7.02/10.702 Spring 2005

PBC Day 1 Recitation Notes

Agenda:

I. Why purify proteins
II. Why does purification work?
III. β-galactosidase Intro
IV. Module Overview
V. β-galactosidase activity assay

I. Why purify proteins

- structure determination
- enzymatic activity
- to determine binding partners in the cell
- antibody production

II. Why does protein purification work (what properties can we take advantage of?)

- proteins have different charges
- proteins have different hydrophobicity
- proteins have different substrates (and binding affinity to those substrates)
- proteins have different sizes and quaternary structures
- proteins have different solubilities

III. β-galactosidase intro

- functions as a tetramer
- breaks down lactose in the cell (see overhead)

IV. Module Overview and Day 1 techniques (see handout)

V. β-galactosidase activity assay (see handout as well)

- Information that you can get from this assay:
  - total activity of a sample
  - yield (how much at each step of a purification)
  - total activity + total protein--> specific activity--> measure of purity
- Can be quantitative (using spec) or qualitative (by eye)
- When doing assays, need to time accurately!

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Please see:-
Voet, D., and J. Voet. *Biochemistry*. New York: J. Wiley & Sons, 2004. ISBN: 0471250902.

Figures removed due to copyright reasons.

Help Aliaa with the β-galactosidase Purification Scheme!

Bacterial cell pellet

↓ Thaw @ 37°C

A. Disrupt Cells
1. Freeze thaw
2. Lysozyme
3. Triton X-100 + ddH_2O

↓

B. Treat to Remove DNA
1. DNAse I

↓

Centrifuge — Removes insoluble material

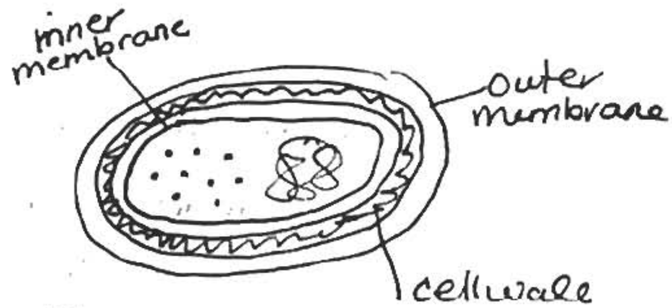
↓

Crude Lysate

↙ Other proteins + βgal
↘ from Lysis cells

Notes

Two strains CSH36 or H15461



Freeze thaw – outer membrane

lysozyme – breaks down cell wall

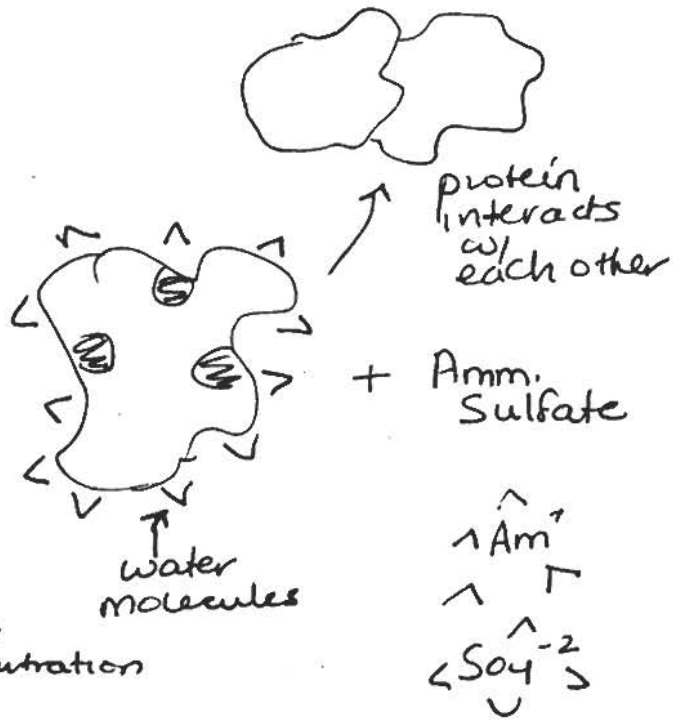
Triton X-100 + ddH_2O – bursts inner membrane – osmolysis

DNAse – breaks down genomic DNA

Crude Lysate

C. Precipitate Proteins
1. Ammonium Sulfate (45%)

different proteins ppt @ different Concentration



D. Column purification
1. PD-10 (desalting)

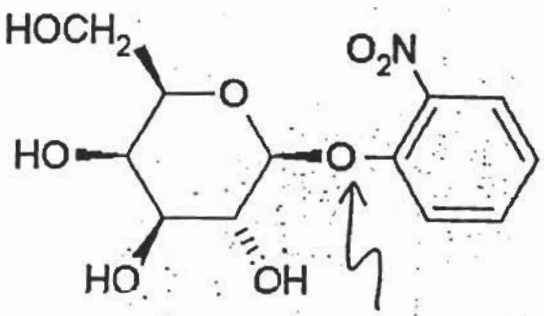
1. DEAE (ion exchange)

1. APTG-affinity
2. PD-10

Purified β-gal

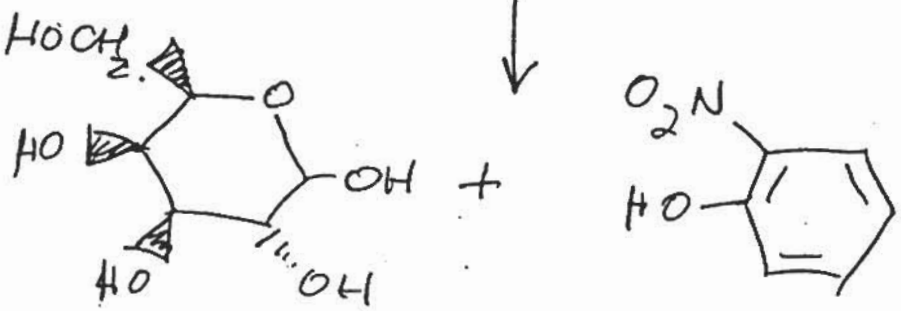
ONPG (Substrate for β-gal)

2 – Nitrophenyl-β-D- galactopyranoside



ONPG

β-gal cleaves here



Galactose

o-nitrophenol (ONP)
YELLOW
absorbs @ 420nM.

βgal. ∝ ONPG cleavage ∝ ONP product