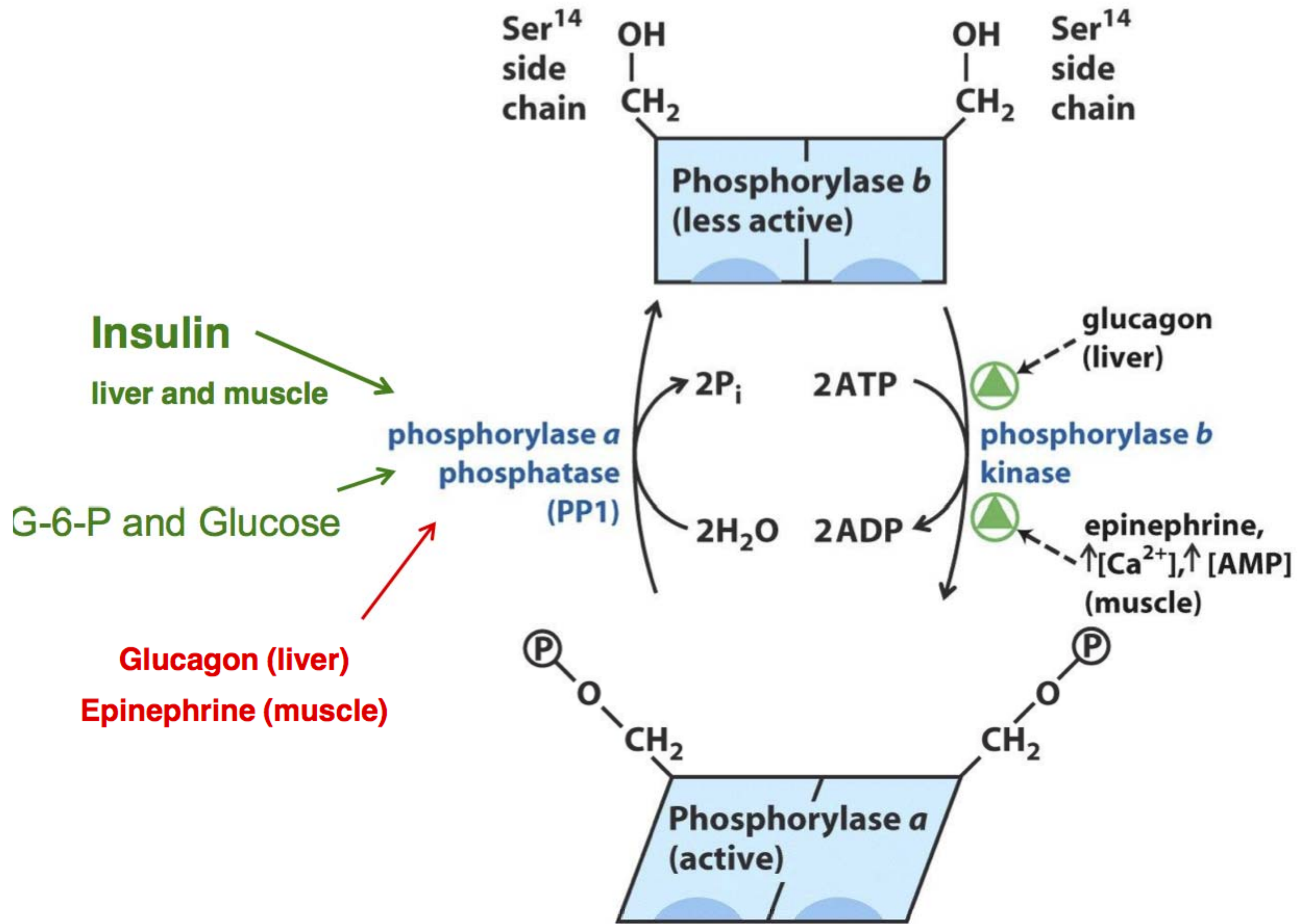


FIGURE 6-30 Some enzyme modification reactions.



