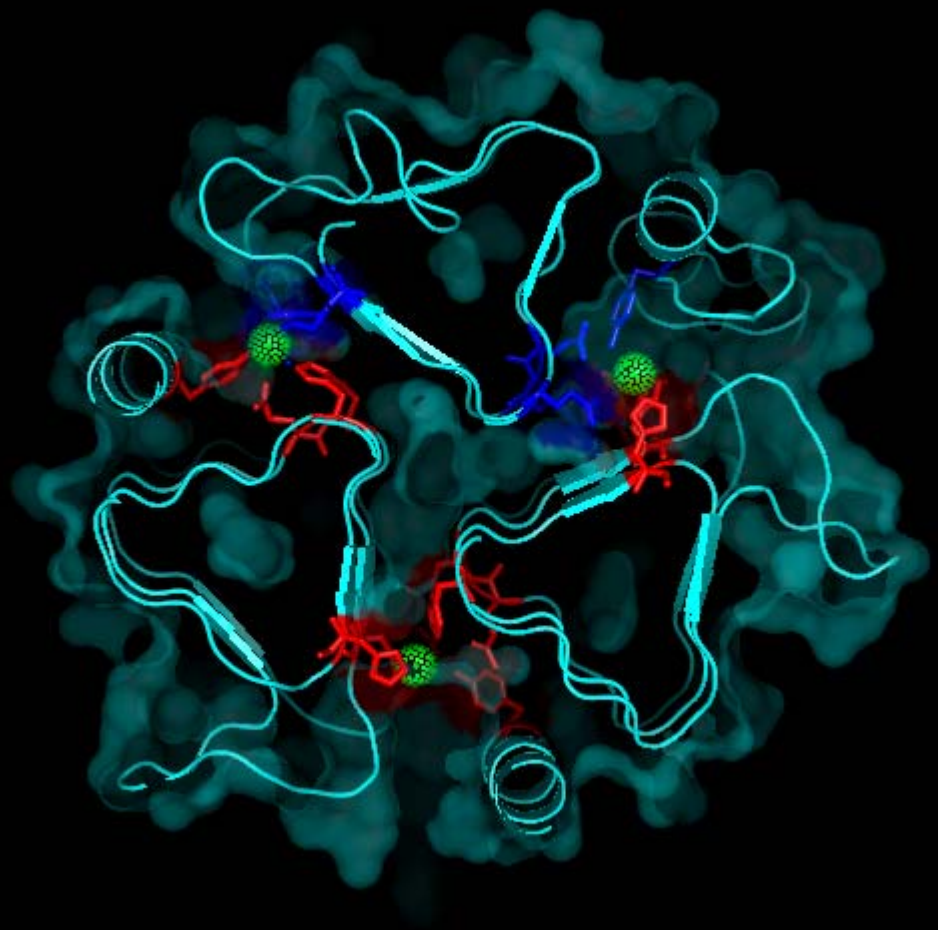
Introducción al estudio de las proteínas



Sur l'existence de la matiere albumineuse dans les vegetaux

Antoine Francois de Fourcroy (1789) Annales de Chimie 3, 352.

1755-1809

Estudia 3 tipos de sustancias : albúmina, fribina y gelatina

"A consistency often thick and flowing, insipid taste, solubility in cold water, precipitation by heat, solubility in alkalis and above all in ammonia, separating itself at the temperature of boiling water from all these liquids in which it is dissolved, passing into putrefaction without acidity, such are the properties which characterize albuminous substance"

On the composition of some animal substances

Gerrit Jan Mulder (1839) Journal für praktische Chemie 16, 129

Se concentra en las llamadas “albuminas”: fibrina, albumina de huevo, de suero bovino y de trigo

	Fibrin	Albumin v. Eiern	Albumin v. Serum
Kohlenstoff .	54,56 —	54,48 —	54,84
Wasserstoff .	6,90 —	7,01 —	7,09
Stickstoff .	15,72 —	15,70 —	15,83
Sauerstoff .	22,13 —	22,00 —	21,23
Phosphor .	0,33 —	0,43 —	0,33
Schwefel .	0,36 —	0,38 —	0,68

Fig. 1.2 Table of Mulder’s analytical results, reproduced from his paper in 1838.[14] The formula calculated by Mulder for fibrin or egg albumin, on the basis of the content of phosphorus and sulphur, was $C_{400}H_{620}N_{100}O_{120}P_1S_1$. For serum albumin it was the same, but with two atoms of sulphur.

