Chapter 11 Enzymatic Catalysis

Enzymes are biological catalysts that increase the rates of biochemical reactions. In some cases, the enzyme-catalyzed reaction is nearly 10^{15} times faster than the uncatalyzed reaction. Enzymes are proteins (or in some cases, RNA) with very specific functions and are active under mild conditions. Enzymes function by lowering the free energy of the reaction's transition state. This chapter first discusses the various types of enzymes, their substrate specificity, the roles of coenzymes and cofactors, and the reaction coordinate. Next, the six modes of catalysis are described in detail. The chapter ends with full descriptions of the catalytic activity of lysozyme and serine proteases.

Essential Concepts

General Properties of Enzymes

1. Enzymes differ from ordinary catalysts in their higher reaction rates, their action under milder reaction conditions, their greater reaction specificities, and their capacity for regulation.

2. The IUBMB enzyme classification system divides enzymes into six groups (each with subgroups and sub-subgroups) based on the type of reaction catalyzed. A set of four numbers identifies each enzyme.

3. Enzymes are highly specific for their substrates and reaction products. Hence, the enzyme and its substrate(s) must have geometric, electronic, and stereospecific complementarity. Enzymes, for example, yeast alcohol dehydrogenase, can distinguish prochiral groups.

4. Many enzymes require cofactors for activity. Cofactors may be metal ions or organic molecules known as coenzymes. Many vitamins are coenzyme precursors. Coenzymes may be cosubstrates, which must be regenerated in a separate reaction, or prosthetic groups, which are permanently associated with the enzyme. An enzyme without its cofactor(s) is called an apoenzyme and is inactive, and the enzyme with its cofactor(s) is a holoenzyme and is active.

Activation Energy and the Reaction Coordinate

5. According to transition state theory, the reactants of a reaction pass through a short-lived high-energy state that is structurally intermediate to the reactants and products. This so-called transition state is the point of highest free energy in the reaction coordinate diagram. The free energy difference between the reactants and the transition state is the free energy of activation, $\Delta G^{\ddagger}$. The reaction rate decreases exponentially with the value of $\Delta G^{\ddagger}$; that is, the greater the value of $\Delta G^{\ddagger}$, the slower the reaction. A catalyst provides a reaction pathway

14. In the Phillips mechanism for lysozyme, the NAM residue that binds in the D site is distorted toward the half-chair conformation. Glu 35 then transfers a proton to O1 to cleave the C1—O1 bond of the substrate (general acid catalysis). The resulting oxonium ion intermediate is stabilized by the ionized carboxyl group of Asp 52 (electrostatic catalysis). The addition of water completes the catalytic cycle. Because the substrate is distorted toward the transition-state conformation on binding, transition state stabilization is an important catalytic mechanism.

Serine Proteases

15. Serine proteases are a widespread family of enzymes that have a common mechanism. The active-site Ser (identified through its inactivation by diisopropylphosphofluoridate), His (identified through affinity labeling with a chloromethylketone substrate analog), and Asp (identified by X-ray crystallography) form a hydrogen-bonded catalytic triad. Nonhomologous serine proteases have developed the same catalytic triad through convergent evolution.

16. The differing substrate specificities of trypsin, chymotrypsin, and elastase depend in part on the shapes and charge distribution of the substrate-binding pocket near the active site.

17. Catalysis by serine proteases is a multistep process in which nucleophilic attack on the scissile bond by Ser 195 (using the chymotrypsinogen numbering system) results in a tetrahedral intermediate that decomposes to an acyl–enzyme intermediate. The replacement of the amine product with water is necessary for the formation of a second tetrahedral intermediate, which yields the carboxyl product and regenerated enzyme.

18. Serine proteases use acid–base catalysis (involving the Ser-His-Asp triad), covalent catalysis (formation of the tetrahedral intermediates), and catalysis through binding of the transition state in the oxyanion hole. The tight binding of bovine pancreatic trypsin inhibitor to trypsin inhibits the enzyme by preventing full attainment of the tetrahedral intermediate as well as the entry of water into the active site.

