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Catalog No. AP1768

Publication No. 1768

Physical Properties of Proteins

Student Laboratory Kit

Introduction

The effects of acids and bases, inorganic salts, organic solvents, and temperature on the physical properties of proteins can help us understand the structures of proteins and how they fulfill their vital biological functions.

Biological and Chemical Concepts

- Protein folding
- Denaturation
- Native structure
- Salting out

Background

Structure often relates to function—nowhere is this relationship more evident than in the description of the structures, physical properties, and biological roles of proteins. The structure of hemoglobin allows it to bind to oxygen and deliver oxygen to body tissues. The structure of a specific antibody protein allows it to recognize, bind, and destroy a potentially harmful foreign substance. The structure of collagen makes skin both elastic and strong.

The biological activity of a protein depends on its three-dimensional shape. All proteins have a common structural "backbone"—amino acid building blocks joined by chemical bonds called peptide linkages. There are more than 20 different, naturally occurring amino acids; they differ in the types of atoms that are attached to the main polypeptide backbone. These amino acid side chains—which can be large or small, polar or nonpolar, acidic or basic, positively or negatively charged—interact through a variety of forces. These forces include hydrogen bonding involving side chain –OH groups, dipole interactions among polar amino acids, ionic "salt bridges" between positively and negatively charged side chains, and hydrophobic effects that stabilize large, nonpolar side chains. All of these forces cause protein chains to twist and fold back on themselves into globular or spherical shapes.

Protein folding is the name given to the process by which proteins naturally coil around or fold in on themselves in order to form a stable three-dimensional structure. Since every protein has a unique sequence of amino acids, every protein also has a unique shape—called its *native structure*—that makes the protein both stable and functional.

The forces that maintain the structure of proteins are illustrated schematically in Figure 1.

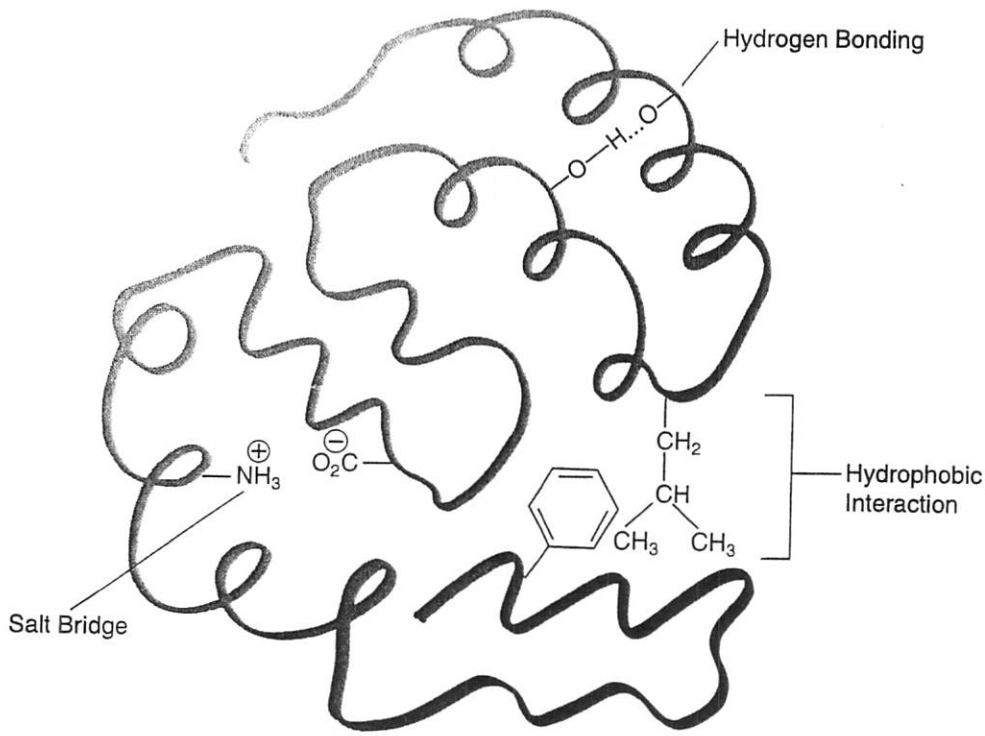

Figure 1.

Denaturation

Any factor that disrupts the native structure of a protein will destroy its function. Destruction of the three-dimensional shape of a protein by physical or chemical means is called *denaturation*. Proteins become denatured by any action which breaks hydrogen bonds, destroys salt bridges, or interferes with hydrophobic interactions. Denaturation causes protein molecules to clump together and precipitate out of solution; the resulting loss of biological activity is generally irreversible. Heating, freezing, and agitation are physical processes that result in protein denaturation. Chemical agents that cause protein denaturation include strong acids and bases, organic solvents, and heavy metal salts.

