

THE MASS OF PROTEINS CAN BE PRECISELY DETERMINED BY ELECTROSPRAY MASS SPECTROMETRY

The very low volatility of proteins was a barrier for many years to using mass spectrometry

Electrospray ionization mass spectrometry: It can accurately determine the mass of a protein to about 1 part in 10^4 which means that the mass of a 10-kd protein can be measured to within 1 Dalton

- peptide mass fingerprinting

AMINO ACID SEQUENCES CAN BE DETERMINED BY AUTOMATED EDMAN DEGRADATION

Ala-Gly-Asp-Phe-Arg-Gly

To determine the amino acid composition of a peptide:

1. The peptide is hydrolyzed by heating it in 6N HCl at 100 °C for 24 h
2. The amino acids in hydrolysates are separated by ion-exchange chromatography on columns of sulfonated polystyrene
3. The separated amino acids are quantitated by reacting them with ninhydrin. All amino acids except proline give an intense blue color; proline gives a yellow color
4. The concentration of amino acid in a solution is proportional to its optical absorbance

Result: (Ala, Arg, Asp, Gly₂, Phe)

ELUTION PROFILE OF PEPTIDE HYDROLYSATE

Absorbance

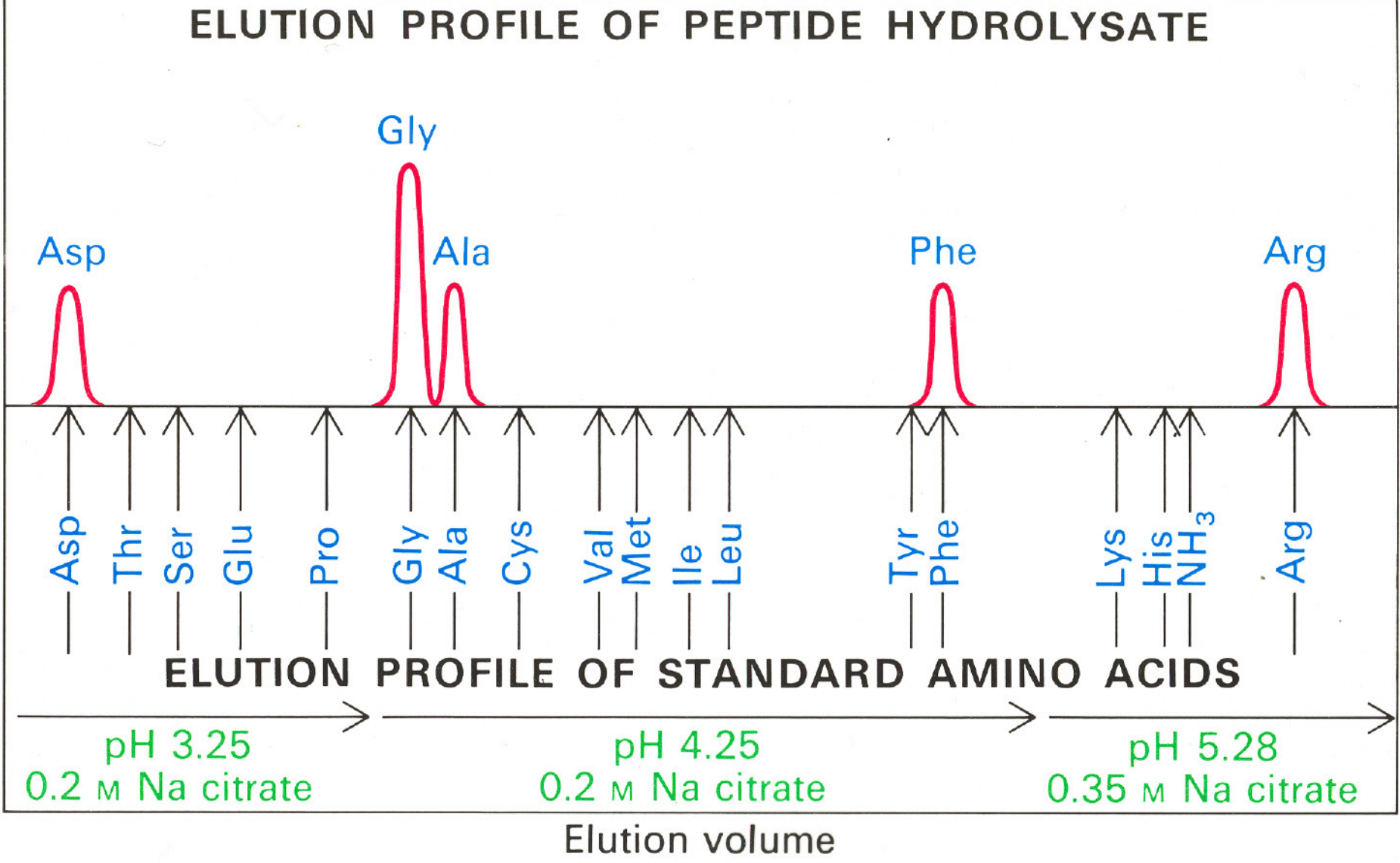


Figure 3-16, page 54

To determine the amino-terminal residue of a protein: (Sanger method)

1. Label the amino-terminal residue by reacting it with fluorodinitrobenzene (FDNB) or dabsyl chloride
2. Hydrolysis of the labeled peptide in 6N HCl
3. Identification of the labeled amino acid by its chromatographic properties

To determine the amino acid sequence in a protein: (Edman method)

1. Phenyl isothiocyanate reacts with the uncharged terminal ~~amion~~
amino group of the peptide
 2. The cyclic derivative is liberated under mildly acidic conditions
 3. The cyclic compound (PTH-amino acid) can be identified by chromatographic procedures
- ❖ The same procedure can be repeated on the shortened peptide (Edman Method)

Sequencers: automated instruments for the determination of amino acid sequence. Sensitive and quick way to sequence proteins

