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Physical Properties Of A Protein

Teacher's Guidebook

(Cat. # BE-401)

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MATERIALS INCLUDED

This kit has enough materials and reagents for 24 students (six groups of four students).

- 1 vial Protein: Protein-Bo
- 1 vial Protein: Protein-Ca
- 1 vial Protein: Protein-Ge
- 6 vials Tissue Extract & Pestle
- 6 vials Precipitant-Acd
- 6 vials Salt Solution (Amm. Sulfate)
- 6 vials Precipitant-Org
- 6 vials Detergent Solution (20%SDS)
- 1 vial Alk. Solution (1N)
- 2 bottles Protein Dilution Buffer
- 80 1.5ml Tubes

SPECIAL HANDLING INSTRUCTIONS

- Store Protein-Bo, Protein-Ca, Protein-Ge and Tissue Extracts at 4ºC.
- All other reagents can be stored at room temperature.
- Briefly centrifuge all small vials before opening to prevent waste of reagents.

The majority of reagents and components supplied in the *BioScience Excellence*™ kits are non toxic and are safe to handle, however good laboratory procedures should be used at all times. This includes wearing lab coats, gloves and safety goggles.

For further details on reagents please review the Material Safety Data Sheets (MSDS).

The following items need to be used with particular caution.

Part #	Name	Hazard
A041	Alk. Solution (1N)	Corrosive
P341	Precipitant-Org	Flammable
P331	Precipitant-Acd	Corrosive

ADDITIONAL EQUIPMENT REQUIRED

- 10 & 50ml Tubes
- 100°C Hot water bath
- Low speed microcentrifuge for 1-2ml tubes

TIME REQUIRED

- 2-4 hours

OBJECTIVES

- Explore physical properties of proteins.
- Explore protein behavior in aqueous solution.
- Study the effects of pH, temperature, salt, detergents, and organic solvent on protein solution.
- Learn how to alter physical properties of proteins.
- Explore protein and detergent interactions.
- Explore protein fractionation using a complex tissue extract.

BACKGROUND

Proteins are the building blocks of life and there are estimated to be almost one million different proteins in a normal animal cell. Each protein has very different and unique physical properties. The Physical Properties of Proteins kit is a lab activity that enables students to investigate the physical properties of several different proteins.

Students will learn about protein solubility and the role played by water and charge interaction and how these are affected by various parameters, including temperature, pH, salt and dielectric constant. They will understand about protein precipitation due to low pH, high salt and in the presence of organic solvents and about protein denaturation as a result of high temperature. In addition, the kit will demonstrate how non-protein agents, such as detergents alter the physical properties of protein molecules and as a result understand the importance of detergents in protein solubilization, research and analysis.

This lab activity involves analysis of solubility of three different types of pure proteins and then students alter some of these properties with an anionic detergent - sodium dodecyl sulfate (SDS, $C_{12}H_{25}NaO_4S$) and re-examine physical properties of these proteins. Students are challenged to consider how physical properties of protein molecules can be exploited for purification and characterization of proteins and apply their findings on a test sample of complex tissue extract.

TEACHER'S PRE EXPERIMENT SET UP

Wear gloves when making protein solutions.

Wear heat protective gloves while using hot water bath.

1. Transfer Protein-Bo, Protein-Ge and Protein-Ca into three separate 50ml tubes. Add 30ml Protein Dilution Buffer to each tube.

2. ***Tube Containing Protein-Bo:*** Invert or vortex the tube to dissolve it completely.

3. ***Tube Containing Protein-Ge:*** Put the tube in a hot water bath and gently vortex or invert the tube periodically until the Protein-Ge is completely dissolved. Do not shake vigorously.

4. ***Tube Containing Protein-Ca:*** Slowly and drop wise add Alkaline Solution (50-100:l each time) to the tube containing Protein-Ca and vortex or invert the tube until Protein-Ca is completely dissolved.

5. Aliquot 4ml of each protein solution for each student group.

MATERIALS FOR EACH GROUP

Supply each group with the following components. Several components are shared by the whole class and should be kept on a communal table.

