

Objective

To study chemical and physical properties of proteins from natural sources (egg and milk) and some chemical reactions of amino acid residues in these proteins, as well as the effects of denaturing agents on these proteins.

Introduction

Proteins are polymers of the 20 common amino acids. The amino acids are linked through their α -amino and α -carboxyl groups to form peptide bonds. The proteins you will study are casein (milk protein) and albumin (in this case, from egg whites). Casein and egg albumin are soluble in water in their native or natural state. However, they may precipitate from solution in the presence of acids, alkali, heavy metals and other denaturing agents. Many of the chemical properties of proteins are due to the characteristics of the R groups of the amino acids that make up the protein. For example, acidification of ionized groups in proteins may neutralize their ionic charges resulting in decreased solubility in water or alterations in their interactions with other ionic and polar groups, causing them to precipitate. Heavy metals may bind to ionic or polar groups on the surface of proteins, causing them to aggregate and diminishing their interactions with water molecules and rendering them insoluble in water. Some of the amino acids in proteins are aromatic, such as tyrosine and tryptophan. You will study the nitration of these amino acids using nitric acid and show that the reactions can also occur when these amino acids are part of a protein chain. You will also study the reaction of sodium nitrite with the phenol group in tyrosine to produce a pink or red product. Other chemical reactions used to characterize proteins are due to interactions with the peptide bond, such as the complexation of copper (Cu^{2+}) ions in the presence of strong base, which is the basis of the biuret test for protein.

Reagents

Skim milk, egg, 0.5% glycine, 0.5% L-tyrosine, 0.5% L-tryptophan, vinegar or 5% acetic acid solution, 10% sodium hydroxide (NaOH), concentrated nitric acid (HNO_3), 1.0 M perchloric acid (HClO_4), 1 M ammonium sulfate ($(\text{NH}_4)_2\text{SO}_4$), 5% sodium nitrite (NaNO_2), 95% ethanol, 0.1 M copper (II) sulfate, 0.1 M mercury (II) chloride, 0.1 M lead (II) nitrate.

Procedure

Fill a 400 mL beaker about half full with water and heat it for a warm (40°C) water bath for the beginning of part A. Later it will be heated to higher temperatures for other parts.

The instructor or teaching assistant will demonstrate how to separate egg white from egg yolk and prepare a solution of one egg white in 500 mL deionized water for the egg albumin solution that will be used in this laboratory.

You will be using about 20 mL of egg white solution and about 20 mL of milk for the

following tests. You should add approximately this amount of each solution (egg white solution and skim milk) to two separate beakers to avoid congestion in the area where these are located. Do not pour any solutions back into their containers. If you have too much in your beaker, you can dispose of it in the sink at the end of class.

Part A. Precipitation of Casein from Milk and Albumin from Egg White.

Proteins are denatured (lose their natural structure and solubility) by a variety of denaturing agents, such as organic solvents, acids, bases, heavy metals (e.g., mercury or lead) and high salt concentration.

1. Place 2 mL of egg white solution in each of six clean large test tubes and place 2 mL of skim milk in each of six clean small test tubes.
2. Place one test tube containing egg white solution and one test tube containing milk in a warm water bath at about 40°C. After the solution in the test tubes has warmed for a few minutes, add about 1 mL of vinegar to each of the test tubes and notice whether any precipitate forms in the test tube. Record your observations on the Report Sheet.

You will have to heat the water bath to boiling for the 5th step below.

3. Add 1 mL (about 20 drops) of 1 M HClO₄ (perchloric acid) to a second tube containing egg white solution and do the same to a second tube containing milk, mix each tube well and record your observations on the Report Sheet.
4. Add 3 mL of 95% ethanol to the third tube of egg white solution and 3 mL of 95% ethanol to the third tube of milk, mix each tube well and notice whether any precipitate forms. Record your observations on the Report Sheet.
5. Place another test tube containing egg white solution and one tube containing milk in a boiling water bath for 5 min and record your observations on the Report Sheet noting whether there is any precipitation or clumping of protein in either tube.

You can dispose the contents of these test tubes in the sink. Do not mix them with the next ones.

CAUTION: HgCl₂ (mercuric chloride) and Pb(NO₃)₂ (lead nitrate) are poisonous. Do not spill it on you. Wash off any areas of contact with water. Do NOT dispose of these solutions in the sink.

6. To one test tube containing egg white solution and one test tube containing milk, carefully add 10 drops of mercury(II) chloride (HgCl₂) solution, mix well and record your observations on the Report Sheet.
7. To the sixth tube containing egg white solution and the final tube containing milk, carefully add 10 drops of lead nitrate (Pb(NO₃)₂) solution, mix well and record your

observations on the Report Sheet.

NOTE: Dispose the contents of these last 4 test tubes containing lead and mercury in the bottle labeled Toxic Metal Waste located in the fume hood. Wash these test tubes immediately.

Part B. The Xanthoproteic Test.

Aromatic rings in the amino acids react with nitric acid, as do all aromatic compounds, forming yellow nitrated products, which often turn deeper yellow in strong base.

CAUTION!!! Nitric acid is very corrosive to skin and clothing. Work with care in the hood and wash immediately with plenty of water if any gets on the skin.