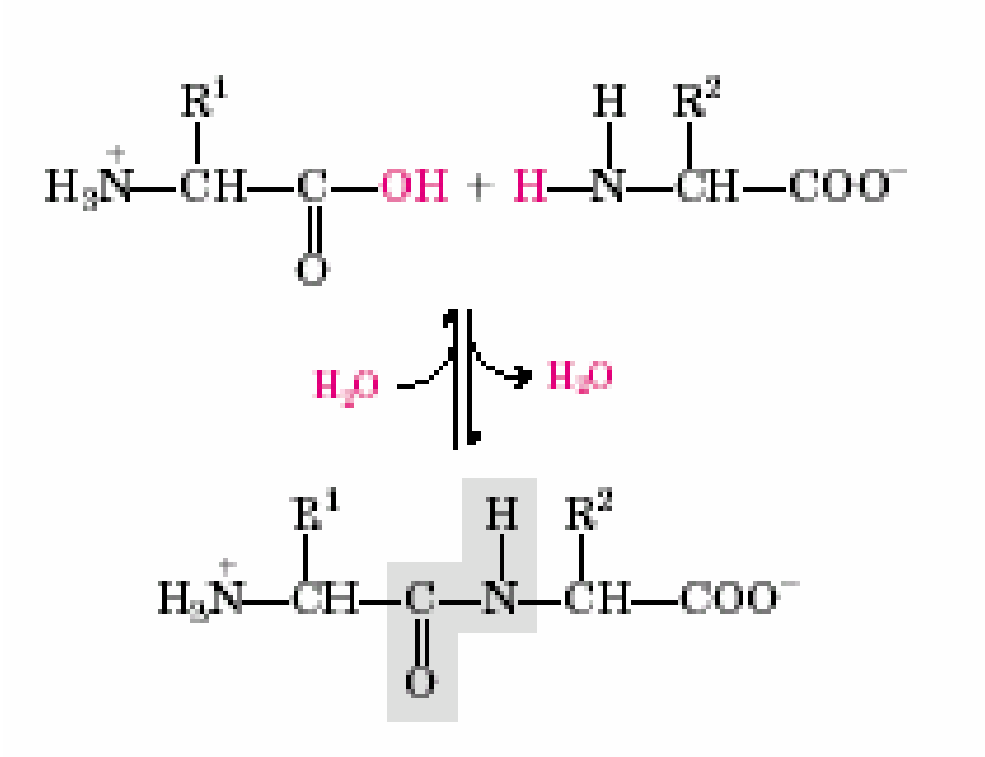
En 1838 siguiendo una sugerencia de Jacob Berzelius propone el término “proteína” para las sustancias “albuminosas”.
Otra consecuencia del estudio de Mulder es que las proteínas son “macromoléculas”.

El enlace peptídico

22 de Septiembre de 1902 en el 74th Annual Meeting of the Gesellschaft der deutschen Naturforschen und Ärzte (Society of German Naturalists and Physicians) in Karlsbad, Bohemia

Franz Hofmeister
1850-1922

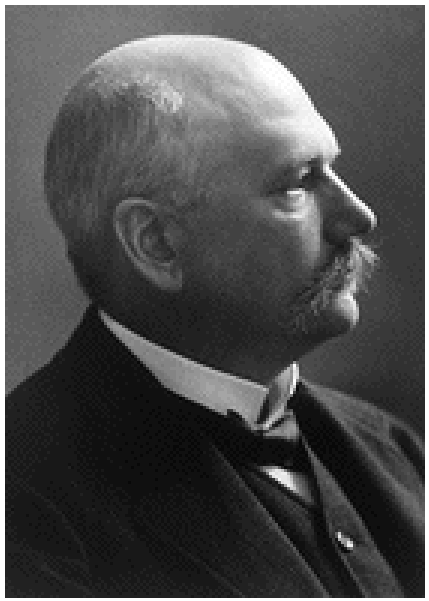
Hermann Emil Fischer
1852-1919



(1) La mayoría del N de las proteínas está unido como amina/imina
(2) Las proteínas dan la reacción de Biuret positiva
(3) Los aminoácidos pueden unirse en forma sintética para dar oligopéptidos formando un enlace amida
(4) Las proteinas son hidrolizadas en ácidos fuertes (enlace amida) y "fermentos"

Las proteínas son *Macromoleculas* o agregados?

Teoría del coloide (1880-1930)



