BIOCHEMISTRY: LS2101

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Enzyme, vitamins and coenzymes

Most Enzymes Are Proteins

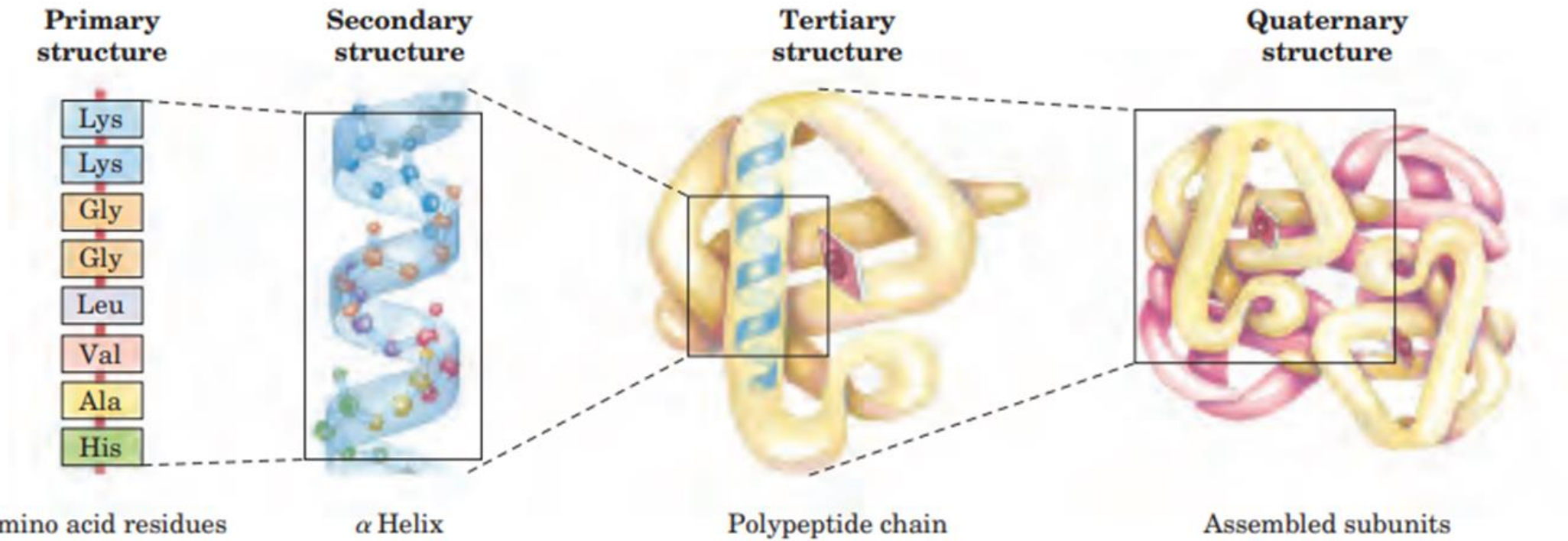


FIGURE 3–16 Levels of structure in proteins. The *primary structure* consists of a sequence of amino acids linked together by peptide bonds and includes any disulfide bonds. The resulting polypeptide can be coiled into units of *secondary structure,* such as an α helix. The helix is a part of the *tertiary structure* of the folded polypeptide, which is itself one of the subunits that make up the *quaternary structure* of the multisubunit protein, in this case hemoglobin.

