



BIOCHEMISTRY REVIEW

Overview of Biomolecules

Chapter 4

Protein Sequence

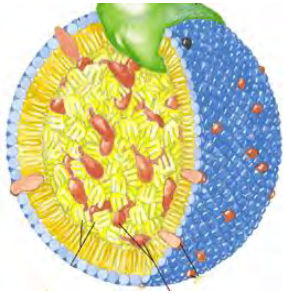
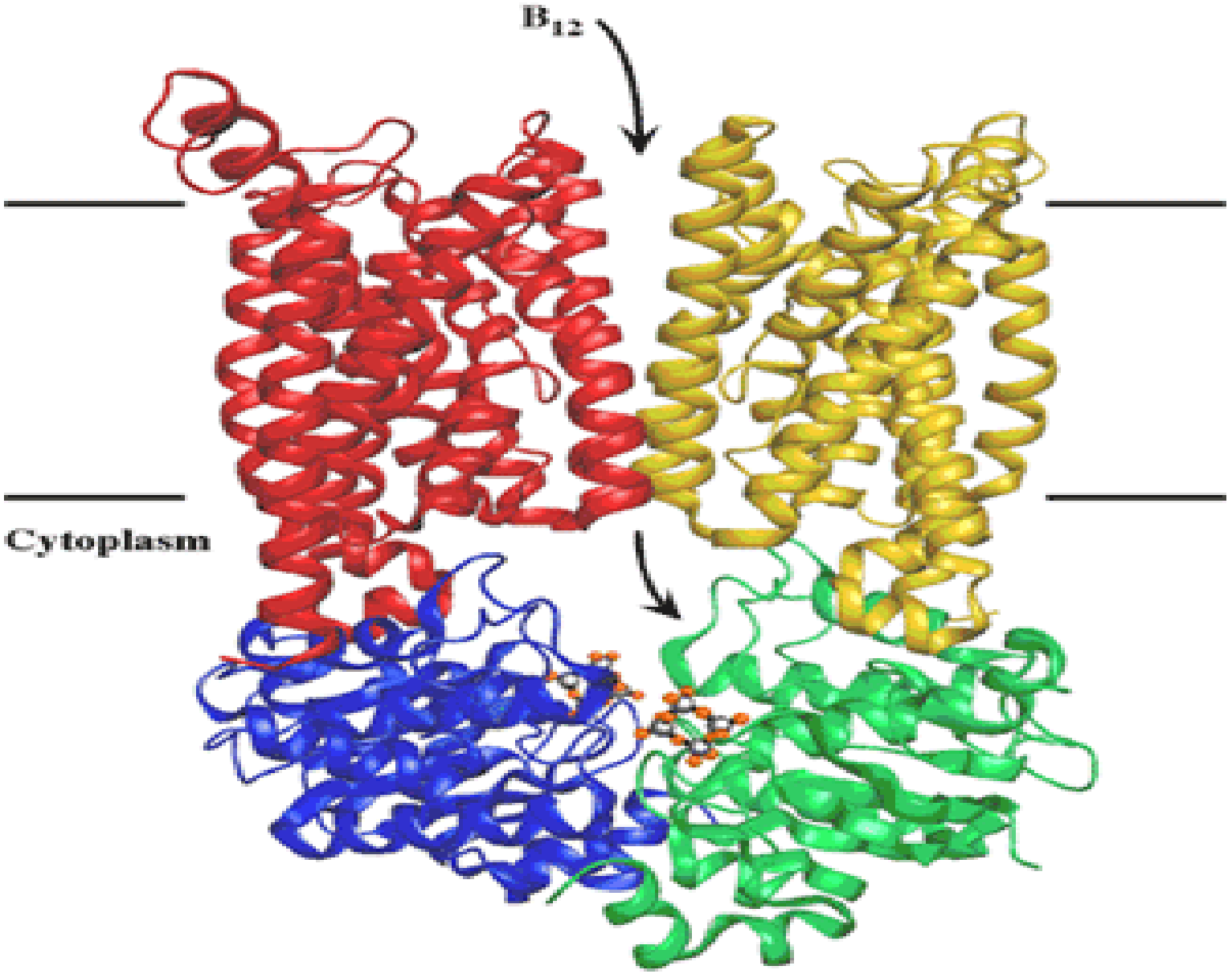




TABLE 3–2 Molecular Data on Some Proteins

	Molecular weight	*Number of residues*	*Number of polypeptide chains*
Cytochrome *c* (human)	13,000	104	1
Ribonuclease A (bovine pancreas)	13,700	124	1
Lysozyme (chicken egg white)	13,930	129	1
Myoglobin (equine heart)	16,890	153	1
Chymotrypsin (bovine pancreas)	21,600	241	3
Chymotrypsinogen (bovine)	22,000	245	1
Hemoglobin (human)	64,500	574	4
Serum albumin (human)	68,500	609	1
Hexokinase (yeast)	102,000	972	2
RNA polymerase (*E. coli*)	450,000	4,158	5
Apolipoprotein B (human)	513,000	4,536	1
Glutamine synthetase (*E. coli*)	619,000	5,628	12
Titin (human)	2,993,000	26,926	1




B_{12}
Cytoplasm



Are You Getting It??



A molecule of hemoglobin is compared with a molecule of lysozyme. Which characteristics do they share?

(multiple answers)

a) Both contain the same number of subunits.
b) Both contain the common amino acids.
c) Both have the same molecular weight.
d) Both contain the same type of peptide bonds.
e) Both have the same number of pKa values.



Are You Getting It??



Answer

A molecule of hemoglobin is compared with a molecule of lysozyme. Which characteristics do they share?

