Protein Characterization

BIT 230
Methods

Many of these methods were covered through this course
Understand purpose!
Amino Acid Composition

Treat with HCl (hydrolysis)

Separate individual aa by ion exchange chromatography

Analyze with HPLC
Amino Acid Sequencing
Primary Structure

- **Edman Degradation**
  - only works with 50 aa fragments
  - cut protein with cyanogen bromide (CNBr)
    - cuts on carboxyl side of mets
  - Phenylisothiocyanate
    - binds to and releases N term residue

- Chromatography against known standards
EDMAN DEGRADATION - AMINO ACID SEQUENCING

phenylisothiocyanate

mild alkaline conditions, pH 9

anhydrous HF

thiazolinone derivative

aqueous H+

PTH-Amino Acid
phenylthiohydantooin-AA
3D Structure Determination

- X Ray Diffraction
  - need to crystalize (Difficult)

- NMR
  - small proteins 25kD

- Electron Micrograph
  - poor resolution
X-ray Crystallography

X-ray diffraction

• beam of x rays directed at protein
• beam is diffracted by electrons of atoms in protein
• these beams hit a film detector
• computer analysis to create electron density map
NMR
Nuclear Magnetic Resonance

• apply magnetic field to protein

• atomic nuclei spin - create their own magnetic field

• emit radiation
Electron Micrograph

Any of a class of microscopes that use electrons rather than visible light to produce magnified images, especially of objects having dimensions smaller than the wavelengths of visible light, with linear magnification approaching or exceeding a million ($10^6$).
Methods of Stabilization of Proteins

Correct pH

Maintain temperatures (usually low)

Minimize processing times

Minimize agitation

Minimize denaturing chemicals

Add protease inhibitors

Add reducing agents (Oxidation can cause inactivation typically intracellular proteins)
Examples of Stabilizers

A. These reduce free water levels by hydrogen bonding with $\text{H}_2\text{O}$

- Glycerol
- Sugar
- Polyethene Glycol

B. BSA Bovine serum albumin
added to proteins which are at LOW concentration
Storage of Proteins

Similar conditions apply as with Stability

Freezing (and thaw) is typically OK

How long?

Concentration of contaminants
Lyophilization

Drying of protein

- Freeze protein
- Increase temperature
- Apply vacuum
- Remove water vapor
Mass Spectrometry

Molecular Mass determination

More accurate than SDS PAGE

Less protein needed for analysis than SDS PAGE

MALDI MS
matrix-assisted laser desorption and ionization
• mix protein with matrix (absorb UV rad)
• bombard mixture with UV photons
• matrix absorbs UV - flight into gas phase
• protein becomes ionized
• electric field pulls protein through analyzer tube
Inclusion Bodies

Define:

- Insoluble protein (precipitates) and RNA aggregates
- Dense granular structures
Why?

A. Incorrect disulfide bond formation

B. Too much protein produced

C. Incorrect folding
   - As protein synthesized - intermediate folding conformations
     Native structure is achieved

In the intermediate conformation, hydrophobic patches may be exposed (normally inside)

At high concentrations, these regions associate with each other before native formation can be formed.
What do we do from here?

A. Fix misfolding
   Dissociate polypeptides
   Solubilize (SDS, urea, guanidine hydrochloride)
   Renature

B. Prevent misfolding
   Lower Temperature
   Hydrophobic interactions decrease at lower temps

   Co Express Chaperones
   Proteins which expend energy to maintain the partially folded proteins in a soluble state