EDMAN DEGRADATION

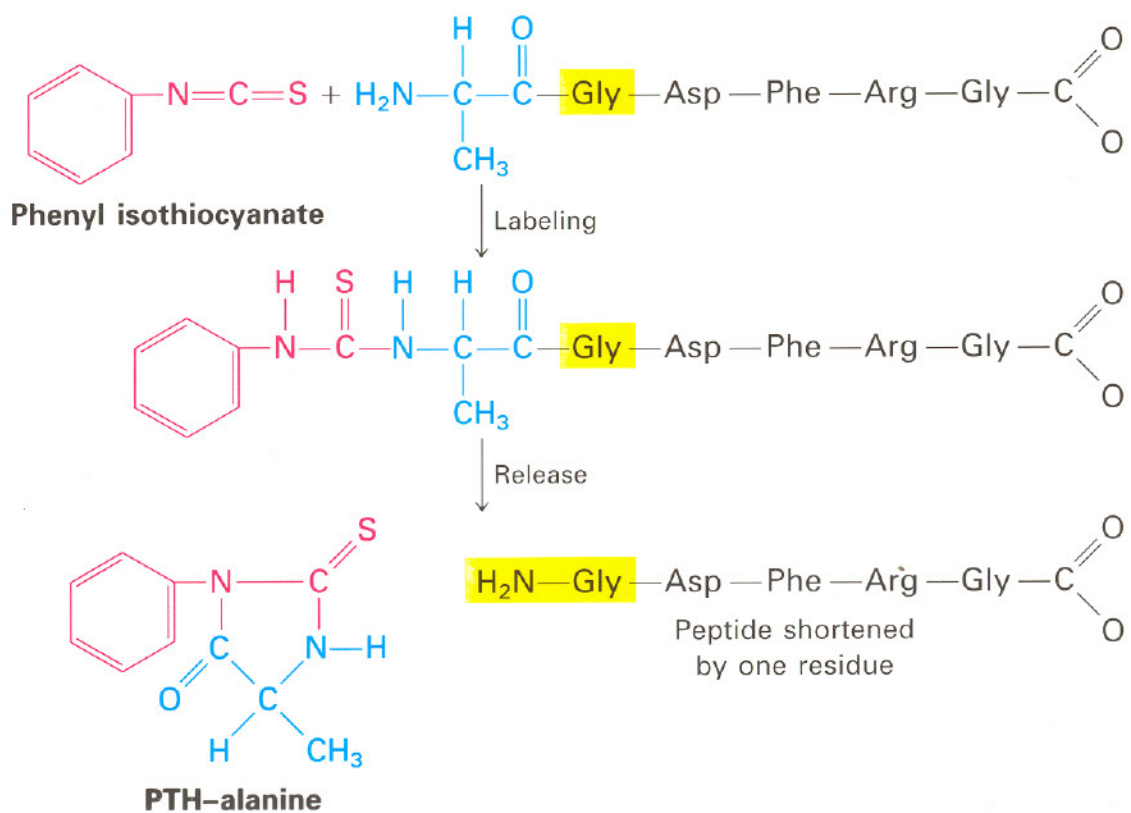
