1. **Keeping the Sweet Taste of Corn** The sweet taste of freshly picked corn (maize) is due to the high level of sugar in the kernels. Store-bought corn (several days after picking) is not as sweet, because about 50% of the free sugar is converted to starch within one day of picking. To preserve the sweetness of fresh corn, the husked ears can be immersed in boiling water for a few minutes ("blanched") then cooled in cold water. Corn processed in this way and stored in a freezer maintains its sweetness. What is the biochemical basis for this procedure?

Answer After an ear of corn has been removed from the plant, the enzyme-catalyzed conversion of sugar to starch continues. Inactivation of these enzymes slows down the conversion to an imperceptible rate. One of the simplest techniques for inactivating enzymes is heat denaturation. Freezing the corn lowers any remaining enzyme activity to an insignificant level.

3. **Rate Enhancement by Urease** The enzyme urease enhances the rate of urea hydrolysis at pH 8.0 and 20 °C by a factor of 10^{14}. If a given quantity of urease can completely hydrolyze a given quantity of urea in 5.0 min at 20 °C and pH 8.0, how long would it take for this amount of urea to be hydrolyzed under the same conditions in the absence of urease? Assume that both reactions take place in sterile systems so that bacteria cannot attack the urea.

Answer

Time to hydrolyze urea

$$= \frac{(5.0 \text{ min})(10^{14})}{(60 \text{ min/hr})(24 \text{ hr/day})(365 \text{ days/yr})}$$

$$= 9.5 \times 10^{8} \text{ yr}$$

$$= 950 \text{ million years!}$$

4. **Protection of an Enzyme against Denaturation by Heat** When enzyme solutions are heated, there is a progressive loss of catalytic activity over time due to denaturation of the enzyme. A solution of the enzyme hexokinase incubated at 45 °C lost 50% of its activity in 12 min, but when incubated at 45 °C in the presence of a very large concentration of one of its substrates, it lost only 3% of its activity in 12 min. Suggest why thermal denaturation of hexokinase was retarded in the presence of one of its substrates.

Answer One possibility is that the ES complex is more stable than the free enzyme. This implies that the ground state for the ES complex is at a lower energy level than that for the free enzyme, thus *increasing the height of the energy barrier* to be crossed in passing from the native to the denatured or unfolded state.

An alternative view is that an enzyme denatures in two stages: reversible conversion of active native enzyme (N) to an inactive unfolded state (U), followed by irreversible conversion to inactivated enzyme (I):

$$N \rightleftharpoons U \longrightarrow I$$

If substrate, S, binds only to N, saturation with S to form NS would leave less free N available for conversion to U or I, as the $N \rightleftharpoons U$ equilibrium is perturbed toward N. If N but not NS is converted to U or I, then substrate binding will cause stabilization.

5. Requirements of Active Sites in Enzymes Carboxypeptidase, which sequentially removes carboxyl-terminal amino acid residues from its peptide substrates, is a single polypeptide of 307 amino acids. The two essential catalytic groups in the active site are furnished by Arg^{145} and Glu^{270}.

(a) If the carboxypeptidase chain were a perfect α helix, how far apart (in Å) would Arg^{145} and Glu^{270} be? (Hint: see Fig. 4–4a.)

(b) Explain how the two amino acid residues can catalyze a reaction occurring in the space of a few angstroms.

Answer

(a) Arg^{145} is separated from Glu^{270} by $270 - 145 = 125$ amino acid (AA) residues. From Figure 4–4a we see that the α helix has 3.6 AA/turn and increases in length along the major axis by 5.4 Å/turn. Thus, the distance between the two residues is

$$\frac{(125 \text{ AA})(5.4 \text{ Å/turn})}{3.6 \text{ AA/turn}} = 190 \text{ Å}$$

(b) Three-dimensional folding of the enzyme brings the two amino acid residues into close proximity.

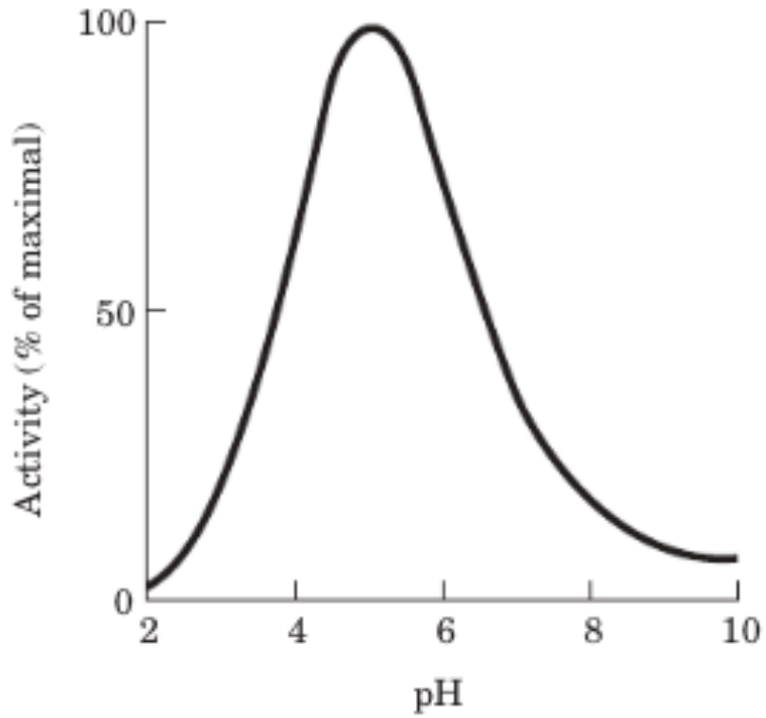
7. Effect of Enzymes on Reactions Which of the following effects would be brought about by any enzyme catalyzing the simple reaction

$$\mathrm{S} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \mathrm{P} \quad \text{where} \qquad K'_{\mathrm{eq}} = \frac{[\mathrm{P}]}{[\mathrm{S}]}\,?$$

(a) Decreased K'_{eq}; (b) Increased k_1; (c) Increased K'_{eq}; (d) Increased $\Delta G^{\ddagger}$; (e) Decreased $\Delta G^{\ddagger}$; (f) More negative $\Delta G'^{\circ}$; (g) Increased k_2.

Answer (b), (e), (g). Enzymes do not change a reaction's equilibrium constant and thus catalyze the reaction in both directions, making (b) and (g) correct. Enzymes increase the rate of a reaction by lowering the activation energy, hence (e) is correct.

21. pH Optimum of Lysozyme The active site of lysozyme contains two amino acid residues essential for catalysis: Glu^{35} and Asp^{52}. The pK_a values of the carboxyl side chains of these residues are 5.9 and 4.5, respectively. What is the ionization state (protonated or deprotonated) of each residue at pH 5.2, the pH optimum of lysozyme? How can the ionization states of these residues explain the pH-activity profile of lysozyme shown below?



Answer At a pH midway between the two pK_a values (pH 5.2), the side-chain carboxyl group of Asp^{52}, with the lower pK_a (4.5), is mainly deprotonated ($—COO^-$), whereas Glu^{35}, with the higher pK_a (5.9; the stronger base), is protonated (—COOH). At pH values below 5.2, Asp^{52} becomes protonated and the activity decreases. Similarly, at pH values above 5.2, Glu^{35} becomes deprotonated and the activity also decreases. The pH-activity profile suggests that maximum catalytic activity occurs at a pH midway between the pK_a values of the two acidic groups, when Glu^{35} is protonated and Asp^{52} is deprotonated.