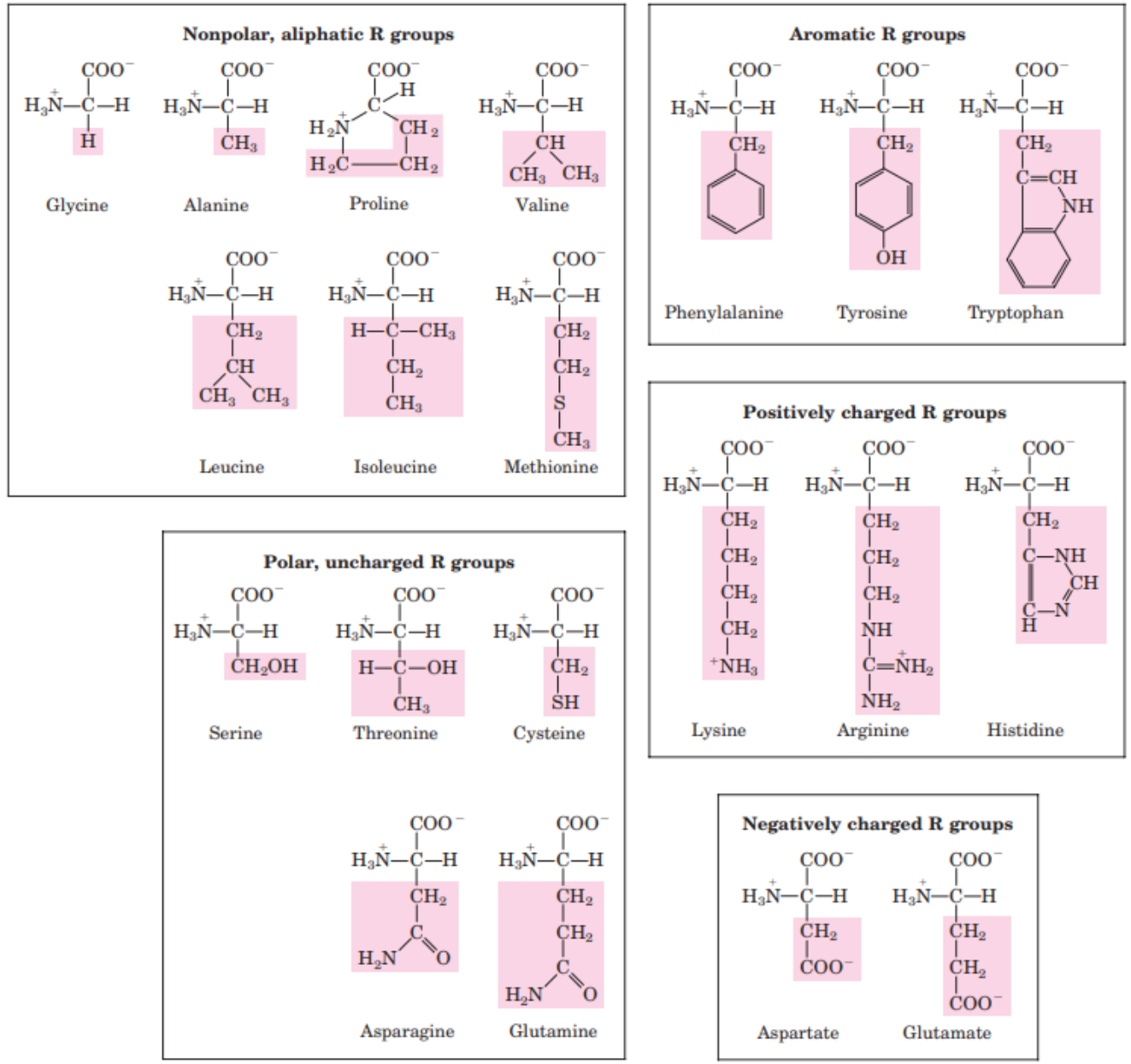


FIGURE 3–5 The 20 common amino acids of proteins. The structural formulas show the state of ionization that would predominate at pH 7.0. The unshaded portions are those common to all the amino acids; the portions shaded in red are the R groups. Although the R group of histidine is shown uncharged, its pK_a (see Table 3–1) is such that a small but significant fraction of these groups are positively charged at pH 7.0.

