Pearson New International Edition

Principles of Biochemistry

Laurence A. Moran Robert A. Horton
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Fifth Edition

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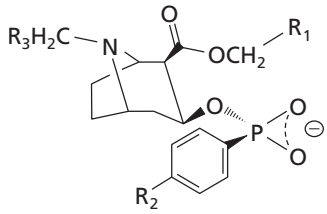
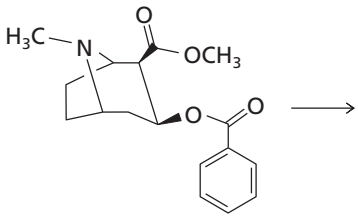
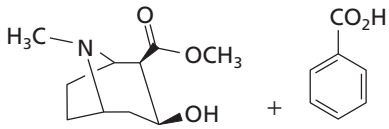
11. Catalytic antibodies are potential therapeutic agents for drug overdose and addiction. For example, a catalytic antibody that catalyzes the breakdown of cocaine before it reached the brain would be an effective detoxification treatment for drug abuse and addiction. The phosphonate analog below was used to raise an anticocaine antibody that catalyzes the rapid hydrolysis of cocaine. Explain why this phosphonate ester was chosen to produce a catalytic antibody.



Phosphonate analog



(−) - Cocaine



Ecgonine methyl ester

Benzoic acid

12. In the chronic lung disease emphysema, the lung's air sacs (alveoli), where oxygen from the air is exchanged for carbon dioxide in the blood, degenerate. α1-Proteinase inhibitor deficiency is a genetic condition that runs in certain families and results from mutations in critical amino acids in the sequence of α1-proteinase inhibitor. The individuals with mutations are more likely to develop emphysema. α1-Proteinase inhibitor is produced by the liver and then circulates in the blood. α1-Proteinase inhibitor is a protein that serves as the major inhibitor of neutrophil elastase, a serine protease present in the lung. Neutrophil elastase cleaves the protein elastin, which is an important component for lung function. The increased rate of elastin breakdown in lung tissue is believed to cause emphysema. One treatment for α1-proteinase inhibitor deficiency is to give the patient human wild-type α1-proteinase inhibitor (derived from large pools of human plasma) intravenously by injecting the protein directly into the bloodstream.

(a) Explain the rational for the treatment with wild-type α1-proteinase inhibitor.

(b) This treatment involves the intravenous administration of the wild-type α1-proteinase inhibitor. Explain why α1-proteinase inhibitor cannot be taken orally.

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General

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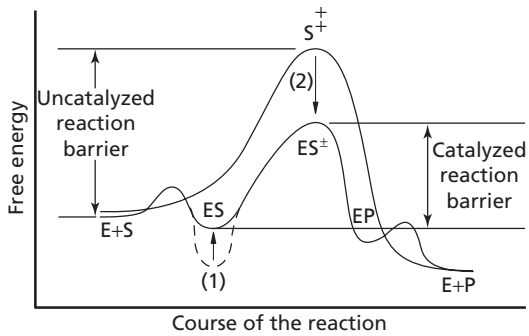
Solutions

1.(a) The major binding forces in ES complexes include charge–charge interactions, hydrogen bonds, hydrophobic interactions, and van der Waals forces. (About 20% of enzymes bind a substrate molecule or part of it covalently.)

(b) Tight binding of a substrate would produce an ES complex that lies in a thermodynamic pit, effectively increasing the activation energy and thereby slowing down the reaction. Tight binding of the transition state, however, lowers the energy of the $ES^{\ddagger}$ complex, thereby decreasing the activation energy and increasing the rate of the reaction.

2. The activation barrier for the reaction is lowered by (1) raising the ground-state energy level (ES) and (2) lowering the transition-state energy level ($ES^{\ddagger}$), resulting in a reaction rate increase.



3. The rate determining step of a multistep reaction is the slowest step, which is the step with the highest activation energy. For Reaction 1, Step 2 is the rate determining step. For Reaction 2, Step 1 is the rate determining step.

