Proteins

Macromolecules built of amino acids.

Huge number (20^n) of possibilities

Classified in many ways:

- solubility
- composition
- shape
- physical properties
- function
- 3-D structure

Shape: Globular *vs*. Fibrous

- Rasmol Demo
- 10:1 ratio arbitrary division

Solubility

Albumins	Soluble in water and salt soln's
Globulins	Sparingly soluble in water but soluble in salt solutions
Prolamines	Soluble in 70-80% EtOH but insol in water and absolute EtOH
Histones	Soluble in salt soln's
Scleroproteins	Insoluble in water or salt soln's

Physical Properties

Not discussed

Composition
Simple *vs*. Conjugated

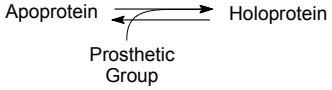
Simple-

Conjugated-

Apoprotein-

Holoprotein-

Prosthetic group-



Function

- Enzymatic catalysts- next chapter
- Transport and storage of molecules- Hb, ferritin
- Mechanical functions- elastin
- Movement- myosin
- Protection- Ab
- Information processing- rhodopsin
- Regulatory- renin
- Other

Structure

Primary (1°)- sequence of amino acids
Secondary (2°)- local 3-D shape
Tertiary (3°)- global 3-D shape
Quaternary (4°)- relation of polypeptides

Homogenization Buffer

- pH buffer
- Osmotic buffer
- Mg^{2+}
- Reducing agent (DTT or ßME)
- Protease inhibitors
- Substances unique to your needs

1° Structure

1° Structure- sequence of amino acids (disulfide bond locations)
MUST have pure protein

Protein Purification Salting-out/salting in

Process
In general, bigger salts work better: $(NH_4)_2SO_4$
In general, bigger proteins ppt at lower [salt]
However, there is not a mathematical relationship, the optimal concentration must be detn'd empirically
Dialysis

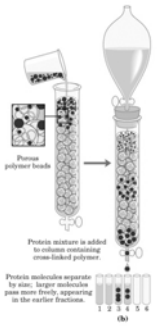
Protein Purification Starting Material

In order to determine what source to use, must have an *assay*
Start with a source very rich in protein: Organism, tissue, cell type
Can you isolate a particular organelle as a starting purification step?

Protein Purification Size exclusion chromatography

Fig. 5-18 (b) Lehninger POB 3rd Ed.

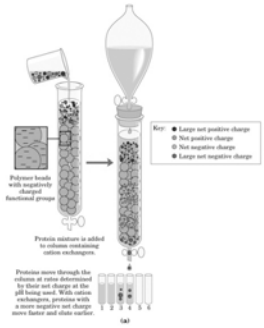
Separate by size (number of amino acids)



Protein Purification
Ion-exchange chromatography

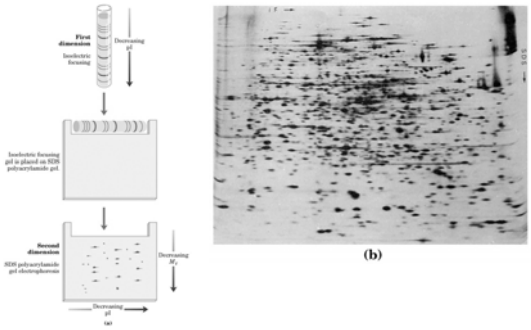
Fig. 5-18 (a) Lehninger POB 3rd Ed.

- Separate on basis of net protein charge
- Positively charged beads
- Negatively charged beads



The Only Honest Silver Stain is an Overloaded Silver Stain

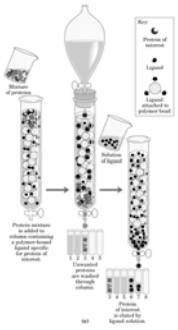
Fig. 5-22 (a and b) Lehninger POB 3rd Ed.



