Amino Acids and Proteins

Performance Goal

35–1 Perform identification tests on amino acids and proteins.

CHEMICAL OVERVIEW

Amino acids are molecules containing two functional groups: the amino group (—NH₂) and the carboxyl group (—COOH). Proteins are large, complex molecules, built from amino acids that are joined by peptide linkages. A peptide bond is formed when an acidic carboxyl group of one amino acid reacts with the basic amino group of another amino acid molecule. The formation of such a bond is shown in Equation 35.1.

Peptide bond

The product still contains a free amino group, which gives basic properties to the molecule, and a free carboxyl group, which gives it acidic properties.

When two amino acids react with each other, the product is a **dipeptide**. When a very large number of amino acids are linked, the **polypeptides** that form are known as **proteins**. These molecules make up our skin, muscles, and enzymes. Hormones, hair, and fingernails are also made of protein. In order for these molecules to function, they must exist in a specific, three-dimensional structure. Biological activity will cease if this structure is destroyed. Extreme temperatures, acids, bases, and heavy metal compounds can break linkages in proteins, causing them to become **denatured**.

In this experiment you will carry out some tests that are characteristic of amino acids and will investigate the various means of coagulating (denaturing) proteins.

1. Biuret test. This test identifies a compound that contains two or more peptide bonds. When these compounds react with Cu²⁺ ions in a basic solution, a pink-violet complex is formed (a positive test). Amino acids

and dipeptides (two amino acids joined by one peptide bond) do not give violet colors with Cu²⁺, but produce a blue solution (a negative test).

- **2. Xanthoproteic test.** Some amino acids and the proteins containing them have aromatic rings. These rings react with concentrated nitric acid to produce a yellow compound, which is intensified in a basic solution. The test is used to determine the presence or absence of aromatic ring structures.
- **3. Hopkins–Cole test.** This is a specific test for the presence of proteins containing triptophan, one of the essential amino acids. Eggs, for example, are high in triptophan. When a triptophan-containing protein solution is layered on top of concentrated sulfuric acid, a purple compound (ring) forms at the interface.
- **4. Unoxidized sulfur test.** This test is to detect the presence of sulfurcontaining amino acids (such as cysteine) or proteins containing these amino acids. In a basic solution inorganic sulfide ions, S²⁻, are produced, which react with lead acetate to give PbS, a black precipitate.

SAFETY PRECAUTIONS AND DISPOSAL METHODS

The reagents used in this experiment contain organic compounds that may irritate skin. Concentrated nitric and sulfuric acids are *very corrosive*. Be sure not to get them on your skin or clothing. If you have spilled concentrated sulfuric acid on your skin, *wipe it off first*, then wash with soap and water. *WEAR EYE PROTECTION THROUGHOUT THE EXPERIMENT*. Also, handle the hot-water bath carefully.

Discard solutions containing heavy metal ions in a stoppered bottle.

PROCEDURE

A boiling-water bath is required for some of the tests in this experiment. Pour about 200 mL of deionized water into a 400-mL beaker and heat it to boiling. Maintain it at that temperature, replenishing the water as it becomes necessary.

1. Biuret Test

Label four clean test tubes. Pour 2 mL of egg albumin solution, 1% gelatin solution, 1% casein solution, and 0.5% alanine solution (an amino acid) into separate test tubes. Now add 3 mL of 10% sodium hydroxide solution to each one and shake carefully to mix. Add 2 drops of 2% copper sulfate solution to each test tube, mix, and observe the color of the solution. Record your observations on the work page.

2. Xanthoproteic Test

Pour 2 mL of egg albumin solution into a test tube and add 10 drops of concentrated nitric acid to it. Mix and heat in a boiling-water bath for 2 minutes. Record any color change. Cool the mixture and add 10% sodium hydroxide solution dropwise until the mixture is basic to litmus (red litmus turns blue). Note any change in color. Record your observations.

3. Hopkins-Cole Test

Label three clean test tubes. Pour 2 mL of egg albumin solution, 1% triptophan solution, and 1% gelatin solution into separate test tubes. Add to each one 2 mL of Hopkins–Cole reagent and mix. Now, holding the first test tube at a 45° angle, VERY CAREFULLY AND SLOWLY add 30 drops of concentrated sulfuric acid down the inside wall of the test tube, so that it forms a layer on the bottom. Avoid shaking the tube or doing anything that will cause mixing. The presence of a purple ring at the interface is a positive test for triptophan. If no ring forms, gently tap the test tube to cause slight mixing at the interface. Record your observations on the work page. Repeat the procedure for the second and third test tubes.