4. The reactive groups in Reaction 2 (—OH and —COOH) are held at close *proximity*. They are oriented in a manner suitable for catalysis by steric crowding of the bulky methyl groups of the ring. The reactive —COOH group cannot rotate away as freely as it can in Reaction 1. Model systems such as these are relevant because they indicate potential rate increases that might be obtained by enzymes that bring substrates and the enzyme's catalytic groups into positions that are optimal for reaction.

5. (1) Binding effects. Lysozyme binds the substrate so that the glycosidic bond to be cleaved is very close to both of the enzyme catalytic groups (Glu-35 and Asp-52). In addition, the energy of the ground-state sugar ring is raised because it is distorted into a half-chair conformation.

(2) Acid–base catalysis. Glu-35 first donates a proton to an oxygen of the leaving sugar (general acid catalysis), and then accepts a proton from the attacking water molecule (general base catalysis).

(3) Transition-state stabilization. Asp-52 stabilizes the developing positive charge on the oxocarbocation intermediate, and subsite D favors the half-chair sugar conformation of this intermediate. The structure proposed for the transition state includes both this charge and sugar conformation in addition to hydrogen bonding to several active-site residues.

6. Serine 195 is the only serine residue in the enzyme that participates in the catalytic triad at the active site of α-chymotrypsin. The resulting increase in the nucleophilic character of Ser-195 oxygen allows it to react rapidly with DFP.

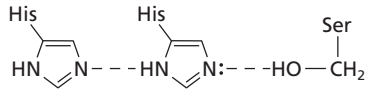
7. (a) The catalytic triad is composed of an aspartate, a histidine, and a serine residue. Histidine acts as a general acid–base catalyst, removing a proton from serine to make serine a more powerful nucleophile in the initial step. Aspartate forms a low-barrier hydrogen bond with histidine, stabilizing the transition state. An acid catalyst, histidine donates a proton to generate the leaving amine group.

(b) The oxyanion hole contains backbone —NH— groups that form hydrogen bonds with the negatively charged oxygen of the tetrahedral intermediate. The oxyanion hole mediates transition-state stabilization since it binds the transition state more tightly than it binds the substrate.

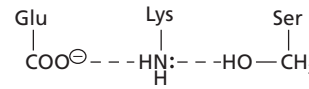
(c) During catalysis, aspartate forms a low-barrier hydrogen bond with the imidazolium form of histidine. Because asparagine lacks a carboxylate group to form the stabilizing hydrogen bond with histidine, enzyme activity is dramatically decreased.

8.

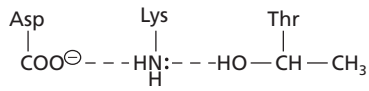
(a) Human cytomegalovirus protease: His, His, Ser



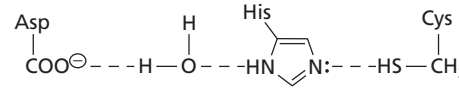
(b) β-Lactamase: Glu, Lys, Ser



(c) Asparaginase: Asp, Lys, Thr



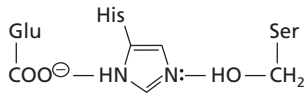
(d) Hepatitis A protease:



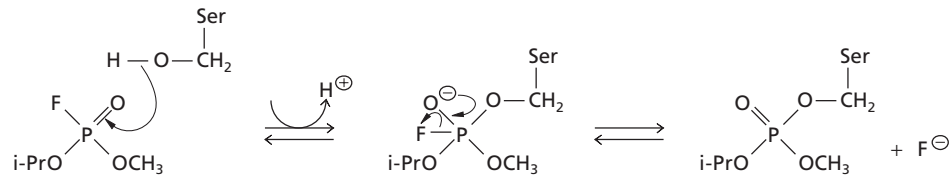
9. When tyrosine was mutated to phenylalanine, the activity of the mutant enzyme was less than 1% of the wild-type enzyme. Thus, the tyrosine residue is involved in the catalytic activity of

DDP-IV. Tyrosine contains an -OH group on the aromatic ring of the side chain. As previously stated, this tyrosine is found in the oxyanion hole of the active site. Hydrogen bonds in the oxyanion hole of serine proteases are known to stabilize the tetrahedral intermediate. Tyrosine with an -OH group on the side chain can form a hydrogen bond and stabilize the tetrahedral intermediate. Phenylalanine does not have a side chain that can form a hydrogen bond. Therefore, the tetrahedral intermediate will not be stabilized resulting in a loss of enzyme activity.