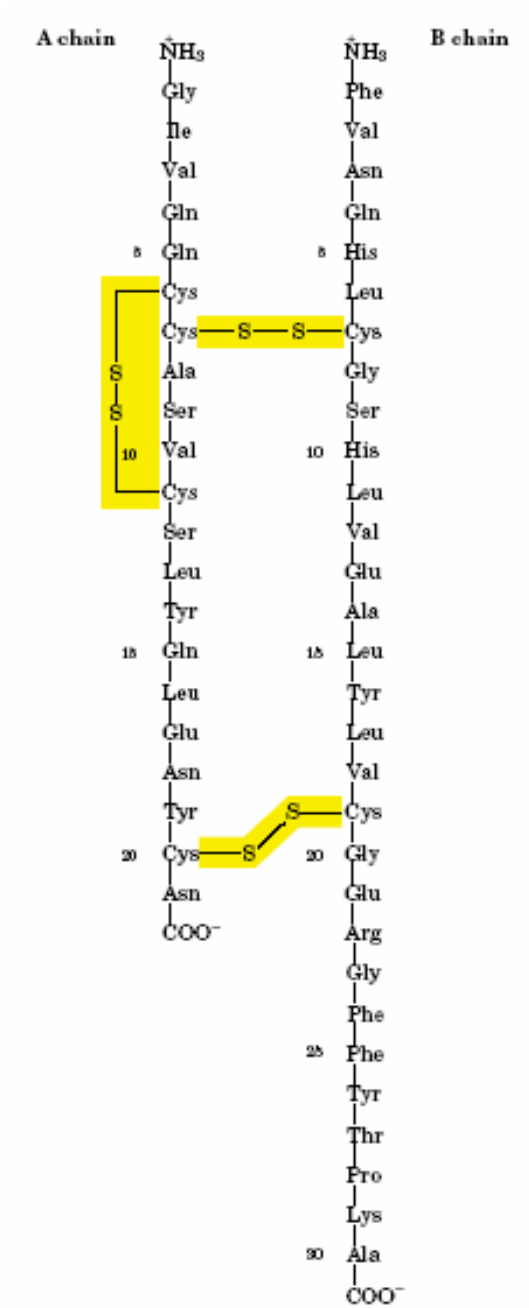
Ludwig Karl Martin Leonhard Albrecht Kossel
1853-1927

"*Amino acids per se* and their *spatial arrangement* within the protein must become the chemical key to understanding of proteins" Kossel, 1900.

Secuenciación

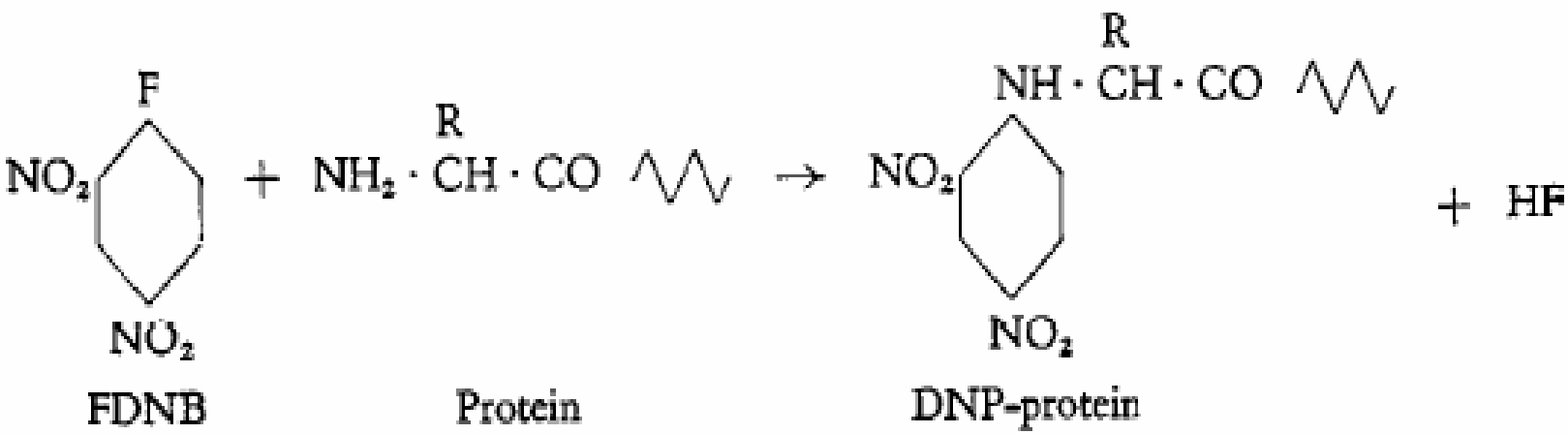
Frederick Sanger
1918-

Nobel Prize (Chemistry, 1958)
Nobel Prize (Chemistry, 1980)



1,2,4 fluorodinitrobenceno

- La reacción se lleva a cabo a temperatura ambiente
- Los DNP derivados tienen color (amarillo)
- Los DNP derivados se pueden separar e identificar por cromatografía



1. Estimación de Aminos en la Insulina

(DNP-insulina seguida de hidrólisis y caracterización por cromatografía)

DNP-Gly, DNP-Phe, DNP-Lys

2. Estimación de Aminos terminales

(DNP-insulina, seguida de hidrólisis, extracción con eter y posterior cromatografía)

DNP-Gly, DNP-Phe

3. Cuantificación de los Amino terminales

Cuantificación de DNP-Gly, DNP-Phe. Si PM 12000 2 Gly, 2 Phe

Sanger, The free amino groups of insulin. Biochemical J. 39:507-515. 1945

Sanger, The terminal peptides of insulin. Biochemical J. 45:563-574. 1949.

