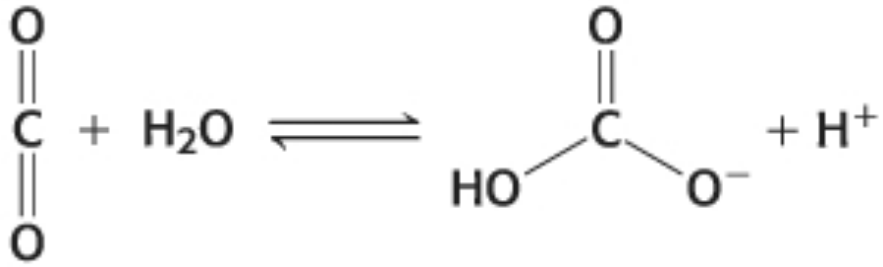
5.1 Enzymes Are Powerful and Highly Specific Catalysts

Agents called **catalysts** speed up chemical reactions without being consumed by them. **Enzymes** are powerful biological catalysts — made by all living organisms — that dramatically enhance and control the rates of chemical reactions. Almost all enzymes are proteins, which are highly effective catalysts for an enormous diversity of chemical reactions because of their capacity to specifically bind a very wide range of molecules. Using the full repertoire of intermolecular forces, enzymes optimally orient reactant molecules to make and break chemical bonds. However, proteins do not have an absolute monopoly on catalysis; the discovery of catalytically active RNA molecules, called **ribozymes**, provides compelling evidence that RNA was a biocatalyst early in evolution. Two remarkable properties of enzymes to consider from the start are their speed and their specificity.

- *Speed*. Enzymes can accelerate reactions by factors of a billion or more (**Table 5.1**). Indeed, most reactions in biological systems do not take place at perceptible rates in the absence of enzymes. Even a reaction as simple as the hydration of carbon dioxide is catalyzed by an enzyme — namely, carbonic anhydrase.



The transfer of CO_2 from the tissues to the blood and then to the air in the alveolae of the lungs would be less complete in the absence of this enzyme. In fact, carbonic anhydrase is one of the fastest enzymes known. Each enzyme molecule can hydrate 10^6 molecules of CO_2 per second. This catalyzed reaction is 10^7 times as fast as the uncatalyzed one. We will consider the mechanism of carbonic anhydrase catalysis in Chapter 6.

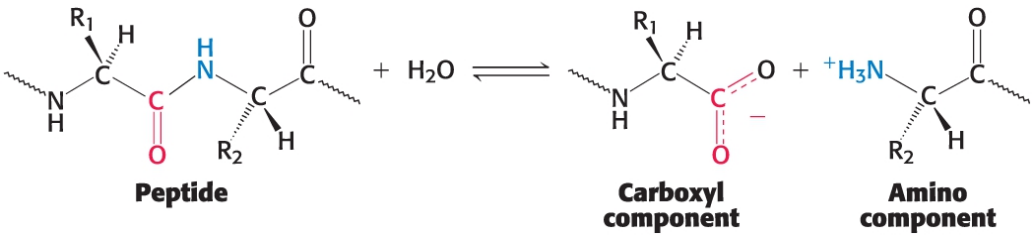
TABLE 5.1 Rate enhancement by selected enzymes

Enzyme	Nonenzymatic half-life	Uncatalyzed rate ($k_{un}s^{-1}$)	Catalyzed rate ($k_{cat}s^{-1}$)	Rate enhancement ($k_{cat}s^{-1}/k_{un}s^{-1}$)
OMP decarboxylase	78,000,000 years	2.8×10^{-16}	39	1.4×10^{17}
Staphylococcal nuclease	130,000 years	1.7×10^{-13}	95	5.6×10^{14}
AMP nucleosidase	69,000 years	1.0×10^{-11}	60	6.0×10^{12}
Carboxypeptidase A	7.3 years	3.0×10^{-9}	578	1.9×10^{11}
Ketosteroid isomerase	7 weeks	1.7×10^{-7}	66,000	3.9×10^{11}