4. Unoxidized Sulfur Test

Pour 2 mL of egg albumin solution into a test tube. Add 5 mL of 10% sodium hydroxide solution and 3 drops of 5% lead acetate solution. Mix and heat in a boiling-water bath for 3 minutes. Observe the results and record them on the work page.

5. Coagulation/ Precipitation of Proteins

Obtain five test tubes and pour 3 mL of egg albumin solution into each one.

- **A.** Heat the first test tube to boiling. Observe any changes and record your results on the work page.
- **B.** To the second test tube add 10 mL of 95% ethanol (ethyl alcohol). Mix and observe changes. Record your observations on the work page.
- **C.** To the third test tube add 2% silver nitrate solution dropwise. Record your observations.
- **D.** Using the fourth test tube, add 5% mercury(II) chloride, MgCl₂, dropwise until you see a change. Record your observations.
- **E.** To the fifth test tube, add dilute tannic acid dropwise until you see a change. Record your observations.

Weiner_5209_ch35, 12/29/8, 18:23, page: 442

 Date	Section
<i>Diffe</i>	Sentin
l amino acids?	
s in contact with conc	entrated nitric acid.
?	
nt before you get a sh	ot?
	metal salt, such as AgNO ₃ o
n antidote?	
	s in contact with conc r?

Weiner_5209_ch35, 12/29/8, 18:23, page: 444

Name	Date	Section

Experiment 35

Work Page

Part 1—Biuret Test

Test Tube	Substance	Yes	No
1	Egg albumin		
2	Gelatin		
3	Casein		
4	Alanine		

If you heated a protein solution with an acid to break the bonds and to produce the	amino acid "building
blocks," would you expect the hydrolysis product to give a positive	or negative
Biuret test? Explain.	

Part 2—Xanthoproteic Test

Original color of solution:	 -
Color after heating with HNO ₃ :	
Color of basic solution	

Part 3—Hopkins-Cole Test

Test Tube	Substance	Observation
1	Egg albumin	
2	Triptophan	
3	Gelatin	

Introduction to Chemical Principles: A Laboratory Approach \blacksquare Weiner and Harrison

446

	xidized Sulfur Test precipitate: Yes; No	
	ion for the reaction, if any.	
1		
Part 5—Coag	gulation/Precipitation of Proteins	
Step	Observation	
5A		
5B		
5C		
5D		
5E		
Questions (C	Ontional)	
	ens to egg albumin when an egg is hard boiled?	
i. What happe	ns to egg albumin when an egg is hard bolled.	
2. A dilute solu	ution of mercury(II) chloride can be used to preserve anatomical specimens. E	xplain why.
3. What happe	ens chemically to proteins in your body during digestion?	

Name	Date	Section	

Experiment 35

Report Sheet

Part 1—Biuret Test

Test Tube	Substance	Yes	No
1	Egg albumin		
2	Gelatin		
3	Casein		
4	Alanine		

If you heated a protein solution with an acid to break the bonds and to produce the am	ino acid ''building
blocks," would you expect the hydrolysis product to give a positive	or negative
Biuret test? Explain.	

Part 2—Xanthoproteic Test

Original color of solution:		
Color after heating with HNO ₃ :	 	
Color of basic solution:		

Part 3—Hopkins-Cole Test

Test Tube	Substance	Observation
1	Egg albumin	
2	Triptophan	
3	Gelatin	

Introduction to Chemical Principles: A Laboratory Approach \blacksquare Weiner and Harrison

448

	xidized Sulfur Test	
Formation of a p	precipitate: Yes; No	
Write the equation	ion for the reaction, if any.	
Part 5—Coag	gulation/Precipitation of Proteins	
Step	Observation	1
5A		
5B		
5C		
5D		
5E		
Questions (C	Optional)	
1. What happen	ns to egg albumin when an egg is hard boiled?	
2. A dilute solu	ation of mercury(II) chloride can be used to preserve anatomical specimens. E	xplain why.
2 What happen	ns chemically to proteins in your body during digestion?	
o. what happen	no enemicany to proteins in your body during digestion:	