1. Add 1 mL of egg white solution to a clean test tube; 1 mL of skim milk to a second test tube; 1 mL of 0.5% tyrosine solution to a third test tube; 1 mL of 0.5% tryptophan solution to a fourth test tube; and 1 mL of 0.5% glycine solution to a fifth test tube. Add 1 mL of concentrated nitric acid (in the hood) to each test tube and mix carefully.
2. After mixing, warm the test tubes in the hot water bath. Notice any changes in color of the solutions and record them on the Report Sheet.
3. Allow each tube to cool and carefully add 10% NaOH solution until each is alkaline to litmus paper (4-5 mL). Record your observations on the Report Sheet.

Part C. Biuret Test for Proteins.

Biuret is obtained by heating urea. Biuret contains amide bonds similar to those in proteins. Biuret reacts with copper (II) ions in basic solution to form a reddish violet complex ion.

1. Add 2 mL of egg white solution to a clean test tube and 2 mL of skim milk to another clean test tube. Add 1 mL of 10% NaOH and 2 drops of copper sulfate solution to each test tube and mix well. Allow these to stand for about 10 minutes and record your observations regarding the color of the solutions on the Report Sheet.
2. Add 2 mL of glycine solution to another clean test tube, add 1 mL of 10% NaOH and 2 drops of copper sulfate solution to this test tube, mix well and compare the results with the previous test solutions containing protein. Record the results on the Report Sheet.

Part D. Millon's Test.

Tyrosine is the only amino acid found in proteins having a phenolic group. On the addition of Millon's reagent, a pink color forms. The presence of Cl^- or NH_4^+ ions interfere with this reaction. You should rinse the test tube with deionized water before performing this test.

1. Add 1 mL of egg white solution to each of two clean test tubes; add 1 mL of skim milk to a separate clean test tubes; 1 mL of 0.5% tyrosine solution to another, and 1 mL of 0.5% glycine solution to a fifth clean test tube. Label each tube.
2. Add 2 drops of 1.0 M ammonium sulfate solution to one of the tubes containing egg white solution to show the effect of NH_4^+ ions on this test.
3. Add 10 drops of Millon reagent to each tube and place the test tubes in a hot water bath for 10 min.
4. Cool the test tubes in an ice bath. Add 10 drops of freshly prepared 5% NaNO_2 solution to each tube and note the color formed on the Report Sheet.

Part E. Test for Sulfur

1. Add 2 mL of egg white solution to a clean test tube, add 2 mL of 0.5% cysteine to another clean test tube and add 2 mL of 0.5% methionine to a third clean test tube.
2. Add 3 mL of 10% NaOH solution to each test tube and heat in a boiling water bath for a few minutes. Add 5 drops of lead nitrate solution to each tube and heat again for a few minutes. Record your observations on the Report Sheet.

Name _____

Section _____

Properties of Proteins

Experiment #9

Data & Report Sheet

Part A. Precipitation of Casein from Milk and Albumin from Egg White.

Record your observations regarding clumping of proteins and whether the clumps settle quickly to the bottom of the tube or remain suspended in the tube for a long time. You should also indicate whether the solution (especially egg white solution, which is clear to begin) becomes cloudy (i.e., small aggregates) or whether there are large aggregates or clumps.

| Vinegar | Mercuric Chloride | Lead Nitrate |
|--------------------|-------------------|--------------|
| | | |
| Boiling Water Bath | Perchloric Acid | Alcohol |
| | | |

A-1. How do you explain any differences between the behavior of egg albumin and casein with respect to precipitation by vinegar and precipitation by heating in boiling water?

Part B. Xanthoproteic Test for Aromatic Groups.

Record the color of the solution after heating with concentrated HNO₃ and then after making the solution alkaline with NaOH.

| Test Sample | Color with HNO ₃ | Change after adding NaOH |
|----------------|-----------------------------|--------------------------|
| Egg White Soln | | |
| Milk | | |
| Tyrosine | | |
| Tryptophan | | |
| Glycine | | |

B-1. Explain why any of the test samples show no color changes.

Part C. Biuret Test.

Indicate the colors observed for each of the test solutions when Cu²⁺ is added.

| Egg White Solution | Milk | Glycine |
|--------------------|------|---------|
| | | |

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C-1. Explain the differences in the color observed for each of the test solutions (recorded in the table above). What color is indicative of a positive biuret test? What color indicates a negative biuret test?

Part D. Millon's Test for Phenols.

Indicate the colors observed for each of the test solutions when Millon's reagent and sodium nitrite are added.

| Glycine | Tyrosine | Egg White (with NH_4^+) | Egg White (No NH_4^+) | Milk |
|---------|----------|--------------------------------------|------------------------------------|------|
| | | | | |

D-1. Explain the observed colors in each of the amino acid or protein samples.

D-2. Would you expect this test to be useful for the analysis of protein in urine? Explain why or why not.

Part E. Sulfur Test.

Record the observed changes in each solution after adding sodium hydroxide and lead nitrate to each sample.

| Egg White Solution | Cysteine | Methionine |
|--------------------|----------|------------|
| | | |

E-1. Although both cysteine and methionine contain sulfur, why would they react differently in this test?

E-2. Lead nitrate was used to denature proteins in part A and is used again here as a test for sulfur. What accounts for the difference in observations between the effects of lead in part A and in part E? Explain any similarities you may have observed (is there denaturation in both parts?) and any differences (are there differences in color?).

Some of the tests you have performed have been used in the past as tests for protein in urine. Why might a physician be interested in determining whether a patient has protein in the urine? What does protein in urine indicate?