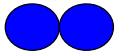
Protein functions

- Enzymatic catalysis
- Transport and storage
- Coordinated motion
- Mechanical support
- Immune protection
- Generation and transmission of nerve impulses
- Control of growth and differentiation
- More

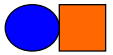
Features of proteins

- Polypeptide chains (linear)
  - Monomeric: single polypeptide; 1 subunit

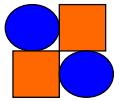
  - Multimeric: ≥ 2 individual polypeptides; multiple subunits



Homodimer, α_2



Heterodimer, $\alpha\beta$



Heterotetramer, $\alpha_2\beta_2$

- Molecular weight (Mr) ~5,000-1,000,00
  ~50 – 10,000 AA res.

Features of proteins

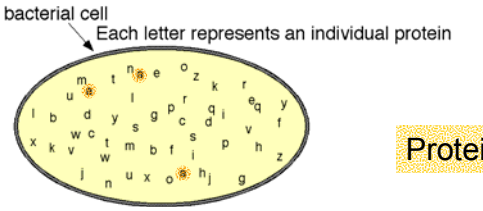
- AA sequence and composition vary
  - *E. coli*: ~3,000 different proteins
  - Human: ~100,000 different proteins

  Structure & function —— AA composition and sequence

- May contain modified amino acids (e.g hydroxyproline, g-carboxyglutamate
- May contain non amino acid components - "prosthetic groups" covalent or noncovalent
  - Lipids, carbohydrates, PO_4^{3-}, metals, nucleotides, etc.

How to study a protein?

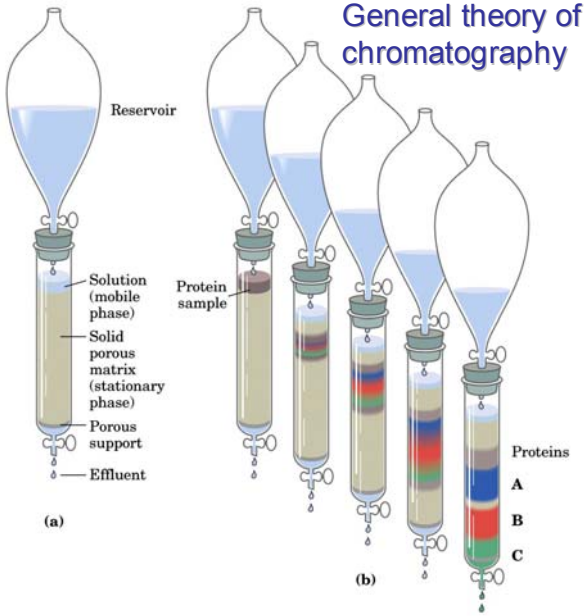
- Identify a protein of interest (function)
  - Enzyme "activity"
- Obtain a source of protein
- Separate protein from other macromolecules (purify)
  - Centrifugation/precipitation
  - Chromatography
    - Ion exchange
    - Size-exclusion
    - affinity
- How do you know which protein to isolate?



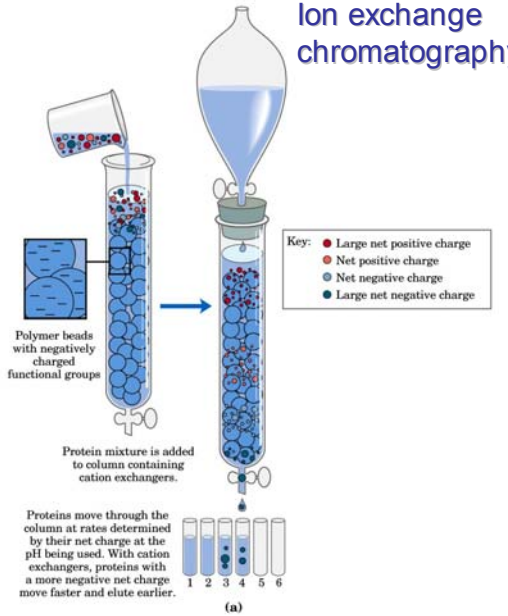
Protein purification

Protein of interest = protein "a"