Ser14 side chain
OH
CH2
OH
CH2
Ser14 side chain
Phosphorylase b
(less active)
Insulin
liver and muscle
G-6-P and Glucose
Glucagon (liver)
Epinephrine (muscle)
phosphorylase a
phosphatase
(PP1)
2Pi
2H2O
2ATP
2ADP
glucagon
(liver)
phosphorylase b
kinase
epinephrine,
↑[Ca2+],↑ [AMP]
(muscle)
P
O
CH2
P
O
CH2
Phosphorylase a
(active)

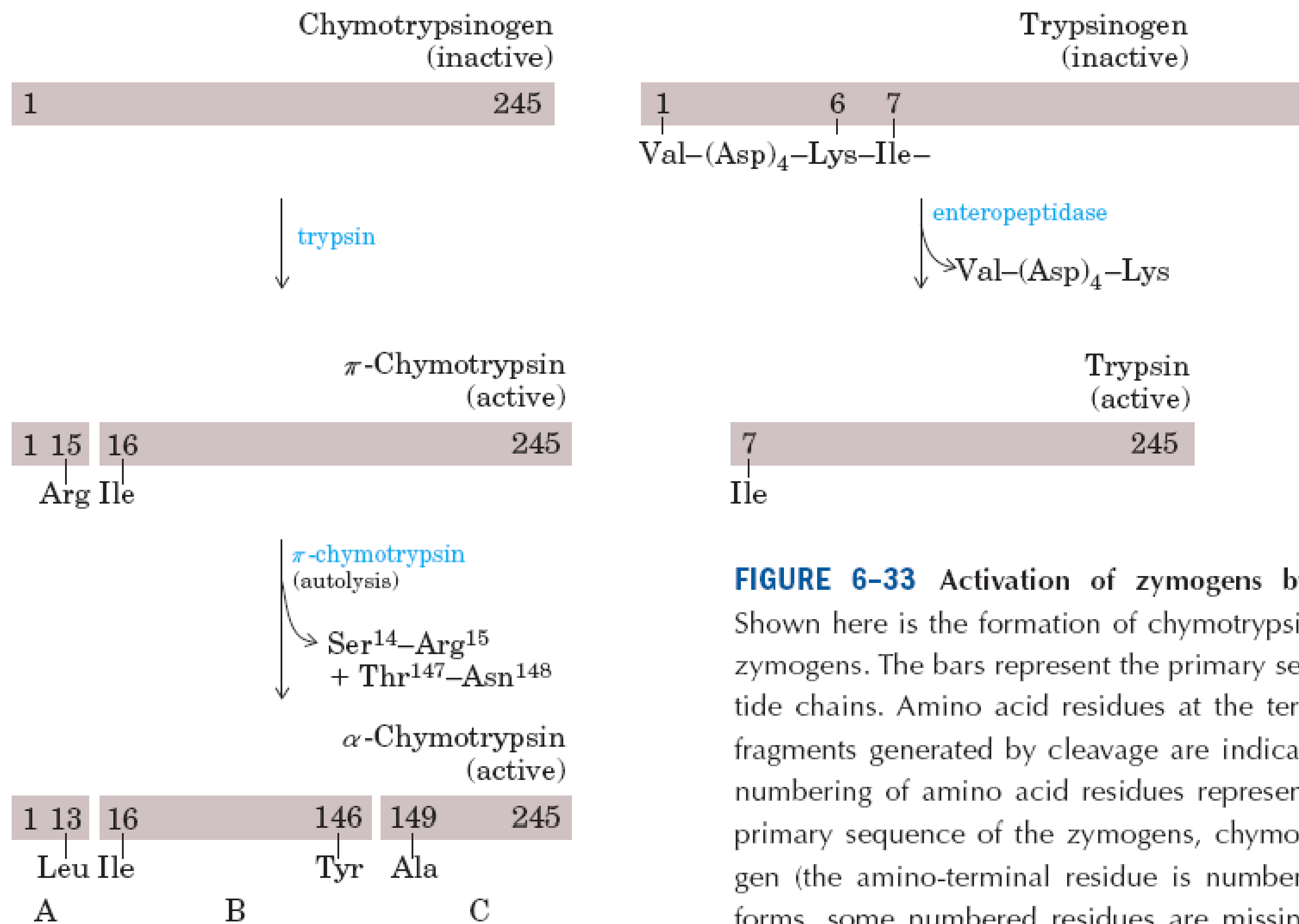


FIGURE 6–33 Activation of zymogens by proteolytic cleavage. Shown here is the formation of chymotrypsin and trypsin from their zymogens. The bars represent the primary sequences of the polypeptide chains. Amino acid residues at the termini of the polypeptide fragments generated by cleavage are indicated below the bars. The numbering of amino acid residues represents their positions in the primary sequence of the zymogens, chymotrypsinogen or trypsinogen (the amino-terminal residue is number 1). Thus, in the active forms, some numbered residues are missing. Recall that the three polypeptide chains (A, B, and C) of chymotrypsin are linked by disulfide bonds (see Fig. 6–18).