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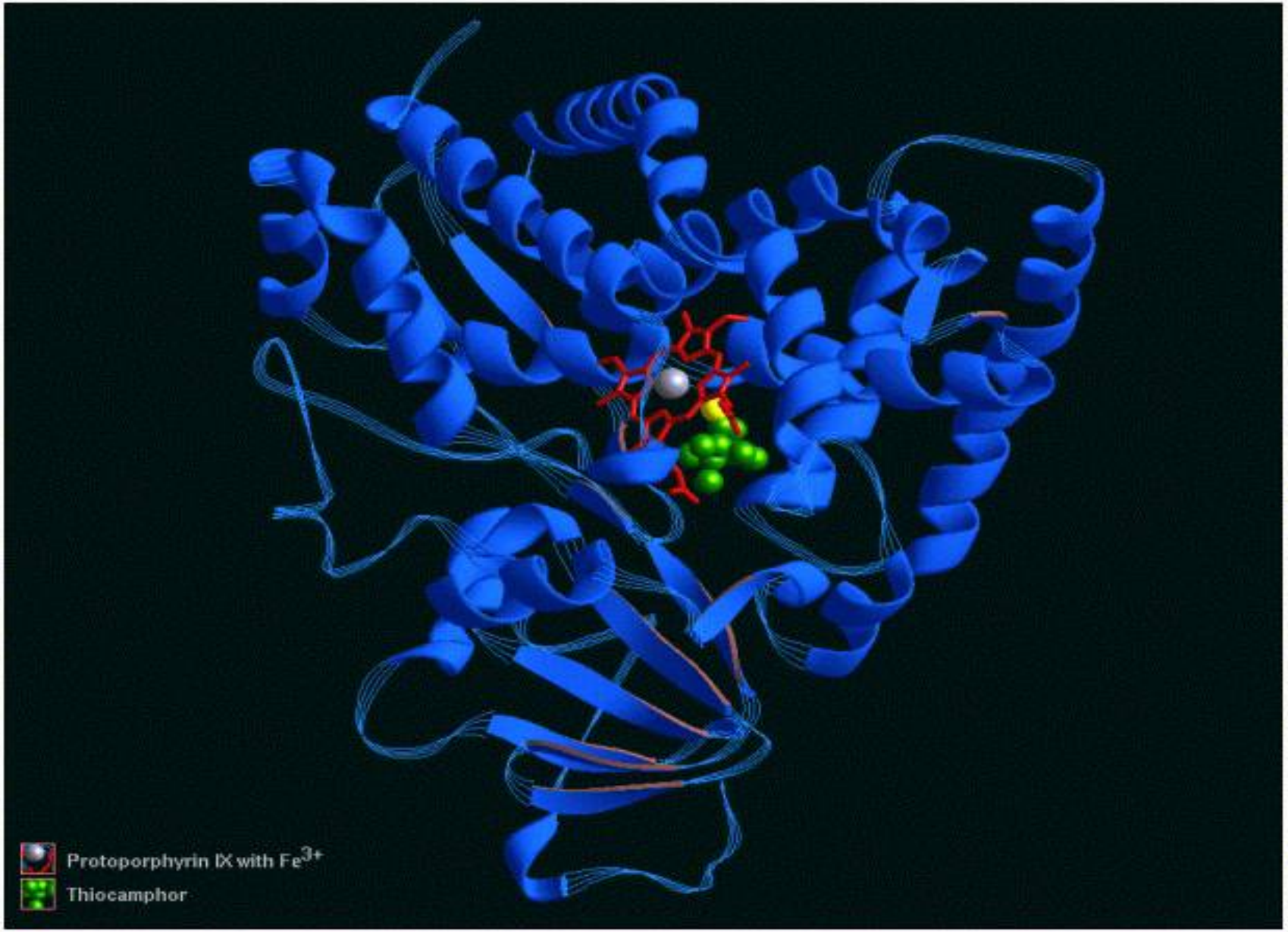
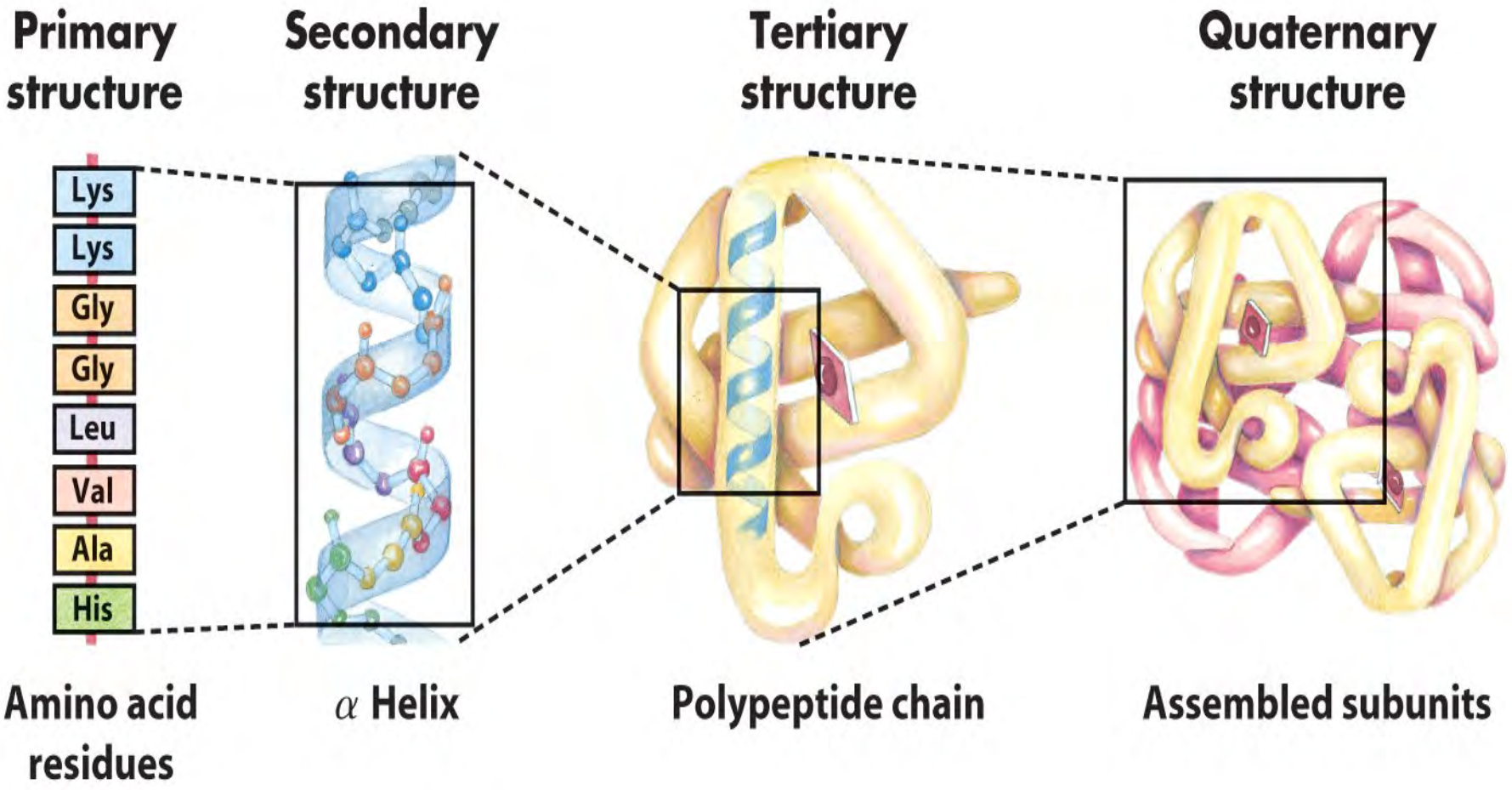

