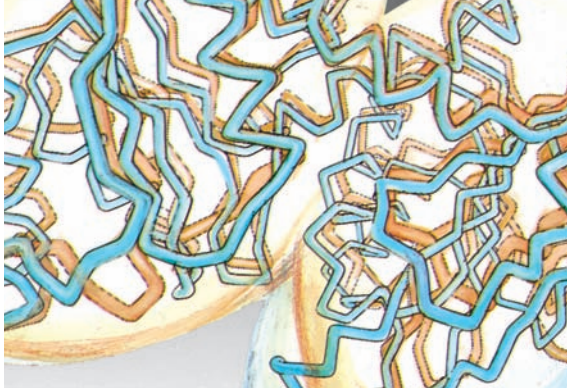


Introduction to Enzymes

CHAPTER 13

The enormous variety of biochemical reactions that comprise life are nearly all mediated by a series of remarkable biological catalysts known as **enzymes.** Although enzymes are subject to the same laws of nature that govern the behavior of other substances, they differ from ordinary chemical catalysts in several important respects:

1. Higher reaction rates: The rates of enzymatically catalyzed reactions are typically factors of 10^6 to 10^{12} greater than those of the corresponding uncatalyzed reactions and are at least several orders of magnitude greater than those of the corresponding chemically catalyzed reactions.

2. Milder reaction conditions: Enzymatically catalyzed reactions occur under relatively mild conditions: temperatures below 100°C, atmospheric pressure, and nearly neutral pH's. In contrast, efficient chemical catalysis often requires elevated temperatures and pressures as well as extremes of pH.

3. Greater reaction specificity: Enzymes have a vastly greater degree of specificity with respect to the identities of both their **substrates** (reactants) and their products than do chemical catalysts; that is, enzymatic reactions rarely have side products. For example, in the enzymatic synthesis of proteins on ribosomes (Section 32-3), polypeptides consisting of well over 1000 amino acid residues are made all but error free. Yet, in the chemical synthesis of polypeptides, side reactions and incomplete reactions presently limit the lengths of polypeptides that can be accurately produced in reasonable yields to ~200 residues (Section 7-5B).

4. Capacity for control: The catalytic activities of many enzymes vary in response to the concentrations of substances other than their substrates and products. The mechanisms of these control processes include allosteric control, covalent modification of enzymes, and variation of the amounts of enzymes synthesized.

Consideration of these remarkable catalytic properties of enzymes leads to one of the central questions of biochemistry: *How do enzymes work?* We address this issue in this part of the text.

In this chapter, following a historical review, we commence our study of enzymes with a discussion of two clear instances of enzyme action: one that illustrates how enzyme specificity is manifested, and a second that exemplifies the control of enzyme activity. These are by no means exhaustive treatments but are intended to highlight these all-important aspects of enzyme mechanism. We shall encounter numerous other examples of these phenomena in our study of metabolism (Chapters 16–28). These two expositions are interspersed with a consideration of the roles of enzymatic cofactors. The chapter ends with a short synopsis of enzyme nomenclature. In Chapter 14 we take up the formalism of enzyme kinetics because the study of the rates of enzymatically catalyzed reactions provides indispensable mechanistic information. Finally, Chapter 15 is a general discussion of the catalytic mechanisms employed by enzymes, followed by an examination of the mechanisms of several specific enzymes.

1 HISTORICAL PERSPECTIVE

The early history of **enzymology,** the study of enzymes, is largely that of biochemistry itself; these disciplines evolved together from nineteenth century investigations of fermentation and digestion. Research on fermentation is widely considered to have begun in 1810 with Joseph Gay-Lussac's determination that ethanol and CO_2 are the principal products of sugar decomposition by yeast. In 1835, Jacob Berzelius, in the first general theory of chemical catalysis, pointed out that an extract of malt known as **diastase** (now known to contain the enzyme **α-amylase;** Section 11-2Db) catalyzes the hydrolysis of starch more efficiently than does sulfuric acid. Yet, despite the ability of mineral acids to mimic the effect of diastase, it was the inability to reproduce most other biochemical reactions in the laboratory that led Louis Pasteur, in the mid-nineteenth century, to propose that the processes of fermentation could only occur in living cells. Thus, as was common in his era, Pasteur assumed that living systems were endowed with a "vital force" that permitted them to evade the laws of nature governing inanimate matter. Others, however, notably Justus von Liebig, argued that biological processes are caused by

the action of chemical substances that were then known as "ferments." Indeed, the name "enzyme" (Greek: *en,* in + *zyme,* yeast) was coined in 1878 by Wilhelm Friedrich Kühne in an effort to emphasize that there is something *in* yeast, as opposed to the yeast itself, that catalyzes the reactions of fermentation. Nevertheless, it was not until 1897 that Eduard Buchner obtained a cell-free yeast extract that could carry out the synthesis of ethanol from glucose (**alcoholic fermentation;** Section 17-3B).

Emil Fischer's discovery, in 1894, that glycolytic enzymes can distinguish between stereoisomeric sugars led to the formulation of his **lock-and-key hypothesis:** *The specificity of an enzyme (the lock) for its substrate (the key) arises from their geometrically complementary shapes.* Yet the chemical composition of enzymes was not firmly established until well into the twentieth century. In 1926, James Sumner, who crystallized the first enzyme, jack bean **urease,** which catalyzes the hydrolysis of urea to NH_3 and CO_2, demonstrated that these crystals consist of protein. Since Sumner's preparations were somewhat impure, however, the protein nature of enzymes was not generally accepted until the mid-1930s, when John Northrop and Moses Kunitz showed that there is a direct correlation between the enzymatic activities of crystalline pepsin, trypsin, and chymotrypsin and the amounts of protein present. Enzymological experience since then has amply demonstrated that enzymes are proteins (although it has more recently been shown that RNA can also have catalytic properties; Section 31-4Ae).

Although the subject of enzymology has a long history, most of our understanding of the nature and functions of enzymes is a product of the last 60 years. Only with the advent of modern techniques for separation and analysis (Chapter 6) has the isolation and characterization of an enzyme become less than a monumental task. It was not until 1963 that the first amino acid sequence of an enzyme, that of **bovine pancreatic ribonuclease A** (Section 15-1Ab), was reported in its entirety, and not until 1965 that the first X-ray structure of an enzyme, that of hen egg white **lysozyme** (Section 15-2A), was elucidated. In the years since then, tens of thousands of enzymes have been purified and characterized to at least some extent, and the pace of this endeavor is rapidly accelerating.

2 SUBSTRATE SPECIFICITY

The noncovalent forces through which substrates and other molecules bind to enzymes are similar in character to the forces that dictate the conformations of the proteins themselves (Section 8-4): Both involve van der Waals, electrostatic, hydrogen bonding, and hydrophobic interactions. In general, a substrate-binding site consists of an indentation or cleft on the surface of an enzyme molecule that is complementary in shape to the substrate (geometric complementarity). Moreover, the amino acid residues that form the binding site are arranged to interact specifically with the substrate in an attractive manner (electronic complementarity; Fig. 13-1). Molecules that differ in shape or functional group distribution from the substrate cannot productively bind to the enzyme; that is, they cannot form enzyme–substrate complexes that lead to the formation of products. The substrate-binding site may, in accordance with the lock-and-key hypothesis, exist in the absence of bound substrate or it may, as suggested by the induced-fit hypothesis (Section 10-4C), form about the substrate as it binds to the enzyme. *X-ray studies indicate that the substrate-binding sites of most enzymes are largely preformed but that most of them exhibit at least some degree of induced fit on binding substrate.*

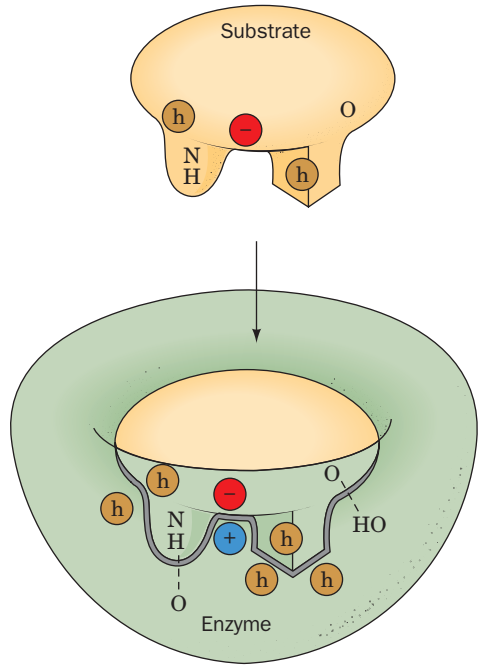


Figure 13-1 **An enzyme–substrate complex illustrating both the geometric and the physical complementarity between enzymes and substrates.** Hydrophobic groups are represented by an h in a brown circle, and dashed lines represent hydrogen bonds.