Guide to Study Exercises (text p. 320)

1. Enzymes, which are either proteins or RNA, differ from other catalysts in that they increase reaction rates to a greater extent; they act under mild conditions (temperatures below 100°C, atmospheric pressure, near-neutral pH); they are much more specific for the substrates and reaction products; and their activity can be regulated. (Section 11-1)

2. An enzyme's substrate specificity depends on the arrangement of amino acid residues and the noncovalent interactions that occur at the substrate-binding site. Although an enzyme may change conformation on binding substrate, it must be complementary to the substrate with respect to geometry, stereochemistry, and charge distribution. (Section 11-1B)

10. Zymogens (inactive precursors) of digestive enzymes are maintained in their inactive state in the pancreas in three ways. (a) Trypsin, which converts trypsinogen, chymotrypsin, and other enzymes to their active forms, is not produced until the zymogens have been secreted into the duodenum, where enteropeptidase cleaves trypsinogen to form trypsin. (b) Trace amounts of trypsin that are generated within pancreatic cells are inhibited by specific inhibitor proteins such as BPTI. (c) Zymogen granules are resistant to proteolytic degradation and thereby encapsulate any inappropriately formed trypsin or other proteases that it has activated. (Section 11-5D)

Questions

General Properties of Enzymes

1. What is an enzyme's EC number?

2. Explain why enzymes are stereospecific.

3. Why is deuterium labeling useful for investigating the stereospecificity of an enzymatic reaction?

4. What is an apoenzyme and how does it differ from a holoenzyme? Which form is active?

5. What is the relationship between vitamins and coenzymes?

6. Proteins can be chemically modified by a variety of reagents that react with specific amino acid residues. How can such reagents be used to identify residues involved in an enzyme's activity? What are the shortcomings of this method?

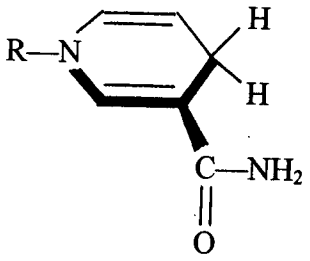
Activation Energy and the Reaction Coordinate

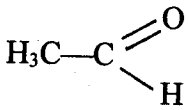
7. What is the rate-determining step of an enzyme-catalyzed reaction?

8. Answer yes or no to the following questions and explain your answer.
   (a) Can the absolute value of ΔG for a reaction be larger than $\Delta G^{\ddagger}$?
   (b) Can $\Delta G^{\ddagger}$ for an enzyme-catalyzed reaction be greater than $\Delta G^{\ddagger}$ for the nonenzymatic reaction?
   (c) In a two-step reaction, such as the one diagrammed in Figure 11-4, must the intermediate (I) have less free energy than the reactant (A)?
   (d) In a multistep reaction, does the transition state with the highest free energy always correspond to the rate-determining step?

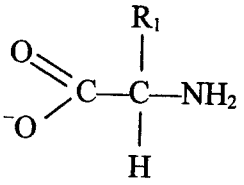
9. An increase in temperature increases the rate of a reaction. How does the temperature affect $\Delta G^{\ddagger}$?

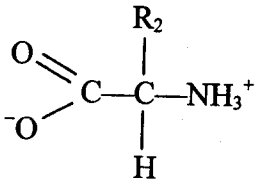
(b) NADH + acetaldehyde ⇌ ethanol + NAD^+





(c) 2 Amino acid ⇌ dipeptide + H_2O





18. What is the role of Zn^{2+} in carbonic anhydrase?

Lysozyme

19. Why does lysozyme appear to bind only NAG residues at subsites C and E?

20. Would $(NAG)_6$ or $(NAM)_6$ be a better substrate for lysozyme and why?

21. Hydrogen bonding of substrates to enzymes often involves the polypeptide backbone rather than amino acid side chains. What backbone–substrate hydrogen bond helps distort NAM in the D subsite of lysozyme? Can this hydrogen bond form when N-acetylxylosamine is in the active site?

22. Draw the resonance forms of the half-chair conformation of the oxonium ion intermediate of the lysozyme reaction.

Serine Proteases

23. What two catalytic residues in chymotrypsin were identified by chemical modification? Could the same reagents used to identify these residues be used to label the catalytic residues of other serine proteases?