Insulina + Ac. Performico

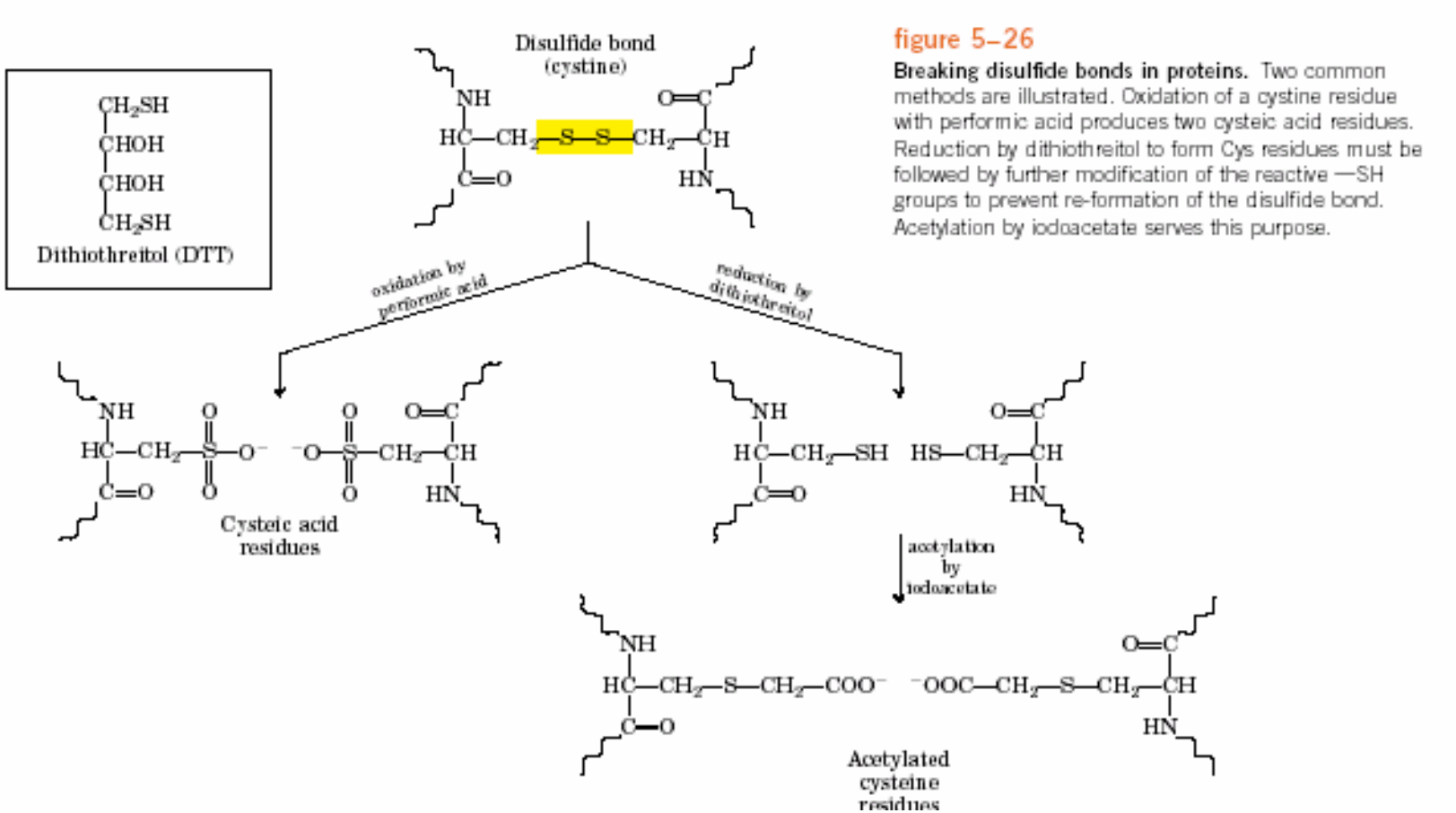


figure 5–26

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.

Harfenist, and Craig. J. Am. Chem. Soc. 74:3087-3089. 1952

Por técnicas de sedimentación encuentran que el PM de la insulina es 6000 y no 12000. Este último peso molecular se debe a la formación de un dímero no-covalente

Sanger, The terminal peptides of insulin. Biochemical J. 45:563-574. 1949.

NH2-G[I V E E] NH2-GIVEQ…… A chain (21aa)

NH2-F[V D E] NH2-FVNQ……. B chain (30 aa)

Las proteínas tienen secuencias específicas

Sanger and Tupy Biochem J. 49: 366-374 1951a

Sanger and Tupy. Biochem J. 49:481-490 1951b

FVNQHLCGSHLVEALYLVCGERGFFYTPKA chain B

Hidrólisis ácida

Proteasas (tripsina, quimotripsina y pepsina)

