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.*

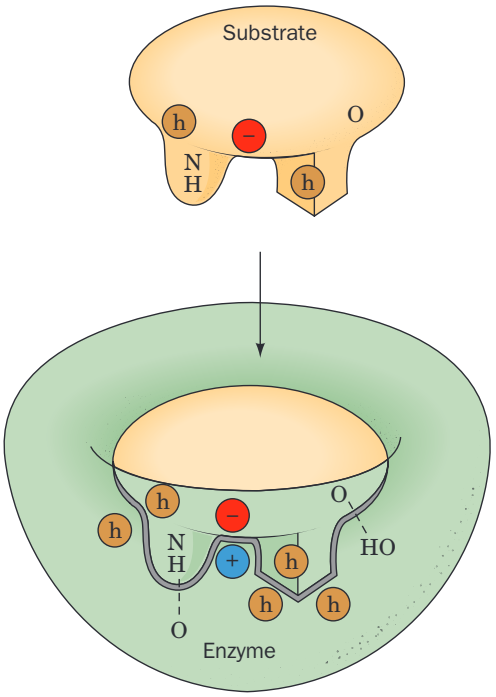


Figure 13-1 **An enzyme–substrate complex illustrating both the geometric and the physical complementarity between enzymes and substrates.** Hydrophobic groups are represented by an h in a brown circle, and dashed lines represent hydrogen bonds.

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

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

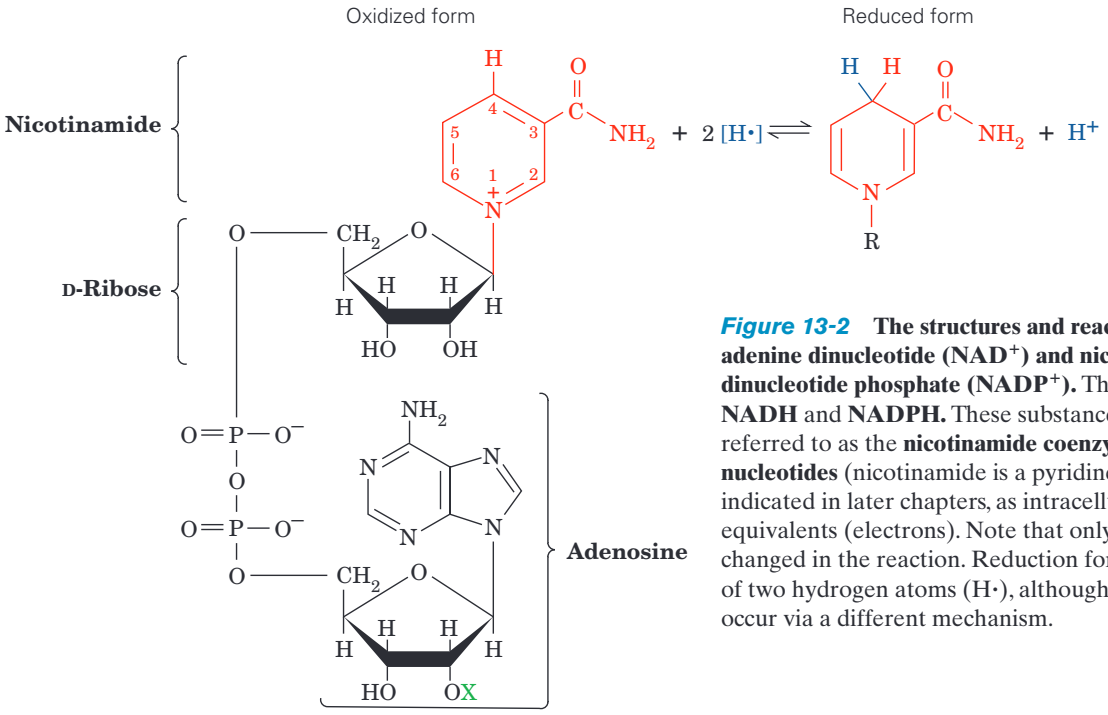
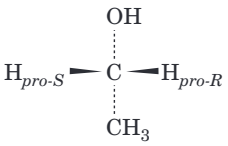


Figure 13-2 **The structures and reaction of nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$).** Their reduced forms are **NADH** and **NADPH.** These substances, which are collectively referred to as the **nicotinamide coenzymes** or **pyridine nucleotides** (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.