5. Many coenzymes are synthesized from precursors that are vitamins (substances that an animal cannot synthesize and must obtain from its diet). However, not all coenzymes have vitamin precursors and not all vitamins are precursors of coenzymes.

6. Protein-modifying reagents can provide clues to the identities of catalytic residues. If chemical modification of a residue does not result in loss of activity, that residue can be ruled out as essential for catalysis. Loss of enzymatic activity on modification of a residue may indicate that the modified residue plays an essential role. However, chemical modification of residues at sites other than the active site may interfere with catalysis nonspecifically by disrupting protein structure.

7. The rate-determining step is the slowest of the steps in the reaction mechanism, the step with the greatest free energy of activation.

8. (a) Yes. ΔG is the difference in free energy between reactants and products, whereas $\Delta G^{\ddagger}$ is the difference in free energy between the reactants and the transition state.
   (b) No. By definition, a catalyst decreases $\Delta G^{\ddagger}$ of a reaction.
   (c) No. The free energy of the intermediate may be greater than that of the reactant. The reaction will proceed as long as the ΔG of the overall reaction A→P is negative.
   (d) No. The rate-determining step is the one whose $\Delta G^{\ddagger}$ is greatest. This does not always correspond to the step with the highest free energy, since $\Delta G^{\ddagger}$ depends on the difference in free energies between a reactant or an intermediate and the following transition state, not just on the free energy of this transition state.

9. $\Delta G^{\ddagger}$ is largely independent of temperature. An increase in temperature increases the rate of a reaction by increasing the number of reacting molecules that reach the transition state.

10. (a) The rate enhancement is

$$\begin{aligned} & e^{\Delta\Delta G^{\ddagger}/RT} \\ = \; & e^{(13\ \text{kJ·mol}-1)/(8.3145\ \text{J·K}-1\text{·mol}-1)(298\text{K})} \\ = \; & e^{5.25} \\ = \; & 190 \end{aligned}$$

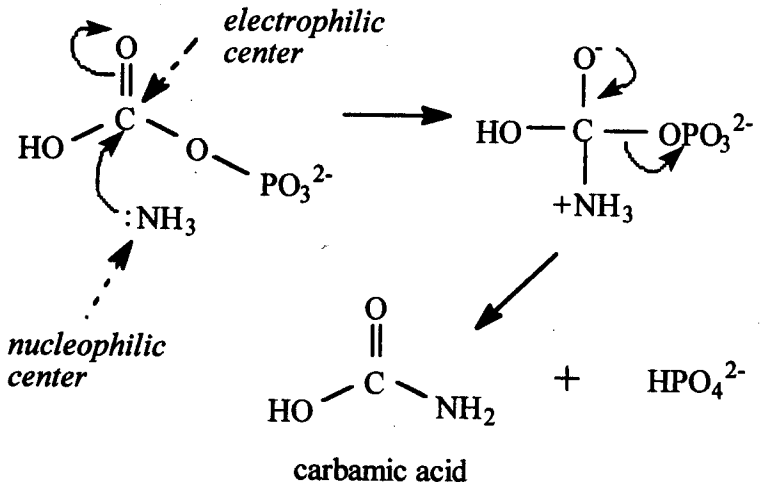
The enzyme-catalyzed reaction therefore proceeds 190 times faster than the uncatalyzed reaction.

