



BIOCHEMISTRY REVIEW

Overview of Biomolecules

Chapter 4

Protein Sequence

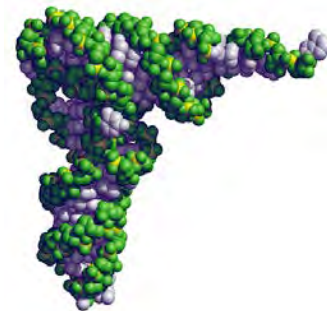
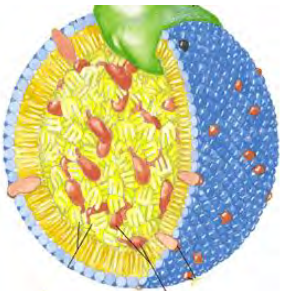
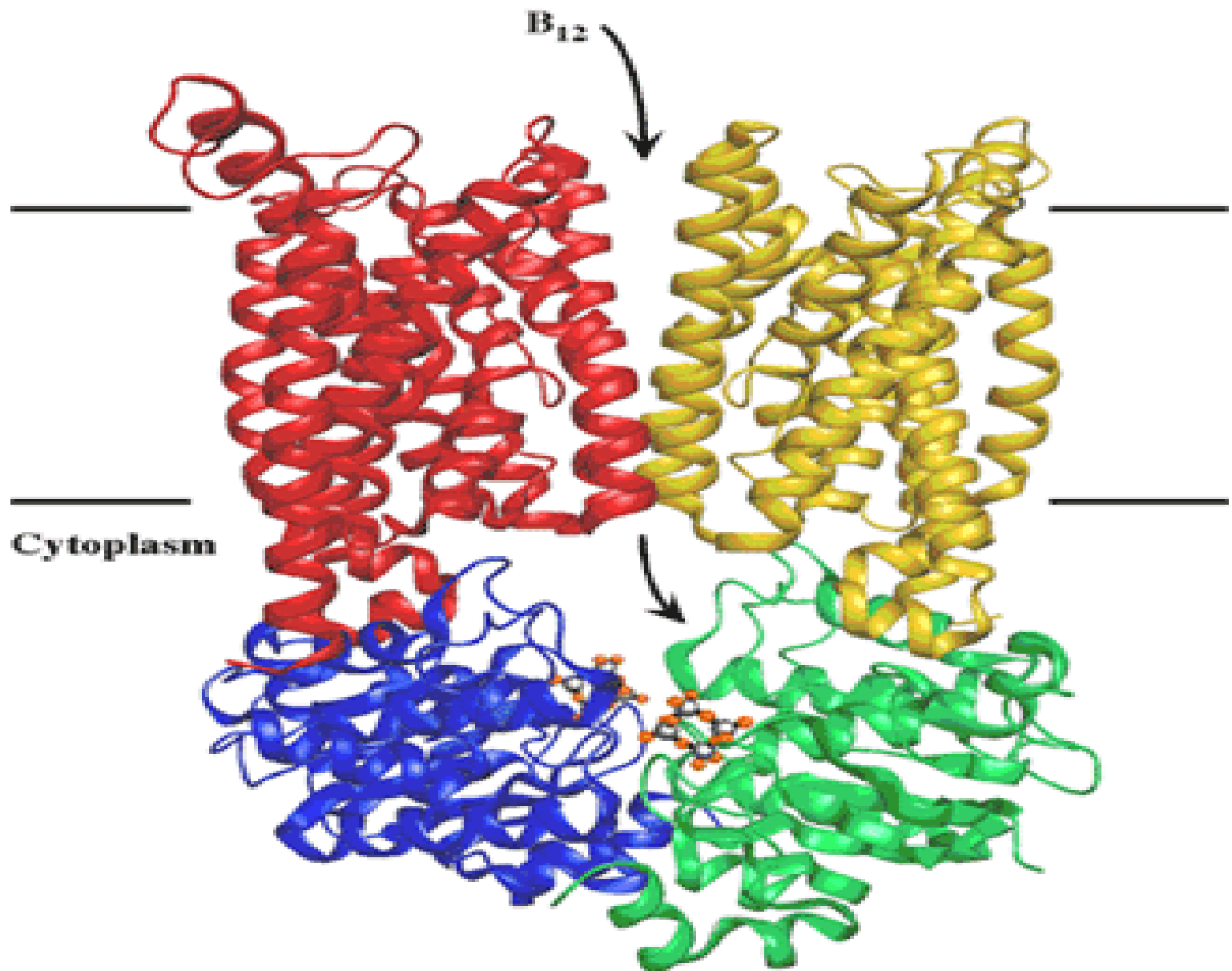


TABLE 3-2 Molecular Data on Some Proteins

	<i>Molecular weight</i>	<i>Number of residues</i>	<i>Number of polypeptide chains</i>
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 (<i>E. coli</i>)	450,000	4,158	5
Apolipoprotein B (human)	513,000	4,536	1
Glutamine synthetase (<i>E. coli</i>)	619,000	5,628	12
Titin (human)	2,993,000	26,926	1







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.