- 4ml Protein-Bo solution
- 4ml Protein-Ge solution
- 4ml Protein-Ca solution
- 0.5ml Tissue Extract
- 1 vial Precipitant-Acd
- 1 vial Salt solution
- 1 vial Precipitant-Org
- 1 vial Detergent Solution
- 1 vial Tissue Extract & Pestle
- 15 1.5ml Tubes
- 1 bottle Protein Dilution Buffer (shared with whole class)
- Marker Pen

Note: *Instruct students to reuse the 1.5ml tubes. In any assay if there is no precipitate formed, the tubes can be reused by rinsing with distilled water.*

PROCEDURE

Wear gloves throughout the whole experiment.

Wear heat protective gloves while using hot water bath.

I. Acid Treatment

1. Label three tubes for Protein-Bo, Protein-Ge and Protein-Ca.

2. Aliquot 0.5ml of Protein-Bo, Protein-Ge and Protein-Ca solutions into the three labeled tubes.

3. Add 100μl Precipitant-Acd to each tube. Tighten the cap and vortex briefly.

4. Observe the tube to see if there is any precipitate formed. Record the result in Table 1.

NOTE: *If no precipitate formed rinse the tube with distilled water and reuse for subsequent experiments.*

II. Heating Treatment

1. Label three tubes for Protein-Bo, Protein-Ge and Protein-Ca.

2. Aliquot 0.5ml of Protein-Bo, Protein-Ge and Protein-Ca solutions to the three labeled tubes.

3. Tighten the cap and put the tubes in a 100ºC hot water bath. Incubate for 5-10 minutes.

4. Observe the tube to see if there is any precipitate formed. Record the result in Table 1.

NOTE: *If no precipitate formed rinse the tube with distilled water and reuse for subsequent experiments.*

III. Ammonium Sulfate Treatment

1. Label three tubes for Protein-Bo, Protein-Ge and Protein-Ca.

2. Aliquot 0.5ml of Protein-Bo, Protein-Ge and Protein-Ca solutions to the three labeled tubes.

3. Add 200µl Salt Solution (Amm. Sulfate) to each tube. Tighten the cap and vortex briefly.

4. Observe the tube to see if there is any precipitate formed. Record the result in table 1.

NOTE: *If there is no precipitate formed in any tube add more ammonium sulfate solution, as follows.*

5. Add another 200µl Salt Solution (Amm. Sulfate) in to each tube without precipitate. Tighten the cap and vortex briefly.

6. Observe the tube to see if there is any precipitate formed. Record the result in Table 1.

NOTE: *If no precipitate formed rinse the tube with distilled water and reuse for subsequent experiments.*

IV. Organic Solvent Treatment

1. Label three tubes for Protein-Bo, Protein-Ge and Protein-Ca.

2. Aliquot 0.5ml of Protein-Bo, Protein-Ge and Protein-Ca solutions to the three labeled tubes.

3. Add 250µl Precipitant-Org to each tube. Tighten the cap and vortex briefly.

4. Observe the tube to see if there is any precipitate formed. Record the result in Table 1.

NOTE: *If there is no precipitate formed in any tube add more Precipitant-Org solution, as follows.*

5. Add another 250µl Precipitant-Org in to the tubes without precipitate. Tighten the cap and vortex briefly.

6. Observe the tube to see if there is any precipitate formed. Record the result in Table 1.

NOTE: *If no precipitate formed rinse the tube with distilled water and reuse for subsequent experiments.*

V. Test the effects of anionic detergent (SDS) on Protein-Bo, Protein-Ge & Protein-Ca

1. Repeat the above tests I-IV after treating the protein solutions with anionic detergent sodium dodecyl sulfate (SDS), as follows.

2. For each experiment, aliquot 0.45ml of Protein-Bo, Protein-Ge and Protein-Ca solutions to three labeled 1.5ml tubes.

3. Add 50µl SDS solution to each tube. Tighten the cap and vortex briefly.

4. Repeat tests I to IV as instructed above. Record the results in Table 1.

VI. Protein Fractionation from tissue extract.

1. Add 0.3ml Protein Dilution Buffer to the tube containing the tissue extract. Incubate for 5 minutes at room temperature. Grind the tissue using the pestle until a uniform homogenate forms.

2. Centrifuge the tube for 5 minutes at maximum speed in a microcentrifuge. Transfer the supernatant to a new tube.

   OPTIONAL: Instruct students to remove 20µl sample from the tissue extract, label and store it at -20°C for the electrophoresis laboratory session. Electrophoretic analysis of the fractionated sample will reveal additional useful information on the fractionation technique.