(b) When the rate enhancement is 10^5,

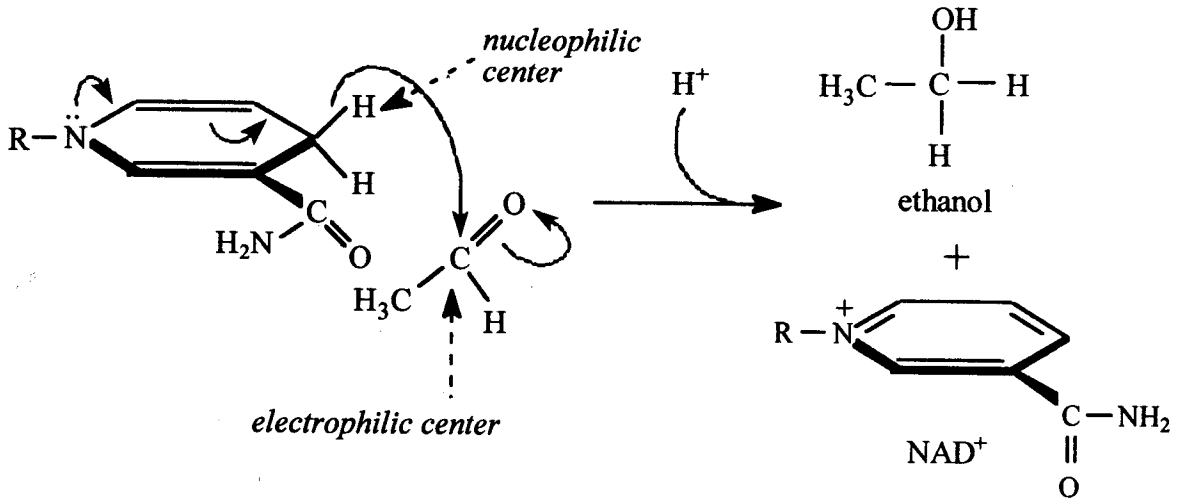
$$\begin{aligned} 100{,}000 &= e^{\Delta\Delta G^{\ddagger}/RT} \\ \Delta\Delta G^{\ddagger} &= RT \ln 100{,}000 \\ &= (8.3145\ \text{J·K}^{-1}\text{·mol}^{-1})(298\text{K})(11.5) \\ &= 28.5\ \text{kJ·mol}^{-1} \end{aligned}$$

11. Aspartate, cysteine, glutamate, histidine, lysine, and tyrosine are likely to participate in general acid–base catalysis. Glycine does not have an ionizable side chain.

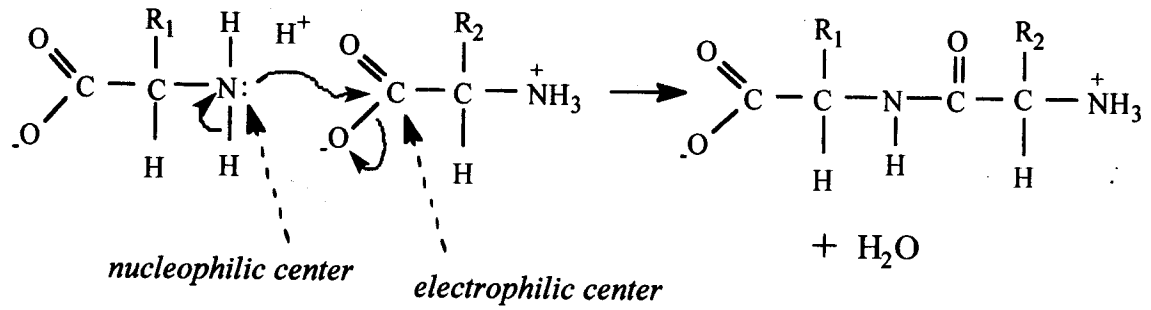
17. (a)


electrophilic center
nucleophilic center
carbamic acid

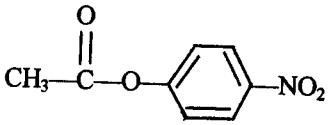
(b)


nucleophilic center
electrophilic center
ethanol
NAD+

(c)


nucleophilic center
electrophilic center

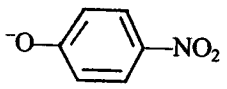
25. Chymotrypsin acts as an esterase, attacking the carbonyl C of the substrate's ester bond to form a tetrahedral intermediate. This is followed by decomposition to an acyl–enzyme intermediate with release of the first product, *p*-nitrophenolate.



p-Nitrophenylacetate

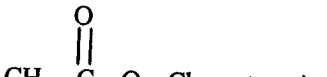
+

HO—Chymotrypsin



p-Nitrophenolate

+



Release of the second product, acetate, via hydrolysis of the acyl-enzyme intermediate, is much slower. Consequently, every active site attacks the substrate and releases the first product in a stoichiometric fashion, accounting for the initial burst of product formation. Because the second phase of the reaction is slow, the enzyme is only slowly regenerated and made available to catalyze additional rounds of the esterolytic reaction.

