CHEM 30B

PROTEIN DETERMINATION AND DENATURATION

Background

Phycocyanin is a pigment carrying protein found in photosynthetic species. A heme-like molecule called phycocyanobilin is covalently bonded to the protein and absorbs visible light in the orange-red range and emits red light through a process called fluorescence. Because it absorbs light in the orange to red range, the protein has a blue-green coloration. Phycocyanin, along with other pigment proteins harvests visible light in the photosynthetic assembly. The photons gathered by these various pigments are ultimately shuttled to a photosynthesis reaction center.

Spirulina is a term used to describe a mixture of two species of blue-green algae found in bodies of brackish and saltwater. Properly classified as cyanobacteria, spirulina is rich in phycocyanin. Algae found at lower depths rely more heavily on the non-blue-green wavelengths of light for photosynthesis. Phycocyanin provides absorption in that range. Spirulina is sold as a dietary supplement owing to its protein, vitamin, and mineral content.

Bradford Reagent

The Bradford reagent is used to detect and quantify proteins in solution. The reagent contains the dye coomassie brilliant blue. When the dye binds to protein, the wavelength at which it absorbs visible light shifts, causing the observed color of the compound to change from a purplish-red to blue.

Objectives

In the first part of the experiment you will test a variety of solutions with the Bradford reagent to determine whether the solution contains protein. In the second part of the experiment, you will attempt to denature solutions containing phycocyanin using heat and a variety of chemical agents. When denatured, the protein loses its capacity to bind with the pigment molecule and changes its color. In cases where denaturation is expected, the Bradford test will be used to determine if the sample continues to contain protein.

Part 1

1. Dispense 10 drops of the Bradford reagent into each of 7 wells in a spot plate.
2. Dispense 2 drops of each of the following solutions into separate wells containing Bradford reagent.
    - Glucose Solution
    - Glycine solution
    - Tyrosine solution
    - Gelatin solution
    - Casein solution
    - Albumin solution
    - Phycocyanin solution
3. Using your glass stirring rod, gently stir each mixture. Rinse the rod with deionized water in between each sample.

4. A color change from purplish-red to blue indicates the presence of protein in the solution. Note the results of the tests on the data sheet.

Part 2

1. Fill a 250 mL beaker about half full with tap water. Place it on a hotplate and heat the water to boiling.
2. While waiting for the water to boil, label 10 test tubes 1-10. Dispense 10 drops of the phycocyanin solution into each of the test tubes.
3. When the water bath comes to a boil, place test tube 1 in the bath for 5 min. Record your observations on the data sheet after the 5 min of heating.
4. In the remaining 9 test tubes dispense up to 20 drops of each of the following substances. Add the substance drop-by-drop, swirling in between each drop added. If a change in the appearance of the solution occurs before adding 20 drops, stop; record your observations on the data paper noting the number of drops dispensed.

Test Tube	**Additive**
2	Deionized water
3	Isopropyl alcohol
4	Acetone
5	Bleach
6	0.1 M NaCl
7	0.1 M $ZnCl_2$
8	Detergent Solution
9	1.0 M HCl
10	1.0 M NaOH

5. If a significant change in the appearance of the solution is observed, continue to perform the Bradford test on that mixture.
6. Add 10 drops of the Bradford reagent into a well on the spot plate and then dispense 2 drops of the solution from the test tube using a clean pipette or dropper. Stir the mixture with a clean glass stirring rod and record the results on the data paper.

Questions

1. Which substances in part A gave a positive Bradford Test for protein?

2. Do these results make sense considering the structures of the compounds? Discuss your response. (You may need to consult your textbook or the internet to learn more the substances under investigation).

3. Which additives from Part B caused a change in the appearance of the phycocyanin solution?

4. For the additives that caused a change in the appearance of the phycocyanin solution which solutions also gave a **negative** test for protein with the Bradford test?

5. Would a positive Bradford test definitively indicate that the protein was **not** denatured? Briefly explain your response.

6. Briefly discuss how each of the substances could potentially serve as a denaturing agent. Which specific intramolecular forces might be affected?

a. Isopropyl alcohol

b. Concentrated NaCl solution

c. Detergent

7. How might the addition hydrochloric acid lead to protein denaturation? Which type(s) of amino acid sidechains could be altered by HCl? Which intramolecular forces might be disrupted?

8. Indicate whether the following descriptions of phycocyanin pertains to the primary, secondary, tertiary, or quaternary structure of the protein.

a. Phycocyanin is a water-soluble globular protein.

b. The structure of phycocyanin contains numerous α-helices.

c. The active form of phycocyanin is composed of two protein subunits.

9. Based on the background information provided above, is phycocyanin a **simple or conjugated** protein. Briefly explain your response.