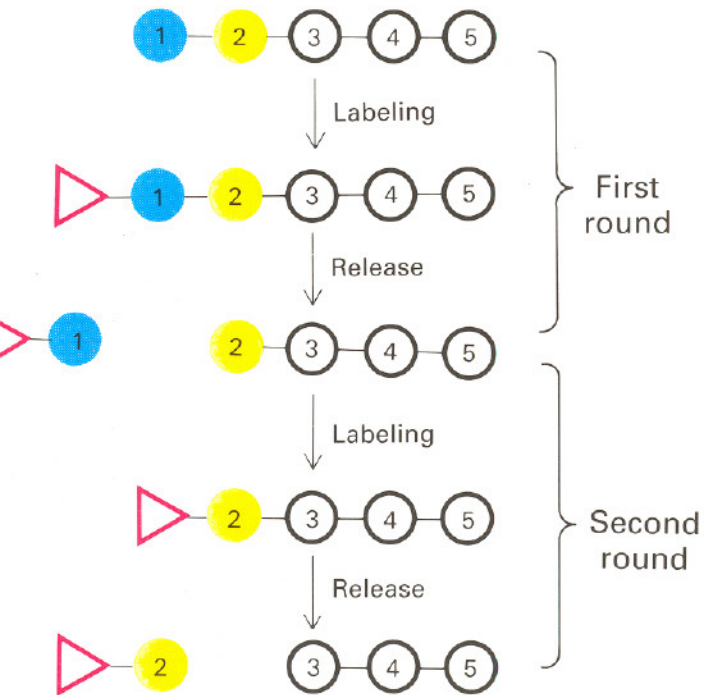
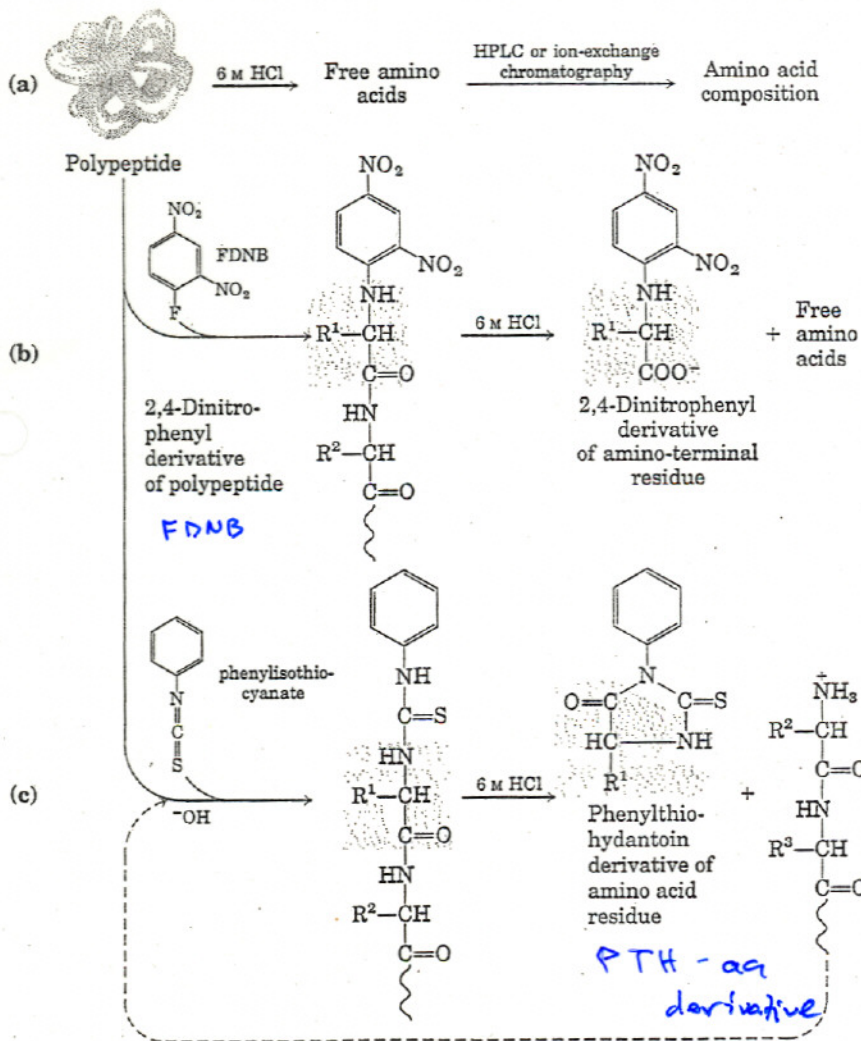