A. *Stereospecificity*

Enzymes are highly specific both in binding chiral substrates and in catalyzing their reactions. This **stereospecificity** arises because enzymes, by virtue of their inherent chirality (proteins consist of only L-amino acids), form asymmetric active sites. For example, trypsin readily hydrolyzes polypeptides composed of L-amino acids but not those consisting of D-amino acids. Likewise, the enzymes involved with glucose metabolism (Section 17-2) are specific for D-glucose residues.

Enzymes are absolutely stereospecific in the reactions they catalyze. This was strikingly demonstrated for the case of **yeast alcohol dehydrogenase (YADH)** by Frank Westheimer and Birgit Vennesland. Alcohol dehydrogenase catalyzes the interconversion of ethanol and acetaldehyde according to the reaction

$$\underset{\textbf{Ethanol}}{CH_3CH_2OH} + NAD^+ \underset{}{\overset{\text{YADH}}{\rightleftharpoons}} \underset{\textbf{Acetaldehyde}}{CH_3\overset{\overset{O}{\|}}{C}H} + NADH + H^+$$

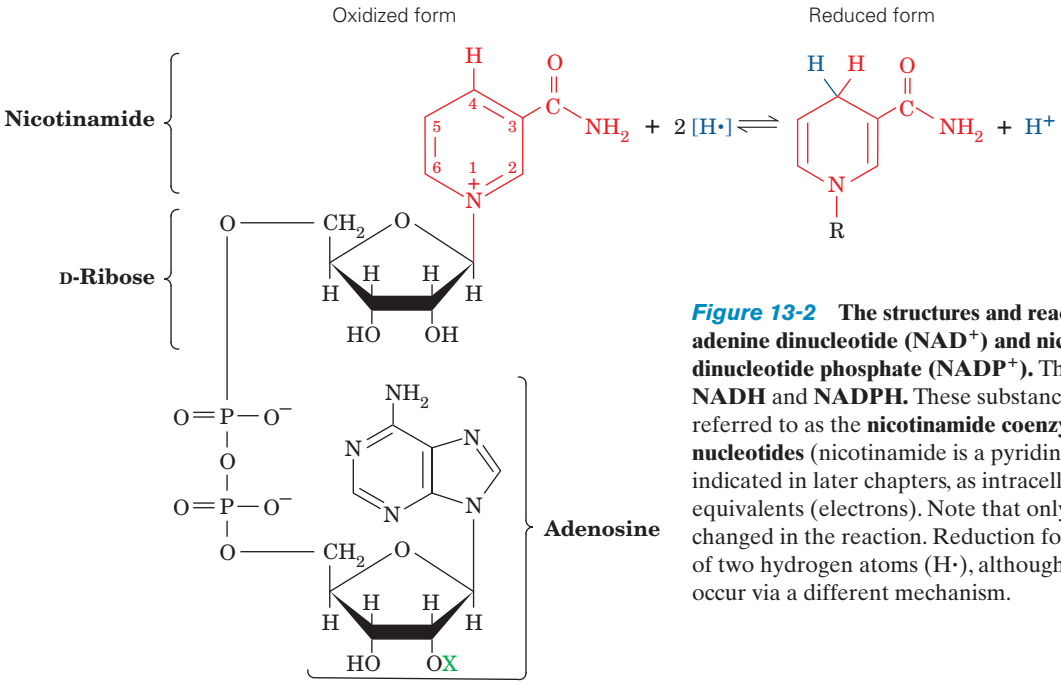
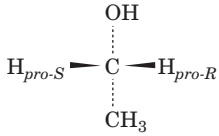


Figure 13-2 **The structures and reaction of nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$).** Their reduced forms are **NADH** and **NADPH.** These substances, which are collectively referred to as the **nicotinamide coenzymes** or **pyridine nucleotides** (nicotinamide is a pyridine derivative), function, as is indicated in later chapters, as intracellular carriers of reducing equivalents (electrons). Note that only the nicotinamide ring is changed in the reaction. Reduction formally involves the transfer of two hydrogen atoms (H·), although the actual reduction may occur via a different mechanism.

X = H **Nicotinamide adenine dinucleotide (NAD^+)**
X = PO_3^{2-} **Nicotinamide adenine dinucleotide phosphate ($NADP^+$)**

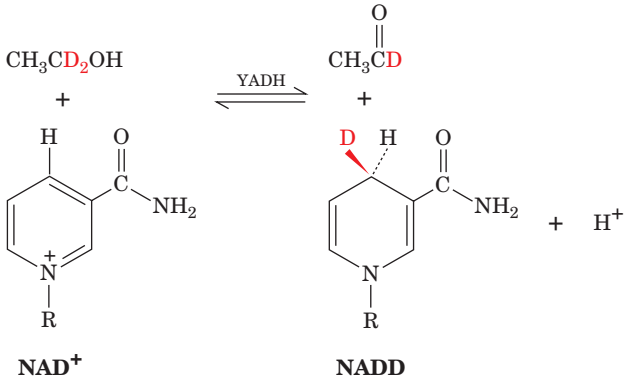
The structures of **NAD^+** and **NADH** are presented in Fig. 13-2. Ethanol, it will be recalled, is a prochiral molecule (see Section 4-2Ca for a discussion of prochirality):



Ethanol's two methylene H atoms may be distinguished if *the molecule is held in some sort of asymmetric jig* (Fig. 13-3). *The substrate-binding sites of enzymes are, of course, just such jigs because they immobilize the reacting groups of the substrate on the enzyme surface.*

Westheimer and Vennesland elucidated the stereospecific nature of the YADH reaction through the following series of experiments:

1. If the YADH reaction is carried out with deuterated ethanol, the product NADH is deuterated:



Note that the nicotinamide ring of NAD^+ is also prochiral.

2. On isolating this NADD and using it in the reverse reaction to reduce normal acetaldehyde, the deuterium is

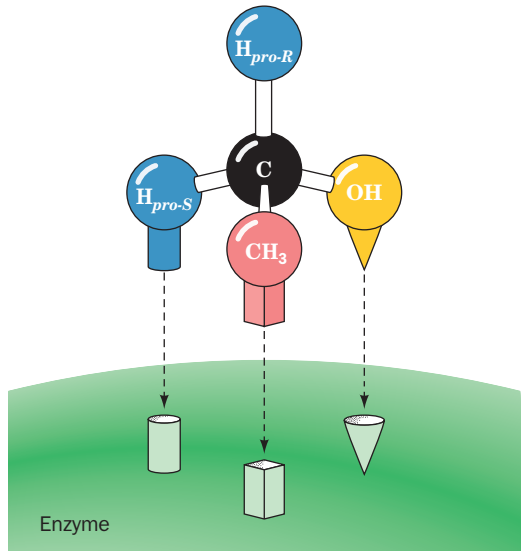
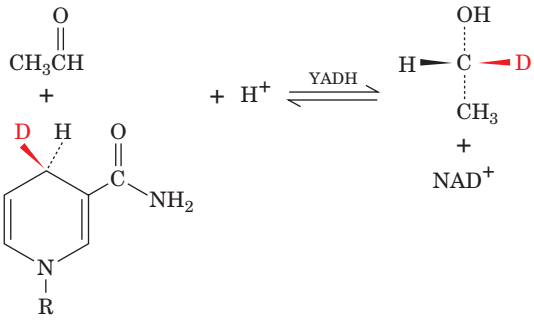
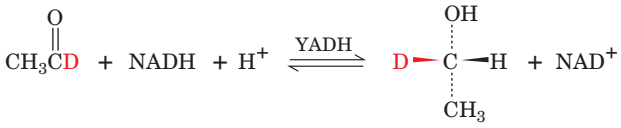