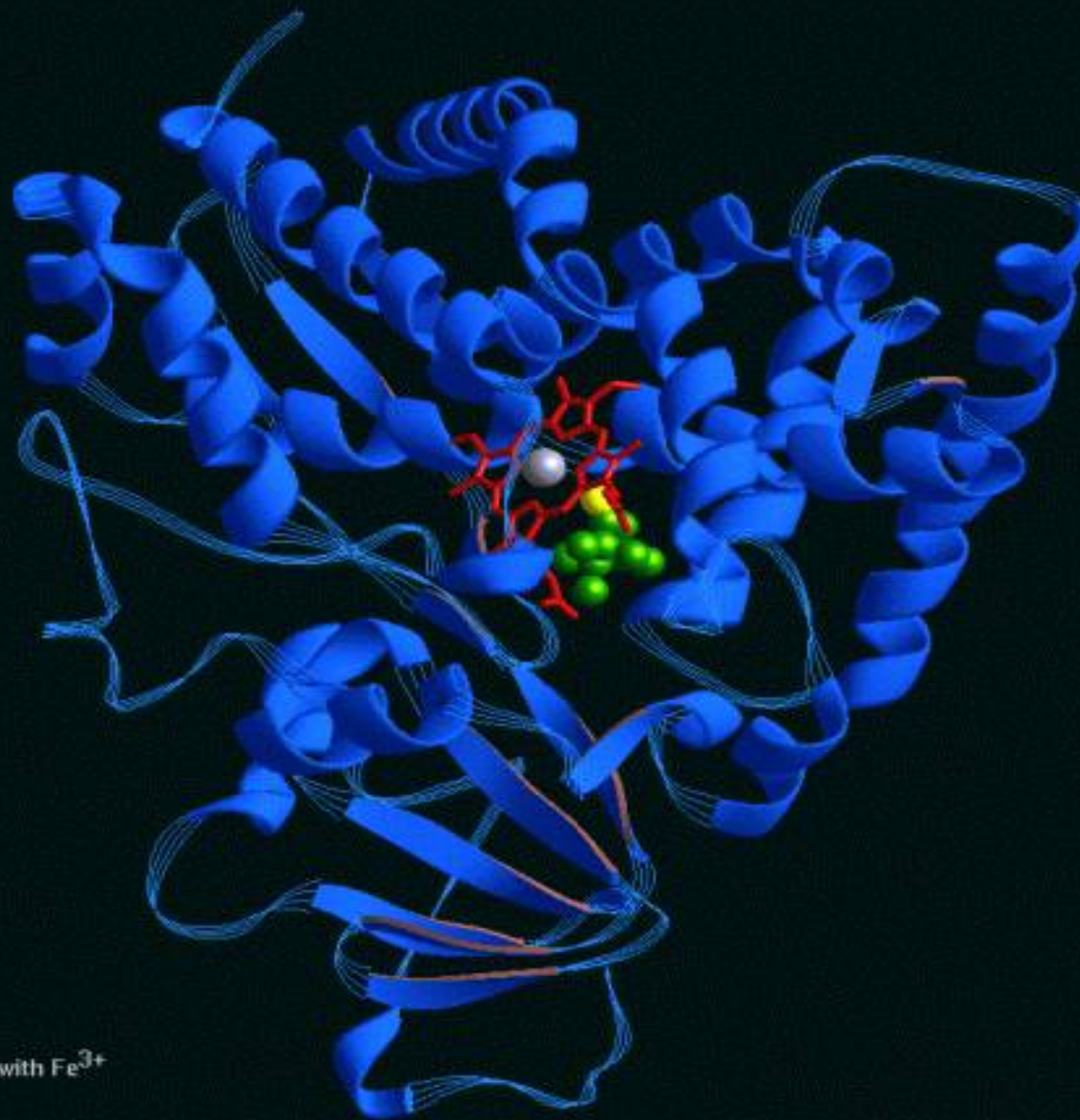
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Answer

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 Protoporphyrin IX with Fe³⁺
 Thiocamphor

TABLE 3-4 Conjugated Proteins

<i>Class</i>	<i>Prosthetic group</i>	<i>Example</i>
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

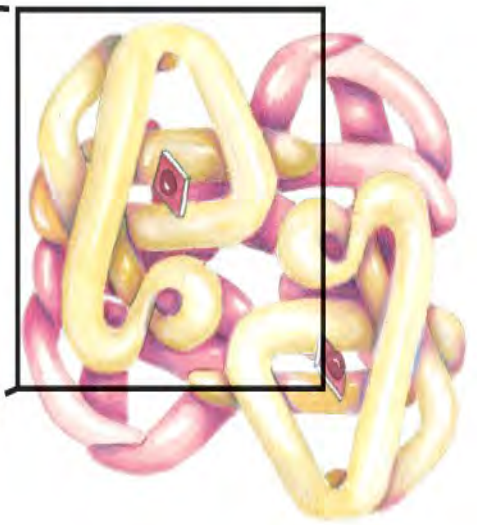
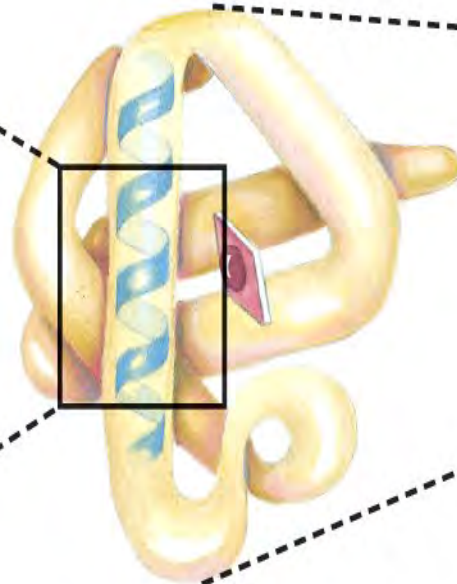
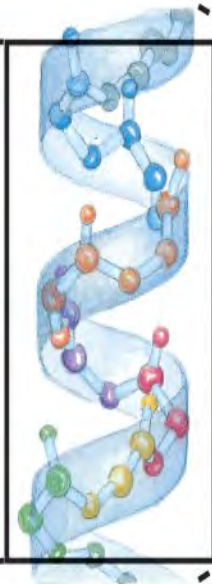
- **ENZYMES/CATALYSIS**
- **TRANSPORT**
- **STORAGE**
- **MOTILITY**
- **STRUCTURE**
- **DEFENSE**
- **REGULATION**