26. Stabilization of the transition state via the oxyanion hole is responsible for the largest portion of chymotrypsin's rate enhancement.

27. Bovine pancreatic trypsin inhibitor binds trypsin so tightly that the complex is too rigid to allow formation of the tetrahedral intermediate, to allow release of the first product, or to allow water to enter the active site of trypsin.

28. Trypsinogen's catalytic activity is too low to activate other trypsinogen molecules at a biologically significant rate. Enteropeptidase cleaves trypsinogen, thereby generating a small amount of trypsin that then commences autocatalytic activation. The role of enteropeptidase is to initiate this process in a controlled manner.

whose $\Delta G^{\ddagger}$ is less than that of the uncatalyzed reaction and hence increases the rate that the reaction achieves equilibrium.

Catalytic Mechanisms

6. Enzymes use several types of catalytic mechanisms, including acid–base catalysis, covalent catalysis, metal ion catalysis, electrostatic catalysis, catalysis by proximity and orientation effects, and catalysis by preferential binding of the transition state.

7. Acid–base catalysis occurs when partial proton transfer from an acid and/or partial proton abstraction by a base lowers the free energy of a reaction's transition state. The catalytic rates of enzymes that use acid–base catalysis are pH-dependent. RNase A has two catalytic His residues, which act as general acid and general base catalysts.

8. In covalent catalysis, the reversible formation of a covalent bond permits the stabilization of the transition state of the reaction through electron delocalization. Nucleophilic attack on the substrate by the enzyme to form a Schiff base intermediate capable of stabilizing (lowering the free energy of) a developing negative charge is an example of covalent catalysis. Common nucleophiles, which are negatively charged or contain unshared electron pairs, include imidazole and sulfhydryl groups.

9. Metalloenzymes tightly bind catalytically essential transition metal ions. Metal-activated enzymes loosely bind alkaline and alkaline earth metal ions that play a structural role. Metal ions may orient substrates for reaction, mediate oxidation–reduction reactions, and electrostatically stabilize or shield negative charges. For example, the Zn^{2+} of carbonic anhydrase makes a bound water molecule more acidic, thereby increasing the concentration of the nucleophile OH^-.

10. Electrostatic catalysis occurs through the proper positioning of charged residues in the active site such that they stabilize the transition state.

11. Enzymes lower the activation energies of the reactions they catalyze by bringing their reactants into proximity, by properly orienting them for reaction, and, most importantly, by freezing out the relative motions of the reactants and the enzyme's catalytic groups.

12. An enzyme's preferential binding of the transition state lowers $\Delta G^{\ddagger}$ and thereby increases the rate of the reaction. For this reason, an unreactive compound that mimics the transition state may be an effective enzyme inhibitor. Similarly, an antibody that binds with high affinity to a transition state analog of a reaction may also catalyze that reaction.

Lysozyme

13. Hen egg white lysozyme, an enzyme that cleaves the glycosidic linkage between NAG and NAM residues in bacterial cell walls, has a substrate-binding cleft that accommodates six sugar residues such that the cleavage occurs between the residues in subsites D and E.

3. Coenzymes are required for reactions that cannot readily be catalyzed by the functional groups that occur in the 20 standard amino acid residues. For example, oxidation–reduction reactions and many types of group-transfer reactions require cofactors such as metal ions and organic molecules (coenzymes). (Section 11-1C)

4. See Figure 11-5.

5. ΔG represents the difference in free energy between the reactants and products of a reaction and can be negative, positive, or zero. $\Delta G^{\ddagger}$, the free energy of activation, represents the difference in free energy between the reactants and the transition state and is always a positive quantity. ΔG indicates whether a reaction can proceed spontaneously (the reaction is favorable only when $\Delta G < 0$), whereas the rate of the reaction decreases exponentially with $\Delta G^{\ddagger}$ (the greater the value of $\Delta G^{\ddagger}$, the slower the rate). An enzyme may alter $\Delta G^{\ddagger}$ for a reaction, but it cannot alter its ΔG. (Section 11-2)