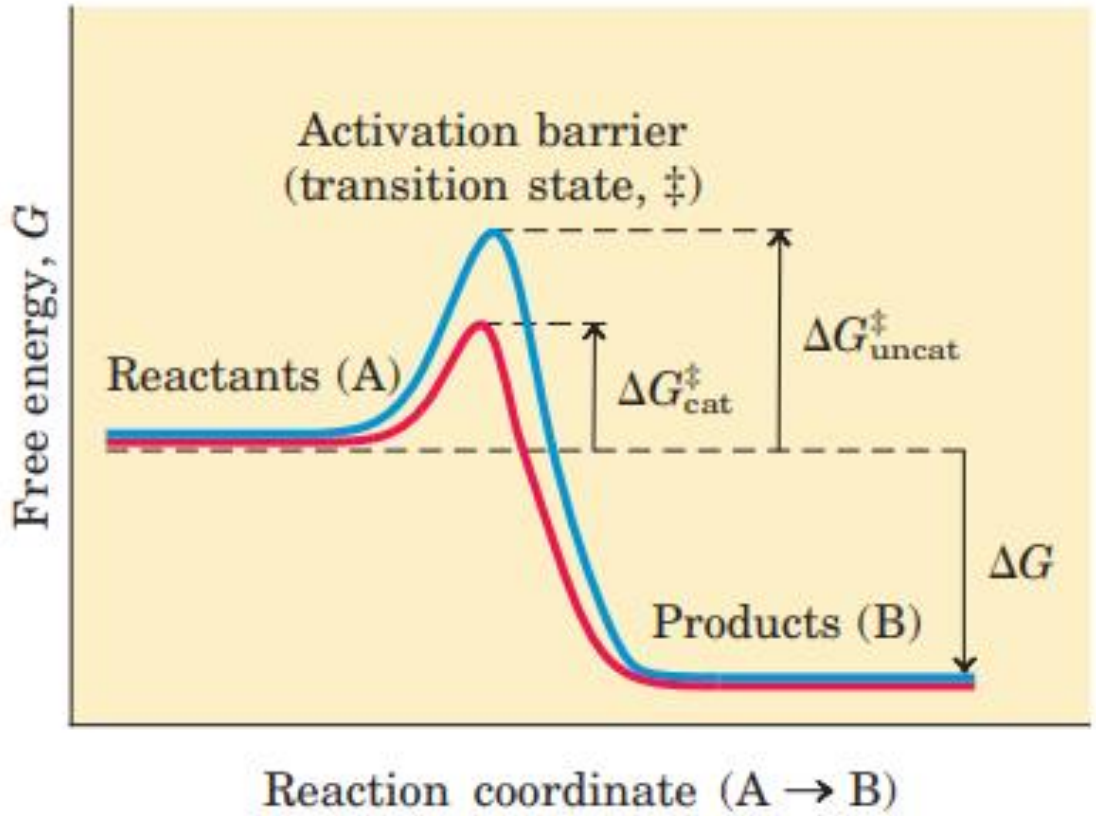


FIGURE 1–27 Energy changes during a chemical reaction. An activation barrier, representing the transition state, must be overcome in the conversion of reactants (A) into products (B), even though the products are more stable than the reactants, as indicated by a large, negative free-energy change (ΔG). The energy required to overcome the activation barrier is the activation energy ($\Delta G^{\ddagger}$). Enzymes catalyze reactions by lowering the activation barrier. They bind the transition-state intermediates tightly, and the binding energy of this interaction effectively reduces the activation energy from $\Delta G^{\ddagger}_{uncat}$ to $\Delta G^{\ddagger}_{cat}$. (Note that activation energy is *not* related to free-energy change, ΔG.)

How Enzymes Work

Enzymes Affect Reaction Rates, Not Equilibria

$$\mathrm{E} + \mathrm{S} \rightleftharpoons \mathrm{ES} \rightleftharpoons \mathrm{EP} \rightleftharpoons \mathrm{E} + \mathrm{P}$$

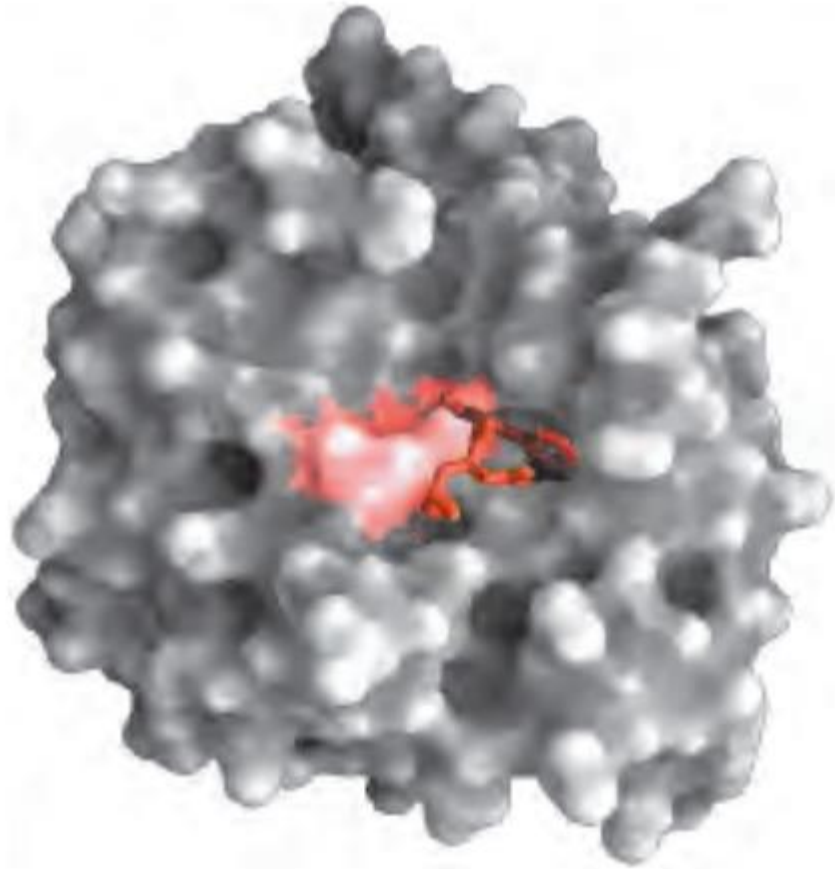


FIGURE 6–1 Binding of a substrate to an enzyme at the active site. The enzyme chymotrypsin, with bound substrate in red (PDB ID 7GCH). Some key active-site amino acid residues appear as a red splotch on the enzyme surface.