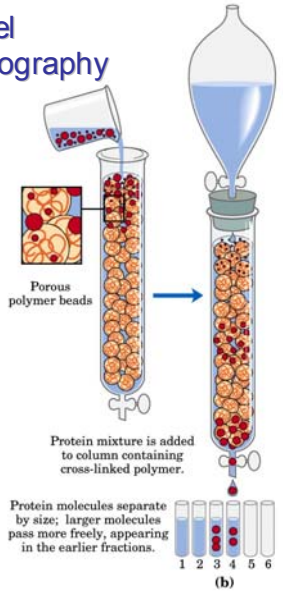
General theory of column chromatography



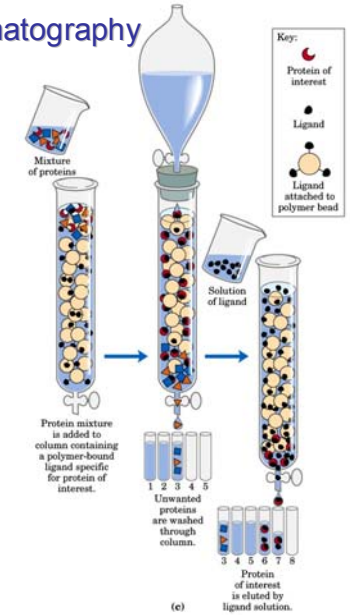
Ion exchange chromatography



Size exclusion (gel filtration) chromatography



Affinity chromatography



Enzyme activity

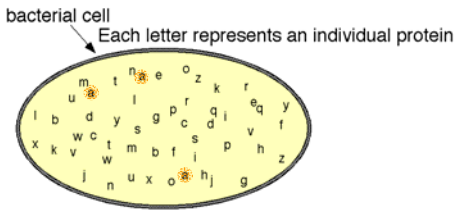
One unit (U) of enzyme activity is the amount of enzyme that will convert 1 μmol of substrate to product per minute

1 U = 1 μmol/min

Specific activity

How many units of activity an enzyme has in each mg of protein sample

SA = "n" μmol/min/mg = "n" units/mg

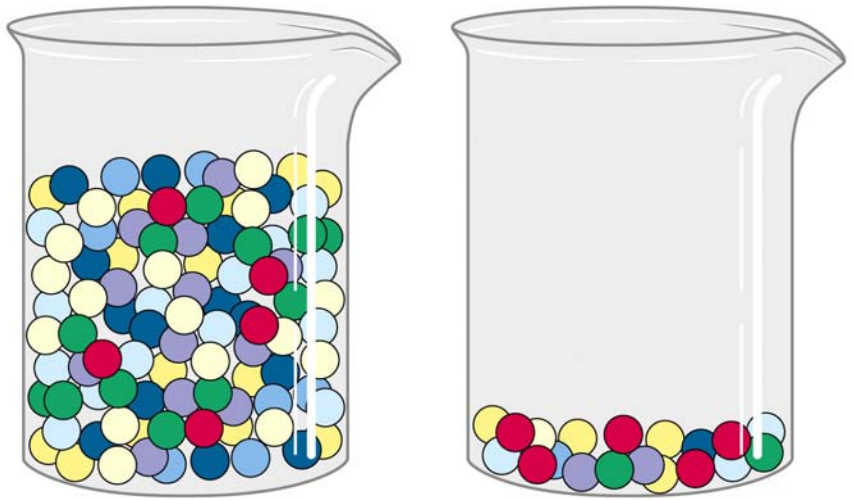


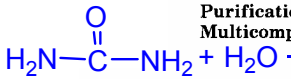
Specific activity of enzyme "a" is low in the cell extract ("n" units/mg total protein)

Isolation (purification) techniques to obtain a homogenous preparation of protein of interest

As we purify enzyme "a", we get rid of other proteins so the sp. act. becomes higher ("m" units/mg protein "a")

a collection of pure protein **a** molecules





THE JOURNAL OF BIOLOGICAL CHEMISTRY
© 1987 by The American Society of Biological Chemists, Inc.

Vol. 262, No. 13, Issue of May 5, pp. 5963-5967, 1987
Printed in U.S.A.