Primary structure

Secondary structure

Tertiary structure

Quaternary structure

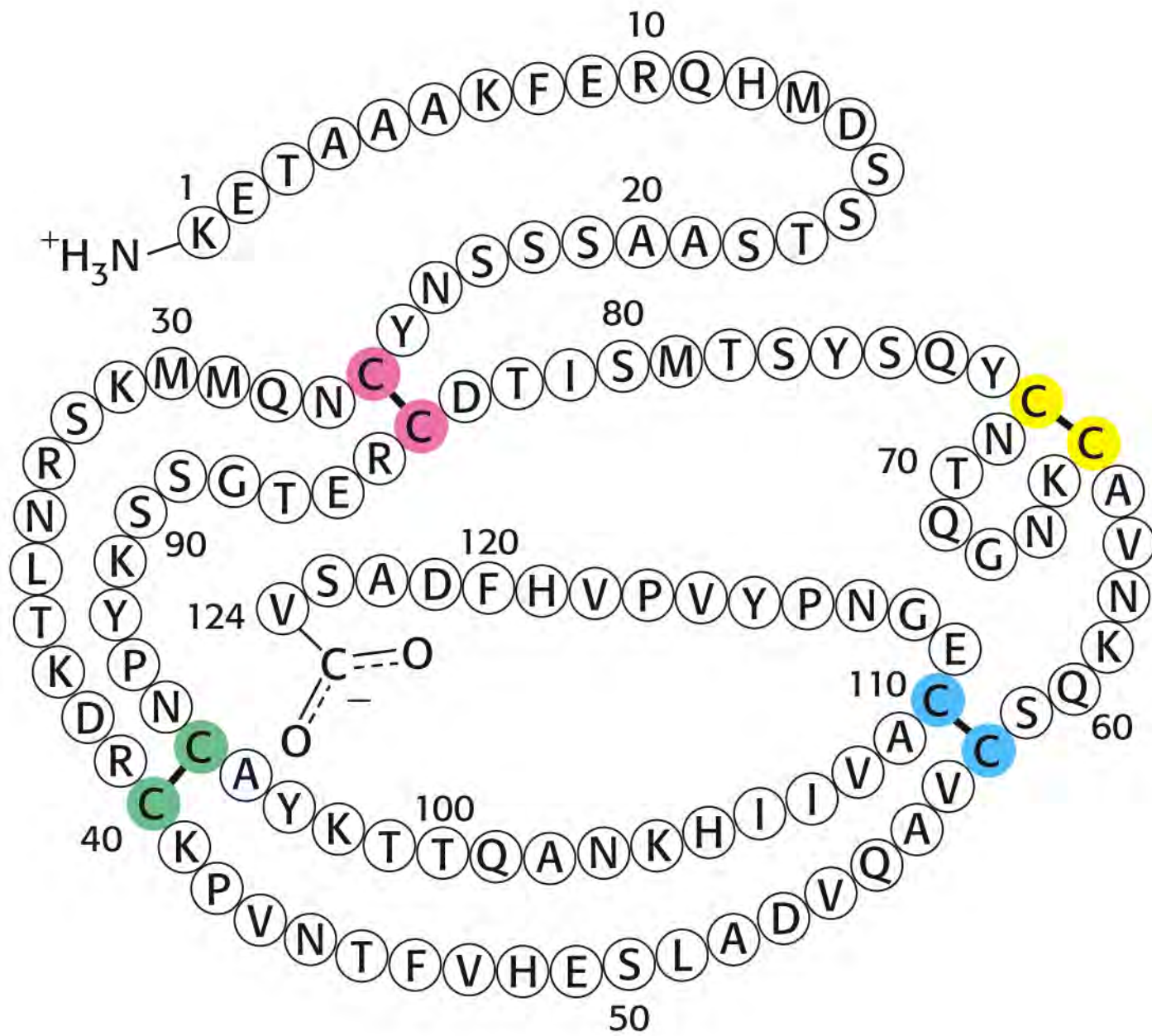


Amino acid residues

α Helix

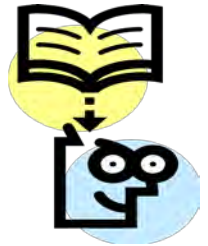
Polypeptide chain

Assembled subunits





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 ACDGMFPT HLS.



Are You Getting It??