Figure 3-18, page 55

figure 5-25

Steps in sequencing a polypeptide. (a) Determination of amino acid composition and (b) identification of the amino-terminal residue are often the first steps in sequencing a polypeptide. Sanger's method for identifying the amino-terminal residue is shown here. The Edman degradation procedure (c) reveals the entire sequence of a peptide. For shorter peptides, this method alone readily yields the entire sequence, and steps (a) and (b) are often omitted. The latter procedures are useful in the case of larger polypeptides, which are often fragmented into smaller peptides for sequencing (see Fig. 5-27).



Determine types and amounts of amino acids in polypeptide.

Identify amino-terminal residue of polypeptide.

Sanger

Identify amino-terminal residue; purify and recycle remaining peptide fragment through Edman process.

Edman

PROTEINS CAN BE SPECIFICALLY CLEAVED INTO SMALL PEPTIDES TO FACILITATE ANALYSIS

Peptides much longer than about 50 residues cannot be reliably sequenced by the Edman Method. To overcome this obstacle, large proteins can be cleaved into peptides not much longer than 50 residues.

There are several reagents that cause specific cleavage of polypeptides.

- e.g. cyanogen bromide (CN BR): cleaves at the carboxyl side of methionine residues**

The peptides obtained by specific chemical or enzymatic cleavage are separated by chromatography. The sequence of each purified peptide is then determined by the Edman Method

The order of the peptide segments is determined by the method of overlap peptides

Proteins containing two or more polypeptide chains held together by noncovalent bonds, are usually treated by a denaturing agent (e.g. urea or guanidine hydrochloride) before they can be sequenced