Purification and Characterization of the Nickel-containing Multicomponent Urease from *Klebsiella aerogenes**

(Received for publication, November 17, 1986)

Table 1

Urease purification from Klebsiella aerogenes

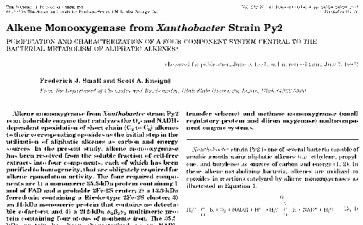
Purification Step	Specific Activity (μmole urea $min^{-1}mg^{-1}$)	Purification (fold)	Total Activity (μmole min^{-1})	Total Protein (mg)	Enzyme Recovery (%)
Cell extracts	2.06	1	19500	9460	100
DEAE-Sepharose	81.1	39	11800	145	60.5
Phenyl-Sepharose	574	279	5420	9.45	27.8
Mono Q	1170	568	5080	4.34	26.1
Superose 6	2200	1070	4770	2.17	24.5

X-fold purification = $\frac{\text{Specific activity, fraction of interest}}{\text{Specific activity, crude extract}}$

Total activity = (specific activity) x (total mg protein in fraction of interest)

Enzyme recovery = $\frac{\text{Total activity, fraction of interest}}{\text{Total activity in crude extract}}$

A good purification step gives a sizable increase in purity (fold) and a reasonable recovery (yield) from the previous step



Alkene Monooxygenase from *Xanthobacter* Strain Py2

PURIFICATION AND CHARACTERIZATION OF A FOUR COMPONENT SYSTEM CENTRAL TO THE BACTERIAL METABOLISM OF ALIPHATIC ALKENES*

Frederick J. Small and Scott A. Ensign

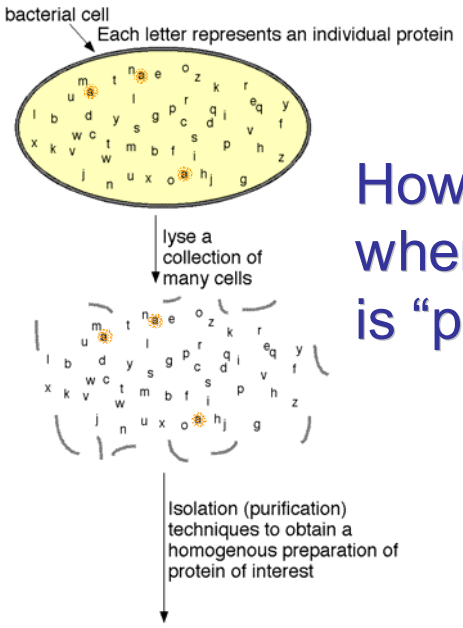
24916 *Four-component Alkene Monooxygenase from Xanthobacter Py2*

TABLE I
Purification of the four alkene monooxygenase components

Step	Volume	Total protein	Total activity[a]	Specific activity	Yield	Fold purification
	ml	*mg*	*milliunits*	*milliunits/mg*	*%*	*x-fold*
Oxygenase						
Extract	417	6,721	111,414	19	100	1
DEAE-Sepharose	205	1,765	57,398	33	52	1.7
Phenyl-Sepharose	140	205	32,896	161	30	8.5
S-300	60	111	28,413	240	26	12.6
Reductase						
Extract	407	17,559	2,050,711	168	100	1
DEAE-Sepharose	1,000	2,243	1,043,100	465	51	3
Reactive Green 19	140	262	873,642	3,336	43	20
S-Sepharose	52	33	451,534	13,589	22	81
S-100	7	3.6	213,158	30,030	10	179
Small protein						
Extract	770	19,846	408,639	20	100	1
DEAE-Sepharose	240	7,109	317,981	44	78	2.2
Phenyl-Sepharose	5	38	113,643	3,034	28	152
S-100	8.5	28	85,863	4,257	21	213
Ferredoxin						
Extract	300	12,943	38,430	6.4	100	1
DEAE-Sepharose	195	220	29,833	135	78	21
Q-Sepharose	47	65	24,771	380	65	59
S-100	4.2	6.8	15,303	2,249	40	351