Answer

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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?**

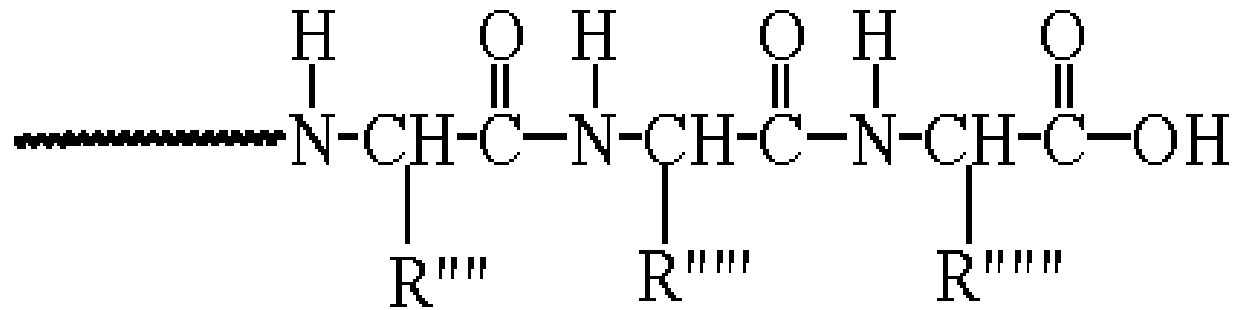
N-TERMINAL ANALYSIS

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

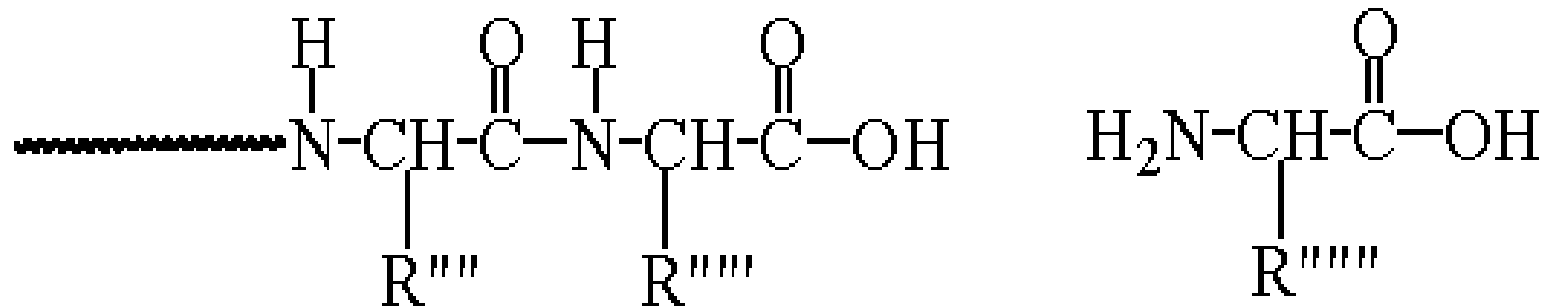
C-TERMINAL ANALYSIS

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

Carboxypeptidase A Method



Carboxypeptidase A





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



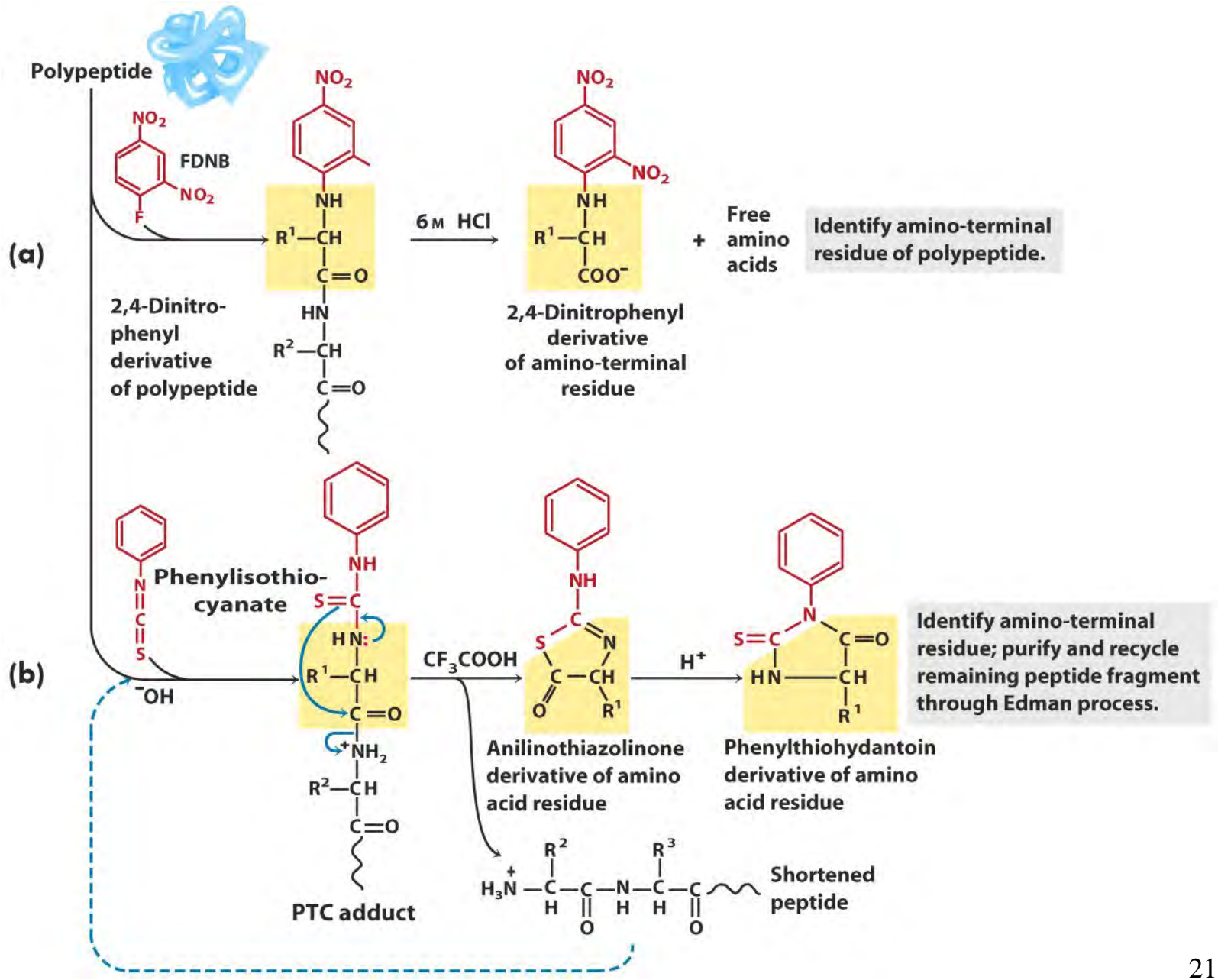
Are You Getting It??



