TABLE 7-6.   TORSION ANGLES (ϕ, ψ) FOR RESIDUES 24 TO 73 OF HEN EGG WHITE
LYSOZYME

Residue Number	Amino Acid	ϕ (deg)	ψ (deg)	Residue Number	Amino Acid	ϕ (deg)	ψ (deg)
24	Ser	−60	147	49	Gly	95	−75
25	Leu	−49	−32	50	Ser	−18	138
26	Gly	−67	−34	51	Thr	−131	157
27	Asn	−58	−49	52	Asp	−115	130
28	Trp	−66	−32	53	Tyr	−126	146
29	Val	−82	−36	54	xxx	67	−179
30	Cys	−69	−44	55	Ile	−42	−37
31	Ala	−61	−44	56	Leu	−107	14
32	Ala	−72	−29	57	Gln	35	54
33	Lys	−66	−65	58	Ile	−72	133
34	Phe	−67	−23	59	Asn	−76	153
35	Glu	−81	−51	60	Ser	−93	−3
36	Ser	−126	−8	61	Arg	−83	−19
37	Asn	68	27	62	Trp	−133	−37
38	Phe	79	6	63	Trp	−91	−32
39	Asn	−100	109	64	Cys	−151	143
40	Thr	−70	−18	65	Asn	−85	140
41	Glu	−84	−36	66	Asp	133	8
42	Ala	−30	142	67	Gly	73	−8
43	Thr	−142	150	68	Arg	−135	17
44	Asn	−154	121	69	Thr	−122	83
45	Arg	−91	136	70	Pro	−39	−43
46	Asn	−110	174	71	Gly	−61	−11
47	Thr	−66	−20	72	Ser	−45	122
48	Asp	−96	36	73	Arg	−124	146

Source: Imoto, T., Johnson, L.N., North, A.C.T., Phillips, D.C., and Rupley, J.A., *in* Boyer, P.D. (Ed.), *The Enzymes* (3rd ed.), Vol. 7, *pp.* 693–695, Academic Press (1972).

probable identity of residue 54? (d) What is the secondary structure of residues 69–71? (e) What additional information besides the torsion angles, ϕ and ψ, of each of its residues are required to define the three-dimensional structure of a protein?

5. Hair splits most easily along its fiber axis, whereas fingernails tend to split across the finger rather than along it. What are the directions of the keratin fibrils in hair and in fingernails? Explain your reasoning.

6. What structural features are responsible for the observations that α keratin fibers can stretch to over twice their normal length, whereas silk is nearly inextensible?

7. What is the growth rate, in turns per second, of the α helices in a hair that is growing 15 cm·year^{-1}?

8. Can polyproline form a collagenlike triple helix? Explain.

9. As Mother Nature's chief engineer, you have been asked to design a five-turn α helix that is destined to have half its circumference immersed in the interior of a protein. Indicate the helical wheel projection of your prototype α helix and its amino acid sequence (see Fig. 7-45).

10. β-Aminopropionitrile is effective in reducing excessive scar tissue formation after an injury (although its use is contraindicated by side effects). What is the mechanism of action of this lathyrogen?

11. Proteins have been classified as α, β, α/β, or $\alpha + \beta$ proteins depending on whether their tertiary structures, respectively, consist of mostly α helices, mostly β sheets, alternating α helices and β sheets, or some α helices and β sheets that tend to aggregate together rather than alternate along the polypeptide chain. By inspection, classify the following proteins according to this nomenclature and, where possible, identify their supersecondary structures: carboxypeptidase A (Fig. 7-19*a*), triose phosphate isomerase (Fig. 7-19*b*), myoglobin (Fig. 7-42), concanavalin A (Fig. 7-43), carbonic anhydrase (Fig. 7-44), glyceraldehyde-3-phosphate dehydrogenase (Fig. 7-47), and prealbumin (Fig. 7-59).

12. The coat protein of tomato bushy stunt virus consists of 180 chemically identical subunits, each of which is composed of ~386 amino acid residues. The probability that a wrong amino acid residue will be biosynthetically incorporated in a polypeptide chain is 1 part in 3000 per residue. Calculate the average number of coat protein subunits that would have to be synthesized in order to produce a perfect viral coat. What would this number be if the viral coat were a single polypeptide chain with the same number of residues that it actually has?

13. State the rotational symmetry of the following objects: (a) a starfish, (b) a square pyramid, (c) a rectangular box, and (d) a trigonal bipyramid.

14. Myoglobin and the subunits of hemoglobin are polypeptides of similar size and structure. Compare the expected ratio of nonpolar to polar amino acid residues in myoglobin and in hemoglobin.