Figure 13-3 **Prochiral differentiation.** The specific attachment of a prochiral center to an enzyme binding site permits the enzyme to differentiate between prochiral groups. Note: If it were possible, the binding of the prochiral molecule's mirror image to the same three sites from the underside of the binding site as pictured here would still result in $H_{pro\text{-}R}$ pointing toward a different position.

quantitatively transferred from the NADD to the acetaldehyde to form the product ethanol:



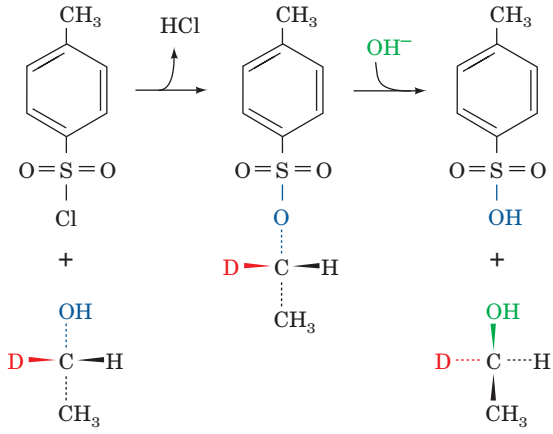
3. If the enantiomer of the foregoing CH_3CHDOH is made as follows:



none of the deuterium is transferred from the product ethanol to NAD^+ in the reverse reaction.

4. If, however, this ethanol is converted to its tosylate and then inverted by S_N2 hydrolysis to yield the enantiomeric ethanol,

***p*-Toluenesulfonyl chloride (tosyl chloride)**



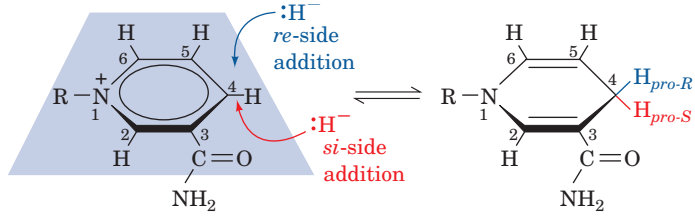
the deuterium is again quantitatively transferred to NAD^+ in the YADH reaction.

The foregoing observations, in addition to showing that there is direct hydrogen transfer in the YADH reaction (Experiments 1 and 2), indicate that the enzyme distinguishes between the *pro-S* and *pro-R* hydrogens of ethanol as well as the *si* and *re* faces of the nicotinamide ring of NAD^+ (Experiments 2–4). It was later demonstrated, by stereospecific syntheses, that YADH transfers the *pro-R* hydrogen of ethanol to the *re* face of the nicotinamide ring of NAD^+ as is drawn in the preceding diagrams.

The stereospecificity of YADH is by no means unusual. As we consider biochemical reactions we shall find that nearly all enzymes that participate in chiral reactions are absolutely stereospecific.

a. Stereospecificity in the NADH-Dependent Dehydrogenases May Have Functional Significance

In our exploration of metabolism, we shall encounter numerous species of NADH-dependent dehydrogenases that function to reduce (or oxidize) a great variety of substrates. These various dehydrogenases are more or less equally distributed between those transferring the *pro-R* (*re*-side) and the *pro-S* (*si*-side) hydrogens at C4 of NADH (also known as A-side and B-side transfers).



Yet, despite the fact that *si*- and *re*-side hydrogen transfers to or from the nicotinamide ring yield chemically identical products, a particular specificity of transfer is rigidly maintained within classes of dehydrogenases catalyzing similar reactions in different organisms. Indeed, dehydrogenases that catalyze reactions whose equilibrium constants with their natural substrates in the direction of reduction are $<10^{-12}\ M$ almost always transfer the nicotinamide's *pro-R* hydrogen, whereas those with equilibrium constants $>10^{-10}\ M$ generally transfer the *pro-S* hydrogen. Why has evolution so assiduously maintained this stereospecificity? Is it simply the result of a historical accident or does it serve some physiological function?

The NADH hydrogen transferred in a given enzymatic reaction is almost certainly that on the side of the nicotinamide ring facing the substrate. It was therefore widely assumed that the stereospecificity in any given class of dehydrogenases simply arose through a random choice made early in evolutionary history. Once made, this choice became "locked in," because flipping a nicotinamide ring about its glycosidic bond in NADH would result, it was presumed, in its carboxamide group obstructing catalytically essential residues on the enzyme.

In an effort to shed light on this matter, Steven Benner mutated YADH in a manner that the X-ray structure of the closely similar enzyme horse **liver alcohol dehydrogenase (LADH)** suggests permits the *si* face of nicotinamide to bind to the enzyme without interfering with catalysis. The resulting mutant enzyme (Leu 182 → Ala) makes one stereochemical "mistake" every 850,000 turnovers versus one mistake every 7 billion turnovers for wild-type (unmutated) YADH. This 8000-fold decrease in stereospecificity indicates that at least some of the side chains responsible for YADH's stereospecificity are not essential for catalysis and hence strengthens the argument that stereospecificity in the dehydrogenases has functional significance.

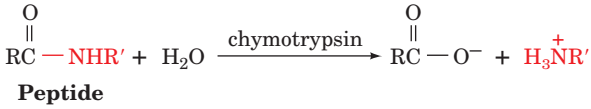
B. *Geometric Specificity*

The stereospecificity of enzymes is not particularly surprising in light of the complementarity of an enzymatic binding site for its substrate. A substrate of the wrong chirality will

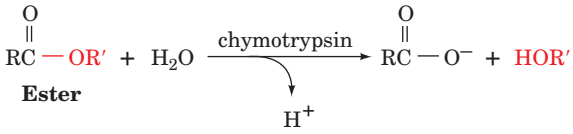
not fit into an enzymatic binding site for much the same reasons that you cannot fit your right hand into your left glove. *In addition to their stereospecificity, however, most enzymes are quite selective about the identities of the chemical groups on their substrates.* Indeed, such **geometric specificity** is a more stringent requirement than is stereospecificity. After all, your left glove will more or less fit left hands that have somewhat different sizes and shapes than your own.

Enzymes vary considerably in their degree of geometric specificity. A few enzymes are absolutely specific for only one compound. Most enzymes, however, catalyze the reactions of a small range of related compounds. For example, YADH catalyzes the oxidation of small primary and secondary alcohols to their corresponding aldehydes or ketones but none so efficiently as that of ethanol. Even methanol and isopropanol, which differ from ethanol only by the deletion or addition of a CH_2 group, are oxidized by YADH at rates that are, respectively, 25-fold and 2.5-fold slower than that for ethanol. Similarly, $NADP^+$, which differs from NAD^+ only by the addition of a phosphoryl group at the 2′ position of its adenosine ribose group (Fig. 13-2), does not bind to YADH. On the other hand, there are many enzymes that bind $NADP^+$ but not NAD^+.

Some enzymes, particularly digestive enzymes, are so permissive in their ranges of acceptable substrates that their geometric specificities are more accurately described as preferences. Carboxypeptidase A, for example, catalyzes the hydrolysis of C-terminal peptide bonds to all residues except Arg, Lys, and Pro if the preceding residue is not Pro (Table 7-1). However, the rate of this enzymatic reaction varies with the identities of the residues in the vicinity of the C-terminus of the polypeptide (see Fig. 7-5). Some enzymes are not even very specific in the type of reaction they catalyze. Thus chymotrypsin, in addition to its ability to mediate peptide bond hydrolysis, also catalyzes ester bond hydrolysis.



Peptide



Ester

Moreover, the acyl group acceptor in the chymotrypsin reaction need not be water; amino acids, alcohols, or ammonia can also act in this capacity. You should realize, however, that such permissiveness is much more the exception than the rule. Indeed, most intracellular enzymes function *in vivo* (in the cell) to catalyze a particular reaction on a specific substrate.