3. Add 5µl Precipitant-Acd to the tube. Tighten the cap and vortex briefly.

4. Centrifuge the tube for 5 minutes at maximum speed in a microcentrifuge.

5. Carefully transfer the supernatant to a new tube without disturbing the pellet. Mark the pellet as Fraction 1.

6. Add 100µl Salt Solution (Amm. Sulfate) to the supernatant. Tighten the cap and vortex briefly. Incubate for 2 minutes. Centrifuge the tube for 5 minutes at maximum speed in a microcentrifuge.

7. Carefully transfer the supernatant to a new tube without disturbing the pellet. Mark the pellet as Fraction 2.

8. Add 400µl Salt Solution (Amm. Sulfate) to the supernatant. Tighten the cap and vortex briefly. Incubate for 2 minutes. Centrifuge the tube for 5 minutes at maximum speed in a microcentrifuge.

9. Carefully transfer the supernatant to a new tube without disturbing the pellet. Mark the pellet as Fraction 3 and the supernatant as Fraction 4.

10. Examine the pellet sizes of each fraction and record in Table 2.

    OPTIONAL:

1. Instruct student to freeze the fractions at -20°C until the electrophoresis laboratory sessions. The pellet can be dissolved in 100-200µl Protein Dilution Buffer. Follow the instructions provided in the Protein Electrophoresis kit. Electrophoresis analysis of the fractionated sample will reveal additional useful information on the fractionation technique.

2. *Perform a protein assay with each fraction and determine the percentage (%) of total tissue extract proteins in each fraction. Use G-Biosciences Protein assay Kit.*

RESULTS, ANALYSIS & ASSESSMENT

Table 1: Reference table of protein precipitation assays

	Without SDS			With SDS		
	Protein-Bo	Protein-Ge	Protein-Ca	Protein-Bo	Protein-Ge	Protein-Ca
Acid Precipitation 100μl	*No*	*No*	*Yes*	*No*	*No*	*No*
Heating Precipitation 100^0C 5-10min	*Yes*	*No*	*No*	*No*	*No*	*No*
Ammonium Sulfate Precipitation 200μl 400μl	*No* *No*	*Yes* *Yes*	*Yes* *Yes*	*Yes* *Yes*	*Yes* *Yes*	*Yes* *Yes*
Precipitant-Org Precipitation 250μl 500μl	*No* *Yes*	*No* *Yes*	*No* *No*	*No* *Yes*	*No* *Yes*	*No* *No*

Table 2: Fractionation of protein in a complex tissue extract

Fraction	I	II	III	IV
	20μl Acid	100μl Ammonium Sulfate	400μl Ammonium Sulfate	Supernatant
Fraction size	*Small*	*Large*	*Smaller*	*Liquid*

Q. Briefly explain the behavior of a protein in an aqueous solution and the role played by water molecules. If a solvent such as Precipitant-Org is added how does the influence of water change and what is the consequences on proteins in solution.

A. In an aqueous solution protein molecules tend to seek their native structure and it is the water molecules that determine the structure of protein molecules. Water molecules form hydrogen bonds within protein molecules (with peptide bonds and charged groups of amino acids), which determine the structure of protein molecules in solution.

Solvents such as Precipitant-Org compete for the protein bond water molecules (i.e. structured water) depriving the proteins of the water molecules, this increases the protein-to-protein interaction and results in protein aggregation, which in turn leads to precipitation. The concentration of Precipitant-Org that triggers protein precipitation is dependent on the primary structure of the protein molecules.

Q. How and why do proteins precipitate in acidic solutions? Briefly describe what will happen if an alkali is added to the protein solutions?

A. When protein solutions are acidified the net positive charge on the protein molecules increase, increasing the similar charge repulsion. The structural disturbance created within the protein molecules due to the similar charge repulsion leads to increase in the protein-to-protein interaction and consequently proteins precipitate.

When alkali is added to the protein solution it will increase the net negative charge on the protein molecule and a similar structural disturbance will lead to protein precipitation.

Q. How and why do proteins precipitate when a protein solution is heated?

A. When a protein solution is heated, the thermal energy increases the rotation within the peptide bond leading to unfolding of the protein molecules followed by increases in the protein-to-protein interaction and protein precipitation.

Q. Describe the role of salts (ammonium sulfate) on protein solubility. Briefly explain why different proteins precipitate at different concentrations of ammonium sulfate.