6. A nucleophile, which is an electron-rich group that is negatively charged or contains unshared electrons, can attack an electrophilic (electron poor) group to form a covalent bond. When the nucleophilic group of an enzyme attacks an electrophilic substrate, the resulting covalent bond allows the catalytic group (now an electrophile) to withdraw electrons, thereby facilitating the chemical transformation of the substrate. Because the covalent bond is unstable, the catalytic group is quickly eliminated, releasing the reaction product and the enzyme in its original nucleophilic form. (Section 11-3B)

7. Nonenzymatic catalysts commonly act via all modes of chemical catalysis except catalysis through stabilization of the transition state. This is because such a catalyst is unlikely to have the complex structure of an enzyme, whose binding site and arrangement of functional groups allow extensive interactions with the substrate throughout the catalytic process. (Section 11-3E)

8. Lysozyme accelerates the cleavage of its substrate through acid–base catalysis and electrostatic catalysis and by preferential binding of the transition state. Glu 35, a general acid catalyst, transfers its proton to O1 of the substrate's D ring to promote cleavage of the C1—O1 bond. Asp 52 acts as an electrostatic catalyst to stabilize the developing positive charge of the oxonium ion reaction intermediate. The D ring has already assumed a planar half-chair conformation, the conformation of the oxonium ion intermediate, in its initial binding to the enzyme, primarily through steric interference and through hydrogen bonding between O6 and the backbone NH of Val 109. Thus, lysozyme facilitates the reaction through stabilization of the transition state. (Section 11-4B)

9. The oxyanion hole of serine proteases refers to a portion of the active site that preferentially binds the tetrahedral reaction intermediate, thereby promoting catalysis through stabilization of the transition state. The substrate cannot occupy the oxyanion hole until it has been attacked by Ser 195. The resulting tetrahedral intermediate has a charged carbonyl oxygen that then moves into the hole to form three hydrogen bonds that are not present in the initial enzyme–substrate complex. (Section 11-5C)

10. $\Delta\Delta G^{\ddagger}$ for an enzymatic reaction at 25°C is 13 kJ/mole. (a) Calculate the rate enhancement. (b) What is $\Delta\Delta G^{\ddagger}$ when the rate enhancement is 10^5?

Catalytic Mechanisms

11. List the amino acid residues that are most likely to participate in general acid–base catalysis. Why isn't glycine among those listed?

12. If a protonated His residue acts as the proton donor in an acid-catalyzed enzymatic reaction, what happens to the enzyme's activity as the pH increases to a value that exceeds the pK_R of that residue?

13. The pK values of two essential catalytic residues in RNase A are 5.4 and 6.4. (a) Which corresponds to His 12 and which to His 119 (see Figure 11-8)? (b) Draw a titration curve for these two residues.

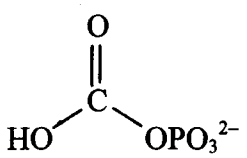
14. What is the difference between nucleophilic catalysis and general base catalysis?

15. A good covalent catalyst is highly nucleophilic and can form a good leaving group. What structural properties support these seemingly opposite characteristics?

16. Classify each of the following groups as an electrophile or nucleophile:
    (a) amine
    (b) carbonyl
    (c) cationic imine
    (d) hydroxyl
    (e) imidazole

17. For each of the following reactions, indicate the nucleophilic center and the electrophilic center. Draw curved arrows to indicate the movement of electrons and draw the reaction products.

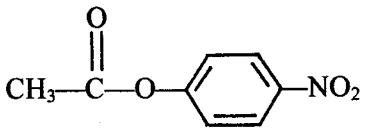
    (a) Carbonyl phosphate + ammonia $\rightleftharpoons$ carbamic acid + P_i



:NH_3

24. The different substrate specificities of chymotrypsin and trypsin have been attributed to the presence of different amino acid residues in the binding pocket. What problems can arise when site-directed mutagenesis is used to test predictions about the roles of such residues in substrate specificity?

