Effect of pH, surfactant, and Organic Solvent on Protein Solubility

Jacob Kaplan

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Florida Institute of Technology

**Introduction:**

Protein solubility is defined as “thermodynamic parameter defined as the concentration of protein in a saturated solution that is in equilibrium with a solid phase, either crystalline or amorphous, under a given set of conditions.” (Kramer et al., 2012) The question for this report to answer is what the given conditions for the protein are to keep normal configuration vs aggregation. Protein aggregation occurs when protein homeostasis is broken by environmental factors, which causes misfolding protein, and the structures hydrophobic residues are exposed of the proteins causing a precipitant to form. (Schramm et al., 2019)

In the experiment acids and different organic solvents, buffers were used to experiment to determine the protein (lysozyme mixed with glycine and NaCl) solubility is given in different environments. The purpose of using different solutions to add to the protein is to experiment with the proteins' stress levels of aggregation. For some solutions, the protein and enzyme will be able to carry out normal functioning, while in others that are too stressful the protein will aggregate and form a precipitant. Dialysis was used to predict protein solubility by dismissing smaller ions and hopefully refolding the aggregated proteins. (Pesarrodona et al., 2014)

The predictions due to protein stability are expected not to work at a certain pH around 5.2. (Venkataramani et al., 2013) It can be predicted in the protein will aggregate in TCA an acid, and SDS a strong base.

**Materials and Methods:**

This experiments protocol called to prepare protein and stock of the following. Lysozyme 20mg/mL in a 10mM Glycine and 150mL NaCl pH of 3 solution. Also, to make 2 buffers, the first one a 10mM glycine and 150 mM NaCl pH of 3 buffer solution. The second is a 100nM NaHCO3 pH of 8.5 solution buffer. Lasty, dilute a .1M HCl acidic solution.

The experimental protocol calls to transfer 1 mL of Lysozyme solution into 5 different 10mL conical tubes and label them 1-5. Tube 1 add 100μL of 1% SDS solution. Tube 2 add 3mL of 95% ethanol. Tube 3 add 300μL of 40% TCA. Tube 4 add 1mL of buffer 2 from stock. Tube 5 add 1mL of buffer 2 first then 1mL of .1 M HCl. Sit and let reactions to take place photograph and document results. Once done pipet solutions into 5 separate dialysis tubes and let sit in 1L of buffer overnight.

**Results:A picture containing cup, indoor, drink, plastic

Description automatically generated**

Figure 1: Results from experimental Protocol

A group of cards on a table

Description automatically generated with low confidence Figure 2: Results from Dialysis (Tube 1)

Results from the lab showed in Figure 1 that tube 1 and 3 fully aggregated while tube 2 partially aggregated. In dialysis it’s shown all aggregated of the solution in the tube has been resolved and resulted back to its non-aggregated form.

**Discussion:**

The results from the lab were more or less expected to turn out the way they did. As hypothesized lysozyme protein is heavily affected by pH level, so in Tubes 1 and 3 in Figure 1 it was shown that those acidic and basic solutions caused the protein to aggregate and clump up into a white cloudy precipitant. For tube 2 some outside research suggests EtOH induced aggregation is possible by “EtOH consumption disrupts hepatic lysosome biogenesis and inhibits the UPS by impeding hepatic proteasome activity,” (Donohue et al., 2019) which caused the protein lysosome to half aggregate since tube 2 did not contain a buffer solution. Several upon several other publishing’s agreed with the hypothesized results that lysozyme aggregation potential is heavily affected by its pH environment, such as in the case for tubes 1 and 3.

For dialysis, it was slightly shocking to see the results of all of the aggregated solutions return to normal folding configuration. All 5 tubes were able to be unfolded to normal configurations due to separating colloidal particles from smaller ions and particles.

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