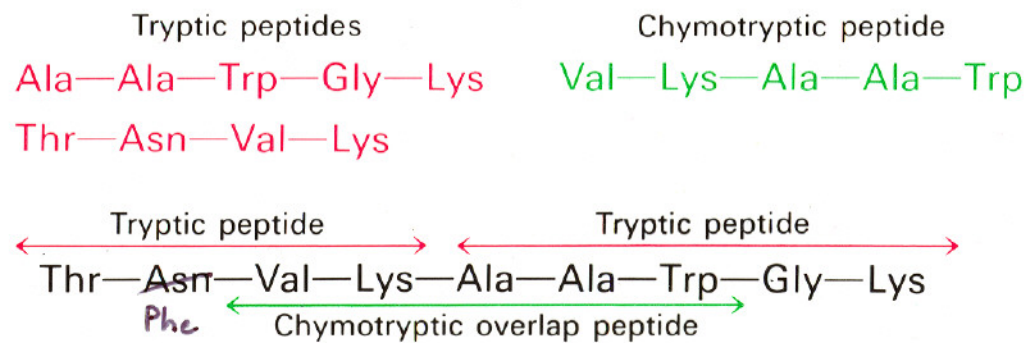
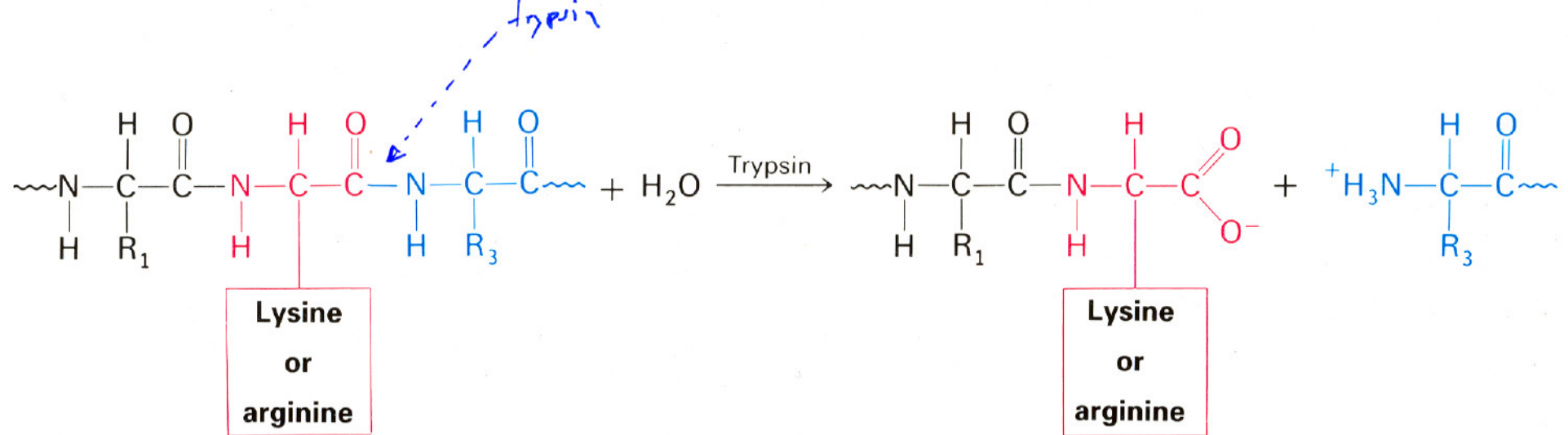


Figure 3-21, page 56; Figure 3-22, page 57

figure 5-26

Breaking disulfide bonds in proteins. Two common methods are illustrated. Oxidation of a cystine residue with performic acid produces two cysteic acid residues. Reduction by dithiothreitol to form Cys residues must be followed by further modification of the reactive —SH groups to prevent re-formation of the disulfide bond. Acetylation by iodoacetate serves this purpose.