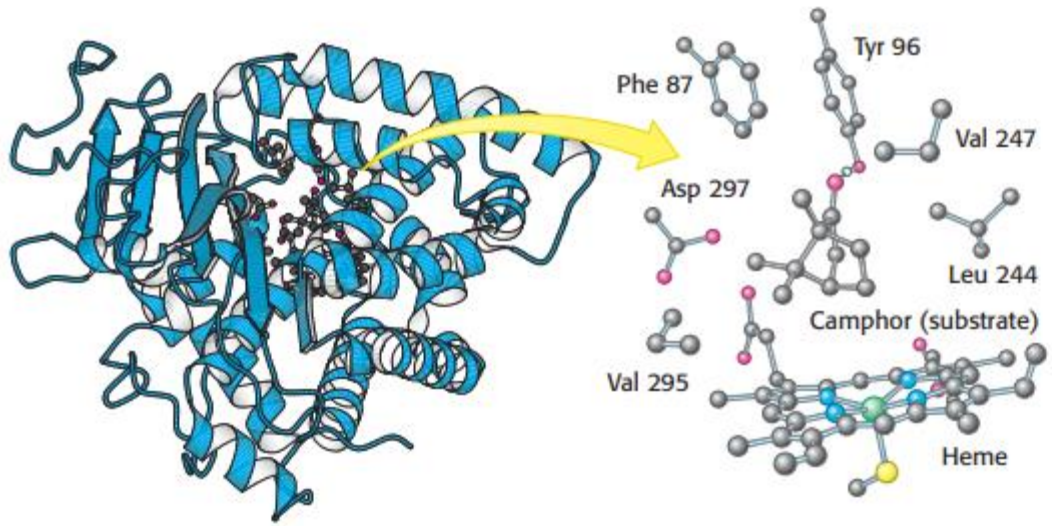


Figure 8.5 Structure of an enzyme–substrate complex. (Left) The enzyme cytochrome P450 is illustrated bound to its substrate camphor. (Right) *Notice* that, in the active site, the substrate is surrounded by residues from the enzyme. Note also the presence of a heme cofactor. [Drawn from 2CPP.pdb.]

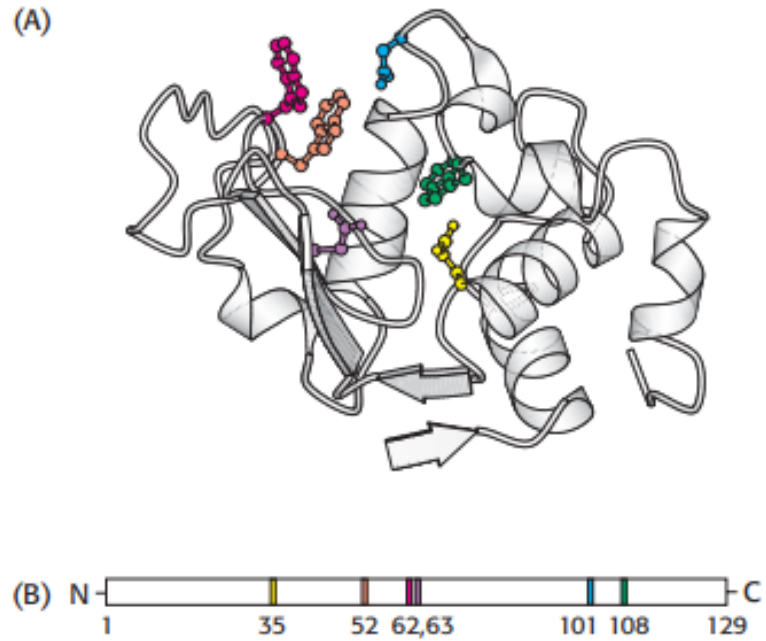


Figure 8.7 Active sites may include distant residues. (A) Ribbon diagram of the enzyme lysozyme with several components of the active site shown in color. (B) A schematic representation of the primary structure of lysozyme shows that the active site is composed of residues that come from different parts of the polypeptide chain. [Drawn from 6LYZ.pdb.]

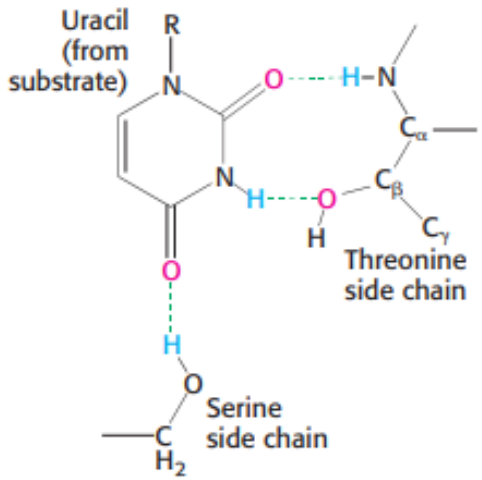


Figure 8.8 Hydrogen bonds between an enzyme and substrate. The enzyme ribonuclease forms hydrogen bonds with the uridine component of the substrate. [After F. M. Richards, H. W. Wyckoff, and N. Allewell. In *The Neurosciences: Second Study Program*, F. O. Schmidt, Ed. (Rockefeller University Press, 1970), p. 970.]

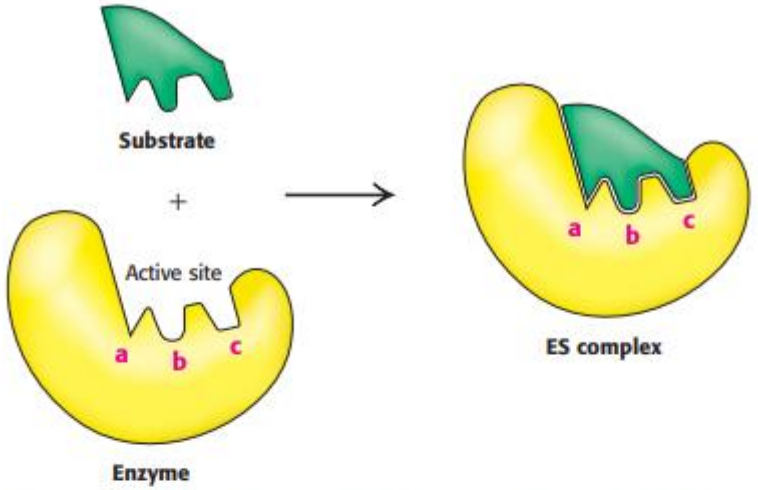


Figure 8.9 Lock-and-key model of enzyme–substrate binding. In this model, the active site of the unbound enzyme is complementary in shape to the substrate.

