VIVEKANANDA COLLEGE
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NAAC ACCREDITED 'A' GRADE

Topic:Protein primary structure sequencing and obtaining overlapping peptides

Course Title:Proteins and Enzymes

Paper:GE-2(CC2)

Unit:2

Semester:2

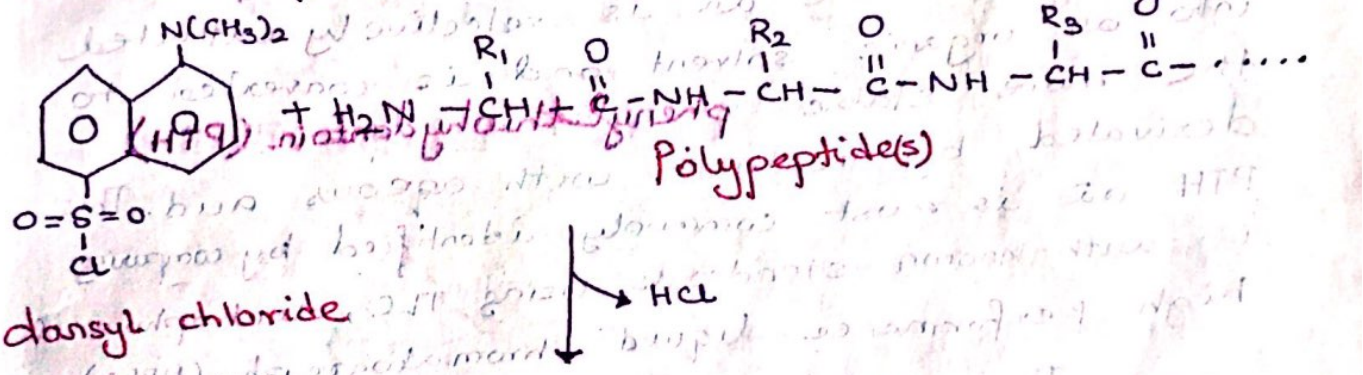
Name of the Teacher:Nineesha Sen Banerjee