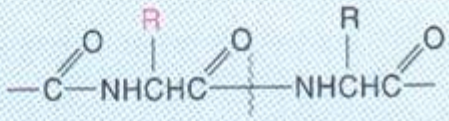
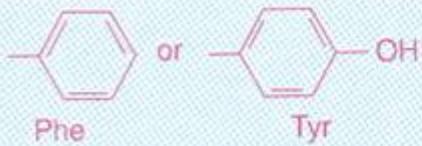
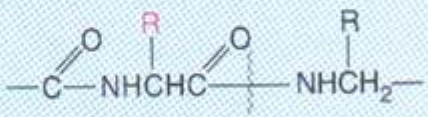
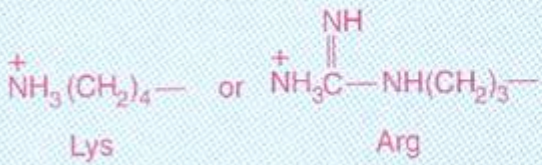
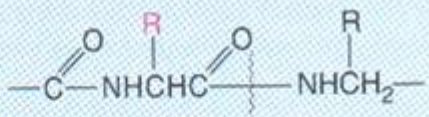
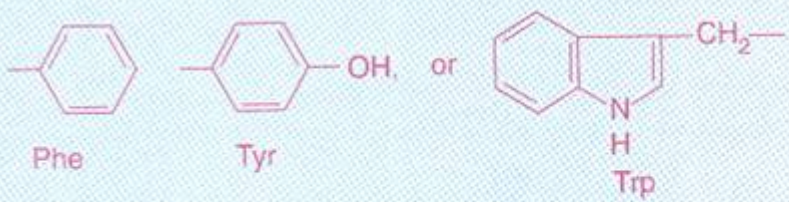
table 5–7

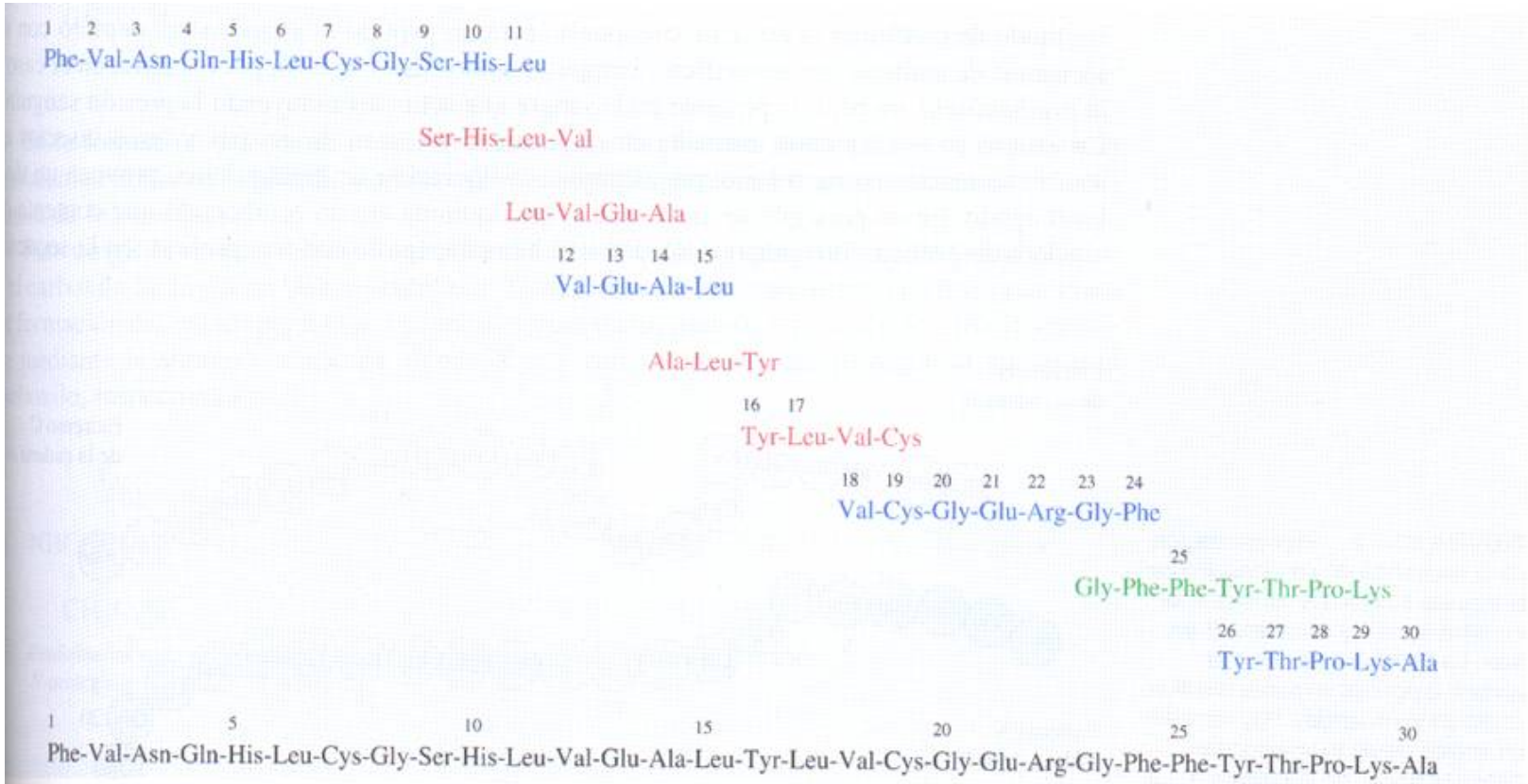
The Specificity of Some Common Methods for Fragmenting Polypeptide Chains