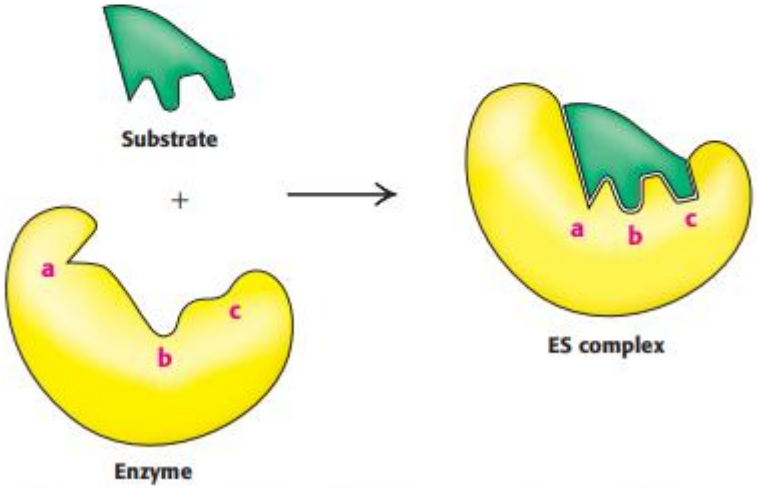


Figure 8.10 Induced-fit model of enzyme–substrate binding. In this model, the enzyme changes shape on substrate binding. The active site forms a shape complementary to the substrate only after the substrate has been bound.

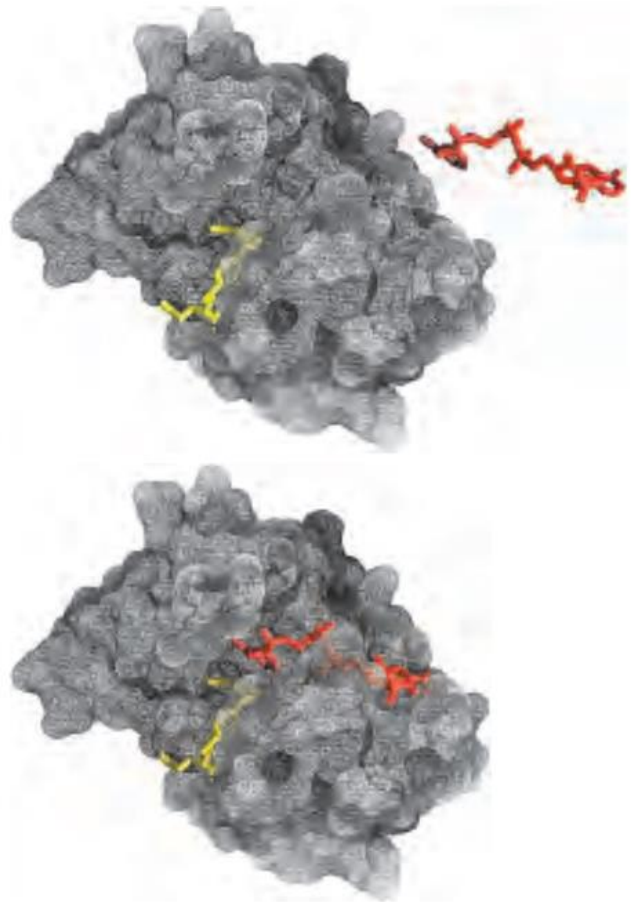


FIGURE 6–4 Complementary shapes of a substrate and its binding site on an enzyme. The enzyme dihydrofolate reductase with its substrate $NADP^+$ (red), unbound (top) and bound (bottom). Another bound substrate, tetrahydrofolate (yellow), is also visible (PDB ID 1RA2). The $NADP^+$ binds to a pocket that is complementary to it in shape and ionic properties. In reality, the complementarity between protein and ligand (in this case substrate) is rarely perfect, as we saw in Chapter 5. The interaction of a protein with a ligand often involves changes in the conformation of one or both molecules, a process called induced fit. This *lack* of perfect complementarity between enzyme and substrate (not evident in this figure) is important to enzymatic catalysis.