3 COENZYMES

Enzymes catalyze a wide variety of chemical reactions. Their functional groups can facilely participate in acid–base reactions, form certain types of transient covalent bonds, and take part in charge–charge interactions (Section 15-1). They are, however, less suitable for catalyzing oxidation–reduction reactions and many types of group-transfer processes. Although enzymes catalyze such reactions, they mainly do so in association with small molecule **cofactors,** which essentially act as the enzymes' "chemical teeth."

Cofactors may be metal ions, such as the Zn^{2+} required for the catalytic activity of carboxypeptidase A, or organic molecules known as **coenzymes,** such as the NAD^+ in YADH (Section 13-2A). Some cofactors, for instance NAD^+, are but transiently associated with a given enzyme molecule, so that, in effect, they function as cosubstrates. Other cofactors, known as **prosthetic groups,** are essentially permanently associated with their protein, often by covalent bonds. For example, the heme prosthetic group of hemoglobin is tightly bound to its protein through extensive hydrophobic and hydrogen bonding interactions together with a covalent bond between the heme Fe^{2+} ion and His F8 (Sections 10-1A and 10-2B).

Coenzymes are chemically changed by the enzymatic reactions in which they participate. Thus, in order to complete the catalytic cycle, the coenzyme must be returned to its original state. For prosthetic groups, this can occur only in a separate phase of the enzymatic reaction sequence. For transiently bound coenzymes, such as NAD^+, however, the regeneration reaction may be catalyzed by a different enzyme.

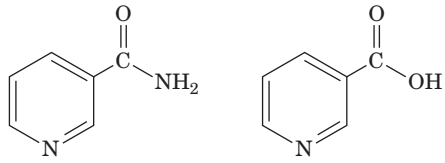
A catalytically active enzyme–cofactor complex is called a **holoenzyme** (Greek: *holos*, whole). The enzymatically inactive protein resulting from the removal of a holoenzyme's cofactor is referred to as an **apoenzyme** (Greek: *apo,* away); that is,

$$\text{Apoenzyme } (\textit{inactive}) + \text{cofactor} \rightleftharpoons \text{holoenzyme } (\textit{active})$$

Table 13-1 lists the most common coenzymes together with the types of reactions in which they participate. We shall

Table 13-1 **The Common Coenzymes**

Coenzyme	Reaction Mediated	Section Discussed
Biotin	Carboxylation	23-1A
Cobalamin (B_{12}) coenzymes	Alkylation	25-2E
Coenzyme A	Acyl transfer	21-2A
Flavin coenzymes	Oxidation–reduction	16-2C
Lipoic acid	Acyl transfer	21-2A
Nicotinamide coenzymes	Oxidation–reduction	13-2A
Pyridoxal phosphate	Amino group transfer	26-1A
Tetrahydrofolate	One-carbon group transfer	26-4D
Thiamine pyrophosphate	Aldehyde transfer	17-3B



Nicotinamide (niacinamide) **Nicotinic acid (niacin)**

Figure 13-4 **Structures of nicotinamide and nicotinic acid.** These vitamins form the redox-active components of the nicotinamide coenzymes NAD^+ and $NADP^+$ (compare with Fig. 13-2).

describe the structures of these substances and their reaction mechanisms in the appropriate sections of the textbook.

a. Many Vitamins Are Coenzyme Precursors

Many organisms are unable to synthesize certain portions of essential cofactors and therefore these substances must be present in the organism's diet; thus they are **vitamins.** In fact, many coenzymes were discovered as growth factors for microorganisms or substances that cure nutritional deficiency diseases in humans and animals. For example, the NAD^+ component **nicotinamide** (alternatively known as **niacinamide**) or its carboxylic acid analog **nicotinic acid (niacin;** Fig. 13-4), relieves the dietary deficiency disease in humans known as **pellagra.** Pellagra, which is characterized by diarrhea, dermatitis, and dementia, was endemic in the rural southern United States in the early twentieth century. Most animals, including humans, can synthesize nicotinamide from the amino acid tryptophan (Section 28-5A). The corn-rich diet that was prevalent in the rural South, however, contained little available nicotinamide or tryptophan from which to synthesize it. [Corn actually contains significant quantities of nicotinamide but in a form that requires treatment with base before it can be intestinally absorbed. The Mexican Indians, who are thought to have domesticated the corn plant, customarily soak corn meal in lime water—dilute $Ca(OH)_2$ solution—before using it to make their staple food, tortillas.]

Table 13-2 **Vitamins That Are Coenzyme Precursors**

Vitamin	Coenzyme	Human Deficiency Disease
Biotin	Biocytin	*a*
Cobalamin (B_{12})	Cobalamin (B_{12}) coenzymes	Pernicious anemia
Folic acid	Tetrahydrofolate	Megaloblastic anemia
Nicotinamide	Nicotinamide coenzymes	Pellagra
Pantothenate	Coenzyme A	*a*
Pyridoxine (B_6)	Pyridoxal phosphate	*a*
Riboflavin (B_2)	Flavin coenzymes	*a*
Thiamine (B_1)	Thiamine pyrophosphate	Beriberi

[a]No specific name; deficiency in humans is rare or unobserved.

The vitamins in the human diet that are coenzyme precursors are all **water-soluble vitamins** (Table 13-2). In contrast, the **lipid-soluble vitamins,** such as **vitamins A** and **D,** are not components of coenzymes, although they are also required in trace amounts in the diets of many higher animals. The distant ancestors of humans probably had the ability to synthesize the various vitamins, as do many modern plants and microorganisms. Yet, since vitamins are normally available in the diets of higher animals, which all eat other organisms, or are synthesized by the bacteria that normally inhabit their digestive systems, it is believed that the then superfluous cellular machinery to synthesize them was lost through evolution.

4 CONTROL OF ENZYMATIC ACTIVITY

An organism must be able to control the catalytic activities of its component enzymes so that it can coordinate its numerous metabolic processes, respond to changes in its environment, and grow and differentiate, all in an orderly manner. There are two ways that this may occur:

1. ***Control of enzyme availability:*** *The amount of a given enzyme in a cell depends on both its rate of synthesis and its rate of degradation.* Each of these rates is directly controlled by the cell. For example, *E. coli* grown in the absence of the disaccharide lactose (Fig. 11-13) lack the enzymes to metabolize this sugar. Within minutes of their exposure to lactose, however, these bacteria commence synthesizing the enzymes required to utilize this nutrient (Section 31-1Aa). Similarly, the various tissues of a higher organism contain different sets of enzymes, although most of its cells contain identical genetic information. How cells achieve this control of enzyme synthesis is a major subject of Part V of this textbook. The degradation of proteins is discussed in Section 32-6.

2. ***Control of enzyme activity:*** *An enzyme's catalytic activity may be directly controlled through conformational or structural alterations.* The rate of an enzymatically catalyzed reaction is directly proportional to the concentration of its enzyme–substrate complex, which, in turn, varies with the enzyme and substrate concentrations and with the enzyme's substrate-binding affinity (Section 14-2A). The catalytic activity of an enzyme can therefore be controlled through the variation of its substrate-binding affinity. Recall that Sections 10-1 and 10-4 detail how hemoglobin's oxygen affinity is allosterically controlled by the binding of ligands such as O_2, CO_2, H^+, and BPG. These homotropic and heterotropic effects (ligand binding that, respectively, alters the binding affinity of the same or different ligands) result in cooperative (sigmoidal) O_2-binding curves such as those of Figs. 10-6 and 10-8. *An enzyme's substrate-binding affinity may likewise vary with the binding of small molecule effectors, thereby changing the enzyme's catalytic activity.* In this section we consider the allosteric control of enzymatic activity by examining one particular example: **aspartate transcarbamoylase (ATCase)** from *E. coli.* (The activities of many enzymes are similarly controlled through