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)

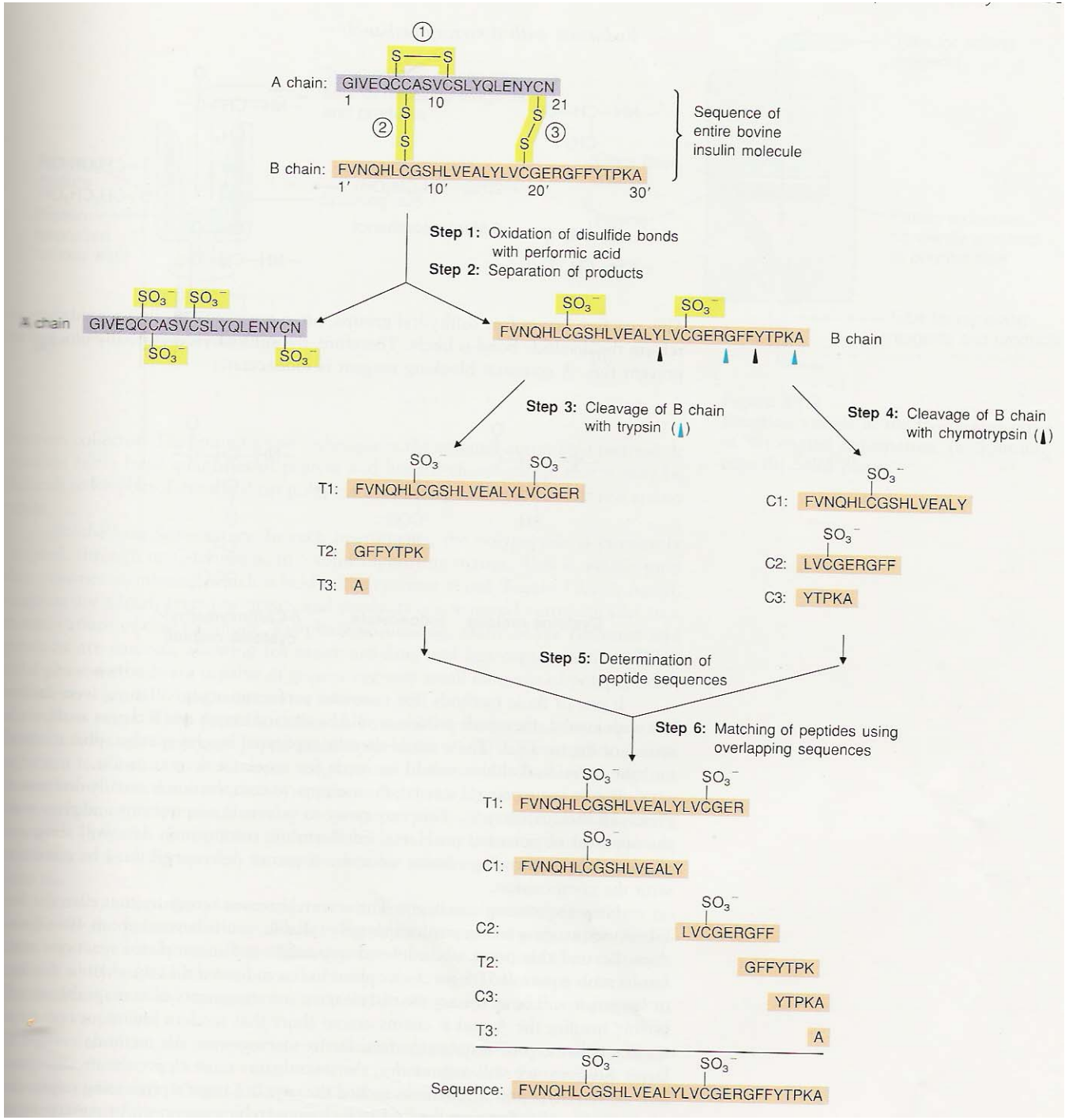
*All except cyanogen bromide are proteases. All are available from commercial sources.

†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.