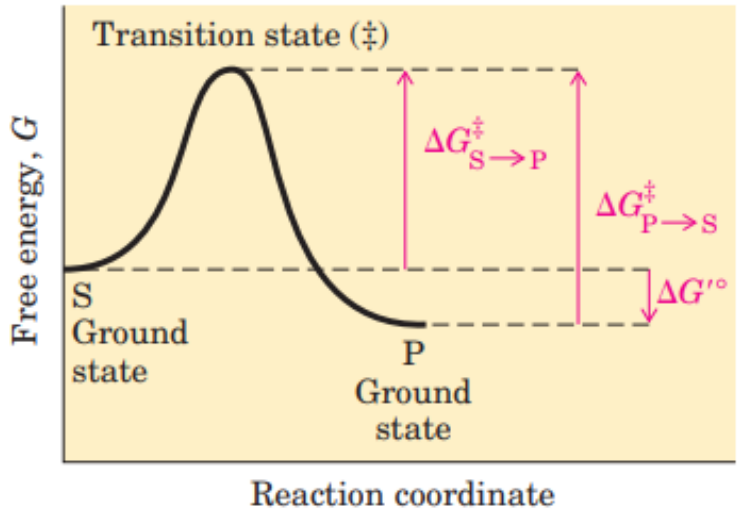


FIGURE 6–2 Reaction coordinate diagram for a chemical reaction. The free energy of the system is plotted against the progress of the reaction S ⟶ P. A diagram of this kind is a description of the energy changes during the reaction, and the horizontal axis (reaction coordinate) reflects the progressive chemical changes (e.g., bond breakage or formation) as S is converted to P. The activation energies, $\Delta G^{\ddagger}$, for the S ⟶ P and P ⟶ S reactions are indicated. $\Delta G'^{\circ}$ is the overall standard free-energy change in the direction S ⟶ P.

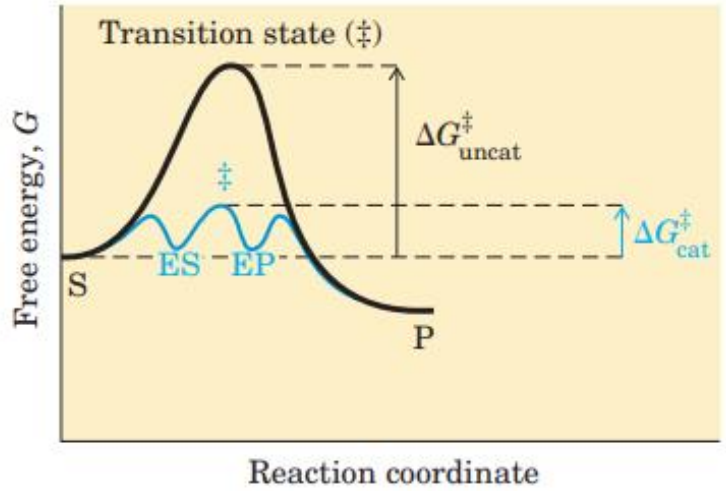


FIGURE 6–3 Reaction coordinate diagram comparing enzyme-catalyzed and uncatalyzed reactions. In the reaction S ⟶ P, the ES and EP intermediates occupy minima in the energy progress curve of the enzyme-catalyzed reaction. The terms $\Delta G^{\ddagger}_{uncat}$ and $\Delta G^{\ddagger}_{cat}$ correspond to the activation energy for the uncatalyzed reaction and the overall activation energy for the catalyzed reaction, respectively. The activation energy is lower when the enzyme catalyzes the reaction.

Acid-Base Catalysis

Covalent Catalysis

Metal Ion Catalysis

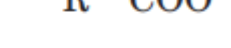
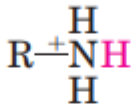
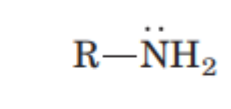
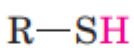
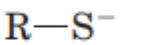
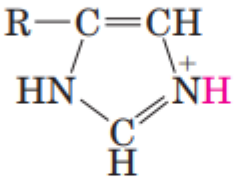
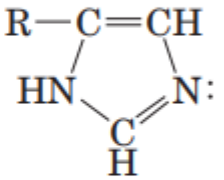
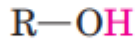
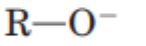
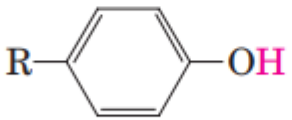
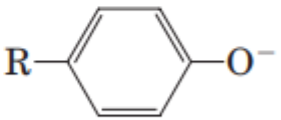
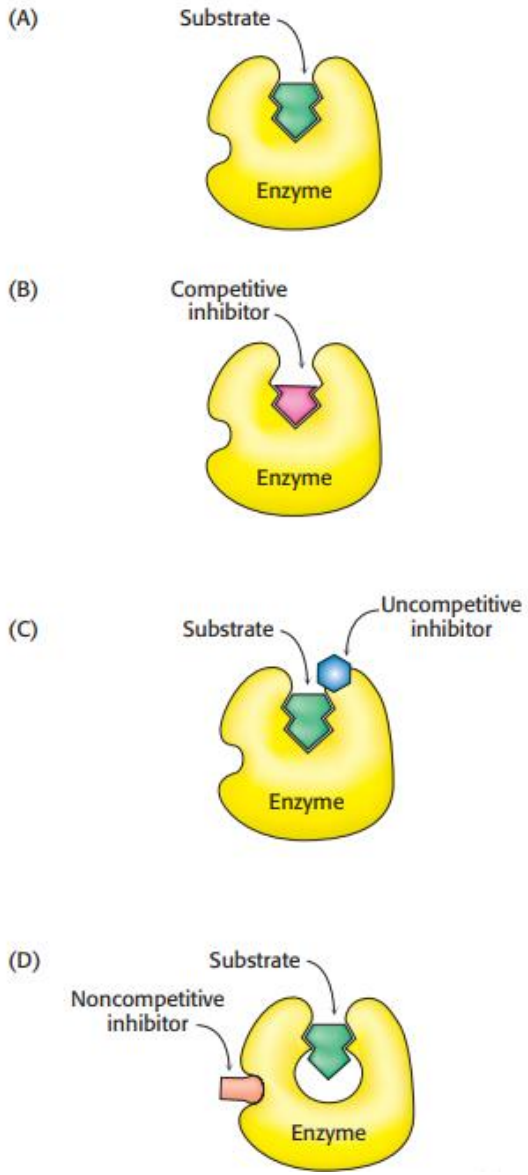
Amino acid residues	General acid form (proton donor)	General base form (proton acceptor)
Glu, Asp		
Lys, Arg		
Cys		
His		
Ser		
Tyr		