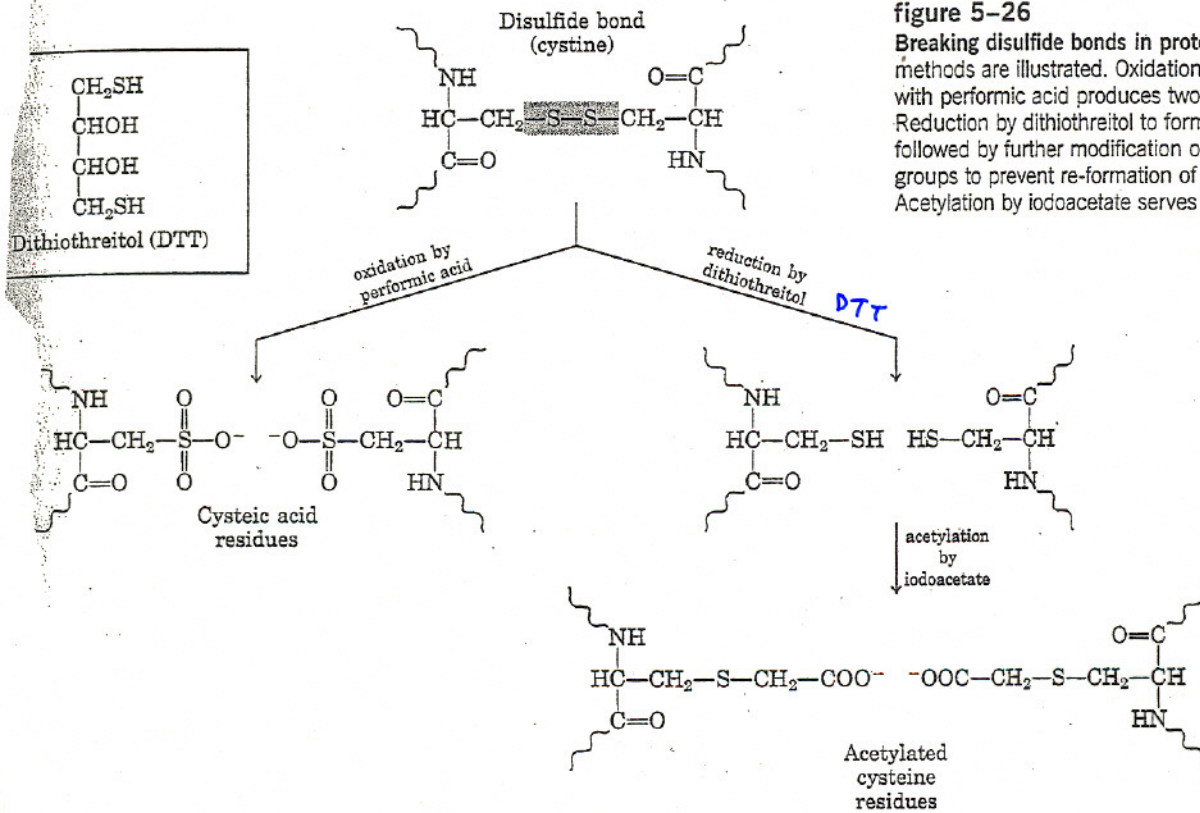


table 5-7

The Specificity of Some Common Methods for Fragmenting Polypeptide Chains

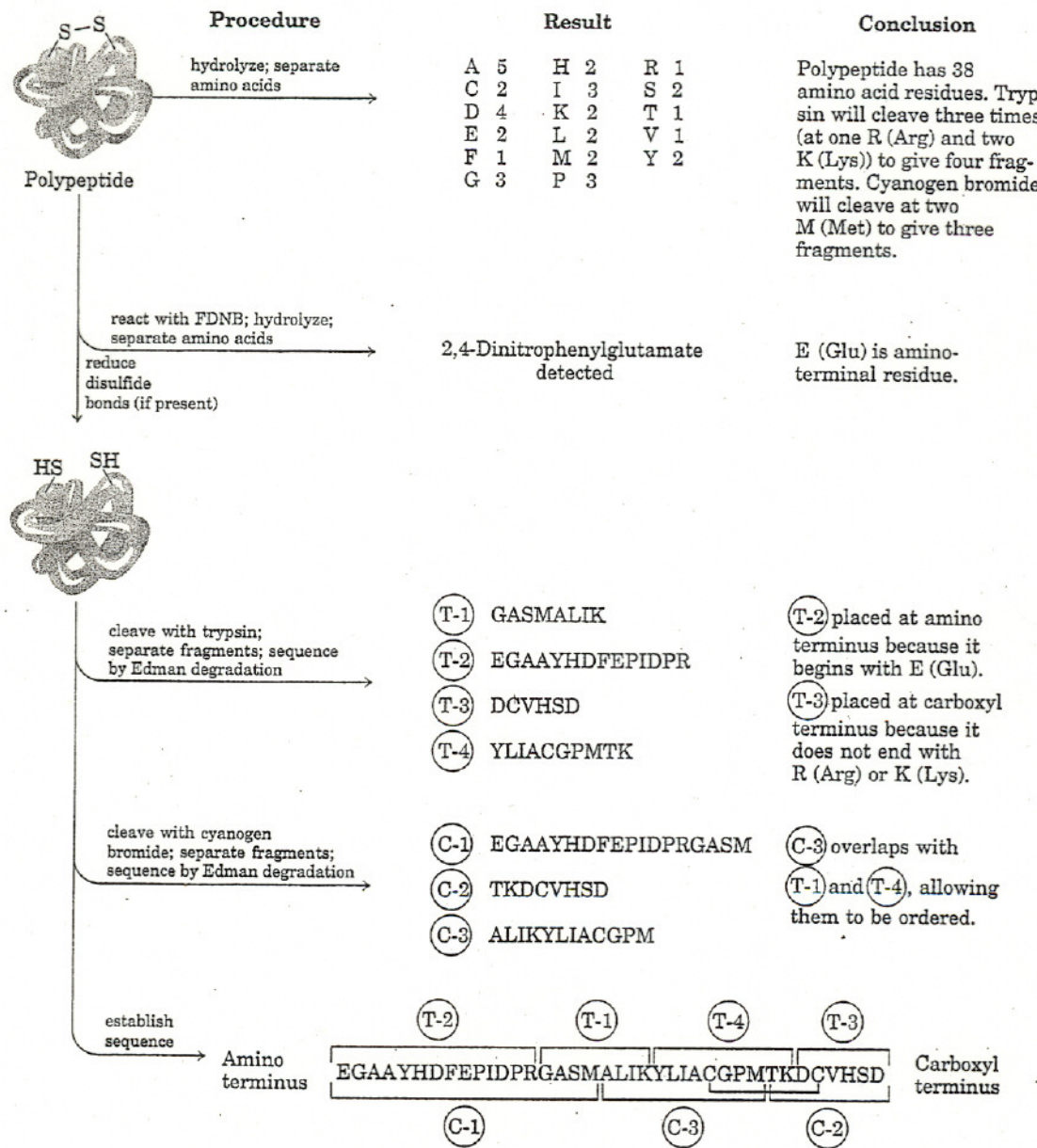
Treatment*	Cleavage points†
Trypsin	Lys, Arg (C)
<i>Submaxillaris</i> protease	Arg (C)
Chymotrypsin	Phe, Trp, Tyr (C)
<i>Staphylococcus aureus</i> V8 protease	Asp, Glu (C)
Asp-N-protease	Asp, Glu (N)
Pepsin	Phe, Trp, Tyr (N)
Endoproteinase Lys C	Lys (C)
Cyanogen bromide	Met (C)

*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.

figure 5-27

Cleaving proteins and sequencing and ordering the peptide fragments. First, the amino acid composition and amino-terminal residue of an intact sample are determined. Then any disulfide bonds are broken prior to fragmenting so that sequencing can proceed efficiently. In this example, there are only two Cys (C) residues, and thus only one possibility for location of the disulfide bond. In polypeptides with three or more Cys residues, the position of disulfide bonds can be determined as described in the text. (The one-letter abbreviations for amino acids are given in Table 5-1.)