15. Why are London dispersion forces always attractive?

16. Membrane-bound proteins are generally closely associated with the nonpolar groups of lipid molecules (Section 11-3A). Explain how detergents affect the structural integrity of membrane bound proteins in comparison to their effects on normal globular proteins.

17. Sickle-cell hemoglobin (HbS) differs from normal human adult hemoglobin (HbA) by a single mutational change, Glu $\beta 6 \rightarrow$ Val, which causes the HbS molecules to aggregate under proper conditions (Section 6-3A). Under certain conditions, the HbS filaments that form at body temperature disaggregate when the temperature is lowered to 0°C. Explain.

18. Indicate experimental evidence that is inconsistent with the hypothesis that urea and guanidinium ion act to denature proteins by competing for their internal hydrogen bonds.

19. Proteins in solution are often denatured if the solution is shaken violently enough to cause foaming. Indicate the mechanism of this process. (*Hint:* The nonpolar groups of detergents extend into the air at air–water interfaces.)

20. An oligomeric protein in a dilute buffer at pH 7 dissociates to its component subunits when exposed to the following agents. Which of these observations would not support the contention that the quaternary structure of the protein is stabilized exclusively by hydrophobic interactions? Explain. (a) $6M$ guanidinium chloride, (b) 20% ethanol, (c) $2M$ NaCl, (d) temperatures below 0°C, (e) 2-mercaptoethanol, (f) pH 3, and (g) $0.01M$ SDS.

*21. What are the relative amounts of each isozyme formed when equimolar amounts of pure heart and muscle lactate dehydrogenase (H_4 and M_4) are hybridized?

*22. The SDS–polyacrylamide gel electrophoresis of a protein yields two bands corresponding to molecular masses of 10 and 17 kD. After cross-linking this protein with dimethylsuberimidate under sufficient dilution to eliminate intermolecular cross-linking, SDS–polyacrylamide gel electrophoresis of the product yields 12 bands with molecular masses 10, 17, 20, 27, 30, 37, 40, 47, 54, 57, 64, and 74 kD. Assuming that dimethylsuberimidate can cross-link only contacting subunits, diagram the quaternary structure of the protein.
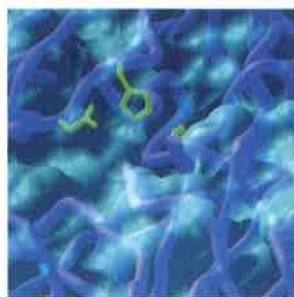
14

Enzymatic Catalysis

Enzymes, as we have seen, cause rate enhancements that are orders of magnitude greater than those of the best chemical catalysts. Yet, they operate under mild conditions and are highly specific as to the identities of both their substrates and their products. These catalytic properties are so remarkable that many nineteenth century scientists concluded that enzymes have characteristics that are not shared by substances of nonliving origin. To this day, there are few enzymes for which we understand in more than cursory detail how they achieve their enormous rate accelerations. Nevertheless, it is now abundantly clear that the catalytic mechanisms employed by enzymes are identical to those used by chemical catalysts. Enzymes are simply better designed.

In this chapter we consider the nature of enzymatic catalysis. We begin by discussing the underlying principles of chemical catalysis as elucidated through the study of organic reaction mechanisms. We then embark on a detailed examination of the catalytic mechanisms of several of the best characterized enzymes: **lysozyme,** the **serine proteases,** and **glutathione reductase.** Their study should lead to an appreciation of the intracacies of these remarkably efficient catalysts as well as of the experimental methods used to elucidate their properties.

1. CATALYTIC MECHANISMS

Catalysis is a process that increases the rate at which a reaction approaches equilibrium. Since, as we discussed in Section 13-1C, the rate of a reaction is a function of its free energy of activation ($\Delta G^{\ddagger}$), a catalyst acts by lowering the height of this kinetic barrier; that is, a catalyst stabilizes the transition state with respect to the uncatalyzed reaction. There is, in most cases, nothing unique about enzymatic mechanisms of catalysis in comparison to nonenzymatic mechanisms. *What apparently make enzymes such powerful catalysts are two related properties: their specificity of substrate binding combined with their optimal arrangement of catalytic groups.* An enzyme's arrangement of binding and catalytic groups is, of course, the product of eons of evolution: Nature has had ample opportunity to "fine tune" the performances of most enzymes.

The types of catalytic mechanisms that enzymes employ have been classified as:

1. Acid–base catalysis.
2. Covalent catalysis.
3. Metal ion catalysis.
4. Electrostatic catalysis.
5. Proximity and orientation effects.
6. Preferential binding of the transition state complex.