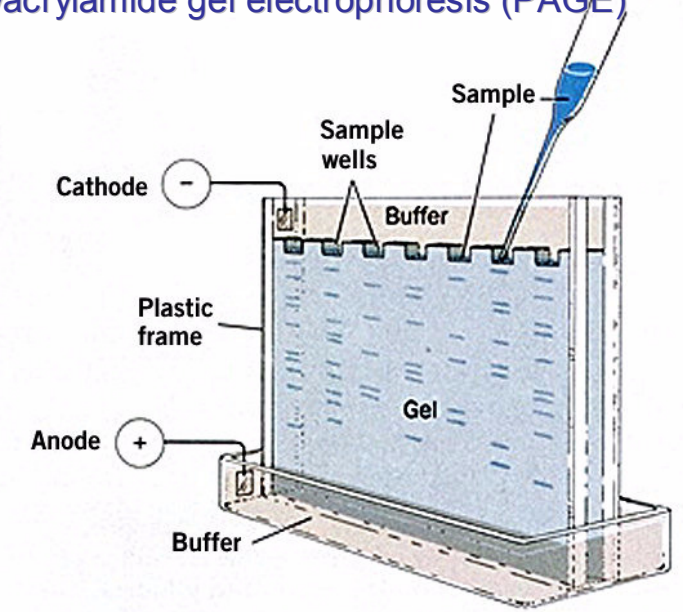
[a] A unit of activity is defined as 1 μmol of propylene degraded per minute at 30 °C.

What useful information does a purification table provide?



How do you know when your enzyme is "pure"?

Polyacrylamide gel electrophoresis (PAGE)

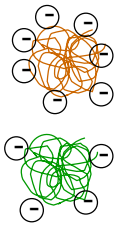


Nondenaturing PAGE

- Rate of migration ∝ size and charge
  - Protein 1: M_r = 100,000
  - Protein 2: M_r = 100,000

Which moves faster?

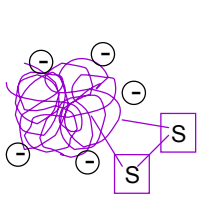
  - Protein A: M_r = 50,000
  - Protein B: Mr = 50,000

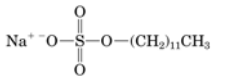


Which moves faster?

Denaturing PAGE

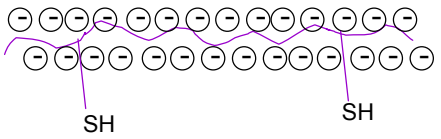
- Add detergent (SDS) and reductant (R-SH) to protein



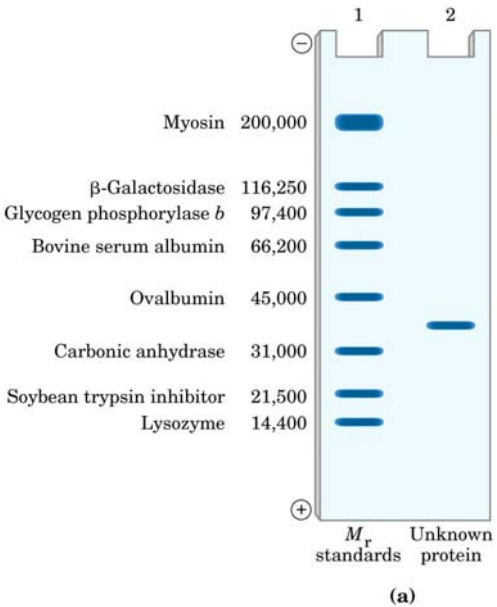


2-mercaptoethanol

1 SDS bound/2 AA residues

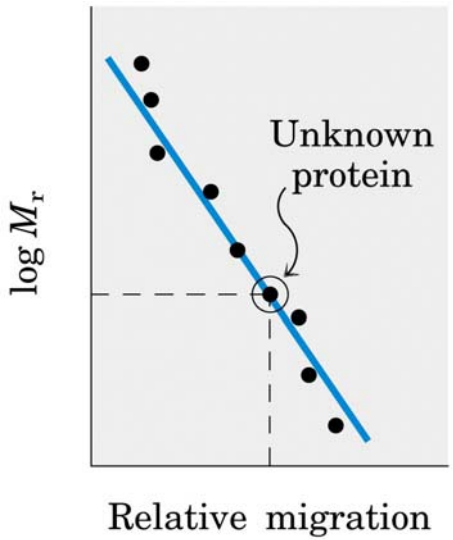


- Separation by SDS-PAGE ∝ M_r *only*



(a)

SDS-PAGE of a newly-discovered monomeric protein



(b)

Proc. Natl. Acad. Sci. USA
Vol. 94, pp. 8456–8461, August 1997
Biochemistry

Purification and characterization of acetone carboxylase from *Xanthobacter* strain Py2

