Experiment 3: Purification and Characterization of α-Lactalbumin, a Milk Protein

In this experiment, you will use a particular type of affinity chromatography to isolate lactalbumin from milk whey. Please make note of the omissions in this lab. You will NOT be performing parts A and C. There will be a copy of the gel results from part C to analyze the relative mobilities talk about in the analysis section. The first week of this lab will be used to do part B and the following week will be committed to part D. **Remember to keep some of your crude whey sample for later comparison**. Rather than use the Bradford assay outlined in part D, you will be performing the Biuret protein assay (outlined below).

Part B.

- The details of the experiment procedure are as written in the text. Note, however, that the columns to be used are fitted with fritted disks at the top of the resin bed. This prevents the chromatography solvent from eluting below the top of the resin bed and thus drying the resin. You will collect fractions (**2 mL**) by hand.
- Rather than use spectrophotometers to locate your protein fractions, you will be using a colorimetric test based on the BCA protein assay. Aliquot 1 mL of BCA solution into separate test tubes corresponding to your column fractions. Add a 0.1 mL aliquot from each fraction to one of the BCA solution containing tubes. Incubate for 30 minutes in a 37°C water bath. The presence of protein should result in a significant color change from green to purple.
- Once you have loaded the whey onto the column, elute with 30 mL of buffer A to ensure removal of unwanted proteins. Collect 1 mL fractions upon eluting with buffer B and locate protein by the BCA procedure outlined above.
- Once you have collected your final protein fraction, place it in a 15 mL polypropylene centrifuge tube label with name and lab section and place it in the rack in the freezer. Elute your column with the EDTA solution, cap the bottom (DO NOT CAP THE TOP!) and bring it back to the from of the lab to be reused.

Part D. Biuret Assay

Set up 10 microcentrifuge tubes and add water, protein standard or sample as shown in the table below. Once all tubes have been prepared, add 0.1 mL of 100% TCA (trichloroacetic acid) solution to each tube. Cap all tubes and shake vigorously for a few seconds. Centrifuge the tubes for 1 minute at 5000 rpm. Using a pasteur pipet, remove the supernatant from each of the tubes and add 1 mL of Biuret reagent to each tube. Include another sample of just Biuret solution to be used as a blank. Incubate at room temperature for 10-15 minutes with occasional shaking and read the A_{540} of each sample. The standard samples should make for a standard curve against which the protein concentration of the purified lactalbumin and whey can be measured.

Revised Table for Biuret Protein Assay

Sample Tube #

Reagent	1	2	3	4	5	6	7	8	9	10
Protein standard (1 mg/mL)	0.2	0.4	0.6	0.8	1.0	-----	-----	-----	-----	-----
α-Lactalbumin fraction	-----	-----	-----	-----	-----	0.5	1.0	-----	-----	-----
Whey	-----	-----	-----	-----	-----	-----	-----	0.1	0.2	-----
Water	0.8	0.6	0.4	0.2	-----	0.5	-----	-----	-----	1.0

*all volumes are in mL

PRELABORATORY EXERCISE

MSDS Search:

imidazole
Copper (II) sulfate
trichloroacetic acid

Questions:

Given the properties of the resin being used for this laboratory, what assumptions can be made about the amino acid composition of α-lactalbumin?

What type of effect do you suspect trichloroacetic acid has on the protien and why that preferred over the use of simple acetic acid?

Protein assays like the BCA, Lowry and Biuret all depend on the binding of copper to the peptide (amide) nitrogens. Why would this method be ineffective for the detection of amino acids under similar conditions?