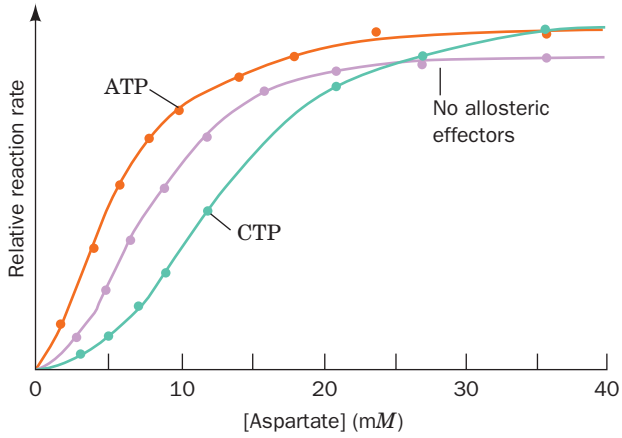
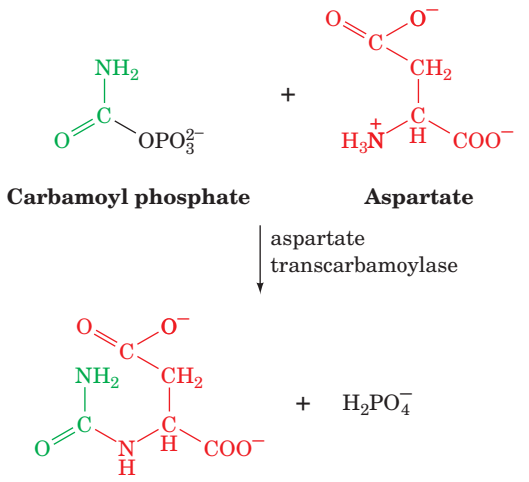


Figure 13-5 **The rate of the reaction catalyzed by ATCase as a function of aspartate concentration.** The rates were measured in the absence of allosteric effectors, in the presence of 0.4 m*M* CTP (inhibition), and in the presence of 2.0 m*M* ATP (activation). [After Kantrowitz, E.R., Pastra-Landis, S.C., and Lipscomb, W.N., *Trends Biochem. Sci.* **5,** 125 (1980).] **See the Animated Figures**

their reversible covalent modification, usually by the phosphorylation of a Ser residue. We study this form of enzymatic control in Section 18-3.)

a. The Feedback Inhibition of ATCase Controls Pyrimidine Biosynthesis

Aspartate transcarbamoylase catalyzes the formation of ***N*-carbamoylaspartate** from **carbamoyl phosphate** and aspartate:



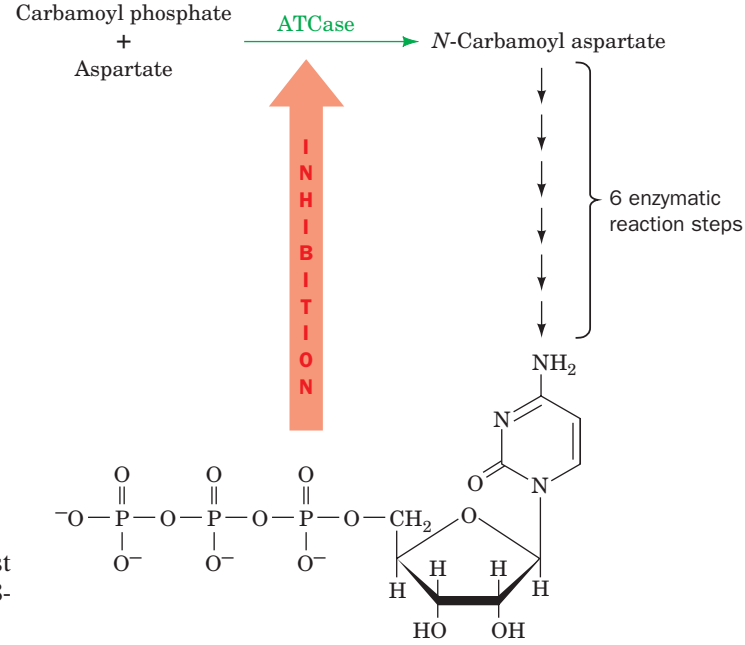
***N*-Carbamoylaspartate**

Arthur Pardee demonstrated that this reaction is the first step unique to the biosynthesis of pyrimidines (Section 28-2A), major components of nucleic acids.

The allosteric behavior of *E. coli* ATCase was investigated by John Gerhart and Howard Schachman, who demonstrated that this enzyme exhibits positive homotropic cooperative binding of both its substrates, namely, aspartate and carbamoyl phosphate. Moreover, ATCase is heterotropically inhibited by **cytidine triphosphate (CTP),** a pyrimidine nucleotide, and is heterotropically activated by **adenosine triphosphate (ATP),** a purine nucleotide. CTP therefore decreases the enzyme's catalytic rate, whereas ATP increases it (Fig. 13-5).

CTP, a product of the pyrimidine biosynthesis pathway (Fig. 13-6), is a nucleic acid precursor (Section 5-4). Consequently, when rapid nucleic acid biosynthesis has depleted a cell's CTP pool, this effector dissociates from ATCase through mass action, thereby deinhibiting the enzyme and increasing the rate of CTP synthesis. Conversely, if the rate of CTP synthesis outstrips its rate of uptake, the resulting excess CTP inhibits ATCase, which, in turn, reduces the rate of CTP synthesis. *This is an example of* ***feedback inhibition,*** *a common mode of metabolic control in which the concentration of a biosynthetic pathway product controls the activity of an enzyme near the beginning of that pathway.*

The metabolic significance of the ATP activation of ATCase is that it tends to coordinate the rates of synthesis of purine and pyrimidine nucleotides for nucleic acid biosynthesis. For instance, if the ATP and CTP concentrations are out of balance with ATP in excess, ATCase is activated to synthesize pyrimidines until balance is achieved. (Note: The ATP concentration in cells is normally greater than the CTP concentration because ATP is in greater demand. Hence the ATP concentration required to activate ATCase is higher than the CTP concentration required to inhibit it by an equal amount.) Conversely, if CTP is in excess, the resulting CTP inhibition of ATCase permits purine biosynthesis to attain this balance.



Cytidine triphosphate (CTP)

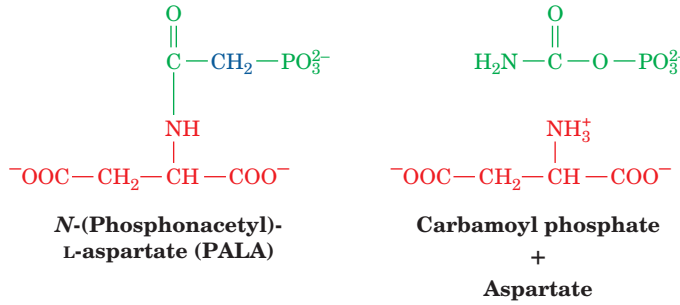
Figure 13-6 **Schematic representation of the pyrimidine biosynthesis pathway.** CTP, the end product of the pathway, inhibits ATCase, which catalyzes the pathway's first step.

b. Allosteric Changes Alter ATCase's Substrate-Binding Sites

E. coli ATCase (309 kD) has the subunit composition c_6r_6, where c and r represent its catalytic and regulatory subunits (311 and 153 residues). The X-ray structure of ATCase (Fig. 13-7), determined by William Lipscomb, reveals that the catalytic subunits are arranged as two sets of trimers (c_3) in complex with three sets of regulatory dimers (r_2) to form a molecule with the rotational symmetry of a trigonal prism (D_3 symmetry; Section 8-5B). Each regulatory dimer joins two catalytic subunits in different c_3 trimers.