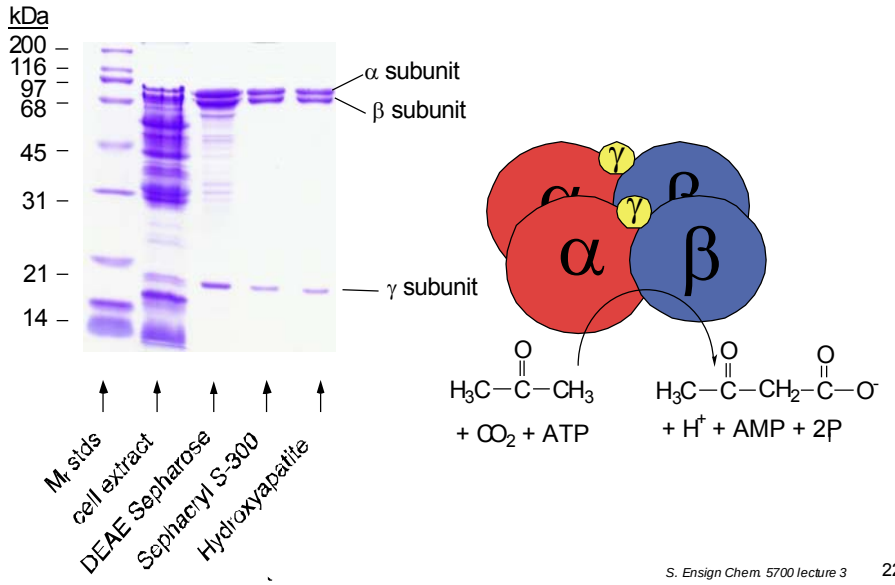
MIRIAM K. SLUIS AND SCOTT A. ENSIGN*

Department of Chemistry and Biochemistry, Utah State University, Logan, UT 84322-0300

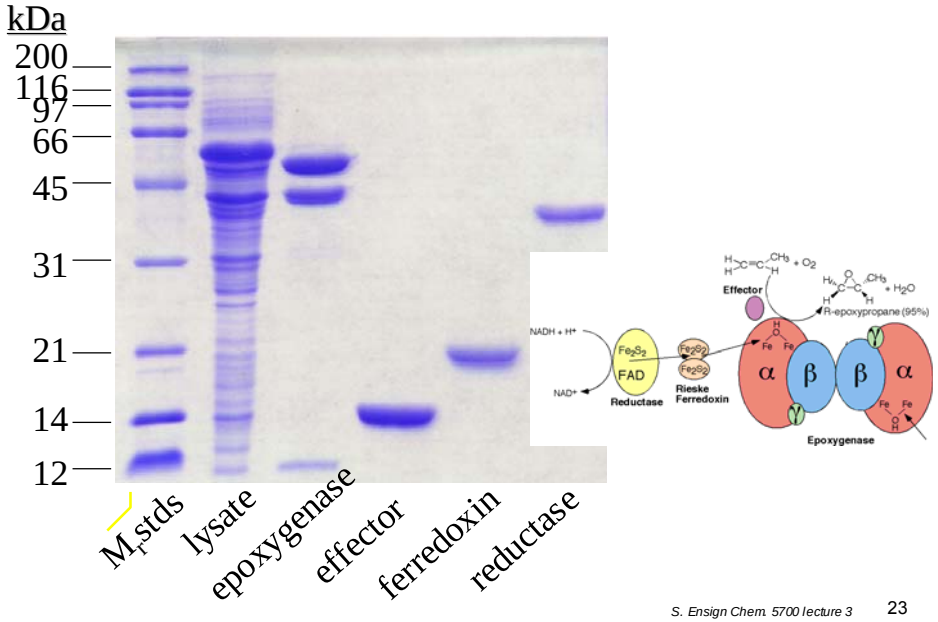
Communicated by R. H. Burris, University of Wisconsin, Madison, WI, June 9, 1997 (received for review March 24, 1997)