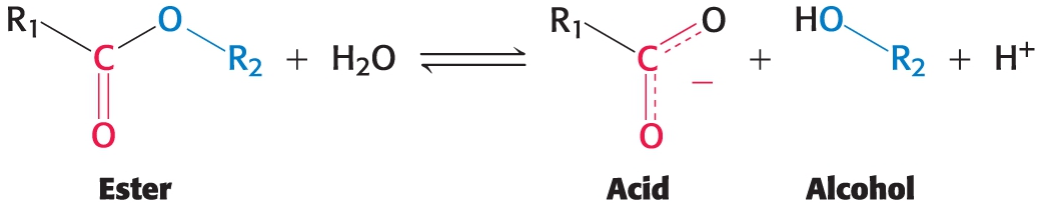
Triose phosphate isomerase	1.9 days	4.3×10^{-6}	4300	1.0×10^{9}
Chorismate mutase	7.4 hours	2.6×10^{-5}	50	1.9×10^{6}
Carbonic anhydrase	5 seconds	1.3×10^{-1}	1×10^{6}	7.7×10^{6}

Abbreviations: OMP, orotidine monophosphate; AMP, adenosine monophosphate.
Source: After A. Radzicka and R. Wolfenden, *Science* 267:90–93, 1995.

- *Specificity*. Enzymes are highly specific both in the reactions that they catalyze and in the reactants they bind, which are called **substrates**. An enzyme usually catalyzes a single chemical reaction or a set of closely related reactions. For example, enzymes known as **proteases** catalyze proteolysis, the hydrolysis of a peptide bond.



Most proteolytic enzymes also catalyze a different but related reaction in vitro — namely, the hydrolysis of an ester bond. Such reactions are more easily monitored than is proteolysis and are useful in experimental investigations of these enzymes.



Proteolytic enzymes differ markedly in their degree of substrate specificity. Papain, which is found in papaya plants, is quite undiscriminating: it will cleave any peptide bond with little regard to the identity of the adjacent side chains. The digestive enzyme trypsin, on the other hand, is quite specific and catalyzes the splitting of peptide bonds only on the carboxyl side of lysine and arginine residues (**Figure 5.1A**). Thrombin, an enzyme that participates in blood clotting (Section 10.4), is even more specific than trypsin. It catalyzes the hydrolysis of Arg–Gly bonds in particular peptide sequences only (**Figure 5.1B**). The specificity of an enzyme is due to the precise interaction of the substrate with the enzyme, which is a result of the intricate three-dimensional structure of the enzyme.