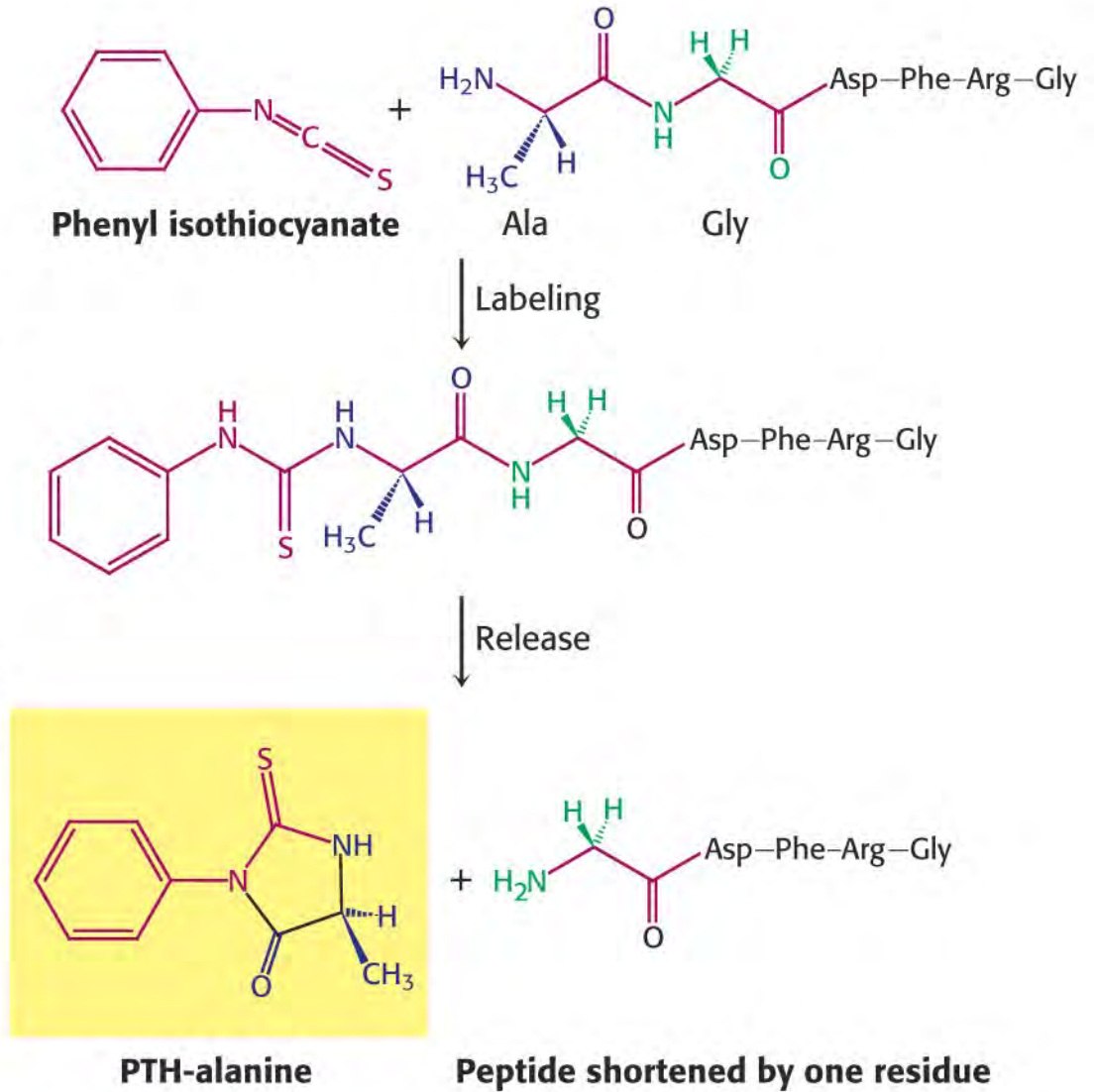
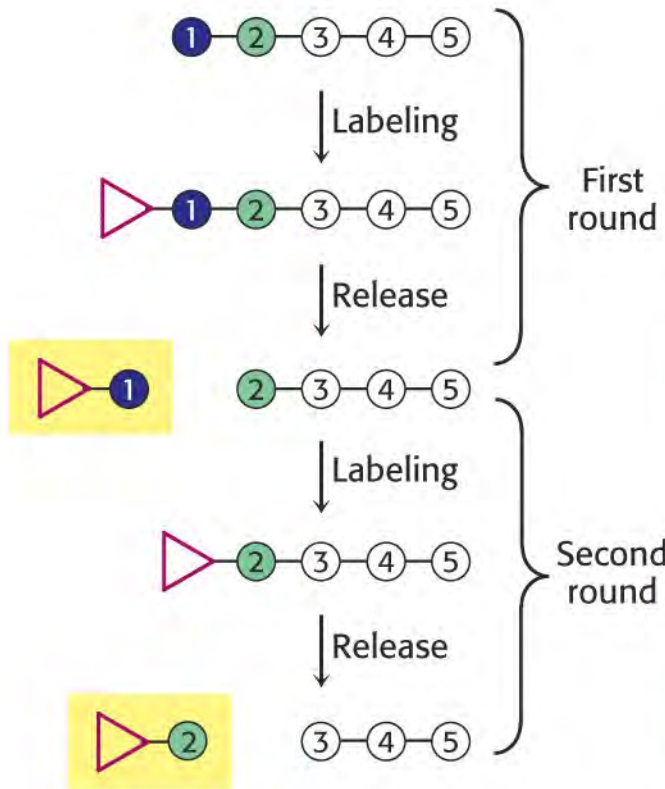
Answer