Dissociated catalytic trimers retain their catalytic activity, exhibit a noncooperative (hyperbolic) substrate saturation curve, have a maximum catalytic rate higher than that of intact enzyme, and are unaffected by the presence of either ATP or CTP. The isolated regulatory dimers bind these allosteric effectors but are devoid of enzymatic activity. Evidently, *the regulatory subunits allosterically reduce the activity of the catalytic subunits in the intact enzyme.*

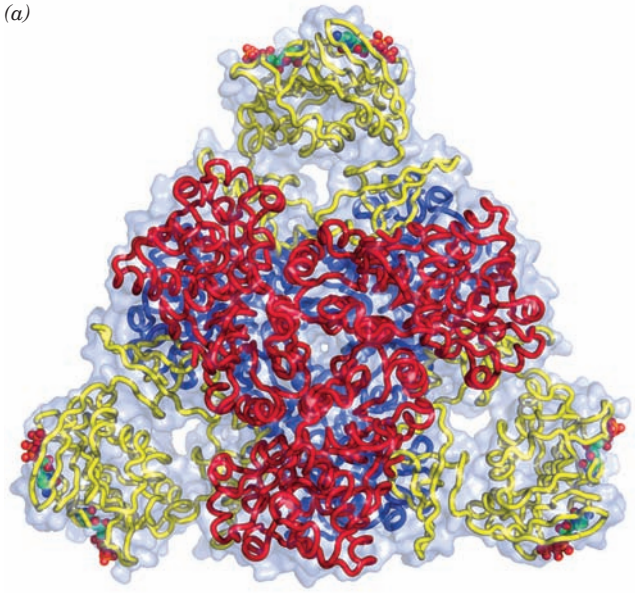
As allosteric theory predicts (Section 10-4), the activator ATP preferentially binds to ATCase's active (R or high substrate affinity) state, whereas the inhibitor CTP preferentially binds to the enzyme's inactive (T or low substrate affinity) state. Similarly, the unreactive bisubstrate analog ***N*-(phosphonacetyl)-L-aspartate (PALA)**

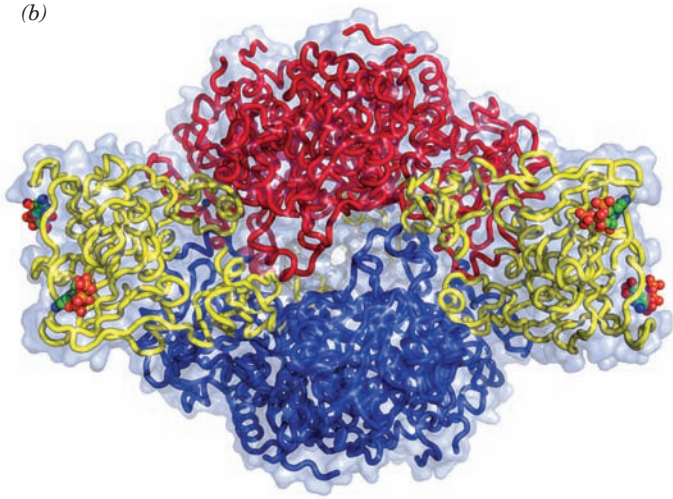


***N*-(Phosphonacetyl)-L-aspartate (PALA)**

Carbamoyl phosphate + Aspartate

binds tightly to R-state but not to T-state ATCase (the use of unreactive substrate analogs is common in the study of enzyme mechanisms because they form stable complexes





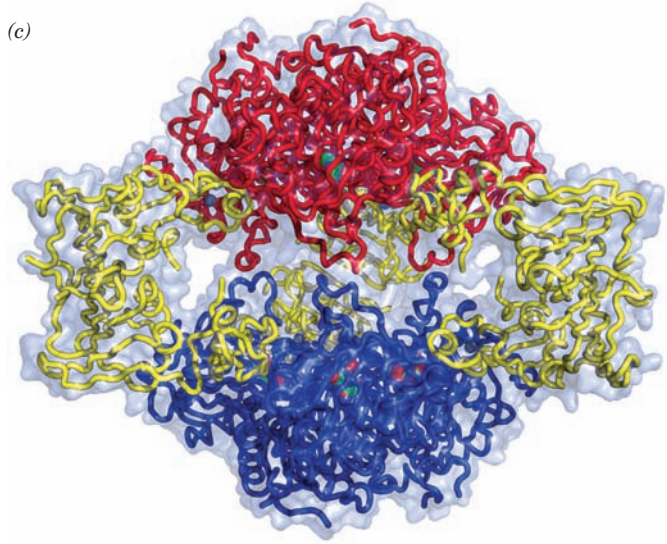


Figure 13-7 **X-ray structure of ATCase from *E. coli*.** The T-state enzyme in complex with CTP is viewed (*a*) along the protein's molecular 3-fold axis of symmetry and (*b*) along a molecular 2-fold axis of symmetry perpendicular to the view in Part *a*. The polypeptide chains are drawn in worm form embedded in their transparent molecular surface. The regulatory dimers (*yellow*) join the upper catalytic trimer (*red*) to the lower catalytic trimer (*blue*). CTP is drawn in space-filling form colored according to atom type (C green, O red, N blue, and P orange). (*c*) The R-state enzyme in complex with PALA viewed as in Part *b*. PALA is drawn in space-filling form. Note how the rotation of the regulatory dimers in the T → R transition causes the catalytic trimers to move apart along the 3-fold axis. [Based on X-ray structures by William Lipscomb, Harvard University. PDBids 5AT1 and 8ATC.] **See Kinemage Exercise 11-1**

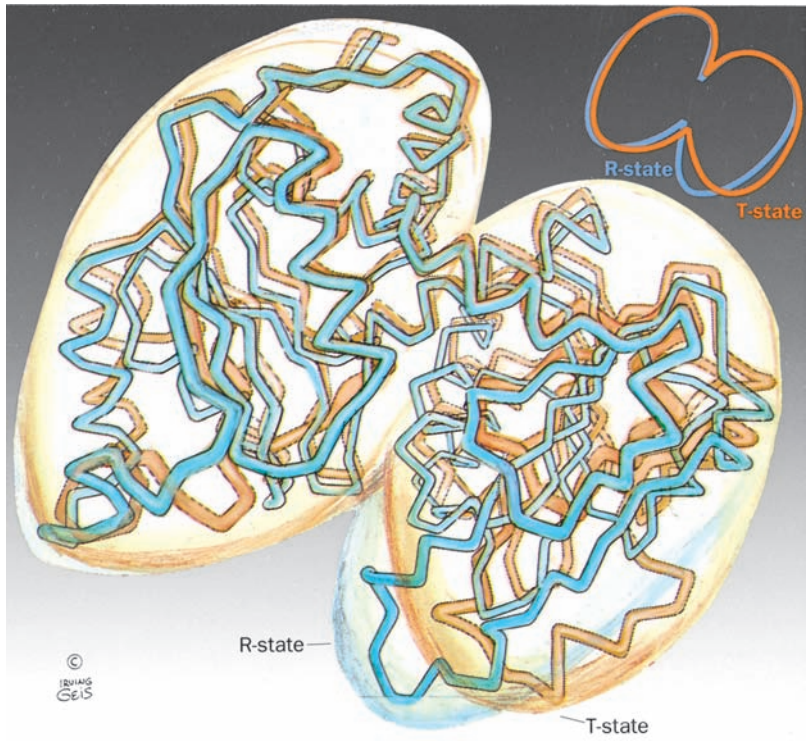


Figure 13-8 **Comparison of the polypeptide backbones of the ATCase catalytic subunit in the T state (*orange*) and the R state (*blue*).** The subunit consists of two domains, with the one on the left containing the carbamoyl phosphate binding site and that on the right forming the aspartic acid binding site. The T → R transition brings the two domains together such that their two bound substrates can react to form product. [Illustration, Irving Geis. Image from the Irving Geis Collection, Howard Hughes Medical Institute. Reprinted with permission.] X-ray structures by William Lipscomb, Harvard University.]

that are amenable to structural study rather than rapidly reacting to form products as do true substrates).

The X-ray structures of the T-state ATCase–CTP complex and the R-state ATCase–PALA complex reveal that the T → R transition maintains the protein's D_3 symmetry. The comparison of these two structures (Fig. 13-7) indicates that in the T → R transition, the enzyme's catalytic trimers separate along the molecular 3-fold axis by ~11 Å and reorient about this axis relative to each other by 12° such that these trimers assume a more nearly eclipsed configuration than is seen in Fig. 13-7*a*. In addition, the regulatory dimers rotate clockwise by 15° about their 2-fold axes and separate by ~4 Å along the 3-fold axis. Such large quaternary shifts are reminiscent of those in hemoglobin (Section 10-2B).