*A. Salt*s, *such as ammonium sulfate, compete for the protein bond water molecules (i.e. structured water) depriving the proteins of water molecules, which increases the protein-to-protein interaction and results in protein aggregation and precipitation. The concentration of salt that triggers protein precipitation is dependent on the primary structure of the proteins.*

Q. Briefly describe the structure of the anionic detergent you have used in these experiments and how the protein properties changed in the present of the anionic detergent. Briefly describe the changes you would expect if you mixed the protein solution with non-ionic and cationic detergents.

A: SDS contains a 12-carbon long hydrophobic tail and a charged (hydrophilic) head, sulfate group. The hydrophobic tails surround and coat the protein molecules and the charged group (sulfate) gives the protein molecules a net negative charge, overriding the native charge of the protein molecules and completely unfolding the protein molecules. Non-ionic detergents will surround and coat the protein without changing net native protein charge and in most cases without disrupting the native protein structure. Cationic detergents will give the protein molecule net positive charge and disrupt the native protein structure.

Q. Protein treated with SDS did not precipitate when heated or boiled, explain why?

A. The anionic detergent, SDS, surrounds and coats the protein molecules giving the protein molecules net negative charge, overriding the native charge of the protein molecules and completely unfolding the protein molecules. The negatively charged protein-SDS complex, due to similar charge repulsion, do not aggregate and form precipitates.

Q: Briefly describe the electrical migration behaviors of proteins and how they will change when treated with non-ionic, cationic and anionic detergents.

A: Protein molecules migrate depending on native net charge, a protein molecule with a net positive charge will migrate towards the anode and a protein molecule with net negative charge will migrate towards the cathode.

When a protein is treated with a non-ionic detergent it will have no effect on protein migration. On the other hand when a protein is treated with an anionic detergent it will render the protein negatively charged forcing the protein molecules to migrate towards the cathode. An anionic detergent will have the opposite effects.

Q. Explained the results you have collected during the fractionation of complex tissue extract.

A: The tissue extract contains a larger number of protein species; each protein has its own distinctive properties. During the fractionation procedure as the solution properties are modified with the addition of reagents, proteins in the solution begin to precipitate depending on their properties resulting in fractionation, enrichment, and partial purification from the crude total protein extract.

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OBJECTIVES

- Explore physical properties of proteins.
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MATERIALS FOR EACH GROUP

Supply each group with the following components. Several components are shared by the whole class and should be kept on a communal table.

- 4ml Protein-Bo solution
- 4ml Protein-Ge solution
- 4ml Protein-Ca solution
- 0.5ml Tissue Extract
- 1 vial Precipitant-Acd
- 1 vial Salt solution
- 1 vial Precipitant-Org
- 1 vial Detergent Solution
- 1 vial Tissue Extract & Pestle
- 15 1.5ml Tubes
- 1 bottle Protein Dilution Buffer (shared with whole class)
- Marker Pen

Note: *Instruct students to reuse the 1.5ml tubes. In any assay if there is no precipitate formed, the tubes can be reused by rinsing with distilled water.*

PROCEDURE

Wear gloves throughout the whole experiment.

Wear heat protective gloves while using hot water bath.

I. Acid Treatment

1. Label three tubes for Protein-Bo, Protein-Ge and Protein-Ca.

2. Aliquot 0.5ml of Protein-Bo, Protein-Ge and Protein-Ca solutions into the three labeled tubes.

3. Add 100μl Precipitant-Acd to each tube. Tighten the cap and vortex briefly.

4. Observe the tube to see if there is any precipitate formed. Record the result in Table 1.

NOTE: *If no precipitate formed rinse the tube with distilled water and reuse for subsequent experiments.*

II. Heating Treatment

1. Label three tubes for Protein-Bo, Protein-Ge and Protein-Ca.

2. Aliquot 0.5ml of Protein-Bo, Protein-Ge and Protein-Ca solutions to the three labeled tubes.

3. Tighten the cap and put the tubes in a 100ºC hot water bath. Incubate for 5-10 minutes.

4. Observe the tube to see if there is any precipitate formed. Record the result in Table 1.

NOTE: *If no precipitate formed rinse the tube with distilled water and reuse for subsequent experiments.*

III. Ammonium Sulfate Treatment

1. Label three tubes for Protein-Bo, Protein-Ge and Protein-Ca.

2. Aliquot 0.5ml of Protein-Bo, Protein-Ge and Protein-Ca solutions to the three labeled tubes.

3. Add 200µl Salt Solution (Amm. Sulfate) to each tube. Tighten the cap and vortex briefly.

4. Observe the tube to see if there is any precipitate formed. Record the result in table 1.

NOTE: *If there is no precipitate formed in any tube add more ammonium sulfate solution, as follows.*

5. Add another 200µl Salt Solution (Amm. Sulfate) in to each tube without precipitate. Tighten the cap and vortex briefly.