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



EDMAN DEGRADATION





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??



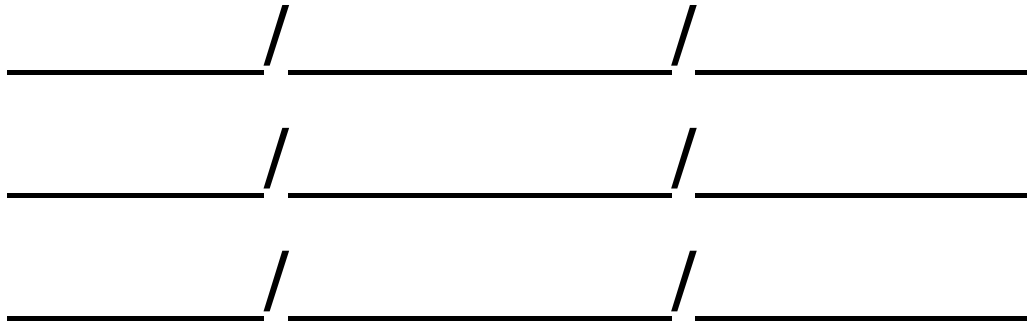
Answer

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

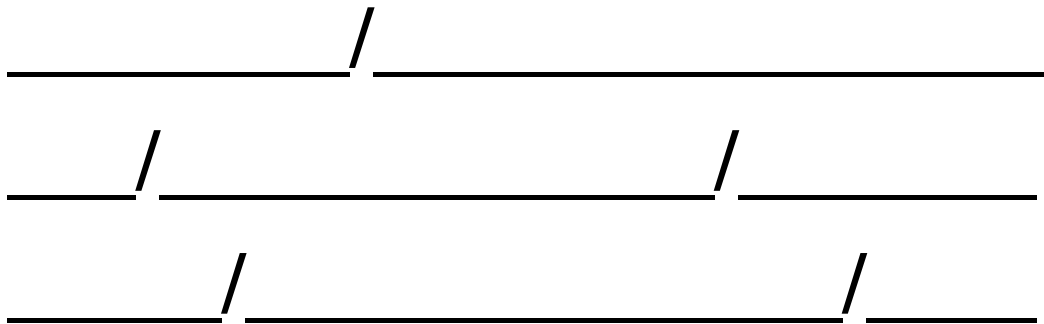
- a) Both react with the N – terminal amino acid.***
- b) Both need strong acid to release the modified amino acid.**
- c) Both form products containing the amino acid R – group.***
- d) Both produce a weakened peptide bond.**
- e) Both can be used to identify the second amino acid in a protein.**

SPECIFIC VS. RANDOM CLEAVAGE

SPECIFIC CLEAVAGE:



RANDOM CLEAVAGE:



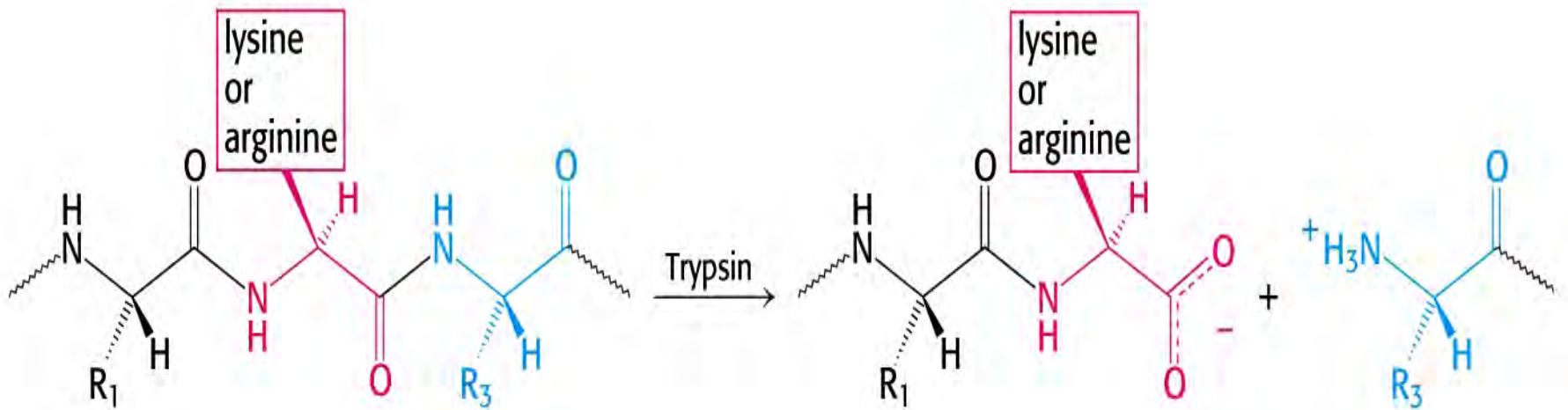


TABLE 3-7 The Specificity of Some Common Methods for Fragmenting Polypeptide Chains

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

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

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- c) cyanogen bromide**
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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
- b) chymotrypsin
- c) cyanogen bromide
- d) V8 protease



Are You Getting It??

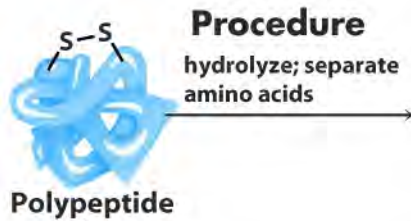


Answer

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

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

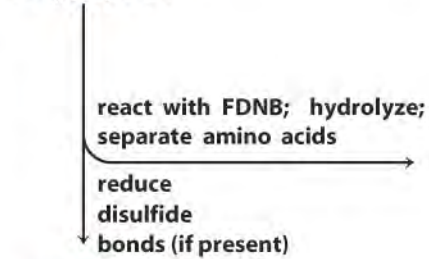
- a) **trypsin**
- b) chymotrypsin
- c) cyanogen bromide
- d) V8 protease



Result

A	5	H	2	R	1
C	2	I	3	S	2
D	4	K	2	T	1
E	2	L	2	V	1
F	1	M	2	Y	2
G	3	P	3		

Conclusion
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. E (Glu) is amino-terminal residue.

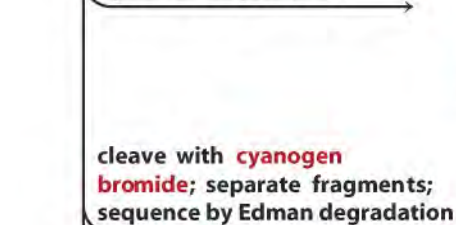


2,4-Dinitrophenylglutamate detected



- (T-1) GASMALIK
- (T-2) EGAAYHDFEPIDPR
- (T-3) DCVHSD
- (T-4) YLIACGPMTK

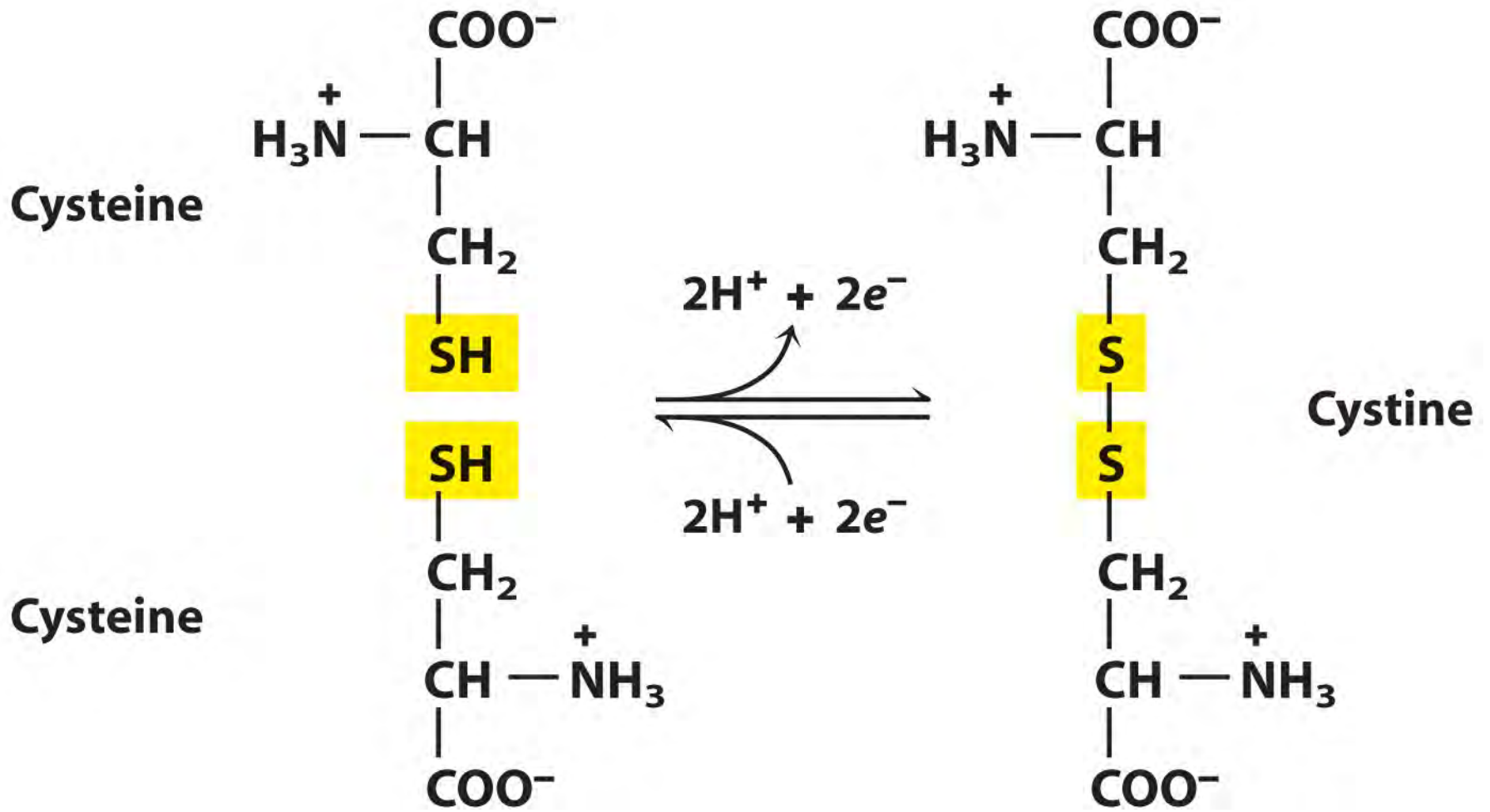
- (T-2) placed at amino terminus because it begins with E (Glu).
- (T-3) placed at carboxyl terminus because it does not end with R (Arg) or K (Lys).