ATCase's substrates, carbamoyl phosphate and aspartate, each bind to a separate domain of the catalytic subunit (Fig. 13-8). The binding of PALA to the enzyme, which presumably mimics the binding of both substrates, induces active site closure in a manner that would bring them together so as to promote their reaction. The resulting atomic shifts, up to 8 Å for some residues (Fig. 13-8), trigger ATCase's T → R quaternary shift. Indeed, *ATCases's tertiary and quaternary shifts are so tightly coupled through extensive intersubunit contacts (see below) that they cannot occur independently* (Fig. 13-9). The binding of substrate to one catalytic subunit therefore increases the substrate-binding affinity and catalytic activity of the other catalytic subunits and hence accounts for the enzyme's positively cooperative substrate binding, much as occurs in hemoglobin (Section 10-2C). Thus, low levels of PALA actually activate ATCase by promoting its T → R transition: ATCase has such high affinity for this unreactive bisubstrate analog that the binding of one molecule of PALA converts all six of its catalytic subunits to the R state. Evidently, *ATCase closely follows the symmetry model of allosterism (Section 10-4B).*

C. The Structural Basis of Allosterism in ATCase

What are the interactions that stabilize the T and R states of ATCase and why must their interconversion be concerted? The region of the protein that undergoes the most profound conformational rearrangement with the T → R transition is a flexible loop composed of residues 230 to 250 in the catalytic (*c*) subunit, the so-called 240s loop [the symmetry-related red and blue loops that lie side by side in the T state (center of Fig. 13-7*b*) but are vertically apposed in the R state (center of Fig. 13-7*c*)]. In the T state, each 240s loop forms two intersubunit hydrogen bonds

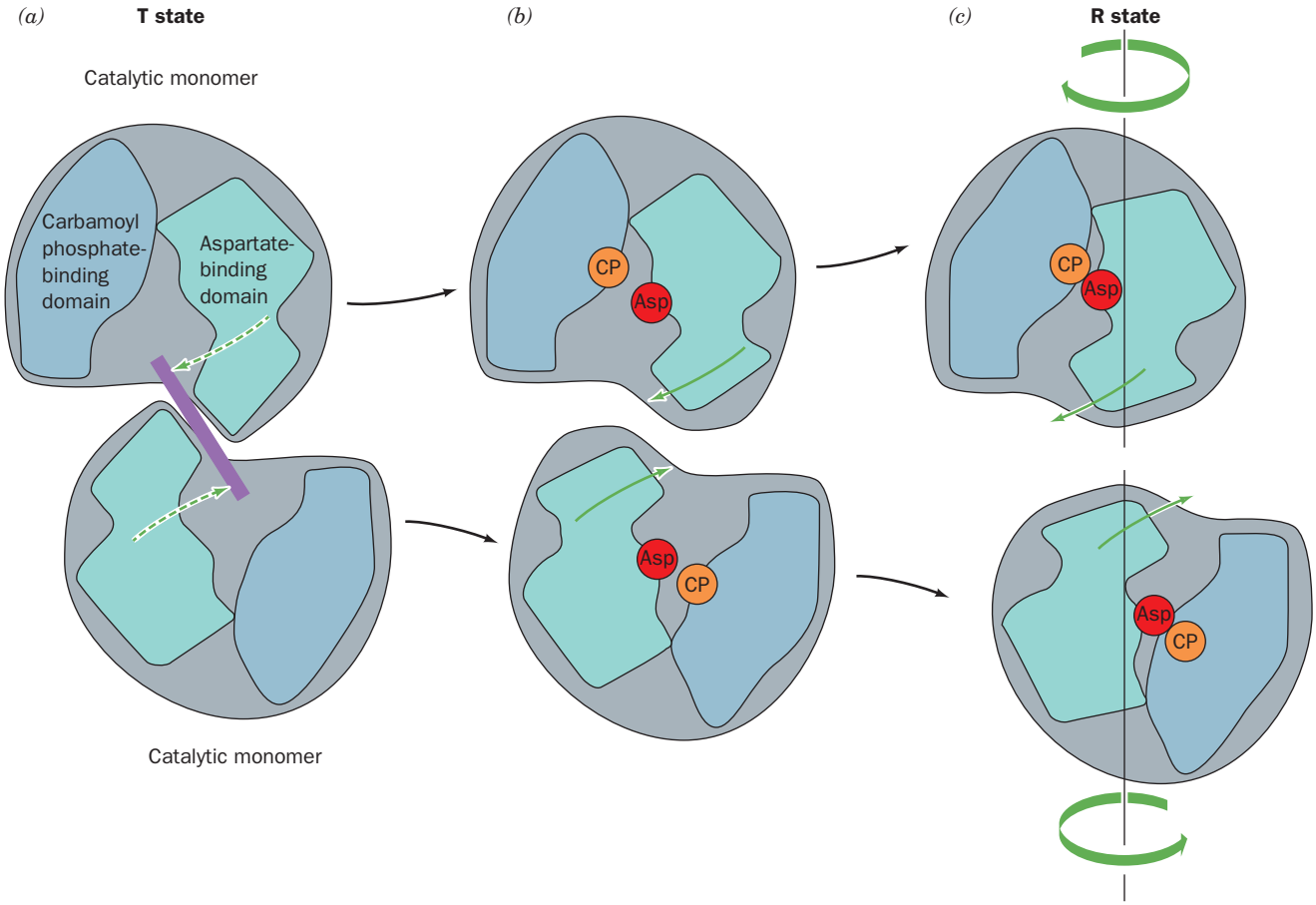


Figure 13-9 **Schematic diagram indicating the tertiary and quaternary conformational changes in two vertically interacting catalytic ATCase subunits.** (*a*) In the absence of bound substrate the protein is held in the T state because the motions that bring together the two domains of each subunit (*dashed arrows*) are prevented by steric interference (*purple bar*) between the contacting aspartic acid binding domains. (*b*) The binding of carbamoyl phosphate (CP) followed by aspartic acid (Asp) to their respective binding sites causes the subunits to move apart and rotate with respect to each other so as to permit the T → R transition. (*c*) In the R state, the two domains of each subunit come together so as to promote the reaction of their bound substrates to form products. [Illustration, Irving Geis. Image from the Irving Geis Collection, Howard Hughes Medical Institute. Reprinted with permission.] **See Kinemage Exercises 11-1 and 11-2**

with the vertically opposite *c* subunit (Fig. 13-7*b*), together with an intrasubunit hydrogen bond. Domain closure as a consequence of substrate binding (Figs. 13-8 and 13-9) ruptures these hydrogen bonds and replaces them, in the R state, with new intrachain hydrogen bonds. The consequent reorientation of the 240s loop is thought to be largely responsible for the quaternary shift to the R state (see below). Since the Glu 239 carboxyl group is the acceptor in all of the above T-state interchain and R-state intrachain hydrogen bonds, this hypothesis is corroborated by the observation that the mutation of Glu 239 to Gln converts ATCase to an enzyme that is devoid of both homotropic and heterotropic effects and that has a quaternary structure midway between those of the R and T states.

What is the structural basis for heterotropic effects in ATCase? Both the inhibitor CTP and the activator ATP bind to the same site on the outer edge of the regulatory (*r*) subunit, about 60 Å away from the nearest catalytic site. CTP binds preferentially to the T state, increasing its stability, while ATP binds preferentially to the R state, increasing its stability. The binding of these effectors to their less favored states also has structural consequences. When CTP binds to R-state ATCase, it reorients several residues at the nucleotide binding site, which induces a contraction in the length of the regulatory dimer (r_2). This distortion, through the interactions of residues at the *r–c* interface, causes the catalytic trimers (c_3) to come together by 0.5 Å (become more T-like, that is, less active, which presumably destabilizes the R state). This, in turn, reorients key residues in the enzyme's active sites, thereby decreasing the enzyme's catalytic activity. ATP has essentially opposite effects when binding to the T-state enzyme: It causes the catalytic trimers to move apart by 0.4 Å (become more R-like, that is, more active, which presumably destabilizes the T state), thereby reorienting key residues in the enzyme's active sites so as to increase the enzyme's catalytic activity. The binding of CTP to T-state ATCase does not further compress the catalytic trimers but, nevertheless, perturbs active

site residues in a way that further stabilizes the T state. Although the X-ray structure of ATP complexed to R-state ATCase has not yet been reported, it is expected that ATP binding perturbs the R state in a manner analogous but opposite to the binding of CTP to T-state ATCase.

d. Allosteric Transitions in Other Enzymes Resemble Those of Hemoglobin and ATCase

Allosteric enzymes are widely distributed in nature and tend to occupy key regulatory positions in metabolic pathways. Three such enzymes, in addition to hemoglobin and ATCase, have had their X-ray structures determined in both their R and T states: **phosphofructokinase** (Sections 17-2C and 17-4F), **fructose-1,6-bisphosphatase** (Section 23-1Ah), and **glycogen phosphorylase** (Section 18-1A). In all five proteins, quaternary changes, through which binding and catalytic effects are communicated among active sites, are concerted and preserve the symmetry of the protein. This is because each of these proteins has two sets of alternative contacts, which are stabilized largely by hydrogen bonds that mostly involve side chains of opposite charge. In all five proteins, the quaternary shifts are primarily rotations of subunits relative to one another with only small translations. Secondary structures are largely preserved in T $\rightarrow$ R transitions, which is probably important for mechanically transmitting heterotropic effects over the tens of Ångstroms necessary in these proteins. The ubiquity of these structural features among allosteric proteins of known structures suggests that the control mechanisms of other allosteric enzymes, by and large, follow this model.