AMINO ACID SEQUENCES PROVIDE MANY KINDS OF INSIGHTS

Amino acid sequences can be highly informative in a variety of ways:

1. The sequence of a protein of interest can be compared with all other known sequences to ascertain whether significant similarities exist. Does this protein belong to one of the established families?
2. Comparison of sequences of the same protein in different species yields a wealth of information about evolutionary pathways
3. Amino acid sequences contain signals that determine the destination of proteins and control their processing
4. Sequence data provide a basis for preparing antibodies specific for a protein of interest
5. Amino acid sequences are also valuable for making DNA probes that are specific for the genes encoding the corresponding proteins
6. Amino acid sequences can be searched for the presence of internal repeats.

ULTRACENTRIFUGATION IS VALUABLE FOR SEPARATING BIOMOLECULES AND DETERMINING THEIR MASS

Centrifugation is a powerful and generally applicable method for separating and analyzing cells, organelles, and biological macromolecules

Zonal (or band) centrifugation: It is used to separate proteins with different sedimentation coefficients.

The mass (molecular weight) of a protein can be directly determined by sedimentation equilibrium, in which a sample is centrifuged at relatively low speed so that sedimentation is counterbalanced by diffusion. It can be used under nondenaturing conditions in which the native structure of multimeric proteins is preserved

The sedimentation coefficient of a particle is a convenient means of quantifying its rate of movement through a liquid medium.

$$s = m(1 - \bar{v}\rho) / f$$

(Svedberg units, s)

m : mass
 \bar{v} : partial specific volume
 ρ : density of medium
 f : frictional coefficient

The sedimentation coefficient of a particle depends on its mass, shape, and density.

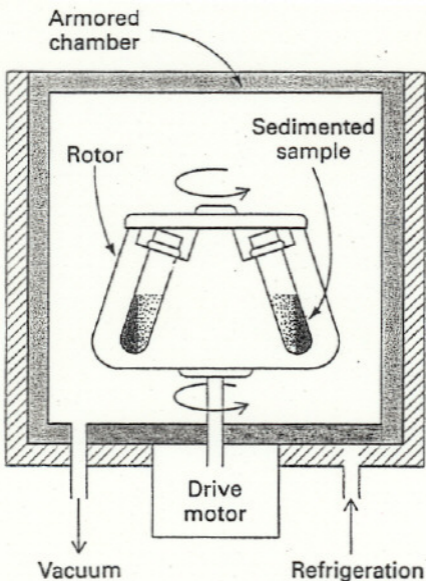


Figure 3-11
Ultracentrifuge rotor containing two sample tubes.

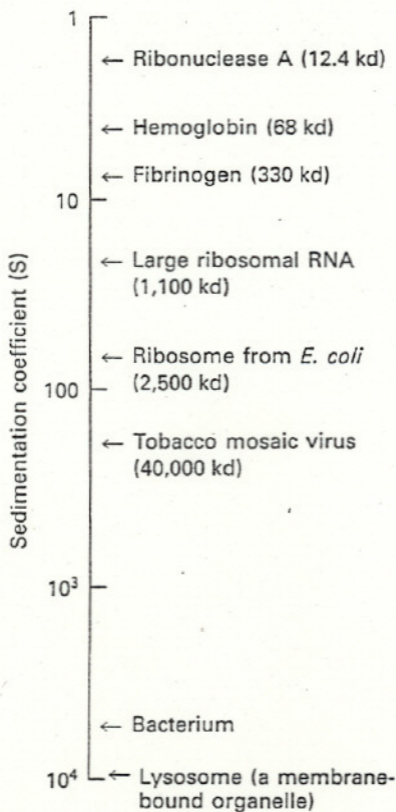


Figure 3-12
Span of S values of biomolecules and cells.