Recitation 1 – 7.02, Fall 2001 - Protein Biochemistry

Ver 1.02

I. Overview of Module

A. Purification of β-Galactosidease

1. Lysis
2. Ammonium Sulfate Precipitation
3. Gel Filtration
4. Ion Exchange Chromatography
5. Affinity Chromatography

B. Analysis

1. Functional: β-gal assay
2. Structural
   a) SDS-Page/Coomasie (what s in sample?)
   b) Immunoblot (where is β-gal?)

II. Today's Lab

A. General Considerations

1. Keep EVERYTING on ice (Always Be Cold). Treat your protein samples as raw meat.
2. Don t vortex shear forces denature foam is bad!
3. Clean but not sterile. Just don t spit in anything and you should be fine. NO fires! If something is burning, it probably means someone is failing — Rob Hagan.
4. Record the volumes of everything (and copious observations).

B. Lysis (Draw cell diagram)

1. Freeze thaw ice crystals damage membranes and cause denaturation fragments outer membrane.
2. Lysozyme hydrolyzes cell wall polymers (n-acetyl glucosamine and n-acetylmuraminic acid linkages) breaks down cell wall.
3. Detergent/Hypotonic Lysis detergent (Triton X-100) solubilizes hydrophobic cell membrane by forming micelles. Triton X-100 is a mild, non-ionic detergent that will not denature the proteins (compared to SDS that will be used later).
4. Low salt concentration in lysis buffer causes cell swelling and rupturing of inner membrane.

C. DNAse Digestion digests chromosomal and plasmid DNA makes lysate less gunky or snotty .

D. Centrifugation remove any insolublie material and unlysed cells from the lysate.

E. Ammonium Sulfate Precipitation salting out (Draw protein)

1. High salt concentration disrupts solvation cage around proteins. Water molecules are attracted to ammonium and sulfate ions and can t solvate protein.

2. Allows surface polar and charged residues on protein to interact.

3. Causes aggregation/precipitation.

4. Add slowly with a lot of stirring!!! Prevent local concentration buildup.

F. β-gal assay.

1. When purifying protein, need an activity to follow (in this case β-galatosidase activity). Must have a good assay.

2. β-gal catalyzes hydrolysis of lactose to glucose and galactose (hydrolyzes 1,4 β-glycosidic bond, hence the β in β-gal.)

3. Hard to monitor sugar formation therefore, use β -gal analog.

4. ONPG (*ortho*-nitrophenyl-β-D-galactoside) can be hydrolized to give galactose and ONP (*ortho*-nitrophenol) which is yellow colored and has an absorbance peak at 420nm. Thus it can be used for a colorometric assay.

5. Reaction can be followed by visually assessing the generation of a yellow color (qualitative) or by a spectrophotometric reading at 420nm (quantitative).

6. Reaction buffer

   a) buffer salts (keep β-gal happy at physiological pH)

   b) DTT to mimic the reducing environment of cell interior, keeps β-gal happy. DTT is a mild reducing agent.

   c) ONPG (for obvious reasons) in whopping excess.

7. Stop solution (NaCO3) raises pH, denatures enzyme, stops the reaction.

8. Take time points (O.D.) readings at 2 and 15. (Draw diagram). Mix ONPG with diluted protein→Remove portion at time t (record time exactly)→Quench in cuvette with stop solution→Read O.D.

9. Run against control (no protein sample added).

10. MAKE SURE O.D. is between 0.1 and 1! If not dilute 1:10 in 1:1 Z-buffer+stop solution.

11. Only need ONE good time point.

12. Express amount of β-gal in terms of Units of activity (1U β-gal=1nmol ONP/min).

13. For day 2 calculate total activity of CL, CL-S, and CL-P samples. (See book for sample calculation)

14. Last thought→Record volumes of EVERYTHING!