In this section, we examine these various phenomena. In doing so we shall frequently refer to the organic model compounds that have been used to characterize these catalytic mechanisms.

A. Acid–Base Catalysis

General acid catalysis is a process in which partial proton transfer from a Brønsted acid (a species that can donate protons; Section 2-2A) lowers the free energy of a reaction's transition state. For example, an uncatalyzed keto–enol tautomerization reaction occurs quite slowly as a result of the high energy of its carbanionlike transition state (Fig. 14-1a). Proton donation to the oxygen atom (Fig. 14-1b), however, reduces the carbanion character of the transition state, thereby catalyzing the reaction. *A reaction may also be stimulated by general base catalysis if its rate is increased by partial proton abstraction by a Brønsted base (a species that can combine with a proton; Fig. 14-1c). Some reactions may be simultaneously subject to both processes: a concerted general acid–base catalyzed reaction.*

**Mutarotation Is Catalyzed by Acids and by Bases**

The mutarotation of glucose provides an instructive example of acid–base catalysis. Recall that a glucose molecule can assume either of two anomeric cyclic forms through the intermediacy of its linear form (Section 10-1B):
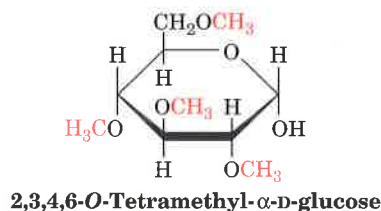

α-D-Glucose
$[\alpha]_D^{20} = 112.2°$

β-D-Glucose
$[\alpha]_D^{20} = 18.7°$

Linear form
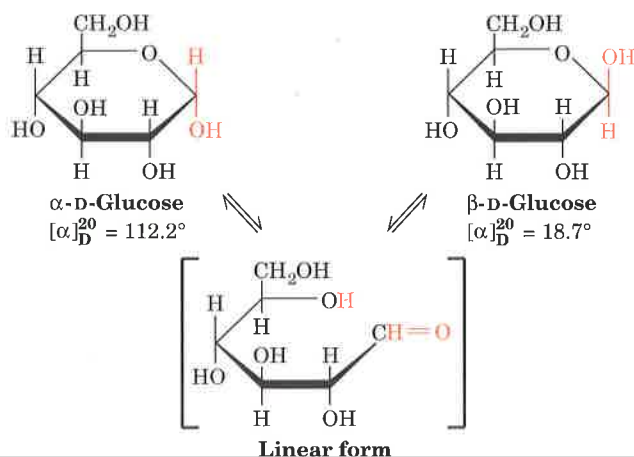
In aqueous solvents, the initial rate of mutarotation of α-D-glucose, as monitored by polarimetry (Section 4-2A), is observed to follow the relationship:

$$v = -\frac{d[\alpha\text{-D-glucose}]}{dt} = k_{obs}\,[\alpha\text{-D-glucose}] \quad [14.1]$$

where k_{obs} is the reaction's apparent first-order rate constant. The mutarotation rate increases with the concentrations of general acids and general bases; they are thought to catalyze mutarotation according to the mechanism:

α-D-Glucose   β-D-Glucose

Linear form
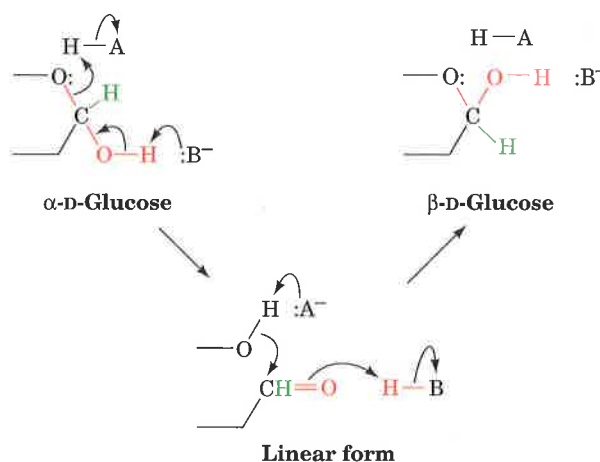
This model is consistent with the observation that in aprotic solvents such as benzene, **2,3,4,6-*O*-tetramethyl-α-D-glucose** (a less polar benzene-soluble analog)

2,3,4,6-*O*-Tetramethyl-α-D-glucose

does not undergo mutarotation. Yet, the reaction is catalyzed by the addition of phenol, a weak benzene-soluble acid, together with pyridine, a weak benzene-soluble base, according to the rate equation:

$$v = k[\text{phenol}]\,[\text{pyridine}]\,[\text{tetramethyl-}\alpha\text{-D-glucose}] \quad [14.2]$$

