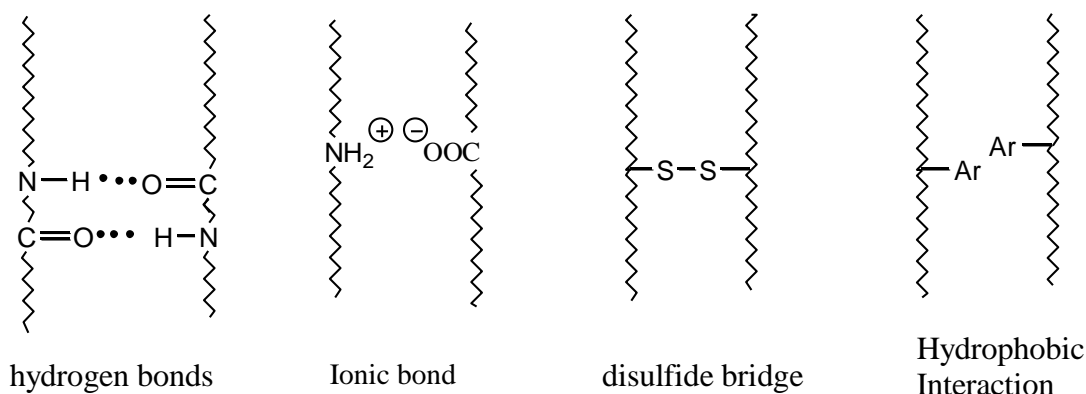


Proteins and Denaturing Agents

Introduction

Proteins are polymers of amino acids. A typical protein may be composed of hundreds of amino acids. The R-groups of the amino acid may be nonpolar, polar, positively charged, or negatively charged. The primary structure of a protein is the sequence of amino acids, and the secondary and tertiary structures of proteins define the proteins folded state. This is called the native conformation and is usually the state in which the protein is most active and functional. Proteins are held in their native conformations by a combination of forces: hydrogen bonds, ionic interactions, disulfide bridges, and hydrophobic interactions.

Changing the conformation of a protein either temporarily or permanently by disrupting these forces is called denaturation. Denaturation results in a loss of protein activity. Since the native conformation is usually the most water soluble, disrupting the secondary and tertiary structures causes changes in solubility and frequently results in the formation of a solid in the solution. Reagents or conditions that can cause denaturation are called denaturing agents; these include heat, pH changes, alcohol, and heavy metal salts.



Heat can supply kinetic energy to protein molecules, causing their atoms to vibrate more rapidly. This will disrupt relatively weak forces such as hydrogen bonds and hydrophobic interactions. The most common example is observed in cooking an egg. Heat is also used in sterilization to denature and hence destroy the enzymes in bacteria.

Extremes of pH can cause a protein to denature. The R-groups in the amino acid chain are often charged and can form ionic bonds with a group of opposite charge. Extremes of pH can change the charges on these positive and negative groups, disrupting ionic bonds.

Some reagents, such as **ethanol**, are capable of forming hydrogen bonds with protein molecules which will disrupt the hydrogen bonding within the molecule. A 70% solution of alcohol can be used as a disinfectant, because the alcohol functions to denature the proteins in bacteria. A 70% solution is used because it will effectively penetrate the bacterial cell wall; a 95% solution coagulates proteins at the surface of the cell wall, forming a crust that prevents the alcohol from penetrating into the cell.

Salts of metal ions such as mercury(II), lead(II), and silver can form strong bonds with disulfide groups of the protein. Thus, they disrupt both disulfide bridges and salt linkages and cause the protein to precipitate out of solution as an insoluble metal-protein salt. This property makes some of the heavy metal salts suitable for use as topical antiseptics. However, most heavy metal salts are toxic when taken internally, because they precipitate the proteins of all the cells with which they come into contact. Substances high in protein, such as egg whites and milk, are used as antidotes for heavy metal poisoning, because their proteins readily combine with the metal ions to form insoluble solids. The resulting insoluble matter must immediately be removed from

the stomach by the use of an emetic to prevent the gastric juices from destroying the protein and once again liberating the poisonous heavy metal ions.

In this exercise, the effect of several denaturing agents on the protein albumin will be studied. Albumin is a simple globular protein. It is soluble in water and dilute salt solutions such as isotonic saline (0.9% NaCl).

PART A: PURPOSE

Record the purpose of this lab in your book:

To examine four different ways of denaturing protein:

- A. Examine the effect of heat on the solubility of albumin
- B. Examine the effect of pH changes on the solubility of albumin
- C. Examine the effects of 95% ethanol on the solubility of albumin
- D. Examine the effects of lead(II) nitrate or silver nitrate on albumin

PART B: HYPOTHESIS

For each laboratory activity (A-D), write a detailed hypothesis outline what you expect to observe and why you expect this result. Include information about the effect of the denaturing agent on the bonding of the protein in your explanation.

PART C: PROCEDURES

1. Prepare a stock albumen solution by adding the white of one egg to 100 mL of distilled water in a beaker. Stir until the albumen is in solution.
2. Place 5 mL of the albumen solution into each of six test tubes. Label the tubes Control, A, B1, B2, C and D.
3. Treat the four solutions as follows:

Control: Let stand at room temperature. Measure the temperature. Determine the pH with pH paper.

A. The effect of heat

Heat it in a hot water bath for a few minutes.

B. The effect of pH changes

In tube B1, add 2 mL of 1M HCl. Record the pH using the pH test strips.

In tube B2, add 2 mL of 10% NaOH. Record the pH using the pH test strips.

C/D. The effects of ethanol and lead(II) nitrate or silver nitrate

In tube C, add 1 mL of 95% ethanol

In tube D, add 5-10 drops of 2% lead(II) nitrate or silver nitrate.

Compare each of the test solutions to the control. Record your observations.

PART D: DATA COLLECTION

For each laboratory activity (A-D), create a relevant data table to record all measurements and observations of your control and experimental groups.

PART E: QUESTIONS

Answer in complete sentences:

1. What was the purpose of the control tubes?
2. What common result occurred in all experimental tubes? Why?