Protoporphyrin IX with Fe^{3+}
Thiocamphor

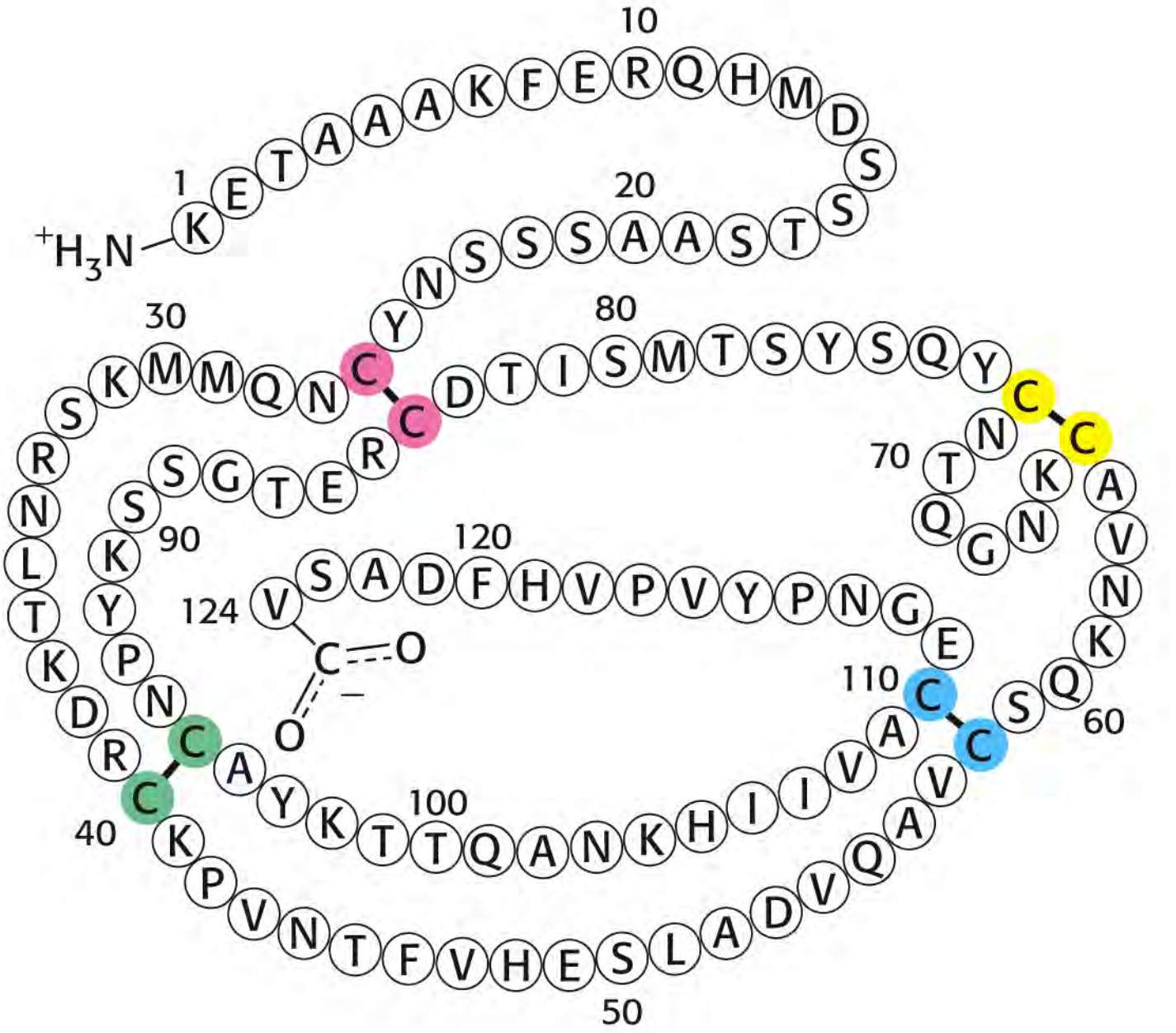
TABLE 3–4 Conjugated Proteins

Class	*Prosthetic group*	*Example*
Lipoproteins	Lipids	β_1-Lipoprotein of blood
Glycoproteins	Carbohydrates	Immunoglobulin G
Phosphoproteins	Phosphate groups	Casein of milk
Hemoproteins	Heme (iron porphyrin)	Hemoglobin
Flavoproteins	Flavin nucleotides	Succinate dehydrogenase
Metalloproteins	Iron	Ferritin
	Zinc	Alcohol dehydrogenase
	Calcium	Calmodulin
	Molybdenum	Dinitrogenase
	Copper	Plastocyanin

PROTEIN FUNCTIONS

- **ENZYMES/CATALYSIS**
- **TRANSPORT**
- **STORAGE**
- **MOTILITY**
- **STRUCTURE**
- **DEFENSE**
- **REGULATION**


Primary structure
Secondary structure
Tertiary structure
Quaternary structure
Lys
Lys
Gly
Gly
Leu
Val
Ala
His
Amino acid residues
α Helix
Polypeptide chain
Assembled subunits


10
1
20
30
80
70
90
120
124
110
40
100
50
60
$^{+}H_3N$



Are You Getting It??



Peptide A and Peptide B are two different peptides, and molecules of both peptides are compared. Which characteristics can they share? ***(multiple answers)***

a) Both peptides could have Thr as the N – terminal.
b) Both peptides could contain a total of 18 amino acids.
c) Both peptides could contain 3 Gly and 2 Asn.
d) Both peptides could have the sequence ACDGMFPTHLS.



Are You Getting It??



Answer

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d) **Both peptides could have the sequence ACDGMFPTHLS.**

TABLE 3–3 Amino Acid Composition of Two Proteins

Amino acid	*Number of residues per molecule of protein**	
	Bovine cytochrome c	*Bovine chymotrypsinogen*
Ala	6	22
Arg	2	4
Asn	5	15
Asp	3	8
Cys	2	10
Gln	3	10
Glu	9	5
Gly	14	23
His	3	2
Ile	6	10
Leu	6	19
Lys	18	14
Met	2	2
Phe	4	6
Pro	4	9
Ser	1	28
Thr	8	23
Trp	1	8
Tyr	4	4
Val	3	23
Total	104	245

*In some common analyses, such as acid hydrolysis, Asp and Asn are not readily distinguished from each other and are together designated Asx (or B). Similarly, when Glu and Gln cannot be distinguished, they are together designated Glx (or Z). In addition, Trp is destroyed. Additional procedures must be employed to obtain an accurate assessment of complete amino acid content.

AMINO ACID COMPOSITION

- **ACID HYDROLYSIS – Trp, Ser, Thr, Tyr?**
- **BASE HYDROLYSIS – Cys, Ser, Thr, Arg?**
- **ENZYMATIC HYDROLYSIS – Complete?**