ABSTRACT Acetone metabolism in the aerobic bacterium *Xanthobacter* strain Py2 proceeds by a carboxylation reaction forming acetoacetate as the first detectable product. In this study, acetone carboxylase, the enzyme catalyzing this reaction, has been purified to homogeneity and characterized. Acetone carboxylase was comprised of three polypeptides with molecular weights of 85,300, 78,300, and 19,600 arranged in an $\alpha_2\beta_2\gamma_2$ quaternary structure. The carboxylation of acetone was coupled to the hydrolysis of ATP and formation of 1 mol AMP and 2 mol inorganic phosphate per mol acetoacetate formed. ADP was also formed during the course of acetone consumption, but only accumulated at low, substoichiometric levels (≈10% yield) relative to acetoacetate. Inorganic pyrophosphate could not be detected as an intermediate or product of acetone carboxylation. In the absence of CO_2, acetone carboxylase catalyzed the acetone-dependent hydrolysis of ATP to form both ADP and AMP, with ADP accumulating to higher levels than AMP during the course of the assays. Acetone carboxylase did not have inorganic pyrophosphatase activity. Acetone carboxylase exhibited a V_{max} for acetone carboxylation of 0.225 μmol acetoacetate formed $min^{-1}mg^{-1}$ at 30°C and pH 7.6 and apparent K_m values of 7.80 μM (acetone), 122 μM (ATP), and 4.17 mM (CO_2 plus bicarbonate). These studies reveal molecular properties of the first bacterial acetone-metabolizing enzyme to be isolated and suggest a novel mechanism of acetone carboxylation coupled to ATP hydrolysis and AMP and inorganic phosphate formation.

SDS-PAGE analysis of acetone carboxylase, a multimeric protein



SDS-PAGE Analysis of Alkene Monooxygenase Components



Other techniques to characterize proteins:

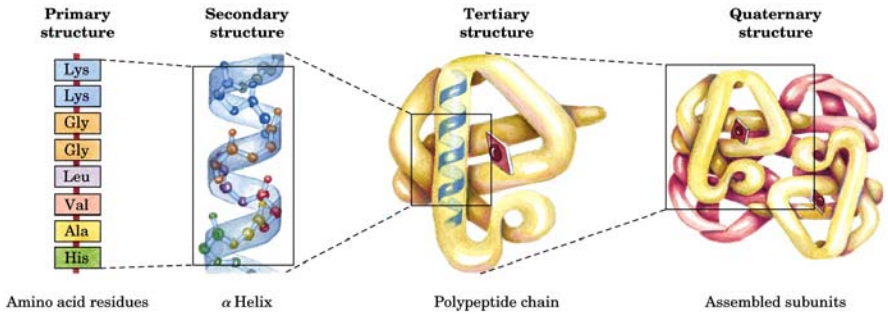
- isoelectric focusing, 2-D electrophoresis
- electrospray mass spectrometry (see text)
- many more!

table 5–6

The Isoelectric Points of Some Proteins

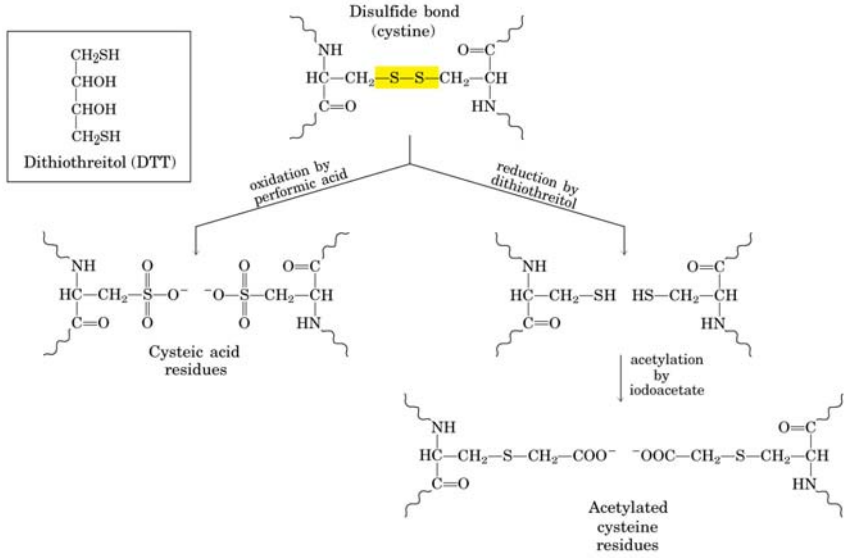
Protein	pI
Pepsin	~1.0
Egg albumin	4.6
Serum albumin	4.9
Urease	5.0
β-Lactoglobulin	5.2
Hemoglobin	6.8
Myoglobin	7.0
Chymotrypsinogen	9.5
Cytochrome *c*	10.7
Lysozyme	11.0