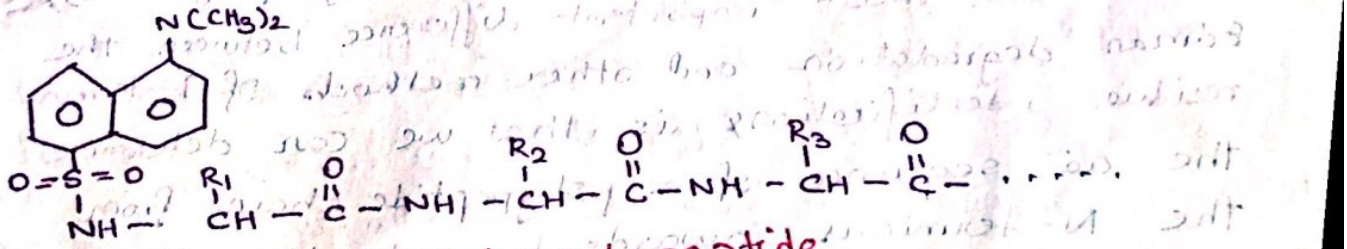
Name of the Department:Biochemistry

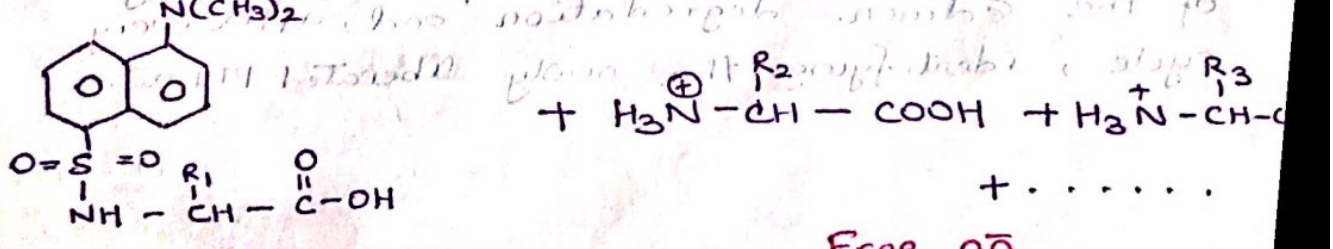
A) End Group Analysis

N-Terminus Identification

There are several effective methods by which a polypeptides N-terminal residue may be identified. 1-Dimethylamino-naphthalene-5-sulfonyl chloride (dansyl chloride) reacts with primary amines (including ε-amino group of lysine) to yield dansylated polypeptides. Acid hydrolysis liberates the N-terminal residue as a dansyl aā, which exhibits such intense yellow fluorescent that it can be chromatographically identified from as little as 100 picomoles (10^{-12} moles).