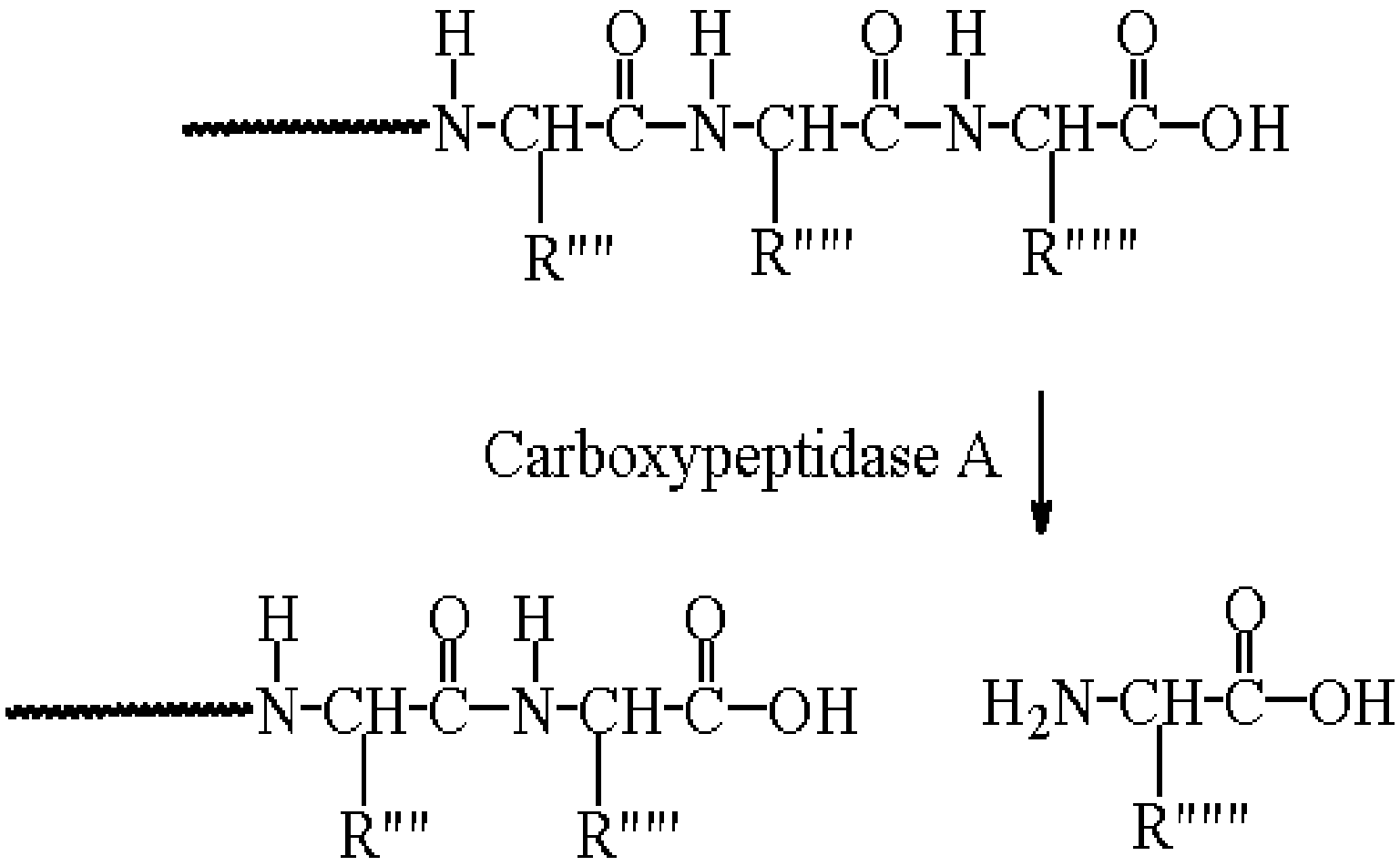
N-TERMINAL ANALYSIS

- **SANGER REAGENT**
- **DANSYL CHLORIDE**

C-TERMINAL ANALYSIS

- **LITHIUM BOROHYDRIDE**
- **HYDRAZINE**
- **CARBOXYPEPTIDASE**

Carboxypeptidase A Method





Are You Getting It??



Which of the following reagents would react only with Ala in the peptide Gly – Met – Phe – Ala?

a) carboxypeptidase
b) strong acid
c) ninhydrin
d) lithium borohydride
e) fluorodinitrobenzene



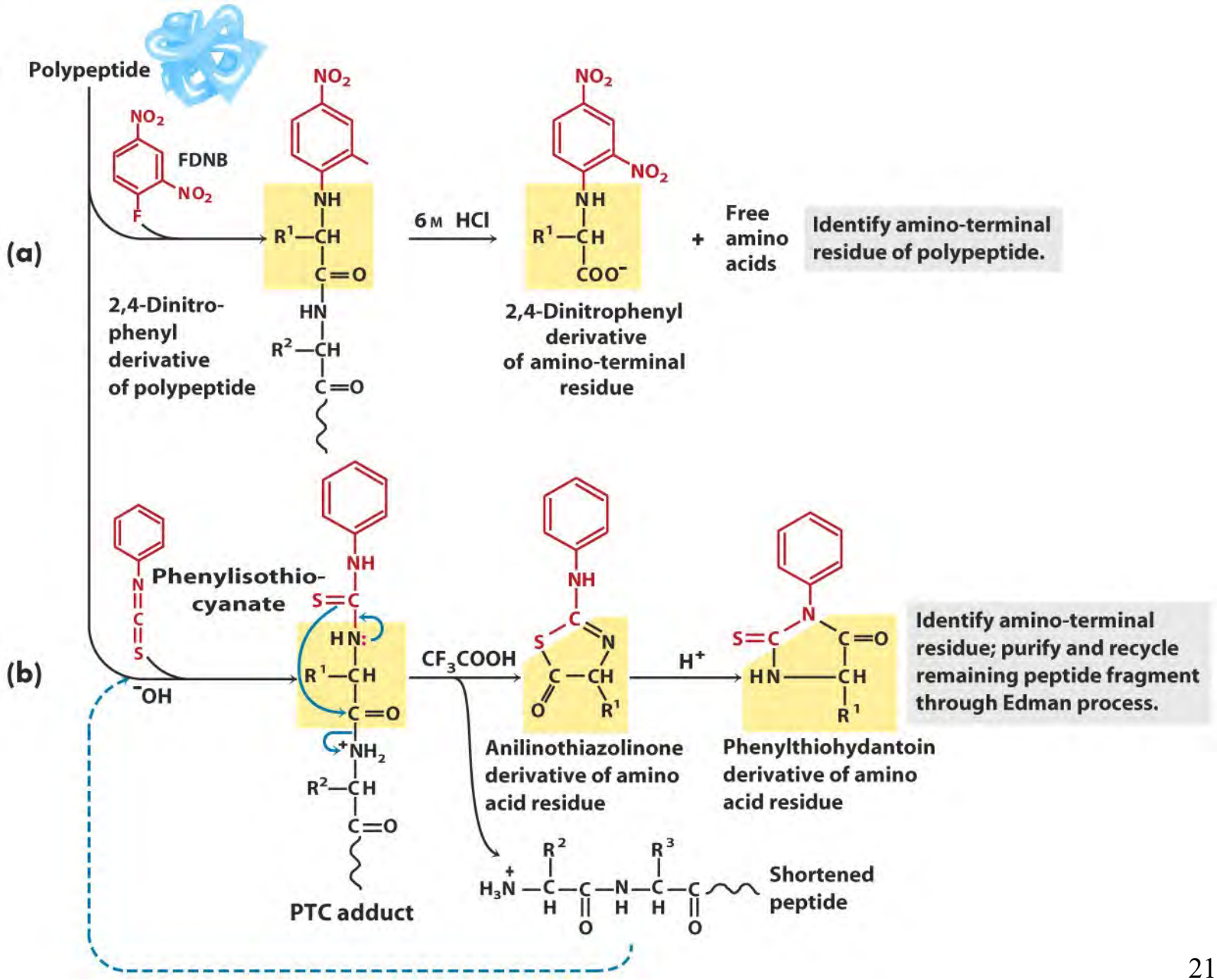
Are You Getting It??

