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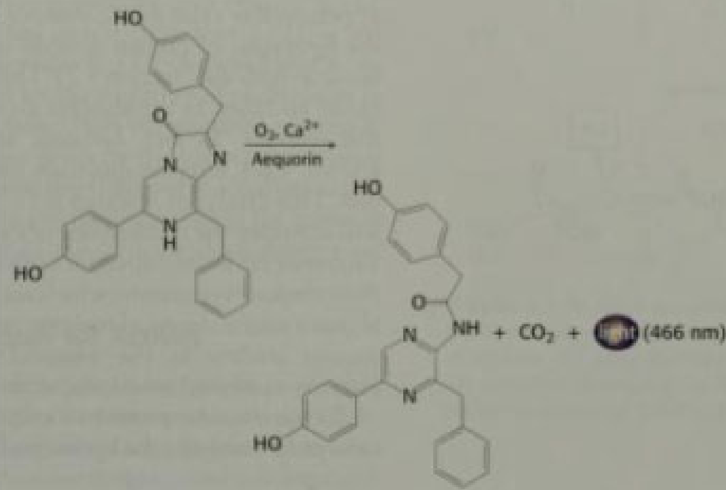
Enzymes: Basic Concepts and Kinetics



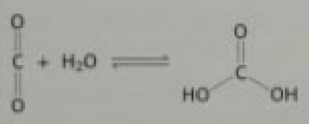
The activity of an enzyme is responsible for the glow of the luminescent jellyfish at left. The enzyme aequorin catalyzes the oxidation of a compound by oxygen in the presence of calcium to release CO_2 and light. [(Left) Fred Bavendam/Peter Arnold.]

Enzymes, the catalysts of biological systems, are remarkable molecular devices that determine the patterns of chemical transformations. They also mediate the transformation of one form of energy into another. The most striking characteristics of enzymes are their *catalytic power and specificity*. Catalysis takes place at a particular site on the enzyme called the *active site*. *Nearly all known enzymes are proteins*. However, proteins do not have an absolute monopoly on catalysis; the discovery of catalytically active RNA molecules provides compelling evidence that RNA was an early biocatalyst (Section 2.2.2).

Proteins as a class of macromolecules are highly effective catalysts for an enormous diversity of chemical reactions because of their capacity *to specifically bind a very wide range of molecules*. By utilizing the full repertoire of intermolecular forces, enzymes bring substrates together in an optimal orientation, the prelude to making and breaking chemical bonds. They catalyze reactions *by stabilizing transition states*, the highest-energy species in reaction pathways. By selectively stabilizing a transition state, an enzyme determines which one of several potential chemical reactions actually takes place.

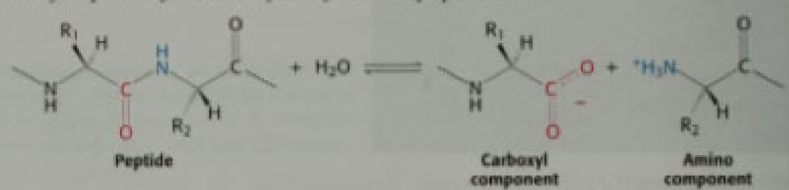
OUTLINE

8.1 ENZYMES ARE POWERFUL AND HIGHLY SPECIFIC CATALYSTS



Enzymes accelerate reactions by factors of as much as a million or more (Table 8.1). 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 (Section 9.2). The transfer of CO_2 from the tissues into the blood and then to the alveolar air 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 9. Enzymes are highly specific both in the reactions that they catalyze and in their choice of reactants, which are called *substrates*. An enzyme usually catalyzes a single chemical reaction or a set of closely related reactions. Side reactions leading to the wasteful formation of by-products are rare in enzyme-catalyzed reactions, in contrast with uncatalyzed ones.

Let us consider *proteolytic enzymes* as an example. In vivo, these enzymes 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 (Section 9.1.2).

Proteolytic enzymes differ markedly in their degree of substrate specificity. Subtilisin, which is found in certain bacteria, is quite undiscriminat-

TABLE 8.1 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}/k_{un})
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}	4,300	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 (1995):90–93.

ing: it will cleave any peptide bond with little regard to the identity of the adjacent side chains. Trypsin, a digestive enzyme, is quite specific and catalyzes the splitting of peptide bonds only on the carboxyl side of lysine and arginine residues (Figure 8.1A). Thrombin, an enzyme that participates in blood clotting, is even more specific than trypsin. It catalyzes the hydrolysis of Arg-Gly bonds in particular peptide sequences only (Figure 8.1B).

DNA polymerase I, a template-directed enzyme (Section 27.2), is another highly specific catalyst. It adds nucleotides to a DNA strand that is being synthesized, in a sequence determined by the sequence of nucleotides in another DNA strand that serves as a template. DNA polymerase I is remarkably precise in carrying out the instructions given by the template. It inserts the wrong nucleotide into a new DNA strand less than one in a million times.

The specificity of an enzyme is due to the precise interaction of the substrate with the enzyme. This precision is a result of the intricate three-dimensional structure of the enzyme protein.

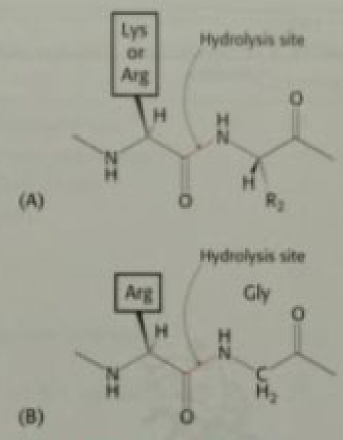


FIGURE 8.1 Enzyme specificity. (A) Trypsin cleaves on the carboxyl side of arginine and lysine residues, whereas (B) thrombin cleaves Arg–Gly bonds in particular sequences specifically.

8.1.1 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. Such 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: metals and small organic molecules (Table 8.2). The enzyme carbonic anhydrase, for example, requires Zn^{2+} for its activity (Section 9.2.1). Glycogen phosphorylase (Section 21.1.5), which mobilizes glycogen for energy, requires the small organic molecule pyridoxal phosphate (PLP).

TABLE 8.2 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′-Deoxyadenosyl cobalamin	Methylmalonyl mutase
Tetrahydrofolate	Thymidylate synthase
Metal	
Zn^{2+}	Carbonic anhydrase
Zn^{2+}	Carboxypeptidase
Mg^{2+}	*Eco*RV
Mg^{2+}	Hexokinase
Ni^{2+}	Urease
Mo	Nitrate reductase
Se	Glutathione peroxidase
Mn^{2+}	Superoxide dismutase
K^{+}	Propionyl CoA carboxylase

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