FIGURE 6–9 Amino acids in general acid-base catalysis. Many organic reactions are promoted by proton donors (general acids) or proton acceptors (general bases). The active sites of some enzymes contain amino acid functional groups, such as those shown here, that can participate in the catalytic process as proton donors or proton acceptors.

Enzyme Inhibitors



Types of Enzymes

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

Note: Most enzymes catalyze the transfer of electrons, atoms, or functional groups. They are therefore classified, given code numbers, and assigned names according to the type of transfer reaction, the group donor, and the group acceptor.

Many Enzymes require cofactors for activity

Apoenzyme + Cofactor = Holoenzyme

Cofactors can be subdivided into two groups:

(1) metals and

(2) (2) small organic molecules called coenzymes

A coenzyme or metal ion that is very tightly or even covalently bound to the enzyme protein is called a **prosthetic group**

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)

Which Vitamins Are Coenzymes?

All of the water-soluble vitamins and two of the fat-soluble vitamins, A and K, function as cofactors or coenzymes.

Coenzymes participate in numerous biochemical reactions involving energy release or catabolism, as well as the accompanying anabolic reactions.

In addition, vitamin cofactors are critical for processes involved in proper vision, blood coagulation, hormone production, and the integrity of collagen, a protein found in bones.

Water - Soluble Vitamins

1. Vitamin B1 - thiamine
    - Converted to thiamine pyrophosphate coenzyme
    - Acts by nucleophilic attack on C = O
    - Permits C - C bond cleavage and formation
2. Vitamin B2 - riboflavin
    - Incorperated in FMN (Flavin MonoNucleotide) and FAD (Flavin Adenine Dinucleotide) coenzymes
    - Acts in Redox Reactions
    - Can accept and donate 2 e^- and 2 H^+
3. Vitamin B6 - pyridoxal (-ol, -amine)
    - Converted to pyridoxal phosphate coenzyme
    - Acts by forming Schiff base with -NH_2 of substrate
    - Permits cleavage of C - C, C - O, C - S, C - H, and C - N bonds in area of attachment
    - Very versatile, but amino group needed in substrate
4. Vitamin B12 - cyanocobalamin
    - Coordination complex of Co+ in a corrin ring
    - Converted to 5'-deoxyadenosylcobalamin coenzyme
    - Acts to exchange -H and another group on adjacent C's
    - Transfers Methyl group from Me-THF to homocycteine to synthesize Met

Water - Soluble Vitamins

1. Nicotinamide (NIacin, nicotinic ACid vimintIN)
   - Converted to NAD and NADP coenzymes
   - Functions in Redox Reactions by accepting and donating $2e^-$ and 1 H^+
2. Pantothenic Acid
   - Converted to Phospho-form coenzyme
   - Activates Acyl moieties for condensation and enolization
3. Biotin (vitamin H)
   - Coenzyme
   - Incorperates CO_2 in *B* carboxylation reactions
   - "Binds the egg white glycoprotein avidin with $K_d = 10^{-15}$ M
   - Vitamins #5-7 are part of the B complex
4. Vitamin C - ascorbic acid ("antiscorbutic") ("anti-scurvy")
   - involved in hydroxylation of proline in collagen, therefore important for wound healing
   - Prevent common cold? How?
5. Folic Acid - pteroylglutamic acid
   - Converted to tetrahydrofolic acid (THF)
   - Carries C-1 groups at all oxidation levels
   - More properly considered a substrate than a coenzyme

Lipid-Soluble Vitamins

1. Vitamin A - trans-retinol
    - Converted to visual pigment cis-retinal
    - (Also contributes to animal growth and development - How? Retinoic acid?)
2. Vitamin D - 7-dehydrocholesterol
    - Converted to hormone 1,25-Dihydroxy vitamin D3
    - (Stimulates gene expression to regulate calcium metabolism)
3. Vitamin E - *a*-tocopherol
    - Antioxidant
    - Prevents sterility - in rats, How?
4. Vitamin K - phylloquinone in plants; menaquinone in aminals and bacteria
    - Cofactor for formation of *gamma*-carboxyglutamic acid in serine proteases of blood clotting cascade, and some other Ca^{++} binding proteins.