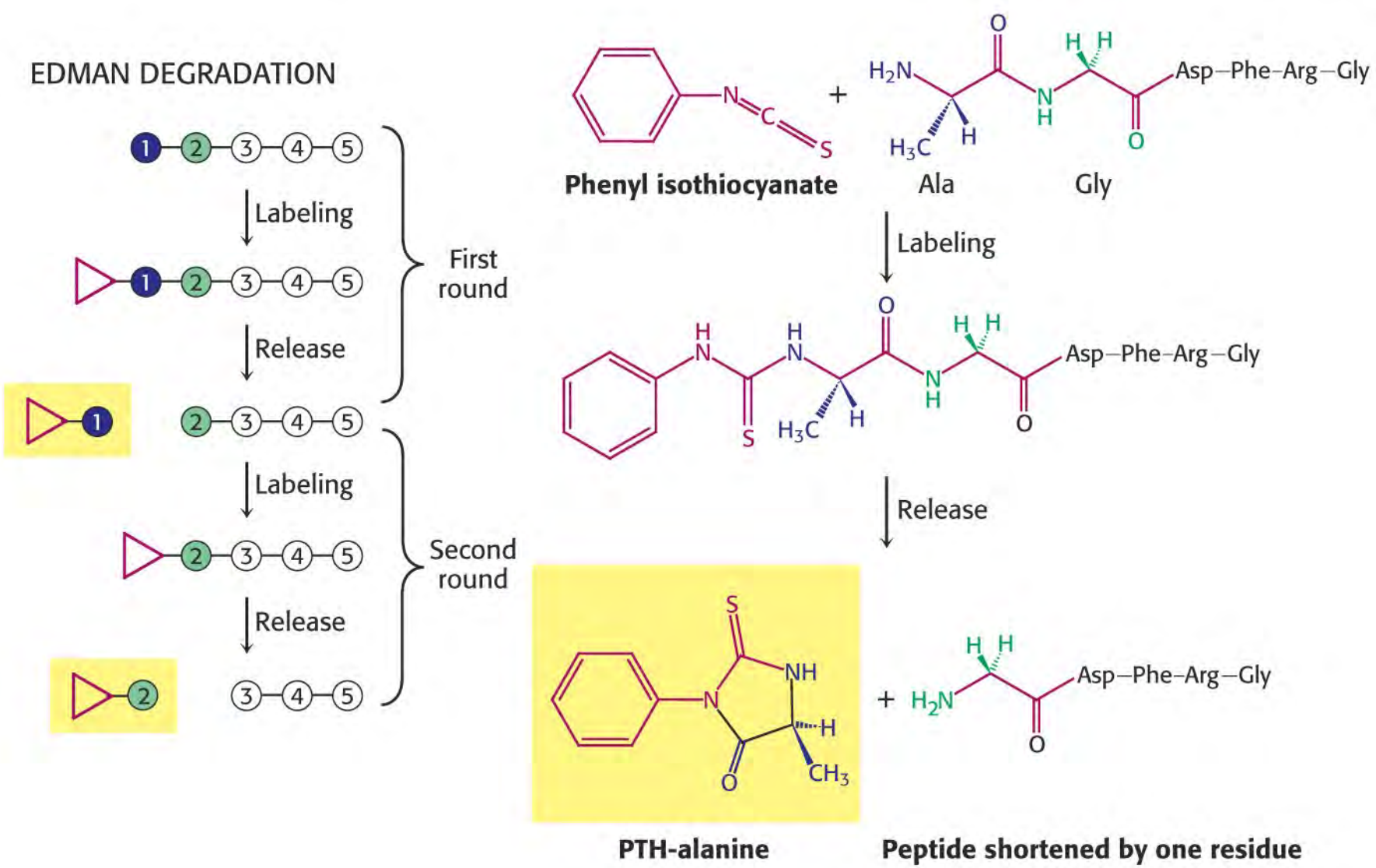


Answer

Which of the following reagents would react only with Ala in the peptide Gly – Met – Phe – Ala?

a) carboxypeptidase
b) strong acid
c) ninhydrin
d) *lithium borohydride*
e) fluorodinitrobenzene


Polypeptide
FDNB
(a)
6 M HCl
Free amino acids
Identify amino-terminal residue of polypeptide.
2,4-Dinitro-phenyl derivative of polypeptide
2,4-Dinitrophenyl derivative of amino-terminal residue
Phenylisothio-cyanate
(b)
$^-$OH
CF_3COOH
H^+
Anilinothiazolinone derivative of amino acid residue
Phenylthiohydantoin derivative of amino acid residue
Identify amino-terminal residue; purify and recycle remaining peptide fragment through Edman process.
PTC adduct
Shortened peptide


EDMAN DEGRADATION
1 2 3 4 5
Labeling
1 2 3 4 5
Release
1
2 3 4 5
First round
Labeling
2 3 4 5
Release
2
3 4 5
Second round
Phenyl isothiocyanate
Ala
Gly
Asp–Phe–Arg–Gly
Labeling
Release
Asp–Phe–Arg–Gly
PTH-alanine
Peptide shortened by one residue
Asp–Phe–Arg–Gly



Are You Getting It??



Compare dansyl chloride and phenylisothiocyanate.
Which properties do they share? *(multiple answers)*

a) Both react with the N – terminal amino acid.
b) Both need strong acid to release the modified amino acid.
c) Both form products containing the amino acid R – group.
d) Both produce a weakened peptide bond.
e) Both can be used to identify the second amino acid in a protein.



Are You Getting It??



Answer

Compare dansyl chloride and phenylisothiocyanate. Which properties do they share?

a) ***Both react with the N – terminal amino acid.***

b) **Both need strong acid to release the modified amino acid.**

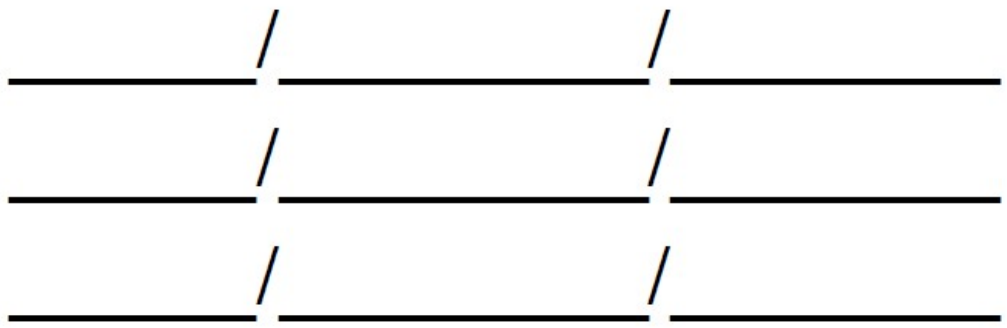
c) ***Both form products containing the amino acid R – group.***

d) **Both produce a weakened peptide bond.**

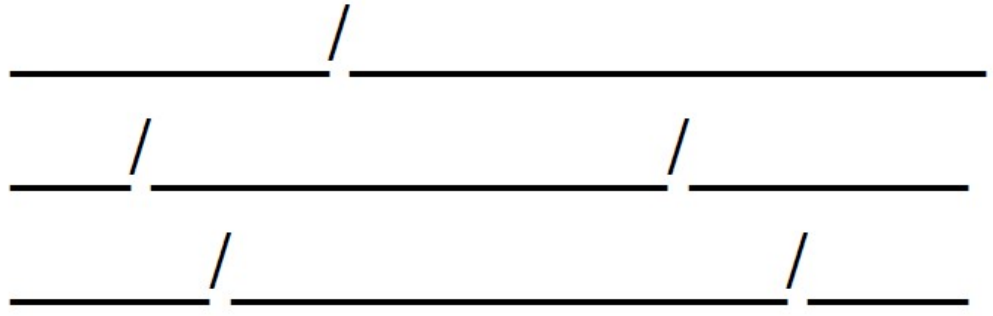
e) **Both can be used to identify the second amino acid in a protein.**