Protein Purification
Affinity chromatography

Fig. 5-18 (c) Lehninger POB 3rd Ed.

- Takes advantage of a specific binding property of the protein.



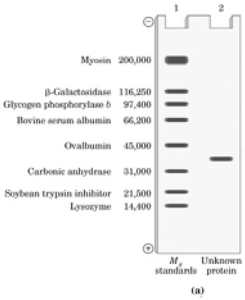
Protein Purification Table

(A) Sample	(B) Protein, mg	(C) Activity, U	(D) Specific Activity, U/mg	(E) Yield, %	(F) Purification, fold
1.			(C)/(B)	C1/C1	D1/D1
2.				C2/C1	D2/D1
3.				C3/C1	D3/D1

Protein Purification
Electrophoresis

Fig. 5-20 (a) Lehninger POB 3rd Ed.

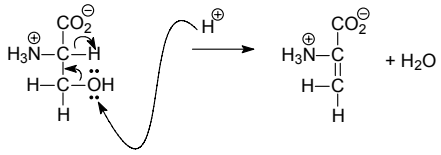
- *Preparative electrophoresis* can be used…
- …but *analytical electrophoresis* used more often to see how pure the protein is.



Manuscript

Protein Sequencing
1. Amino Acid Composition

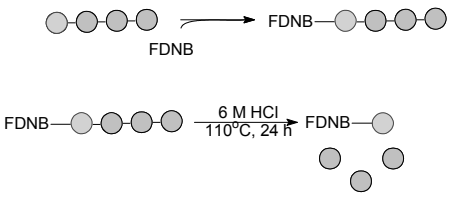
Acid hydrolysis- destroys Ser, others



Base hydrolysis- destroys Gln, others

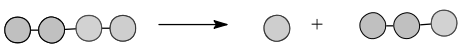
Protein Sequencing
2. What is amino terminus?

Sanger's reagent



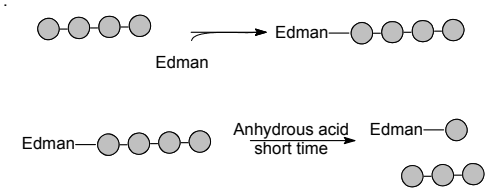
Protein Sequencing
3. What is carboxy terminus?

Limited carboxypeptidase digestion



Protein Sequencing
4. What is sequence?

Edman degradation



Protein Sequencing Continued

5. Fragment protein
   cyanogen bromide
   proteases
6. Align fragments

Protein Sequencing Example

1. Isolated pure protein
2. What is amino acid composition?

Acid hydrolyze, 2D chromatography and detect

Gly- 2	Val- 2	Pro- 2	Ser- 2
His- 1	Phe- 1	Tyr- 1	Trp- 1
Met- 1	Arg- 3	Lys- 3	Glx- 1

Protein Sequencing Example Cont'd

3. What is amino terminus?: Serine
   Sangers reagent
4. What is carboxy terminus?: Valine
   Carboxypeptidase

Limited digestion (short time, low temp) gives a single major a.a.

5. What is sequence?
   Edman degradation

Forces Involved in 1^{o} Structure

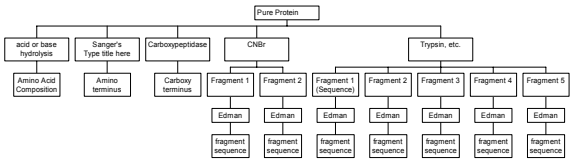
Strong (covalent)
- peptide bond
- disulfide bond

Protein Sequencing Example Cont'd

6. Fragment protein
   cyanogen bromide: 2 pieces
      CNBr1 sequence
      CNBr2 sequence
   Chymotrypsin: 4 pieces
   Trypsin: ? Pieces
7. Align fragments

Ser-...

The Process Just Described is a Huge Amount of Work



- Method of choice today is to sequence DNA: drawback
- Only with prior knowledge do we move forward