6. Observe the tube to see if there is any precipitate formed. Record the result in Table 1.

NOTE: *If no precipitate formed rinse the tube with distilled water and reuse for subsequent experiments.*

IV. Organic Solvent Treatment

1. Label three tubes for Protein-Bo, Protein-Ge and Protein-Ca.

2. Aliquot 0.5ml of Protein-Bo, Protein-Ge and Protein-Ca solutions to the three labeled tubes.

3. Add 250µl Precipitant-Org to each tube. Tighten the cap and vortex briefly.

4. Observe the tube to see if there is any precipitate formed. Record the result in Table 1.

NOTE: *If there is no precipitate formed in any tube add more Precipitant-Org solution, as follows.*

5. Add another 250µl Precipitant-Org in to the tubes without precipitate. Tighten the cap and vortex briefly.

6. Observe the tube to see if there is any precipitate formed. Record the result in Table 1.

NOTE: *If no precipitate formed rinse the tube with distilled water and reuse for subsequent experiments.*

V. Test the effects of anionic detergent (SDS) on Protein-Bo, Protein-Ge & Protein-Ca

1. Repeat the above tests I-IV after treating the protein solutions with anionic detergent sodium dodecyl sulfate (SDS), as follows.

2. For each experiment, aliquot 0.45ml of Protein-Bo, Protein-Ge and Protein-Ca solutions to three labeled 1.5ml tubes.

3. Add 50µl SDS solution to each tube. Tighten the cap and vortex briefly.

4. Repeat tests I to IV as instructed above. Record the results in Table 1.

VI. Protein Fractionation from tissue extract.

1. Add 0.3ml Protein Dilution Buffer to the tube containing the tissue extract. Incubate for 5 minutes at room temperature. Grind the tissue using the pestle until a uniform homogenate forms.

2. Centrifuge the tube for 5 minutes at maximum speed in a microcentrifuge. Transfer the supernatant to a new tube.

3. Add 5µl Precipitant-Acd to the tube. Tighten the cap and vortex briefly.

4. Centrifuge the tube for 5 minutes at maximum speed in a microcentrifuge.

5. Carefully transfer the supernatant to a new tube without disturbing the pellet. Mark the pellet as Fraction 1.

6. Add 100µl Salt Solution (Amm. Sulfate) to the supernatant. Tighten the cap and vortex briefly. Incubate for 2 minutes. Centrifuge the tube for 5 minutes at maximum speed in a microcentrifuge.

7. Carefully transfer the supernatant to a new tube without disturbing the pellet. Mark the pellet as Fraction 2.

8. Add 400µl Salt Solution (Amm. Sulfate) to the supernatant. Tighten the cap and vortex briefly. Incubate for 2 minutes. Centrifuge the tube for 5 minutes at maximum speed in a microcentrifuge.

9. Carefully transfer the supernatant to a new tube without disturbing the pellet. Mark the pellet as Fraction 3 and the supernatant as Fraction 4.

10. Examine the pellet sizes of each fraction and record in Table 2.

RESULTS, ANALYSIS & ASSESSMENT

Table 1: Reference table of protein precipitation assays

	Without SDS			With SDS		
	Protein-Bo	Protein-Ge	Protein-Ca	Protein-Bo	Protein-Ge	Protein-Ca
Acid Precipitation 100µl						
Heating Precipitation 100^0C 5-10min						
Ammonium Sulfate Precipitation 200µl 400µl						
Precipitant-Org Precipitation 250µl 500µl						

Table 2: Fractionation of protein in a complex tissue extract

Fraction	I	II	III	IV
	20µl Acid	100µl Ammonium Sulfate	400µl Ammonium Sulfate	Supernatant
Fraction size				

Q. Briefly explain the behavior of a protein in an aqueous solution and the role played by water molecules. If a solvent such as Precipitant-Org is added how does the influence of water change and what is the consequences on proteins in solution.

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