SPECIFIC VS. RANDOM CLEAVAGE

SPECIFIC CLEAVAGE:



RANDOM CLEAVAGE:



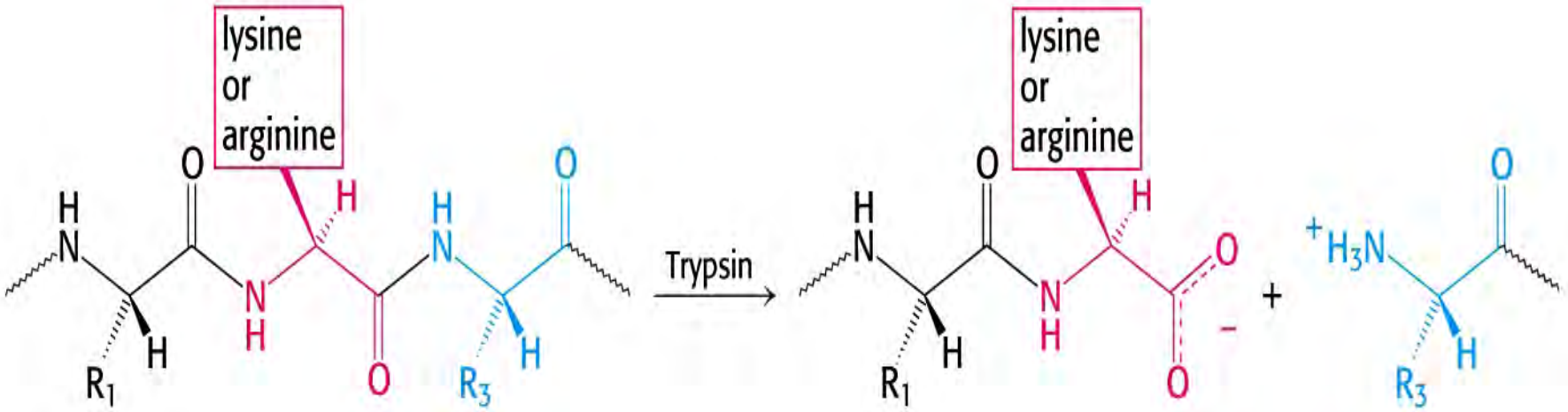

lysine
or
arginine
Trypsin
lysine
or
arginine

TABLE 3–7 The Specificity of Some Common Methods for Fragmenting Polypeptide Chains

*Reagent (biological source)**	*Cleavage points†*
Trypsin (bovine pancreas)	Lys, Arg (C)
Submaxillarus protease (mouse submaxillary gland)	Arg (C)
Chymotrypsin (bovine pancreas)	Phe, Trp, Tyr (C)
Staphylococcus aureus V8 protease (bacterium *S. aureus*)	Asp, Glu (C)
Asp-*N*-protease (bacterium *Pseudomonas fragi*)	Asp, Glu (N)
Pepsin (porcine stomach)	Phe, Trp, Tyr (N)
Endoproteinase Lys C (bacterium *Lysobacter enzymogenes*)	Lys (C)
Cyanogen bromide	Met (C)

*All reagents 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.

TABLE 4.3 Specific cleavage of polypeptides

Reagent	Cleavage site
Chemical cleavage	
Cyanogen bromide	Carboxyl side of methionine residues
O-Iodosobenzoate	Carboxyl side of tryptophan residues
Hydroxylamine	Asparagine–glycine bonds
2-Nitro-5-thiocyanobenzoate	Amino side of cysteine residues
Enzymatic cleavage	
Trypsin	Carboxyl side of lysine and arginine residues
Clostripain	Carboxyl side of arginine residues
Staphylococcal protease	Carboxyl side of aspartate and glutamate residues (glutamate only under certain conditions)
Thrombin	Carboxyl side of arginine
Chymotrypsin	Carboxyl side of tyrosine, tryptophan, phenylalanine, leucine, and methionine
Carboxypeptidase A	Amino side of C-terminal amino acid (not arginine, lysine, or proline)



Are You Getting It??



Which of the following reagents will cleave this peptide into two fragments?

Ser – Trp – Met – Trp – Leu – Thr – Ile– Gln – Ala

a) trypsin
b) chymotrypsin
c) cyanogen bromide
d) V8 protease



Are You Getting It??



Answer

Which of the following reagents will cleave this peptide into two fragments?

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c) *cyanogen bromide*
d) V8 protease



Are You Getting It??



Which of the following reagents will cleave this peptide into two fragments?

Gly – Met – Asp – Phe – Lys – Tyr – Met – Glu – Arg

a) trypsin
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c) cyanogen bromide
d) V8 protease



Are You Getting It??