Moreover, in the presence of **α-pyridone,** whose acid and base groups can rapidly interconvert between two tauto-
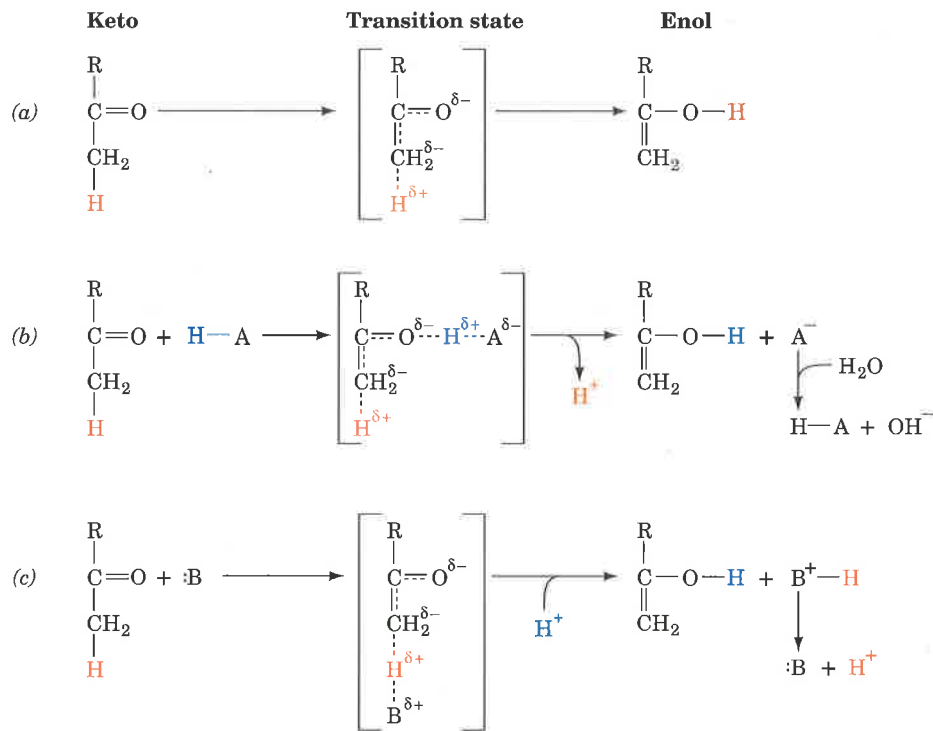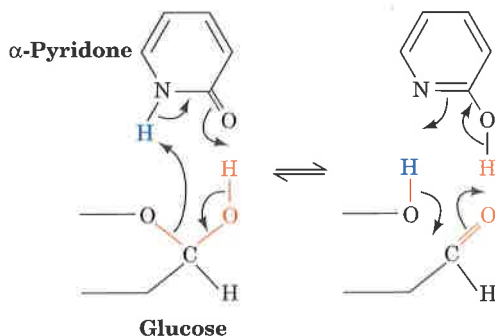
	Keto	Transition state	Enol

FIGURE 14-1. Mechanisms of keto–enol tautomerization: (*a*) uncatalyzed, (*b*) general acid catalyzed, and (*c*) general base catalyzed.

meric forms and are situated so that they can simultaneously catalyze mutarotation,

the reaction follows the rate law

$$v = k'[\alpha\text{-pyridone}][\text{tetramethyl-}\alpha\text{-D-glucose}] \quad [14.3]$$

where $k' = 7000M \times k$. This increased rate constant indicates that α-pyridone does, in fact, catalyze mutarotation in a concerted fashion since $1M$ α-pyridone has the same cata-

lytic effect as impossibly high concentrations of phenol and pyridine (e.g., $70M$ phenol and $100M$ pyridine).

Many types of biochemically significant reactions are susceptible to acid and/or base catalysis. These include the hydrolysis of peptides and esters, the reactions of phosphate groups, tautomerizations, and additions to carbonyl groups. The side chains of the amino acid residues Asp, Glu, His, Cys, Tyr, and Lys have pK's in or near the physiological pH range (Table 4-1) which, we shall see, permits them to act in the enzymatic capacity of general acid and/or base catalysts in analogy with known organic mechanisms. Indeed, the ability of enzymes to arrange several catalytic groups about their substrates makes concerted acid–base catalysis a common enzymatic mechanism.

The RNase A Reaction Incorporates General Acid–Base Catalysis

Bovine pancreatic ribonuclease A (RNase A) provides an illuminating example of enzymatically mediated general acid–base catalysis. This digestive enzyme functions to hydrolyze RNA to its component nucleotides. The isolation of **2′,3′-cyclic nucleotides** from RNase A digests of RNA

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