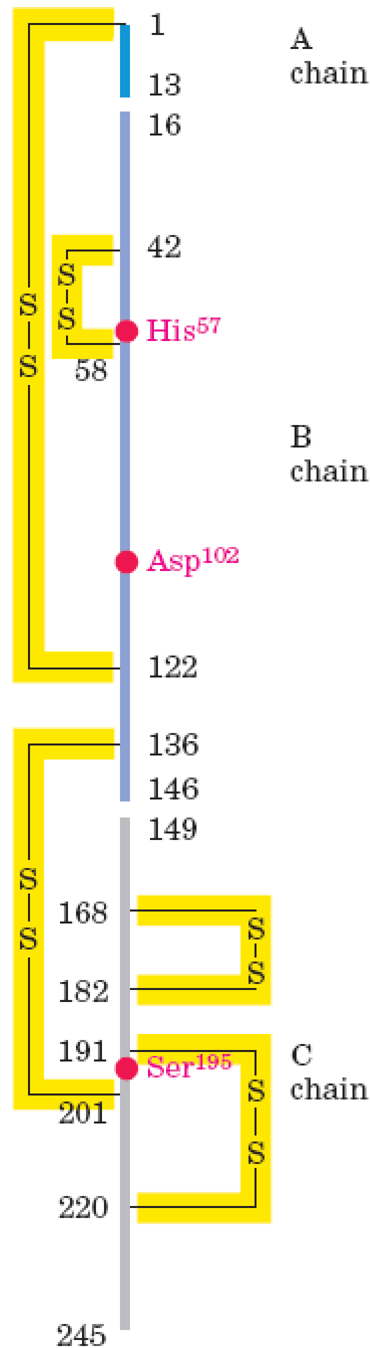
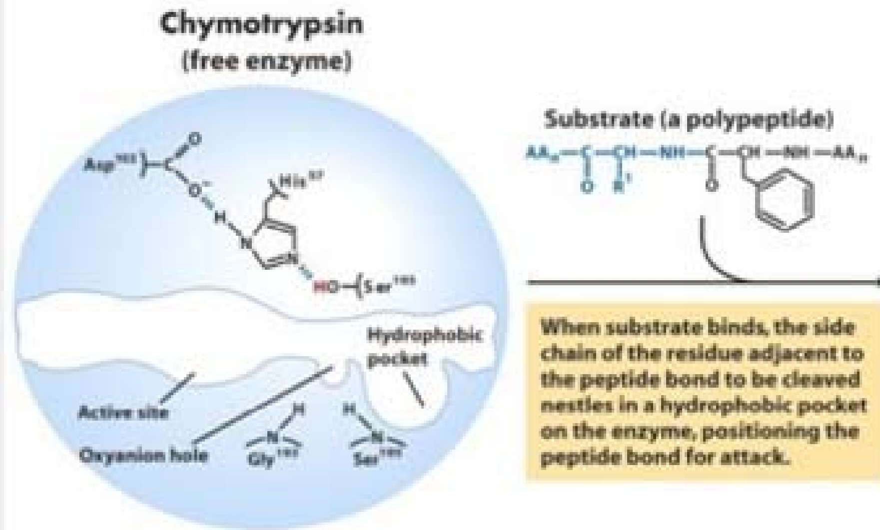
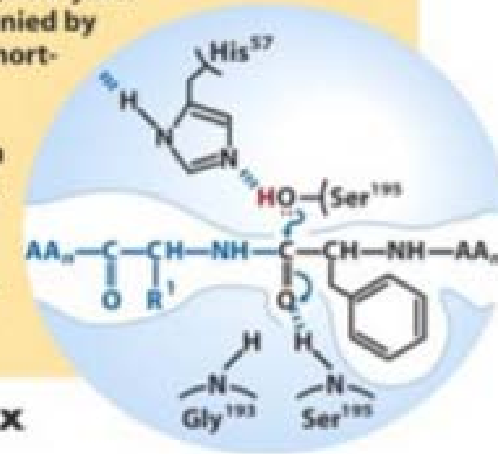