Most proteins are denatured by temperatures above 50 °C (normal body temperature is 37 °C). Cooking an egg provides an everyday example of the changes that occur when a protein solution—the egg white—is heated. Heat supplies excess energy and destabilizes all of the major forces that hold a protein together. Addition of strong acids or bases affects the number of charges on amino acid side chains and interferes with ionic "salt bridge" formation in proteins. Strong acids increase the concentration of H^+ ions in solution, which neutralize negatively charged side chains. Strong bases increase the concentration of OH^- ions that in turn neutralize positively charged side chains. Proteins have an optimal pH range at which they are most stable, most soluble, and most active. Small pH changes around the optimum pH may reduce the solubility of a protein, but these changes are usually reversible. High concentrations of strong acid and strong base, on the other hand, coagulate proteins and lead to total loss of protein structure and function—irreversible denaturation. Proteins can also be denatured by the addition of polar organic solvents, such as alcohols and acetone, that interfere with hydrogen bonding. The poisonous nature of heavy metal salts containing Ag^+, Hg^{2+}, and Pb^{2+} ions is due to protein denaturation as well.

High concentrations of inorganic salts, such as ammonium sulfate, are used to precipitate proteins without loss of protein activity. The solubility of a protein decreases as the concentration of ionic compounds increases and the protein eventually precipitates completely. This process—called *salting out*—results from changes in hydrogen bonding between water molecules and the protein. Because salting out involves mild conditions, the process is generally reversible and thus ideally suited as a means of isolating proteins and purifying them to remove contaminants.

Pre-Lab Questions

1. Define the term denaturation. What is the most common, visible change that indicates denaturation has occurred?
2. Isopropyl alcohol is sold in drugstores as "rubbing alcohol," a disinfectant. What effect might alcohols have on bacterial proteins?

Materials

Albumin, 2% aqueous solution, 22 mL
Ammonium sulfate solution, $(NH_4)_2SO_4$, saturated, 25 mL
Casein, 2% aqueous solution, 2 mL
Copper(II) sulfate solution, $CuSO_4$, 0.1 M, 4 mL
Gelatin, 2% aqueous solution, 2 mL
Hydrochloric acid solution, HCl, 2.5 M, 6 mL
Isopropyl alcohol, $(CH_3)_2CHOH$, 2 mL
Silver nitrate solution, $AgNO_3$, 0.1 M, 2 mL
Sodium hydroxide solution, NaOH, 2.5 M, 5 mL
Water, distilled or deionized
Wash bottle
Beakers, 50- and 250-mL
Beral-type pipets, graduated, 9
Erlenmeyer flask, 125-mL
Filter paper and funnel
Hot plate
Stirring rod
Test tubes, small, 3
Test tube, medium, 1
Test tube clamp
Test tube rack
Thermometer

Safety Precautions

Hydrochloric acid and sodium hydroxide solutions are highly corrosive liquids and can cause skin burns. Silver nitrate solution is a corrosive liquid and toxic by ingestion; it will stain skin and clothes. Isopropyl alcohol is a flammable organic solvent; do not use near flames or other sources of ignition. Ammonium sulfate and copper sulfate solutions are slightly toxic by ingestion. Avoid exposure of all chemicals to eyes and skin. Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron. Wash hands thoroughly with soap and water before leaving the laboratory.

Procedure

Part A. Solubility and Protein Denaturation

1. Label three small test tubes 1–3.
2. Using a clean, graduated Beral-type pipet for each solution, add approximately 1 mL of albumin, casein, and gelatin to test tubes 1, 2, and 3, respectively. Record the initial appearance of each solution in Data Table A.
3. Add 2 drops of 2.5 M HCl to each test tube 1–3. Gently swirl each tube to mix the contents, then record the appearance of the solutions in Data Table A.
4. Add 5 more drops of 2.5 M HCl to each test tube 1–3. Swirl each sample mixture and record its appearance in Data Table A.
5. Add 10 more drops of 2.5 M HCl to each test tube 1–3. Swirl each sample mixture and record its appearance in Data Table A.
6. Add 10 more drops of 2.5 M HCl to each test tube 1–3. Swirl each sample mixture and record its appearance in Data Table A.
7. Wash the contents of each test tube down the drain with excess water and rinse the test tubes twice with distilled water from a wash bottle. Relabel the test tubes 1–3, if necessary.
8. Using the appropriate graduated Beral-type pipet for each solution, add approximately 1 mL of albumin, casein, and gelatin to test tubes 1, 2, and 3, respectively.
9. Add 5 drops of 2.5 M NaOH to each test tube 1–3. Gently swirl each tube to mix the contents and record the appearance of the solutions in Data Table 1.
10. Add 10 more drops of 2.5 M NaOH to each test tube 1–3. Swirl each sample mixture and record its appearance in Data Table A.
11. Wash the test tube contents down the drain with excess water and rinse the test tubes twice with distilled water. Relabel the test tubes 1–3, if necessary.
12. Add 1 mL of 2% albumin solution to each tube.
13. Using a clean, graduated Beral-type pipet for each reagent, add 2 mL of 0.1 M $CuSO_4$ to test tube 1, 2 mL of 0.1 M $AgNO_3$ to test tube 2, and 2 mL of isopropyl alcohol to test tube 3. Swirl each tube gently to mix the contents, then record the appearance of each sample in Data Table A.