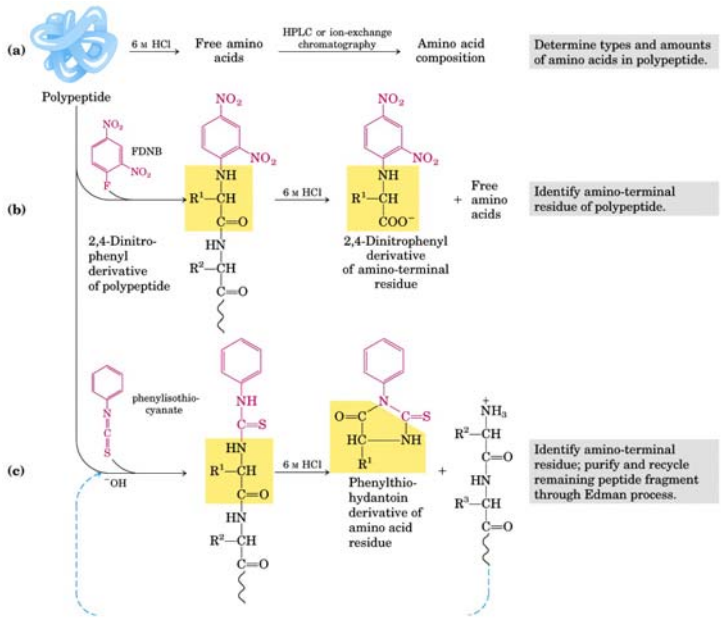
Levels of protein structure



Primary sequence determination: short polypeptide chains (~20 AA or less)

1. Deal with disulfide bonds
2. Determine total amino acid composition
3. Determine N-terminal residue
4. Sequence by Edman degradation





Primary sequence determination: longer polypeptide chains (>20 AA)

1. Deal with disulfide bonds
2. Determine total amino acid composition
3. Determine N-terminal residue
4. Digest polypeptide into smaller peptides suitable for Edman degradation
5. Sequence each fragment by Edman degradation
6. Deduce the order of fragments from overlaps

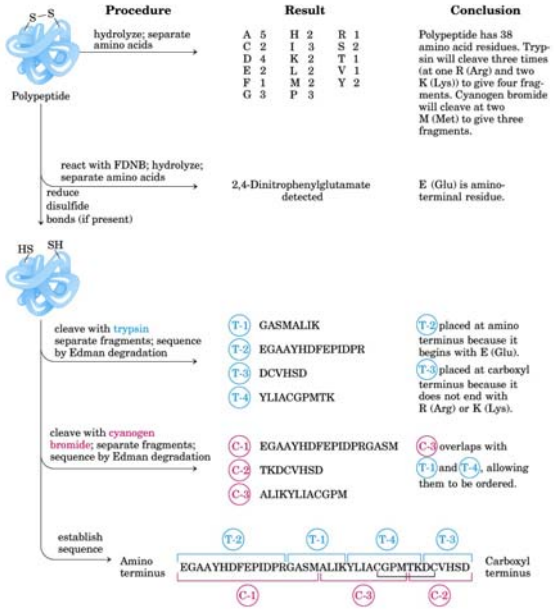
table 5–7

The Specificity of Some Common Methods for Fragmenting Polypeptide Chains

Treatment*	Cleavage points†
Trypsin	Lys, Arg (C)
Submaxillarus protease	Arg (C)
Chymotrypsin	Phe, Trp, Tyr (C)
Staphylococcus aureus V8 protease	Asp, Glu (C)
Asp-*N*-protease	Asp, Glu (N)
Pepsin	Phe, Trp, Tyr (N)
Endoproteinase Lys C	Lys (C)
Cyanogen bromide	Met (C)

*All except cyanogen bromide are proteases. All are available from commercial sources.

†Residues furnishing the primary recognition point for the protease or reagent; peptide bond cleavage occurs on either the carbonyl (C) or the amino (N) side of the indicated amino acid residues.



Primary sequence determination: longer polypeptide chains (>20 AA)

Deduce from gene sequence: molecular biology

Amino acid sequence (protein) Gln–Tyr–Pro–Thr–Ile–Trp

DNA sequence (gene) CAGTATCCTACGATTTGG