FIGURE 6–18 Structure of chymotrypsin. (PDB ID 7GCH) **(a)** A representation of primary structure, showing disulfide bonds and the amino acid residues crucial to catalysis. The protein consists of three polypeptide chains linked by disulfide bonds. (The numbering of residues in chymotrypsin, with "missing" residues 14, 15, 147, and 148, is explained in Fig. 6–33.) The active-site amino acid residues are grouped together in the three-dimensional structure.



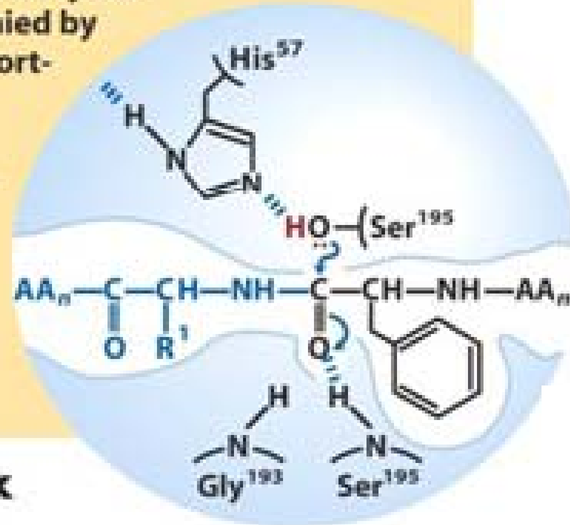
Interaction of Ser[195] and His[57] generates a strongly nucleophilic alkoxide ion on Ser[195]; the ion attacks the peptide carbonyl group, forming a tetrahedral acyl-enzyme. This is accom-panied by formation of a short-lived negative charge on the carbonyl oxygen of the substrate, which is stabilized by hydrogen bond-ing in the oxyanion hole.