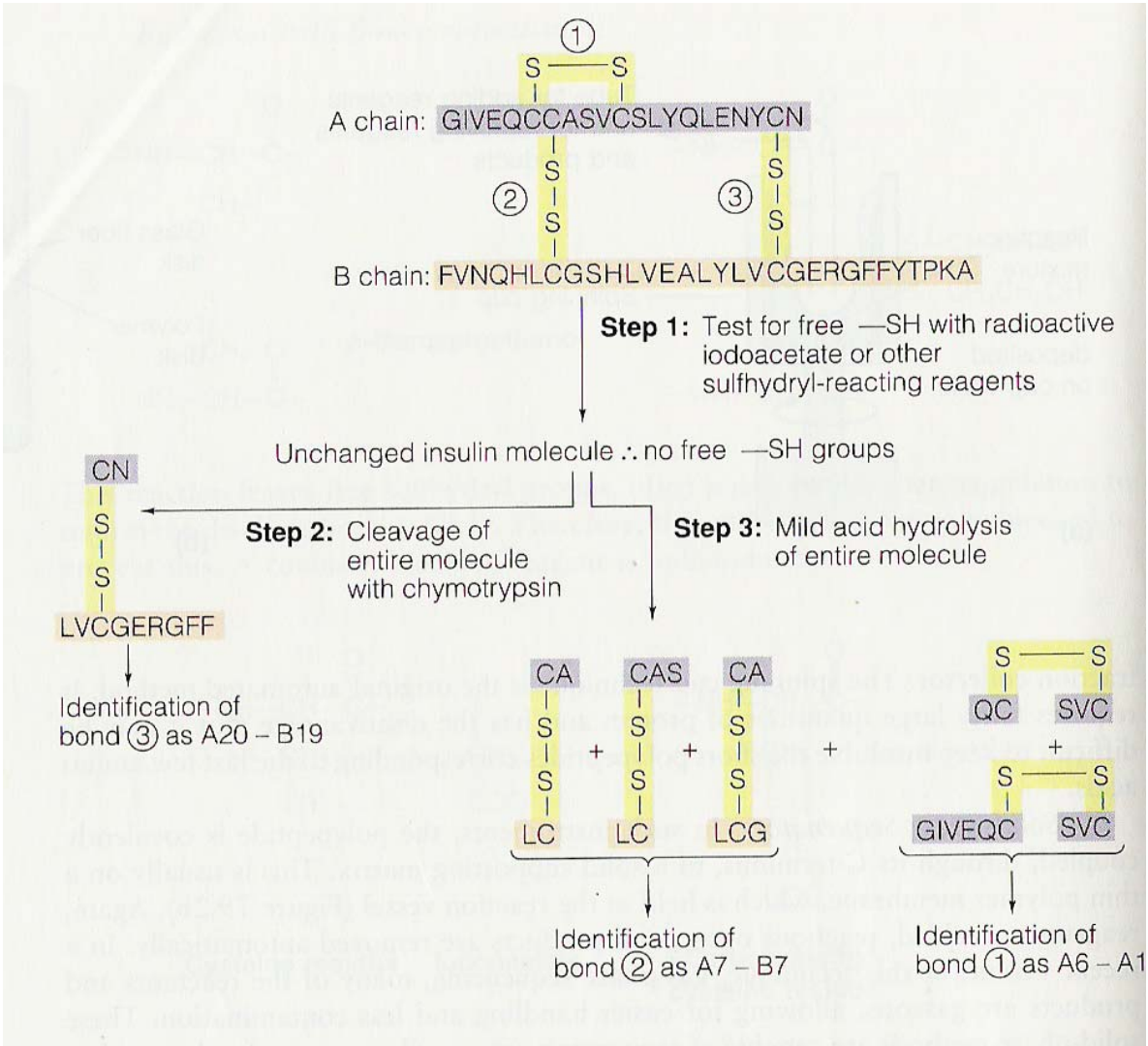
Peptidase	Point of cleavage	Preferred side-chain group (R) in substrate
Pepsin		
Trypsin		
Chymotrypsin		



GURA 27.12. Diagrama que muestra cómo determinar la secuencia de aminoácidos de la cadena B


A chain: GIVEQCCASVCSLYQLENYCN
1 10 21
B chain: FVNQHLCGSHLVEALYLVCGERGFFYTPKA
1' 10' 20' 30'
Sequence of entire bovine insulin molecule
Step 1: Oxidation of disulfide bonds with performic acid
Step 2: Separation of products
A chain GIVEQCCASVCSLYQLENYCN
FVNQHLCGSHLVEALYLVCGERGFFYTPKA B chain
Step 3: Cleavage of B chain with trypsin
Step 4: Cleavage of B chain with chymotrypsin
T1: FVNQHLCGSHLVEALYLVCGER
T2: GFFYTPK
T3: A
C1: FVNQHLCGSHLVEALY
C2: LVCGERGFF
C3: YTPKA
Step 5: Determination of peptide sequences
Step 6: Matching of peptides using overlapping sequences
T1: FVNQHLCGSHLVEALYLVCGER
C1: FVNQHLCGSHLVEALY
C2: LVCGERGFF
T2: GFFYTPK
C3: YTPKA
T3: A
Sequence: FVNQHLCGSHLVEALYLVCGERGFFYTPKA

