Sosnick:Purifying Ubiquitin from expression lysates

From OpenWetWare

(Redirected from Purifying Ubiquitin from expression lysates)

Step 1: Take the expression lysis supernatent and treat with concentrated acetic acid until the pH drops to between 4.5 and 5.0. The solution will turn milky white, since many of the bacterial proteins will fall out of solution. Centrifuge the solution at 10,000 RPM in a Sorvall SS-34 rotor for 10 minutes at 4°C. Then adjust the pH with sodium hydroxide so that it is 5.1. This prevents other proteins from precipitating during your ion exchange step. You can also syringe filter the supernatent in a 0.2 micron filter to remove other precipitated debris.

Step 2: Setup your cation exchange column. Use Pharmacia Fast Flow SP resin in a XK50 or XK26 style column.

Step 3: Make your buffers. A good buffer to use for ubiquitin purification at pH 5.1 is ammonium acetate, since the buffer is volatile and mass spectroscopy can be performed directly on the fractions. Other buffer options are available. Two buffers should be made. Buffer A is usually the low salt buffer (20 mM ammonium acetate, pH 5.1). Buffer B is usually the high salt buffer (0.5 M ammonium acetate, pH 5.1). When you adject the pH, be sure to always use either ammonium hydroxide (NH4OH) or acetic acid, since the goal is to not have any non-volatile metal ions in the solutions.

Step 4: Pre-equilibrate the cation exchange column by first running 2 column volumes of high salt Buffer B, followed by extensive washing by 5 column volumes of low salt Buffer A.

Step 5: Load your protein sample onto the column. Save the "flow through" (the stuff that doesn't stick to the column just in case you had a problem). Wash the column with 2 to 3 column volumes of Buffer A. Then elute the protein from the column with a linear gradient of increasing concentration of ammonium acetate (or Buffer B). Collect the gradient in 25 ml fractions.

Step 6: Analyze your fractions in the major peak of the gradient (determined typically by absorbance at 280 nm) by using mass spectroscopy or gel electrophoresis. Pool the pure fractions together, dialyze against pure water, and lyophilize (or freeze-dry) the protein solution to powder. (An HPLC step using a C18 column with an acetonitrile gradient may be necessary if the fractions are not sufficiently pure).

Notes:

For ubiquitin mutants that have a low pI (6 or below, roughly), the protocol should be altered slightly. In step 1, add the acetic acid until the pH reaches <4. Do not bother restoring it after centrifugation. In step 3, make Buffer A with 0.1% TFA in H_2O; make Buffer B with 0.1% TFA and 1M NaCl in H_2O. Ecukier

If mass spectroscopy is not a concern, one may conserve acetate by making Buffer A with 20 mM ammonium acetate and Buffer B with 20 mM ammonium acetate + 1M NaCl. Ecukier