Part B. "Salting Out" with Ammonium Sulfate

14. Add 10 mL of 2% albumin to a 50-mL beaker, followed by approximately 25 mL of saturated ammonium sulfate solution. Stir the mixture thoroughly using a glass stirring rod. Describe the appearance of the mixture in Data Table B.
15. Set up a gravity filtration apparatus and filter the mixture through a piece of wetted filter paper. Collect the liquid (filtrate) in a clean Erlenmeyer flask.
16. Label three small test tubes 1–3.
    - Add 2 mL of 2% albumin solution to test tube 1.
    - Add 2 mL of the filtrate from step 15 to test tube 2.
    - Remove a small portion of the precipitate from the funnel with the tip of a spatula, and dissolve the wet solid in 2 mL of distilled water in test tube 3.

17. To each test tube 1–3, add 10 drops of 2.5 M NaOH followed by 5 drops of 0.1 M $CuSO_4$ solution. Compare the appearance of the three solutions and record the observations in Data Table B.

Part C. Effect of Heat

18. *Prepare a hot water bath:* Fill a 250-mL beaker half-full with water and heat it on a hot plate at the lowest setting. Place a thermometer in the water bath to record the temperature of the bath.
19. To a medium size test tube, add 5 mL of 2% albumin solution.
20. When the temperature of the hot water bath is 35–40 °C, place the test tube in the bath. Record the initial temperature of the water bath in Data Table C. Adjust the heat setting on the hot plate to a medium-high range to slowly heat the protein solution.
21. Holding the test tube with a test tube clamp, gently swirl the protein solution and observe its appearance. Note the temperature of the bath when the first signs of protein precipitation are observed. Record the temperature and make observatons in Data Table C.
22. Continue heating the protein solution. Record the temperature of the hot water bath and make observations of the protein solution when it first appears milky white (opaque).
23. When the temperature of the hot water bath reaches 85–90 °C, remove the test tube. Record the final appearance of the protein sample in Data Table C.

Disposal

Consult your instructor for appropriate disposal procedures.

Name: _____

Physical Properties of Proteins

Results and Discussion

Data Table A. *Solubility and Protein Denaturation*

Effect of Strong Acid and Base				
Test Tube		**1**	**2**	**3**
Protein Solution		**Albumin**	**Casein**	**Gelatin**
Initial Appearance				
Effect of HCl Addition	**2 drops**			
	5 drops			
	10 drops			
	10 drops			
Effect of NaOH Addition	**5 drops**			
	10 drops			

Effect of Inorganic and Organic Additives			
Test Tube	**1**	**2**	**3**
Additive	**$CuSO_4$**	**$AgNO_3$**	**Isopropyl Alcohol**
Results			

Data Table B. *"Salting Out" with Ammonium Sulfate*

	Observations
Effect of Ammonium Sulfate	
Test Tube 1 (Albumin + $CuSO_4$)	
Test Tube 2 (Filtrate + $CuSO_4$)	
Test Tube 3 (Redissolved solid + $CuSO_4$)	

Data Table C. *Effect of Heat*

	Temperature	Additional Observations
Initial temperature (water bath)		
First signs of precipitate appeared		
Solution appeared milky white		
Final observations		

Post-Lab Questions

Use a separate sheet of paper to answer the following questions.

1. Compare and contrast the effect of strong acid (2.5 M HCl) on albumin, casein, and gelatin. Which protein was most sensitive to the action of strong acid? Least sensitive?
2. Do strong acid and strong base have similar effects on protein solubility and denaturation? Explain.
3. Which metal salts ($CuSO_4$ and $AgNO_3$) caused albumin denaturation? Relate this observation to the fact that silver salts are more toxic than copper salts.
4. You have just been to the doctor's office to receive an inoculation. Before administering the injection, the doctor wipes the area with an alcohol swab. Do your results for the effect of alcohol on albumin denaturation support the use of isopropyl alcohol as a disinfectant?
5. The reaction of $CuSO_4$ with proteins in strong base is used as a color test to identify proteins. What do the results obtained in Part B for the reaction of $CuSO_4$ with albumin and the filtrate tell you about the effectiveness of the "salting out" procedure with ammonium sulfate?
6. Is the denaturation of albumin by ammonium sulfate reversible or irreversible? Explain on the basis of your observations for the reaction of $CuSO_4$ with albumin (test tube 1) and the redissolved precipitate (test tube 3), respectively, in Part B.
7. Based on the results of Part C, suggest a reason why heat is an effective form of sterilization for biological materials and equipment.