ES complex

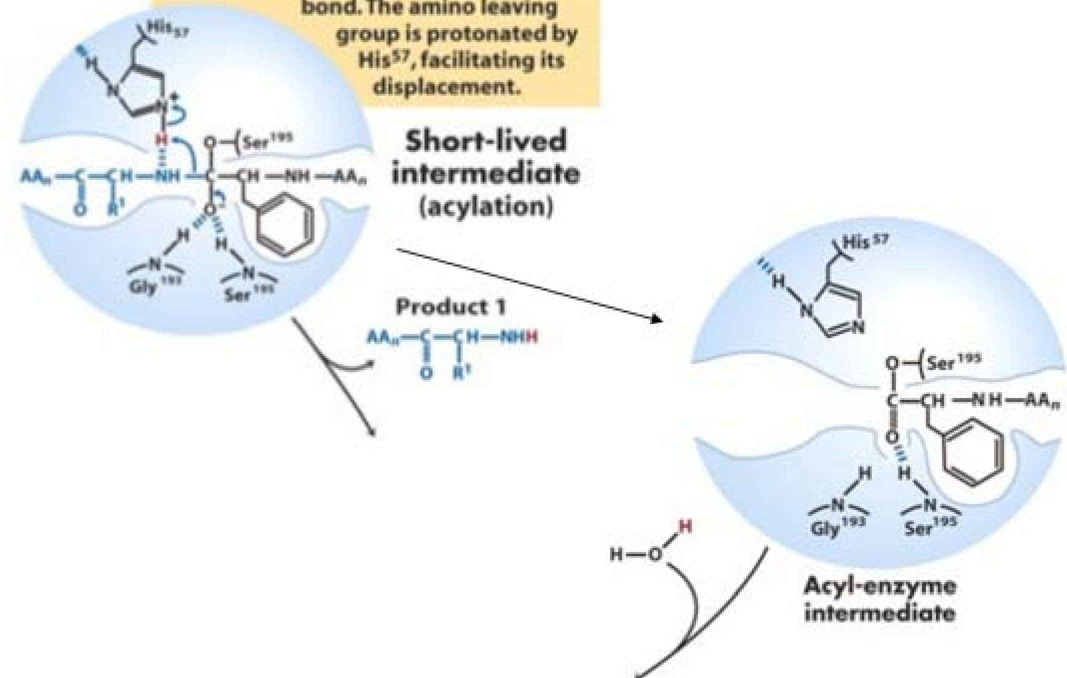
Chymotrypsin Mechanism

Interaction of Ser^{195} and His^{57} generates a strongly nucleophilic alkoxide ion on Ser^{195}; the ion attacks the peptide carbonyl group, forming a tetrahedral acyl-enzyme. This is accom-panied by formation of a short-lived negative charge on the carbonyl oxygen of the substrate, which is stabilized by hydrogen bond-ing in the oxyanion hole.

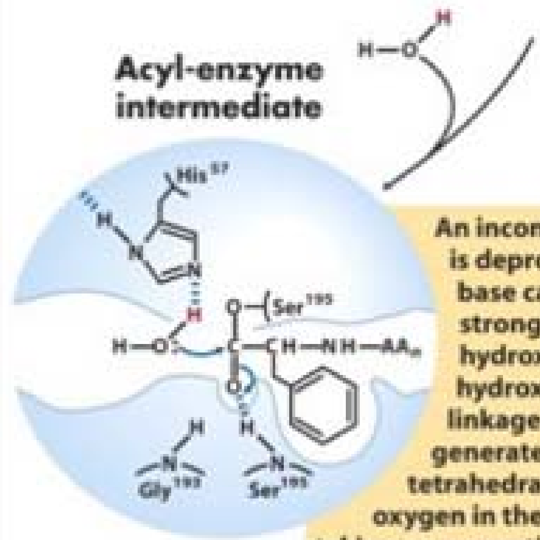


ES complex

Instability of the negative charge on the substrate carbonyl oxygen leads to collapse of the tetrahedral inter-mediate; 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 His^{57}, facilitating its displacement.

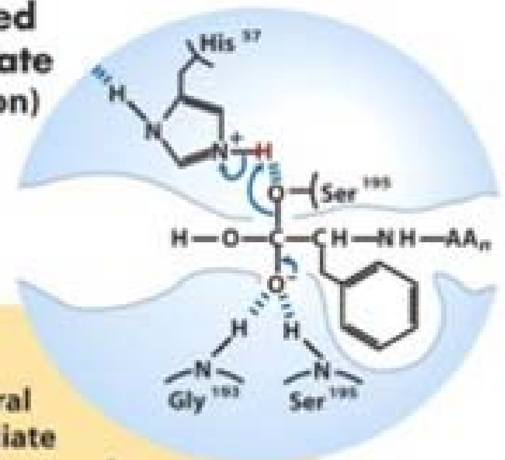


Acyl-enzyme intermediate



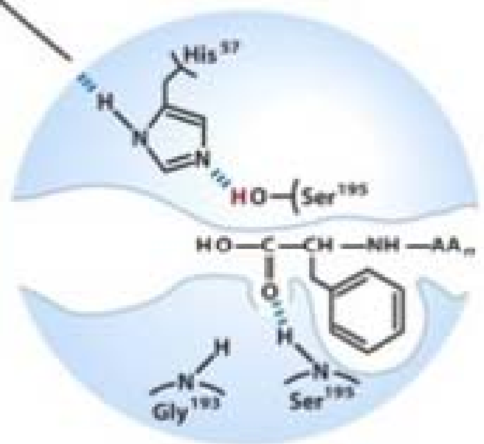
An incoming water molecule is deprotonated by general base catalysis, generating a strongly nucleophilic hydroxide ion. Attack of hydroxide on the ester linkage of the acyl-enzyme generates a second tetrahedral intermediate, with oxygen in the oxyanion hole again taking on a negative charge.

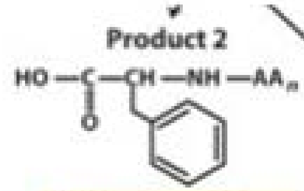
Short-lived intermediate (deacylation)



Collapse of the tetrahedral intermediate forms the second product, a carbohydrate anion, and displaces Ser^{195}.

Enzyme-product 2 complex





Diffusion of the second product from the active site regenerates free enzyme.