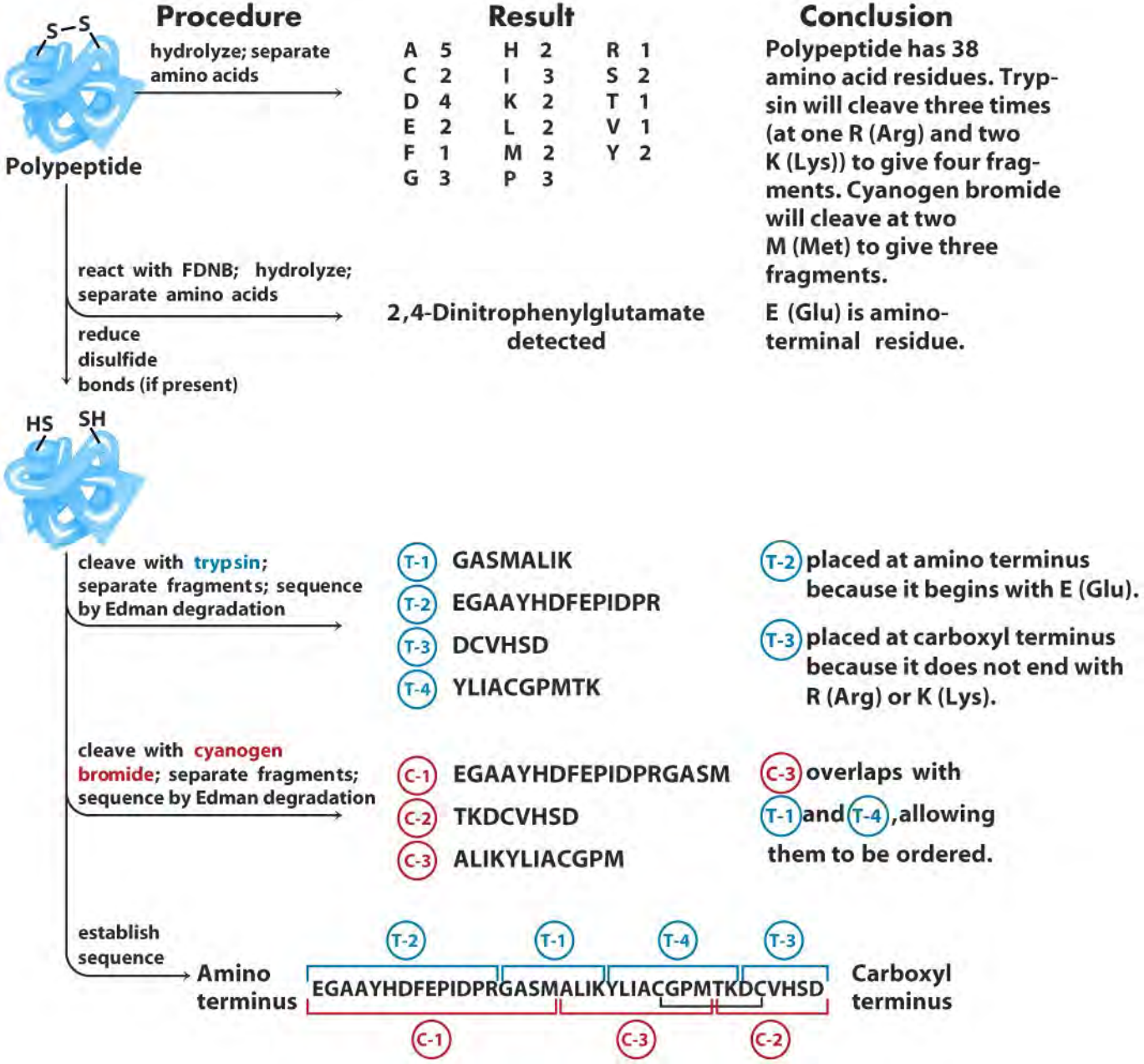


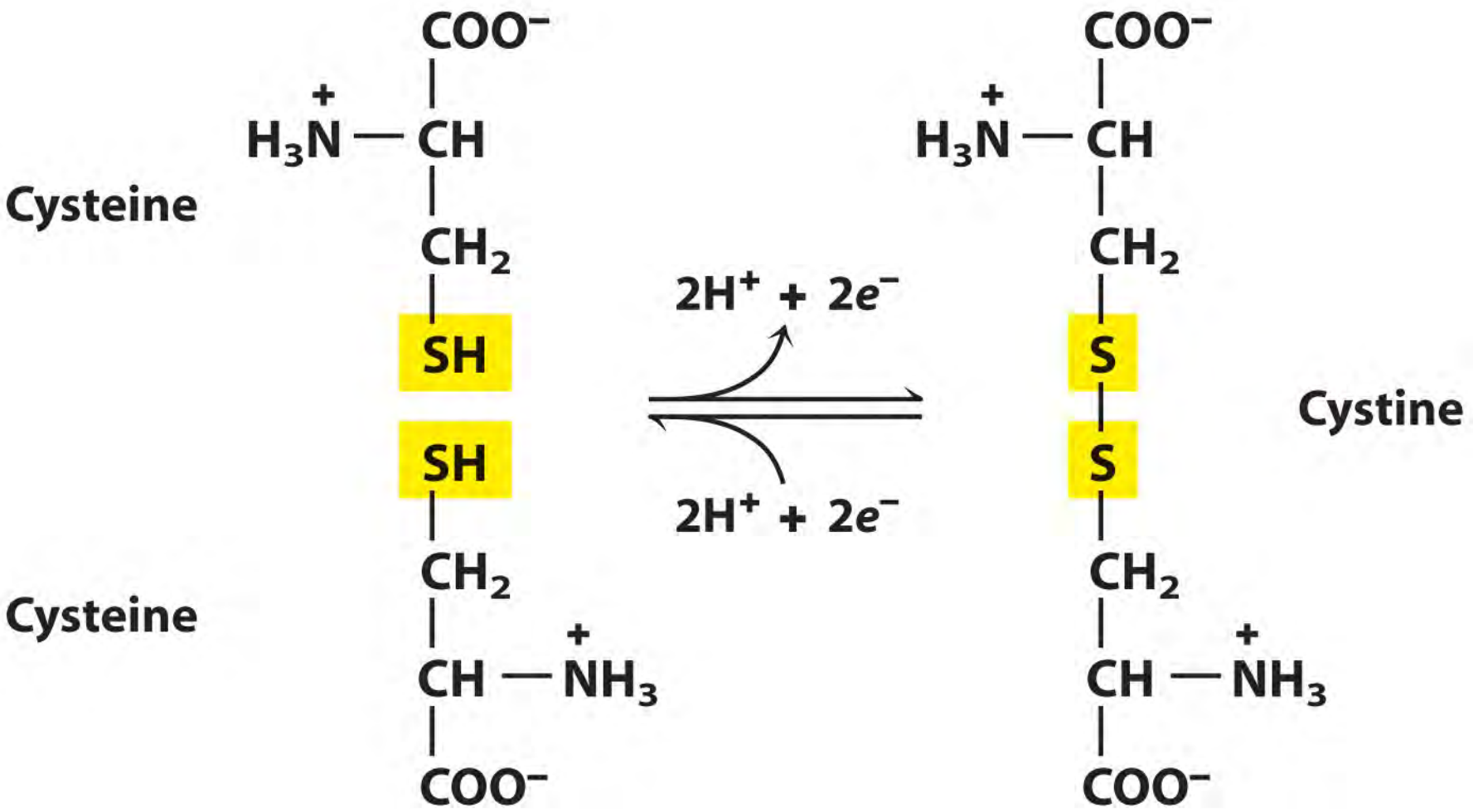
Answer

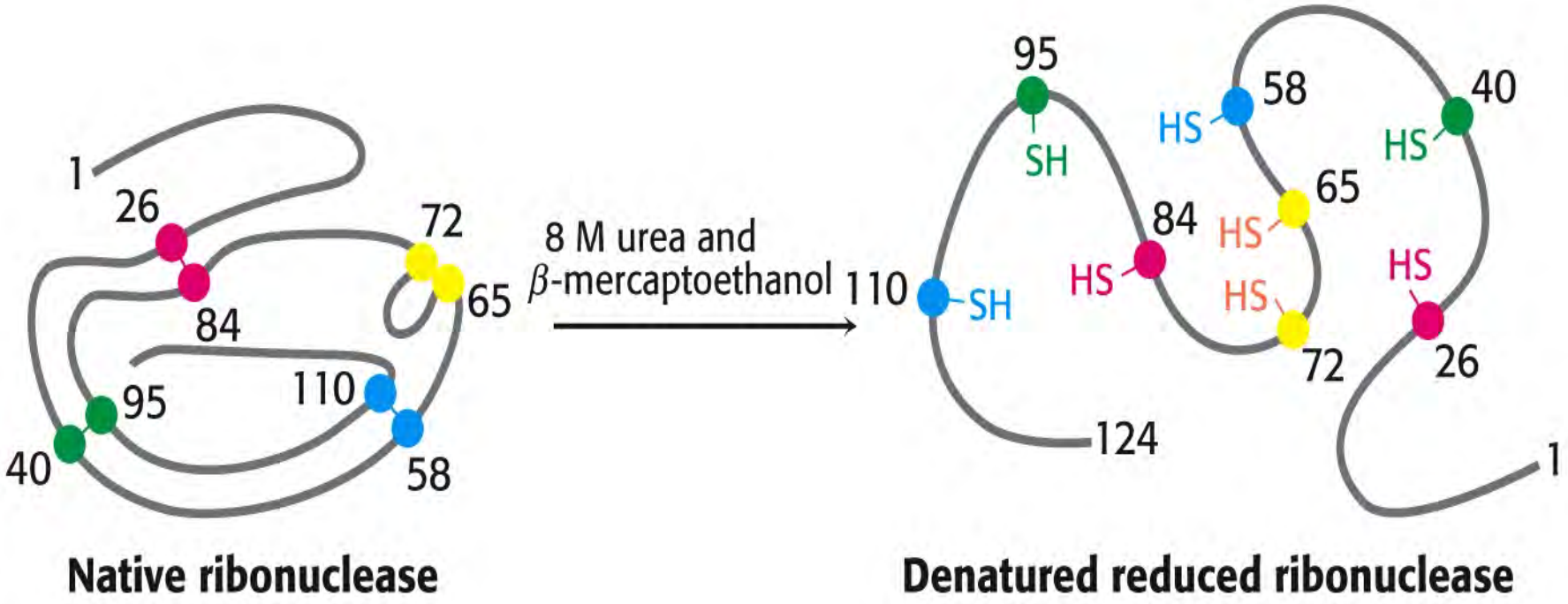
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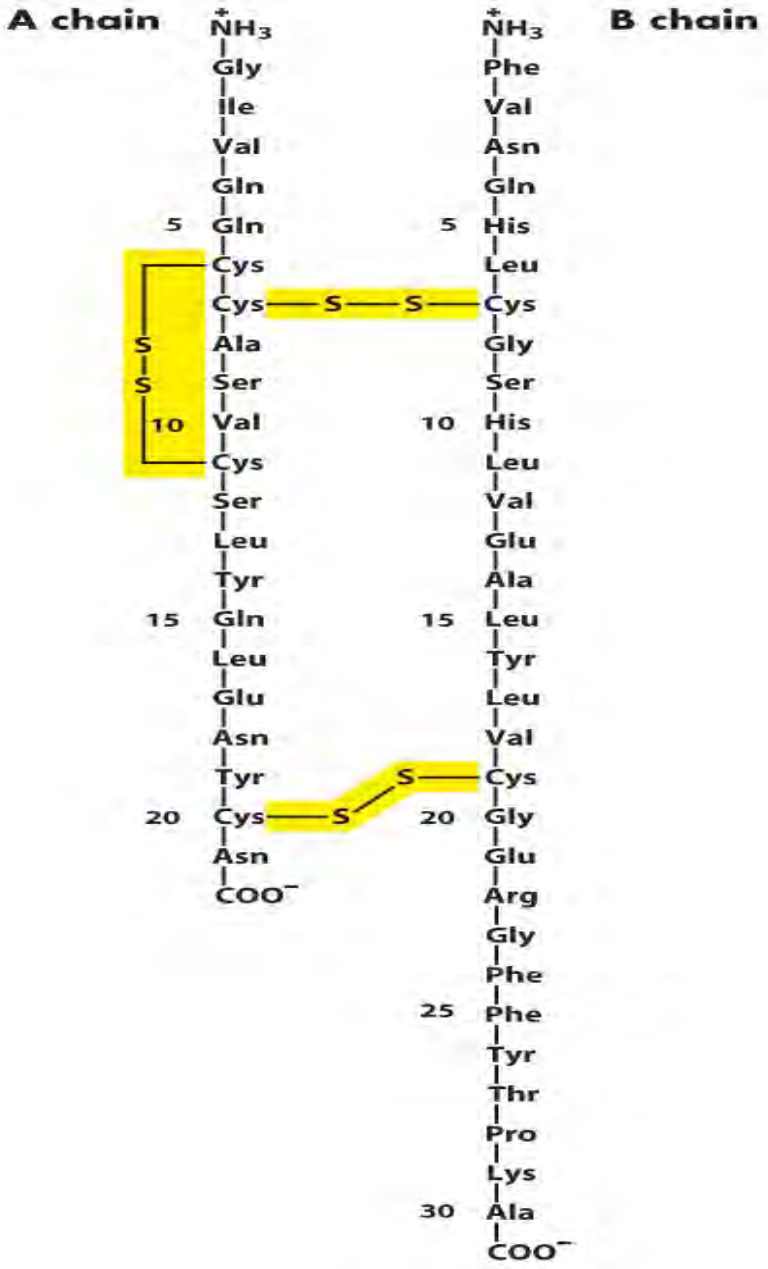
Gly – Met – Asp – Phe – Lys – Tyr – Met – Glu – Arg

a) ***trypsin***
b) **chymotrypsin**
c) **cyanogen bromide**
d) **V8 protease**


Procedure
Result
Conclusion
S–S
hydrolyze; separate amino acids
Polypeptide
A 5
C 2
D 4
E 2
F 1
G 3
H 2
I 3
K 2
L 2
M 2
P 3
R 1
S 2
T 1
V 1
Y 2
Polypeptide has 38 amino acid residues. Trypsin will cleave three times (at one R (Arg) and two K (Lys)) to give four fragments. Cyanogen bromide will cleave at two M (Met) to give three fragments.
react with FDNB; hydrolyze; separate amino acids
2,4-Dinitrophenylglutamate detected
E (Glu) is amino-terminal residue.
reduce disulfide bonds (if present)
HS SH
cleave with trypsin; separate fragments; sequence by Edman degradation
T-1 GASMALIK
T-2 EGAAYHDFEPIDPR
T-3 DCVHSD
T-4 YLIACGPMTK
T-2 placed at amino terminus because it begins with E (Glu).
T-3 placed at carboxyl terminus because it does not end with R (Arg) or K (Lys).