25. The cleavage of the ester *p*-nitrophenylacetate (shown below) by chymotrypsin occurs in two stages. In the first stage, the product *p*-nitrophenolate is released in a burst, in amounts equivalent to the amount of active enzyme present. In the second stage, *p*-nitrophenolate is generated at a steady but much reduced rate. Explain this phenomenon in terms of the catalytic mechanism presented in Figure 11-26.



p-Nitrophenylacetate

26. What catalytic mechanism contributes the most to chymotrypsin's rate enhancement?

27. Lys 15 of bovine pancreatic trypsin inhibitor binds to the active site of trypsin but is not cleaved. Explain why the proteolytic reaction cannot proceed.

28. Since trypsin activation is autocatalytic, what is the role of enteropeptidase in activating trypsin?

Answers to Questions

1. The enzyme commission (EC) number is unique to each enzyme. An enzyme is assigned an EC number according to the type of reaction it catalyzes.

2. The protein (or RNA) enzyme is a chiral molecule whose binding clefts and catalytic residues are arranged in a specific three-dimensional asymmetric array. Hence, only substrates with the appropriate stereochemistry can bind to the enzyme, and the enzyme transforms the substrate to product according to the spatial arrangement of interacting functional groups.

3. Deuterium can be distinguished from hydrogen (usually by mass spectrometry). Substitution of a deuterium atom for a hydrogen atom usually does not significantly affect a reaction and provides a way to label a hydrogen-containing stereoisomer.

4. An apoenzyme is the protein portion of an enzyme that has lost its cofactor (a metal ion or coenzyme). A holoenzyme is an active enzyme containing both the protein and the cofactor.

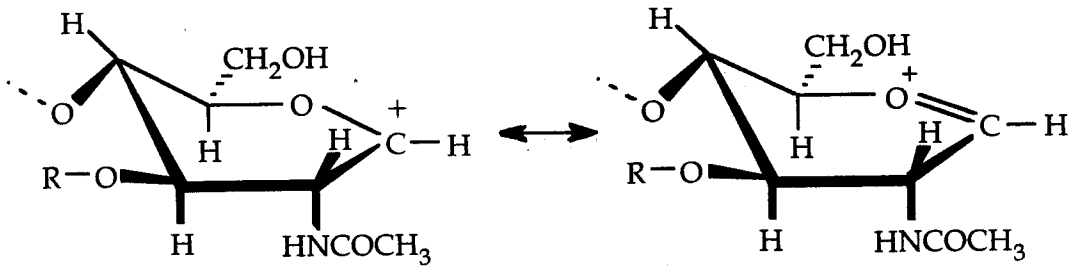
18. The Zn^{2+} in carbonic anhydrase polarizes a water molecule and thereby causes it to ionize. The resulting Zn^{2+}—OH^- is the nucleophile that attacks carbon dioxide, converting it to HCO_3^-. The metal ion stabilizes the negative charge of the OH^-, which would otherwise not form at neutral pH.

19. The lactyl side chain of NAM residues sterically prevents NAM binding to subsites C and E. Hence only NAG residues bind to these subsites.

20. NAG_6 would be better because the C and E residues of NAM_6 would not bind to their subsites.

21. The backbone NH group of Val 109 forms a hydrogen bond with O6 of the D-site residue, helping stabilize its half-chair conformation. *N*-acetylxylosamine lacks O6 and therefore cannot form this hydrogen bond.

22.



23. Ser 195 was identified through the use of diisopropylphosphofluoridate (DIPF), which reacts with the Ser —OH group at the active site of chymotrypsin and irreversibly inactivates the enzyme. His 57 was identified through affinity labeling using the substrate analog tosyl-L-phenylalanine chloromethylketone. DIPF also reacts with the active-site Ser in other serine proteases and therefore would label this residue. However, these other enzymes, not all of which share chymotrypsin's specificity for Phe-containing substrates, would require chloromethylketone analogs that incorporated residues corresponding to their substrate specificities, in order to react with their active-site His residues.

24. Site-directed mutations often change (or fail to change) enzymes in unexpected ways. For example, site-directed mutation of trypsin's Asp 189 to Ser did not change trypsin's specificity to that of chymotrypsin. Several other changes involving amino acids found on the surface loops surrounding the binding pocket were required for trypsin to emulate chymotrypsin.