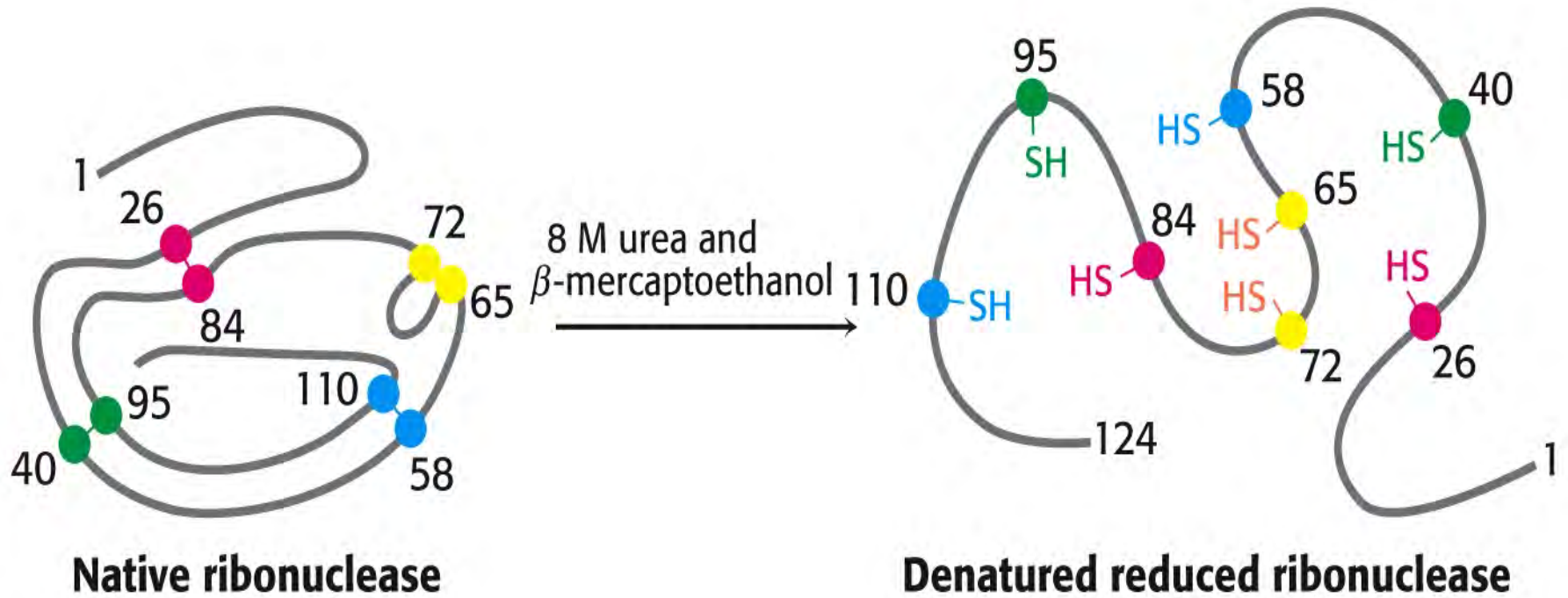


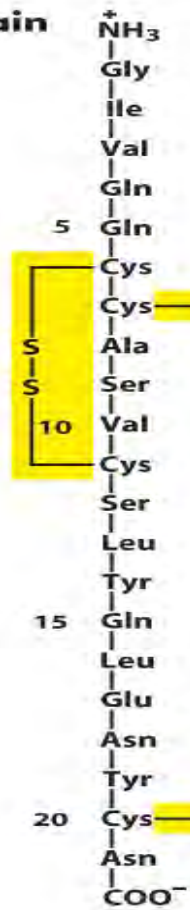
- (C-1) EGAAYHDFEPIDPRGASM
- (C-2) TKDCVHSD
- (C-3) ALIKYLIACGPM

- (C-3) overlaps with (T-1) and (T-4), allowing them to be ordered.







A chain**B chain**



Are You Getting It??



Which is a property of this peptide?

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



- 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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