cleave with cyanogen bromide; separate fragments; sequence by Edman degradation
C-1 EGAAYHDFEPIDPRGASM
C-2 TKDCVHSD
C-3 ALIKYLIACGPM
C-3 overlaps with T-1 and T-4, allowing them to be ordered.
establish sequence
Amino terminus
T-2
T-1
T-4
T-3
EGAAYHDFEPIDPRGASMALIKYLIACGPMTKDCVHSD
C-1
C-3
C-2
Carboxyl terminus


Cysteine
Cysteine
Cystine
2H+ + 2e−
2H+ + 2e−


1
26
72
65
84
95
110
40
58
8 M urea and
β-mercaptoethanol
95
SH
58
HS
40
HS
65
84
HS
HS
110
SH
HS
HS
72
26
124
1
Native ribonuclease
Denatured reduced ribonuclease


A chain
B chain
NH3
Gly
Ile
Val
Gln
Gln
Cys
Cys
Ala
Ser
Val
Cys
Ser
Leu
Tyr
Gln
Leu
Glu
Asn
Tyr
Cys
Asn
COO−
NH3
Phe
Val
Asn
Gln
His
Leu
Cys
Gly
Ser
His
Leu
Val
Glu
Ala
Leu
Tyr
Leu
Val
Cys
Gly
Glu
Arg
Gly
Phe
Phe
Tyr
Thr
Pro
Lys
Ala
COO−
S
5
10
15
20
25
30



Are You Getting It??



Which is a property of this peptide?

Gln – Cys – Lys – Val – His – Cys – Phe – Gly – Asn

S – S

a) It contains two disulfide bonds.
b) Chymotrypsin will cleave it into two fragments.
c) Trypsin will cleave it into two fragments.
d) Mercaptoethanol will cleave it into two fragments.



Are You Getting It??



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