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# BIOCHEMISTRY

## SECOND EDITION

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*Cover Art:* Two paintings of horse heart cytochrome *c* by Irving Geis in which the protein is illuminated by its single iron atom. On the front cover the hydrophilic side chains are drawn in green, and on the back cover the hydrophobic side chains are drawn in orange. The paintings are based on an X-ray structure by Richard Dickerson.

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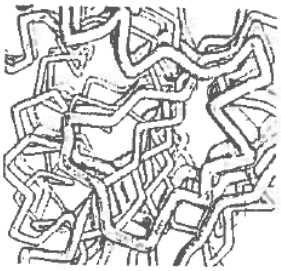
CHAPTER



# 12

# *Introduction to Enzymes*

1. **Historical Perspective**
2. **Substrate Specificity**
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   - B. Geometric Specificity
3. **Coenzymes**
4. **Regulation of Enzymatic Activity**
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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 30-3), 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 ~10 residues (Section 6-4B).
4. **Capacity for regulation:** The catalytic activities of many enzymes vary in response to the concentrations of substances other than their substrates. The mechanisms of these regulatory 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 classic instances of enzyme action: one that illustrates how enzyme specificity is manifested, and a second that exemplifies the regulation 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 15–20). These two expositions are interspersed with a consideration of the role of enzymatic cofactors. The chapter ends with a short synopsis of enzyme nomenclature. In Chapter 13 we take up the formalism of enzyme kinetics because the study of the rates of enzymatically catalyzed reactions provides indispensable mechanistic information. Finally, Chapter

14 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 10-2D) 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 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 Fredrich Wilhelm 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 16-3B).

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 recently been shown that some species of RNA also have catalytic properties; Section 29-4B).

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 latter half of the twentieth century. Only with the advent of modern techniques for separation and analysis (Chapter 5) 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 14-1A), 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 14-2A), was elucidated. In the years since then, several thousand 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 identical in character to the forces that dictate the conformations of the proteins themselves (Section 7-4): 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 (geometrical 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. 12-1). Molecules that differ in shape or func-

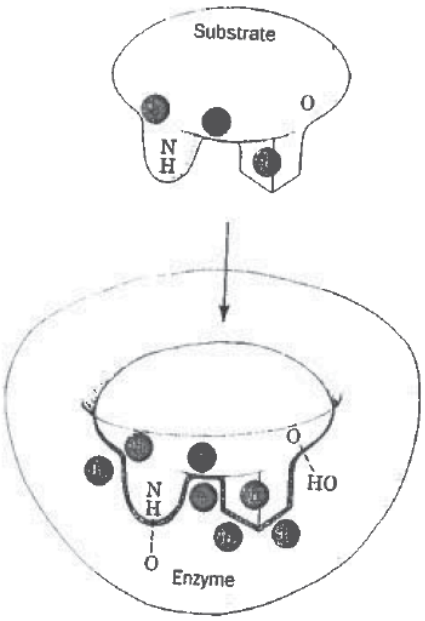



**FIGURE 12-1.** An enzyme–substrate complex illustrating both the geometrical and the physical complementarity between enzymes and substrates. Hydrophobic groups are represented by an h in a brown circle and dashed lines represent hydrogen bonds.

tional 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 9-4C), 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 upon binding substrate.*

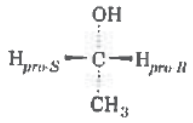
## *A. Stereospecificity*

*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 16-2) 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:

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

The structures of $NAD^+$ and NADH are presented in Fig. 12-2. Ethanol, it will be recalled, is a prochiral molecule (see Section 4-2C 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. 12-3). The substrate-binding sites of enzymes are, of course,*

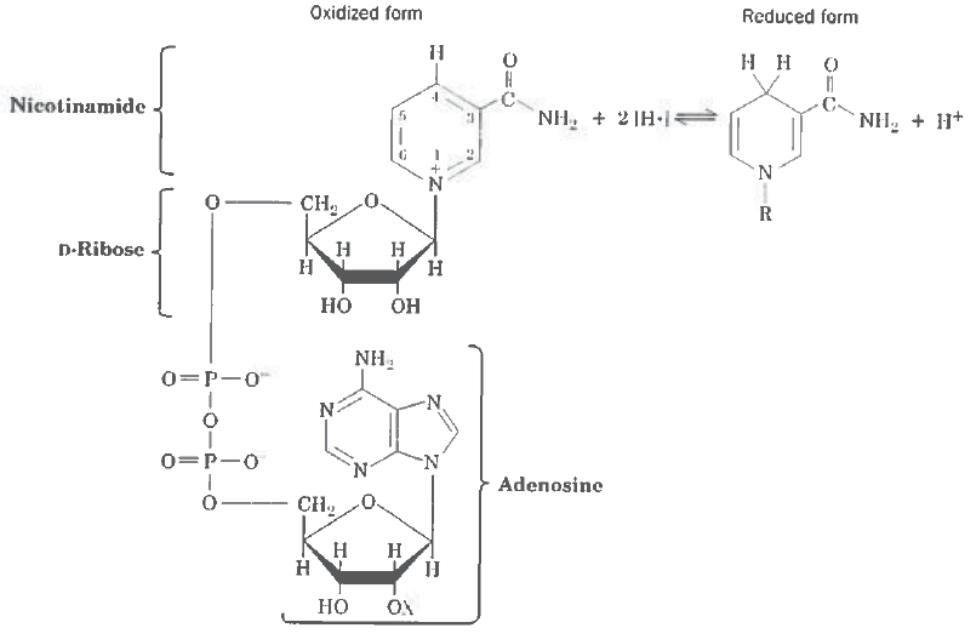



**FIGURE 12-2.** The structures and reactions of **nicotinamide adenine dinucleotide ($NAD^+$)** and **nicotinamide adenine dinucleotide phosphate ($NADP^+$)**. Their reduced forms are **NADH** and **NADPH.** [In the older literature they are termed **diphosphopyridine nucleotide ($DPN^+$)** and **triphosphopyridine nucleotide ($TPN^+$)** and their reduced forms are symbolized **DPNH** and **TPNH.**] 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.

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