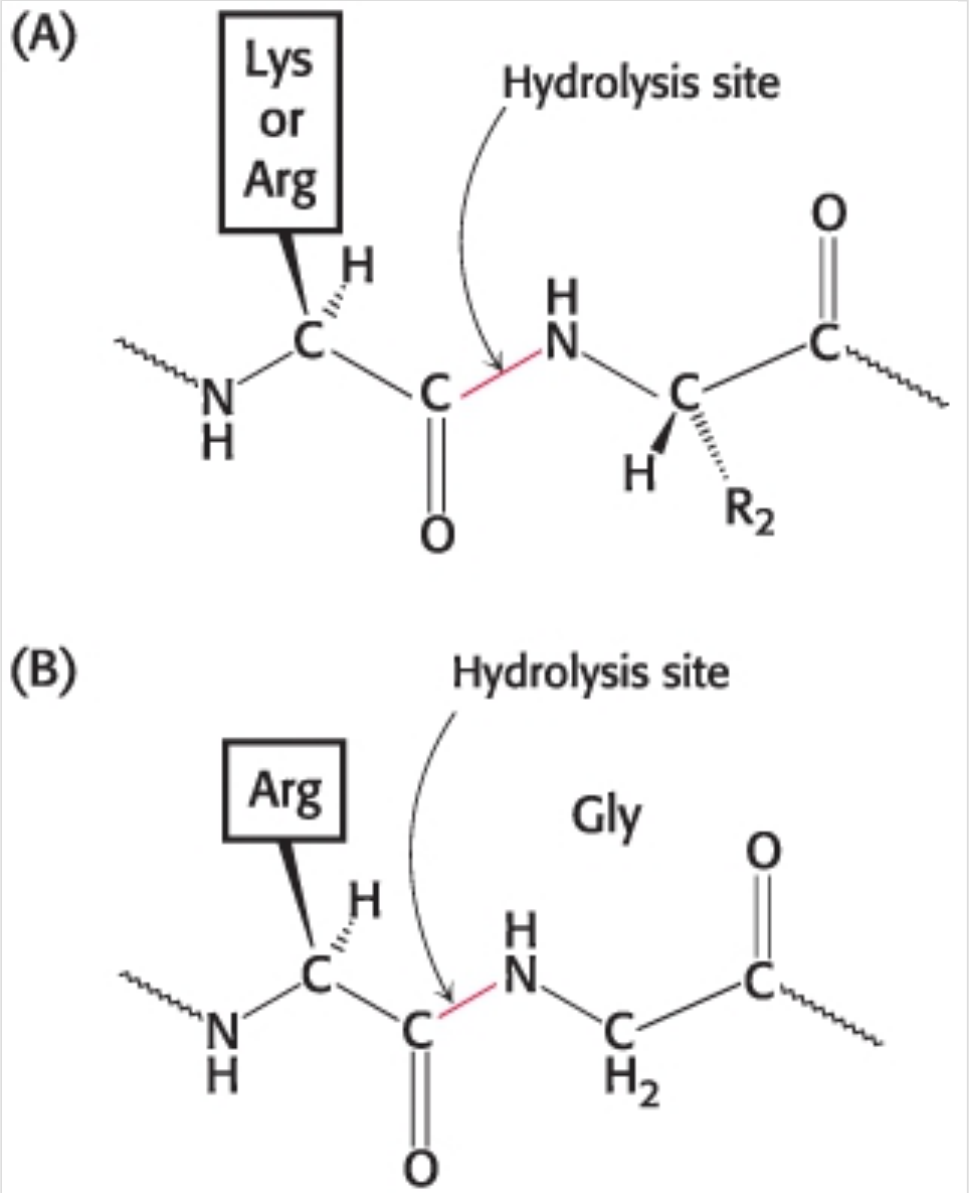


FIGURE 5.1 Enzymes are highly specific to particular substrates and chemical reactions. (A) Trypsin cleaves on the carboxyl side of arginine and lysine residues, whereas (B) thrombin cleaves Arg–Gly bonds in particular sequences only.

Most enzymes are classified by the types of reactions they catalyze

While some enzymes have common names — like papain, trypsin, and thrombin — that provide little information regarding their function, most enzymes are named for one of their substrates and for the reactions that they catalyze, with the suffix "-ase" added. Thus, a peptide hydrolase is an enzyme that hydrolyzes peptide bonds, whereas ATP synthase is an enzyme that synthesizes ATP. It is important to note, however, that enzymes catalyze chemical reactions in both the forward and reverse directions, yet only one direction of the reaction is typically denoted in the name.

To bring some consistency to enzyme nomenclature, a classification system for enzymes — developed by the International Union of Biochemistry — divides reactions into seven major groups (**Table 5.2**). These groups are further subdivided so that a four-number code preceded by the letters *EC* could precisely identify all enzymes. Consider as an example nucleoside monophosphate (NMP) kinase, an enzyme that we will examine in detail in Section 9.4. It catalyzes the following reaction:

$$\mathrm{ATP} + \mathrm{NMP} \rightleftharpoons \mathrm{ADP} + \mathrm{NDP}$$

NMP kinase transfers a phosphoryl group from ATP to any NMP to form a nucleoside diphosphate (NDP) and ADP. Consequently, it is a transferase, or member of group 2. Transferases that shift a phosphoryl group are designated 2.7. If a phosphate is the acceptor,

the transferase is designated 2.7.4, and most precisely EC 2.7.4.4 if a nucleoside monophosphate is the acceptor. Although the common names are used routinely, the classification number is used when the precise identity of the enzyme is not clear from the common name alone.

TABLE 5.2 Seven major classes of enzymes

Class	Type of reaction	Example	Chapter
1. Oxidoreductases	Oxidation–reduction	Lactate dehydrogenase	16
2. Transferases	Group transfer	Nucleoside monophosphate kinase (NMP kinase)	6
3. Hydrolases	Hydrolysis reactions (transfer of functional groups to water)	Chymotrypsin	6
4. Lyases	Addition or removal of groups to form double bonds	Fumarase	17
5. Isomerases	Isomerization (intramolecular group transfer)	Triose phosphate isomerase	16
6. Ligases	Ligation of two substrates at the expense of ATP hydrolysis	Aminoacyl-tRNA synthetase	30
7. Translocases	Movement of ions or molecules across membranes	Na^+–K^+ATPase	12

	or within membranes

Many enzymes require cofactors for activity

The catalytic activity of many enzymes depends on the presence of small molecules termed **cofactors**, although the precise role varies with the cofactor and the enzyme. Generally, these cofactors are able to execute chemical reactions that cannot be performed by the standard set of twenty amino acids. An enzyme without its cofactor is referred to as an **apoenzyme**; the complete, catalytically active enzyme is called a **holoenzyme**.