Non-Vitamin Coenzyme

1.Lipoic Acid

- (Isolated in 1951 by Lester Reed at UT-Austin - he obtained 30mg from 10 tons of liver residue.)
- Relays electrons and acetyl groups between catalytic subunits of pyruvate dehydrogenase complex.

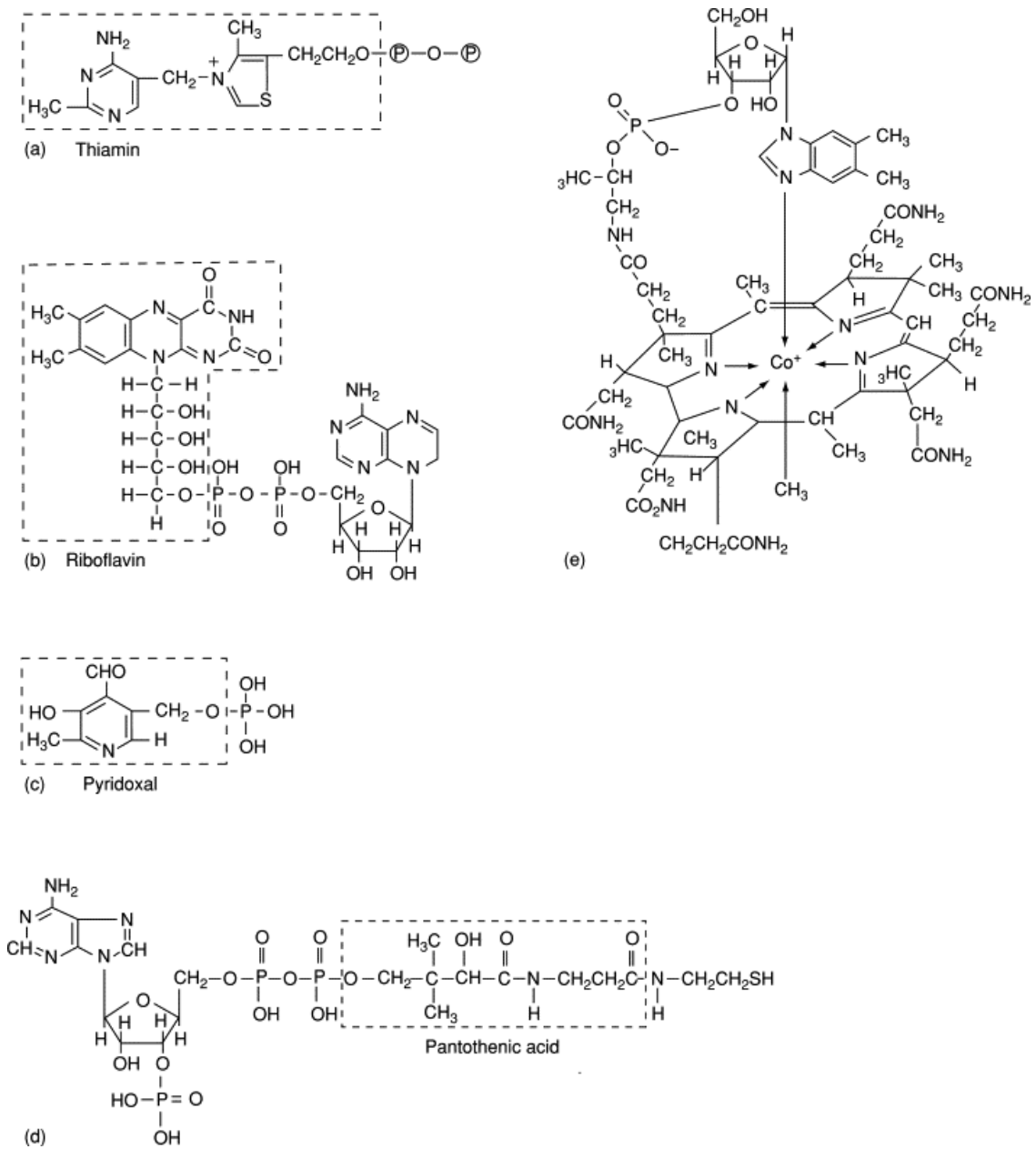


Figure. Selected examples of vitamins as coenzymes: (a) thiamin pyrophosphate; (b) flavin mononucleotide; (c) pyridoxal phosphate; (d) coenzyme A; and (e) methylcobalamin or coenzyme B_{12}. Reproduced from Coenzymes, *Encyclopaedia of Food Science, Food Technology and Nutrition*, Macrae R, Robinson RK, and Sadler MJ (eds), 1993, Academic Press.