Chapter 3
Exploring Proteins and Proteomes

The Proteome is the Functional Representation of the Genome p. 66

The proteome the combination of the functional proteins in a cell.
The genes that have been expression
Proteome varies from one cell type to another

3.1 The Purification of Proteins is an Essential First Step in Understanding Their Function pp. 66-78

"Never waste pure thoughts on an impure protein"

The Assay: How do We recognize the Protein That We Are Looking For? p. 67

When you want to purify a protein you have to know when you have it.

The way we recognize this is called an assay
- With enzymes you can assay their activity
  - Usually given in enzyme units (eu)
    - generally $\frac{\mu mol}{min}$ or $\frac{mmol}{min}$
- Sometimes you can directly measure the reaction

Slide

  - $NADH \rightarrow NAD^+$
    - lactate dehydrogenase
  - Radioactive assay
- Protein Assays - detect protein concentration
  - Bradford assay
  - Lowry assay
  - 280 nm
  - Units in mg
- Specific Activity
  - activity / protein
  - Units - eu/mg
  - rises as purification proceeds
  - Object of purification is to maximize the specific activity

Proteins Must Be Released From the Cell to be Purified pp. 67-68

Break the cell open
- French press
- Chemical lysis
- sonication

Get homogenate

Slide

Centrifugation
- Spin down after breaking cells
- If your protein is soluble just on high speed spin
- If protein is the insoluble or in a membrane
  - Differential centrifugation
    - Spin harder & harder, Collect pellets which the protein is in

Proteins Can Be Purified According to Solubility, Size, Charge, and Binding Affinity pp. 68-71

Questions x3

Purification according to solubility size, charge, and binding affinity

Salting out

Proteins are in general are less soluble at a high salt conc.

Ammonium sulfate

Dialysis

Slide

Proteins can be seperated from small molecules through dialysis

Esp. good for getting rid of salt

Small molecules will pass out through the pours of the dialysis tubing

Gel-filtration chromatography

Slide

separates proteins based on size

beads with tunnels

larger proteins come out first

Playground example

Question x2

Ion exchange chromatography

Slide

separates protein on charge

pI's are important

negative proteins stick to positive colums – anion exchange

positive proteins stick to negative columns- cation exchange

Increasing salt washes out protein

Hydrophobic chromotagraphy (not in book)

Slide

Binds to non-polar patches

start at high [salt] and lower

Affinity chromotagraphy -Best if you can find one that will work

~~Best~~ Takes ~~advantage~~ advantage of affinity some proteins have for certain chemicals

Maybe attach a molecule the protein binds to the column

Concanavalin A + glucose

Nickel columns

High-Pressure Liquid Chromatography

-uses other types of columns

-uses finer beads so needs high pressure to run

-finer beads leads to better seperation

Proteins Can be Separated by Gel Electrophoresis and Displayed pp. 71-74

Not

purification – Evaluation of purification!

How can we tell if our purification worked?

1. Gel electrophoresis
2. Increasing Specific activity

Homework Question

Gel electrophoresis

Slide

Separates macromolecules based on size & charge

Separates them in an electric field

Common gels are polyacrylamide & agarose

Slide
The are large polymers... like one big size exclusion beads
Have holes in them that smaller molecules can get through
Big molecules left behind

Question
Proteins usually separated by SDS-PAGE

Slide
SDS - detergent that denatures the protein & coats them in (-) charge

BME or dithiothreitol breaks disulfide bonds
- roughly 1 SDS molecule per a.a. so the charge is ~~ok~~ to the size of the protein

Slide
Separates the proteins on size only

To visualize coomassie blue, silver stain, or radiolabeled
Coomassie blue detects to 0.1 ug
silver stain can detect to 0.02 ug

Slide
Isoelectric focusing
separates proteins based on pI
passes proteins through a gel w/ a pH gradient
polyampholytes are passed through the gel
The protein stops at its pI

Slide
Two dimensional electrophoresis
Combines SDS-PAGE w/ isoelectric focusing
First IF
Then SDS-PAGE
Slide
Good for when looking for in a large combination

A Protein Purification Scheme Can Be Quantitatively Evaluated pp. 74-75

Protein purification evaluation
Total Protein
Slide
Total activity
Specific Activity
Slide
Yield (total remaining activity)
Purification level (times SA has increased)
Need to have good yield and specific activity

Ultracentrifuation is Valuable for Separating Biomolecules and Determining Their Masses pp. 76-77

Slide When being centrifuged molecules will move through the medium depending on several factors.

Slide
1. The mass – more mass moves through quicker
2. Shape – because of drag – elongated moves slower than spherical
3. Density of the molecule – more dense move faster (combo of 1 & 2)
4. Density of a solution – if the molecule is less dense than the sol'n they won't move through

Sedimentation coefficients are unique to molecules. Usually in Svedberg constant (S)
-seen w/ ribosomes (5S, 16S, etc.)

3.2 Amino Acid Sequences Can Be Determined by Automated Edman Degradation pp. 78-84

Proteins can be sequenced using Edman Degredation Edman Degradation

Protein sequencing is not as important b/c the advent of recombinant DNA technology
DNA is easier to sequence & we get protein from DNA
Doesn't show post translational modifications

3.3 Immunology Provides Important Techniques with Which to Investigate Proteins pp. 84-90

Antibodies to Specific Proteins Can be Generated

Slide Antibody (Immunoglobulin) – protein that binds invaders of the organism

Slide Antigen – invader (protein, nucleic acids polysaccharides)

The binding of the Antibody to the antigen is the beginning of the immune response

Antigenic Determinant or Epitope – specific area recognized on antigen

Making Antibodies