$$\text{apoenzyme} + \text{cofactor} = \text{holoenzyme}$$

Cofactors can be subdivided into two groups: (1) metals, whose importance to enzymatic activity we will explore in Chapter 6, and (2) small organic molecules called **coenzymes** (**Table 5.3**). Often derived from vitamins, coenzymes either can be tightly or loosely bound to the enzyme.

TABLE 5.3 Enzyme cofactors

Cofactor	Enzyme
Coenzyme	
Thiamine pyrophosphate	Pyruvate dehydrogenase

Flavin adenine nucleotide	Monoamine oxidase
Nicotinamide adenine dinucleotide	Lactate dehydrogenase
Pyridoxal phosphate	Glycogen phosphorylase
Coenzyme A (CoA)	Acetyl CoA carboxylase
Biotin	Pyruvate carboxylase
5′-Deoxyadenosylcobalamin	Methylmalonyl mutase
Tetrahydrofolate	Thymidylate synthase
Metal	
Zn^{2+}	Carbonic anhydrase
Zn^{2+}	Carboxypeptidase
Mg^{2+}	*Eco*RV
Mg^{2+}	Hexokinase
Ni^{2+}	Urease
Mo	Nitrogenase
Se	Glutathione peroxidase

Mn	Superoxide dismutase
K^+	Acetyl CoA thiolase

Tightly bound coenzymes are called **prosthetic groups**. Prosthetic groups are catalytic in that they are unchanged in the overall chemical reaction. In contrast, loosely associated coenzymes often behave more like second substrates (cosubstrates) because they bind to the enzyme, are changed by it, and then are released from it. Thus, these are also sometimes called *stoichiometric coenzymes* because they must be present in stoichiometric ratios with other substrates. The use of the same loosely associated coenzyme by a variety of enzymes sets these coenzymes apart from normal substrates, however, as does their source in vitamins (Table 5.3 and Section 15.4). Enzymes that use the same coenzyme usually perform catalysis by similar mechanisms. Throughout the book, we will see how coenzymes and their enzyme partners operate in their biochemical context.

Self-Check Question

Which terms (cofactor, coenzyme, and/or prosthetic group) correctly describe the heme from cytochrome c oxidase, which doesn't dissociate from the protein?

Enzymes can transform energy from one form into another

A key activity in all living systems is the conversion of one form of energy into another. For example, in photosynthesis, light energy is converted into chemical potential energy. In cellular respiration, which takes place in mitochondria, the free energy contained in small molecules derived from food is converted first into the free energy of an ion gradient and then into a different currency — the free energy of adenosine triphosphate. Given their centrality to life, it should come as no surprise that enzymes play vital roles in energy transformation.

After enzymes perform fundamental roles in photosynthesis and cellular respiration, other enzymes can then use the chemical potential energy of ATP in diverse ways. For instance, the enzyme myosin converts the energy of ATP into the mechanical energy of contracting muscles (Section 6.5). Pumps in the membranes of cells and organelles, which can be thought of as enzymes that move substrates rather than chemically alter them, use the energy of ATP to transport molecules and ions across the membrane (Chapter 13). The chemical and electrical gradients resulting from the unequal distribution of these molecules and ions are themselves forms of potential energy that can be used for a variety of purposes, such as sending nerve impulses (Section 13.4).

Recent developments show that the power of enzymes may be harnessed to generate energy for entire communities, as well as reducing landfill. Unsorted municipal waste can be treated with a cocktail of enzymes that includes an array of proteases as well as carbohydrate- and lipid-degrading enzymes, turning much of the waste into a bioliquid of sugars, amino acids, and other biomolecules. The bioliquid can then be used to fuel the growth of

methane-producing bacteria, and the methane harvested and burned to generate electricity. Any waste not degraded by the enzyme cocktail is recycled or incinerated to produce electricity.