Identificación de puentes disulfuro

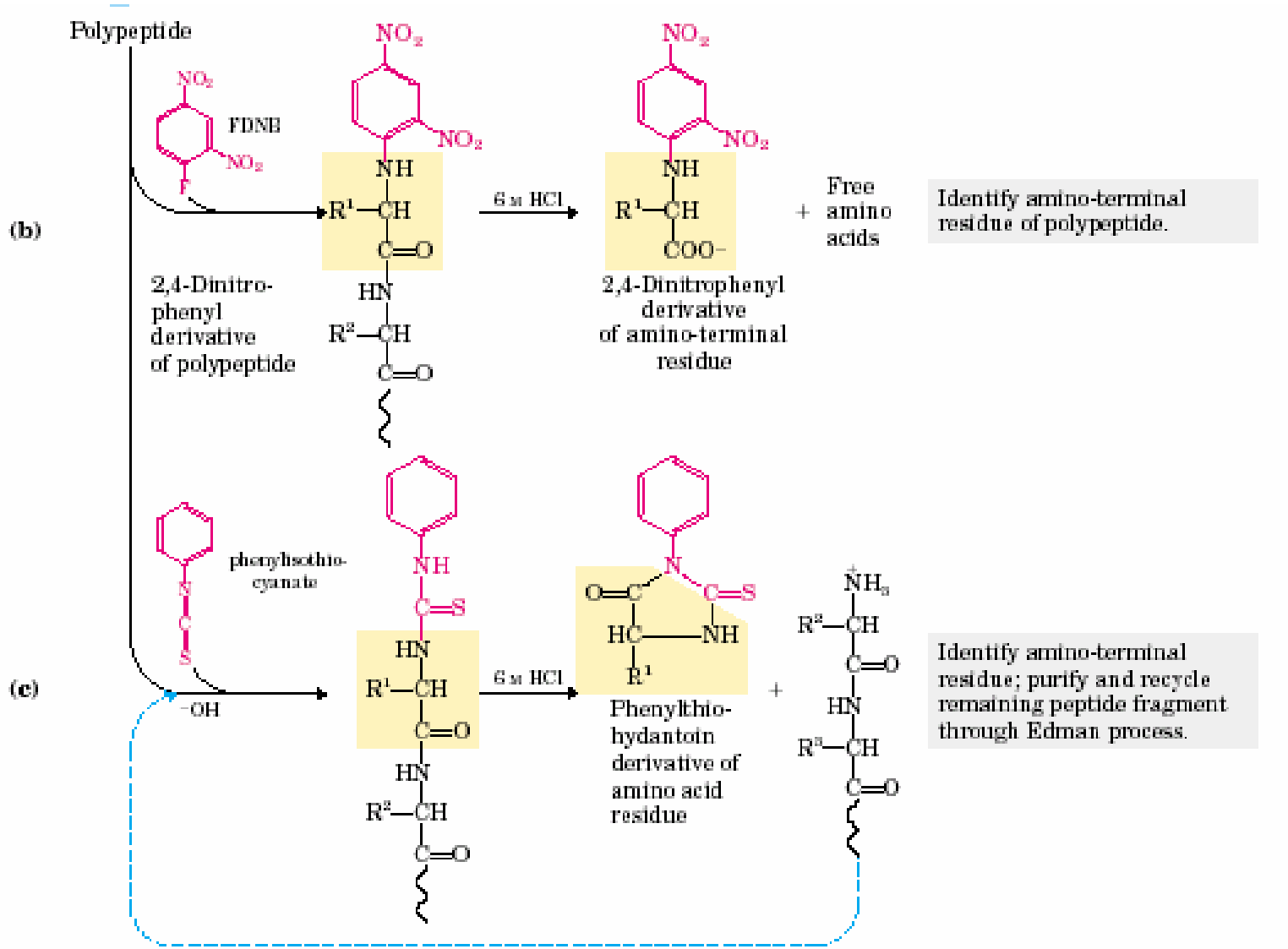


Sanger and Thompson, Biochem. J. 53:353-366. 1953

Sanger and Thompson, Biochem. J. 53:366-374. 1953

It would thus seem that no general conclusions can be drawn from these results concerning the general principles which govern the arrangement of the amino-acid residues in protein chains. In fact, it would seem more probable that there are no such principles, but that each protein has its own unique arrangement; an arrangement which endows it with its particular properties and specificities and fits it for the function that it performs in nature.

Edman, P., 1950 Method for determination of the amino acid sequence in peptides. Acta Chem. Scand. **4:** 283–293.



Resumen

1. Purificación de la proteína o péptido
2. Determinación de la composición y PM
3. Determinación del número de cadenas (ruptura de los puentes disulfuro)
4. Determinación de los extremos amino-terminales y carboxilo-terminales
5. Corte específico de la secuencia principal en fragmentos menores, al menos en dos formas distintas
6. Purificación de cada segmento y secuenciación
7. Determinación de la secuencia total