5 A PRIMER OF ENZYME NOMENCLATURE

Enzymes, as we have seen throughout the text so far, are commonly named by appending the suffix *-ase* to the name of the enzyme's substrate or to a phrase describing the enzyme's catalytic action. Thus urease catalyzes the hydrolysis of urea and alcohol dehydrogenase catalyzes the oxidation of alcohols to their corresponding aldehydes. Since there were at first no systematic rules for naming enzymes, this practice occasionally resulted in two different names being used for the same enzyme or, conversely, in the same name being used for two different enzymes. Moreover, many enzymes, such as catalase, which mediates the dismutation of H_2O_2 to H_2O and O_2, were given names that provide no clue as to their function; even such atrocities as "old yellow enzyme" had crept into use. In an effort to eliminate this confusion and to provide rules for rationally naming the rapidly growing number of newly discovered enzymes, a scheme for the systematic functional classification and nomenclature of enzymes was adopted by the International Union of Biochemistry and Molecular Biology (IUBMB).

Table 13-3 **Enzyme Classification According to Reaction Type**

Classification	Type of Reaction Catalyzed
1. Oxidoreductases	Oxidation–reduction reactions
2. Transferases	Transfer of functional groups
3. Hydrolases	Hydrolysis reactions
4. Lyases	Group elimination to form double bonds
5. Isomerases	Isomerization
6. Ligases	Bond formation coupled with ATP hydrolysis

Enzymes are classified and named according to the nature of the chemical reactions they catalyze. There are six major classes of reactions that enzymes catalyze (Table 13-3), as well as subclasses and sub-subclasses within these classes. Each enzyme is assigned two names and a four-number classification. Its **accepted** or **recommended name** is convenient for everyday use and is often an enzyme's previously used name. Its **systematic name** is used when ambiguity must be minimized; it is the name of its substrate(s) followed by a word ending in *-ase* specifying the type of reaction the enzyme catalyzes according to its major group classification. For example, the Enzyme Nomenclature Database (available from http://www.brenda-enzymes.info/ and from http://www.chem.qmul.ac.uk/iubmb/enzyme/) indicates that the enzyme whose alternative name is lysozyme (Section 11-3Ba) has the systematic name peptidoglycan *N*-acetylmuramoylhydrolase and the Classification Number EC 3.2.1.17. Here "EC" stands for *E*nzyme *C*ommission, the first number (3) indicates the enzyme's major class (hydrolases; Table 13-3), the second number (2) denotes its subclass (glycosylases), the third number (1) designates its sub-subclass (enzymes hydrolyzing *O*- and *S*-glycosyl compounds), and the fourth number (17) is the enzyme's arbitrarily assigned serial number in its sub-subclass. As another example, the enzyme with the recommended name alcohol dehydrogenase has the systematic name alcohol:NAD^+ oxidoreductase and the classification number EC 1.1.1.1. In this text, as in general biochemical terminology, we shall most often use the recomended names of enzymes but when ambiguity must be minimized, we shall refer to an enzyme's systematic name.

CHAPTER SUMMARY

2 Substrate Specificity Enzymes specifically bind their substrates through geometrically and physically complementary interactions. This permits enzymes to be absolutely stereospecific, both in binding substrates and in catalyzing reactions. Enzymes vary in the more stringent requirement of geometric specificity. Some are highly specific for the identity of their substrates, whereas others can bind a wide range of substrates and catalyze a variety of related types of reactions.

3 Coenzymes Enzymatic reactions involving oxidation–reduction reactions and many types of group-transfer processes are mediated by coenzymes. Many vitamins are coenzyme precursors.

4 Control of Enzyme Activity Enzymatic activity may be regulated by the allosteric alteration of substrate-binding affinity. For example, the rate of the reaction catalyzed by *E. coli* ATCase is subject to positive homotropic control by substrates, heterotropic inhibition by CTP, and heterotropic activation by ATP. ATCase has the subunit composition c_6r_6. Its isolated catalytic trimers are catalytically active but not subject to allosteric control. The regulatory dimers bind ATP and CTP. Substrate binding induces a tertiary conformational shift in the catalytic subunits, which increases the subunit's substrate-binding affinity and catalytic efficiency. This tertiary shift is strongly coupled to ATCase's large quaternary T → R conformational shift, thereby accounting for the enzyme's allosteric properties. Other allosteric enzymes appear to operate in a similar manner.

5 A Primer of Enzyme Nomenclature Enzymes are classified according to their recommended name, their systematic name, and their EC classification number, which is indicative of the type of reaction catalyzed by the enzyme.

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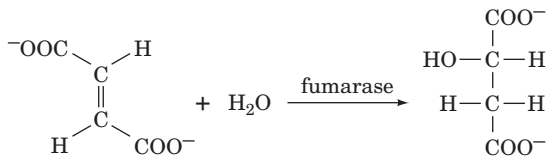
PROBLEMS

1. Indicate the products of the YADH reaction with normal acetaldehyde and NADH in D_2O solution.

2. Indicate the product(s) of the YADH-catalyzed oxidation of the chiral methanol derivative (*R*)-TDHCOH (where T is the symbol for tritium).

3. The enzyme fumarase catalyzes the hydration of the double bond of fumarate:



Fumarate **L-Malate**

Predict the action of fumarase on **maleate,** the cis isomer of fumarate. Explain.

4. Write a balanced equation for the chymotrypsin-catalyzed reaction between an ester and an amino acid.

5. Hominy grits, a regional delicacy of the southern United States, is made from corn that has been soaked in a weak lye (NaOH) solution. What is the function of this unusual treatment?

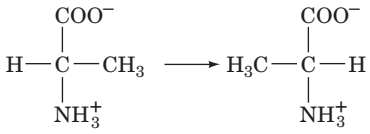
6. Which of the curves in Fig. 13-5 exhibits the greatest cooperativity? Explain.

7. What are the advantages of having the final product of a multistep metabolic pathway inhibit the enzyme that catalyzes the first step?

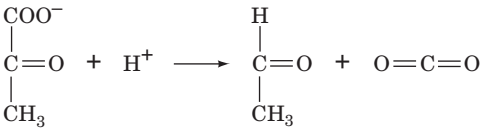
8. What are the systematic names and classification numbers for the enzymes whose recommended names are hexokinase, dihydroorotase, and catalase?

9. Which type of enzyme (Table 13-3) catalyzes the following reactions?

(a)



(b)



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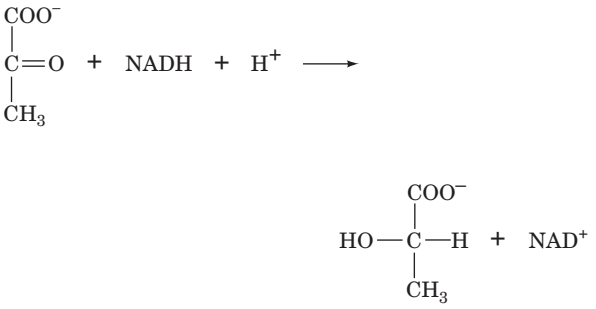

(d)