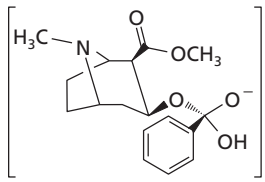
10. (a) Acetylcholinesterase catalytic triad: Glu-His-Ser



(b)



11. Transition-state analogs bound to carrier proteins are used as antigens to induce the formation of antibodies with catalytic activity. The tetrahedral phosphonate ester molecule is an analog of the tetrahedral intermediate structure in the transition state for hydrolysis of the benzyl ester moiety of cocaine. An antibody raised against the phosphonate structure that was able to stabilize the transition state of the cocaine benzyl ester hydrolysis could effectively catalyze this reaction.



Transition state

12. (a) Wild-type α1-proteinase inhibitor is given as treatment to individuals who produce an α1-proteinase inhibitor with substitutions in the amino acid sequence. These changes result in a protein that does not effectively inhibit the protease elastase. Uncontrolled elastase activity leads to increased breakdown of elastin, leading to destructive lung disease. Therefore, these patients are given a functional elastase inhibitor.

(b) The treatment for α1-proteinase inhibitor deficiency is to administer the wild-type protein intravenously. If the protein is given orally, the enzymes present in the digestive tract will cleave the peptide bonds in the α1-proteinase inhibitor. By administering the drug directly into the bloodstream, the protein can circulate to the lungs to act at the site of the neutrophil elastase.

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Carbohydrates

Carbohydrates (also called saccharides) are—on the basis of mass—the most abundant class of biological molecules on Earth. Although all organisms can synthesize carbohydrate, much of it is produced by photosynthetic organisms, including bacteria, algae, and plants. These organisms convert solar energy to chemical energy that is then used to make carbohydrate from carbon dioxide. Carbohydrates play several crucial roles in living organisms. In animals and plants, carbohydrate polymers act as energy storage molecules. Animals can ingest carbohydrates that can then be oxidized to yield energy for metabolic processes. Polymeric carbohydrates are also found in cell walls and in the protective coatings of many organisms. Other carbohydrate polymers are marker molecules that allow one type of cell to recognize and interact with another type. Carbohydrate derivatives are found in a number of biological molecules, including some coenzymes and the nucleic acids.

The name *carbohydrate*, "hydrate of carbon," refers to their empirical formula $(CH_2O)_n$, where n is 3 or greater (n is usually 5 or 6 but can be up to 9). Carbohydrates can be described by the number of monomeric units they contain. **Monosaccharides** are the smallest units of carbohydrate structure. **Oligosaccharides** are polymers of two to about 20 monosaccharide residues. The most common oligosaccharides are disaccharides, which consist of two linked monosaccharide residues. **Polysaccharides** are polymers that contain many (usually more than 20) monosaccharide residues. Oligosaccharides and polysaccharides do not have the empirical formula $(CH_2O)_n$ because water is eliminated during polymer formation. The term *glycan* is a more general term for carbohydrate polymers. It can refer to a polymer of identical sugars (homoglycan) or of different sugars (heteroglycan).

Glycoconjugates are carbohydrate derivatives in which one or more carbohydrate chains are linked covalently to a peptide, protein, or lipid. These derivatives include proteoglycans, peptidoglycans, glycoproteins, and glycolipids.

In this chapter, we discuss nomenclature, structure, and function of monosaccharides, disaccharides, and the major homoglycans—starch, glycogen, cellulose, and

Molecular biology has dealt largely on the triad of DNA, RNA and protein. Biochemistry is concerned with all the molecules of the cell. Excluded from the province of molecular biology have been most of the structures and functions essential for growth and maintenance: carbohydrates, coenzymes, lipids, and membranes.

—Arthur Kornberg
"For the love of enzymes: the odyssey of a biochemist" (1989)

Top: Darkling beetle. The exoskeletons of insects contain chitin, a homoglycan.

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