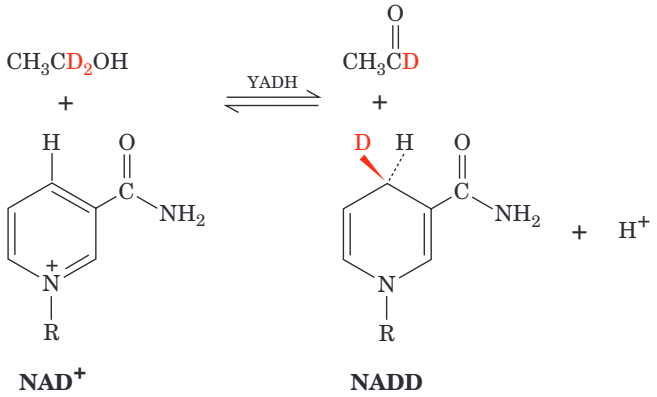
The structures of **NAD^+** and **NADH** are presented in Fig. 13-2. Ethanol, it will be recalled, is a prochiral molecule (see Section 4-2Ca for a discussion of prochirality):



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

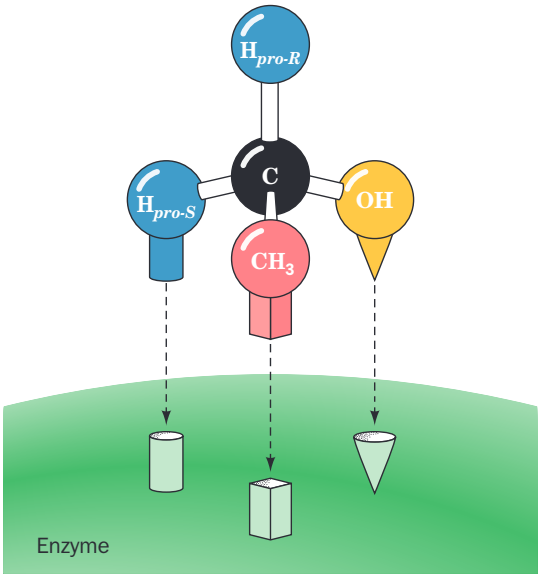
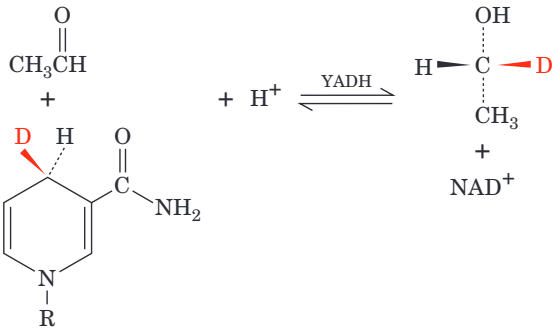
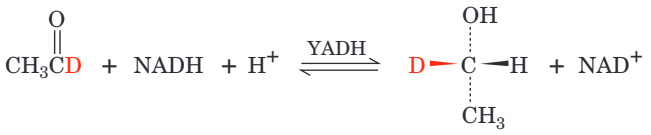


Figure 13-3 **Prochiral differentiation.** The specific attachment of a prochiral center to an enzyme binding site permits the enzyme to differentiate between prochiral groups. Note: If it were possible, the binding of the prochiral molecule's mirror image to the same three sites from the underside of 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:



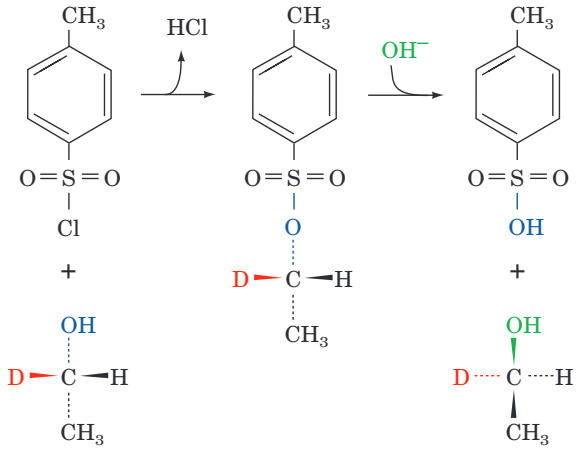
3. If the enantiomer of the foregoing CH_3CHDOH is made as follows:



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)**



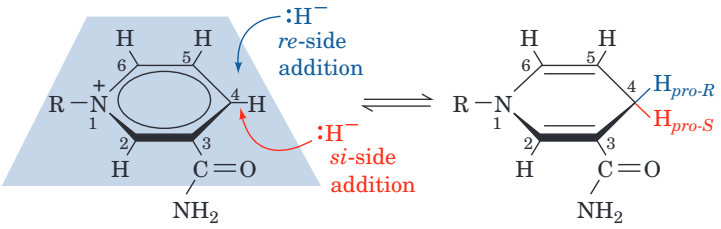
the deuterium is again quantitatively transferred to NAD^+ in the YADH reaction.

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