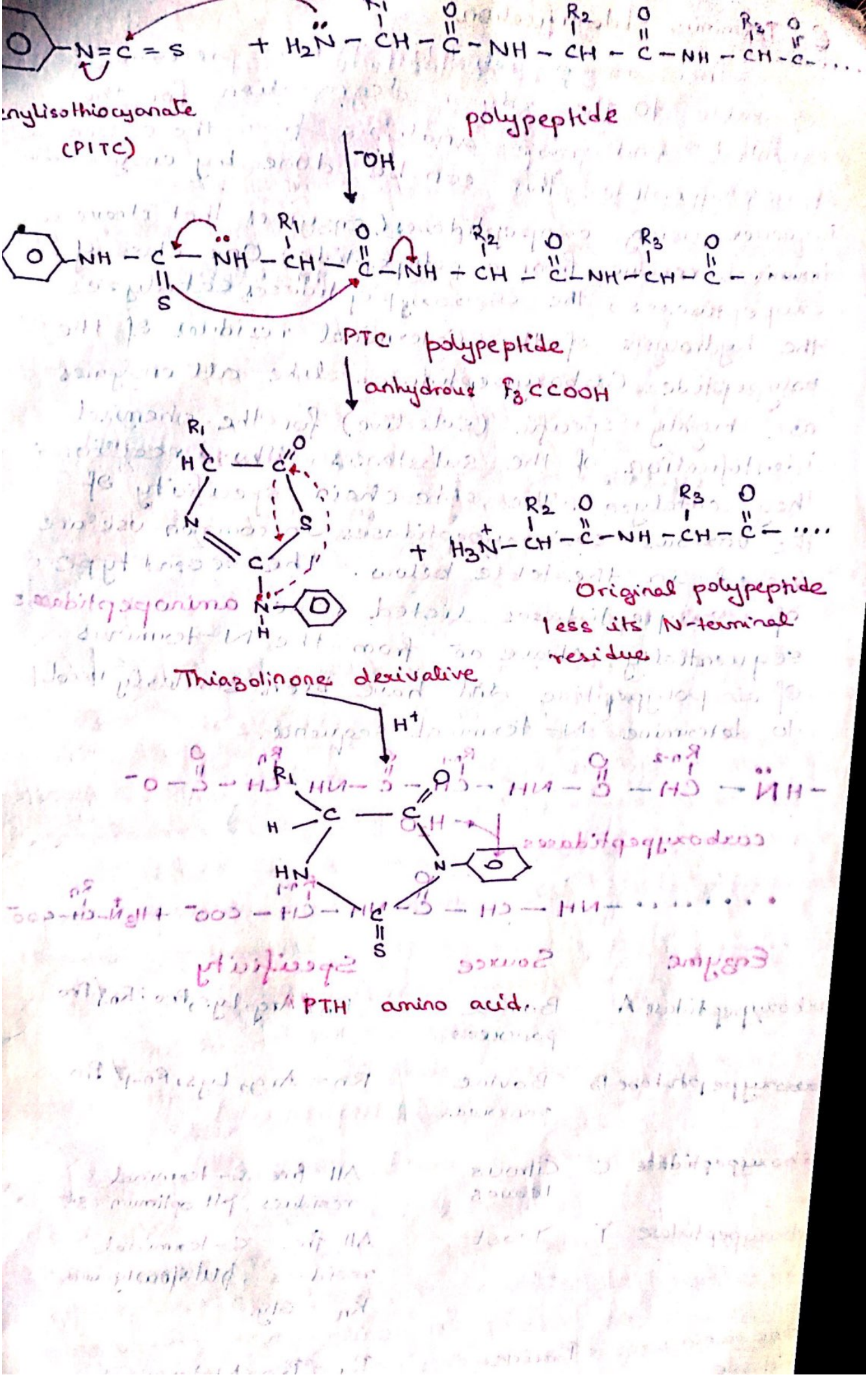






In the most useful method of N-terminal residue identification, the Edman's degradation (named after the inventor Pehr Edman), Phenylisothiocyanate (PITC, Edman's reagent reacts with the N-terminal amino group of protein under mildly alkaline solution to form their phenylthiocarbamyl (PTC) adducts. This product is treated with an anhydrous strong acid such as trifluoroacetic acid which cleaves the N-terminal residue as its thiazolinone derivative but does not hydrolyse other peptide bonds. The Edman degradation releases the N-terminal aa residue but leaves intact the rest of the polypeptide chain. The thiazolinone aa is selectively extracted into an organic solvent and is converted to the more stable phenylthiohydantoin (PTH) derivated by treatment with aqeous acid. This PTH aa is most commonly identified by comparing it with known standards using TLC, electrophoresis, high performance liquid chromatography (HPLC).

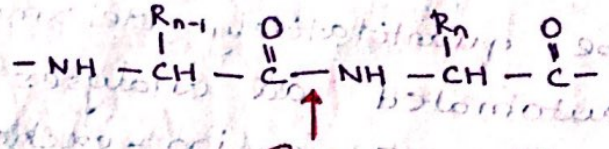
The most important difference between the Edman degradation and other methods of N-terminal residue identification is that we can determine the aa sequence of a polypeptide chain from the N-terminus inwards by subjecting the polypeptide chain (from the N-terminus inwards by subjecting the polypeptide) to repeated cycles of the Edman degradation and, after every cycle, identifying the newly liberated PTH aa.

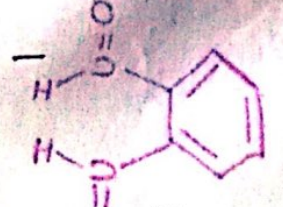


their characteristic elution volumes (retention times on HPLC) and quantitatively estimated from their fluorescence intensities (UV absorbances for PTC-aa). With modern aa analyzers, the complete analysis of a protein digest can be performed in <1h with a sensitivity that can detect as little as 1pmol of each aa.

E) Specific Peptide Cleavage Reactions

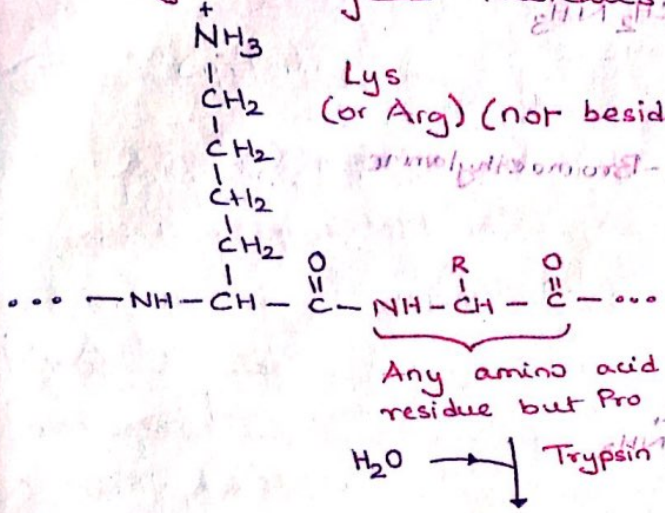
Polypeptides that are longer than 40–100 residue cannot be directly sequenced. Polypeptides of greater length must ∴ be cleaved, either enzymatically or chemically, to fragments small enough to be sequenced. In either case, the cleavage process must be complete and highly specific so that the aggregate sequence of a subunit's peptide fragments, when correctly ordered, is that of the intact subunit.



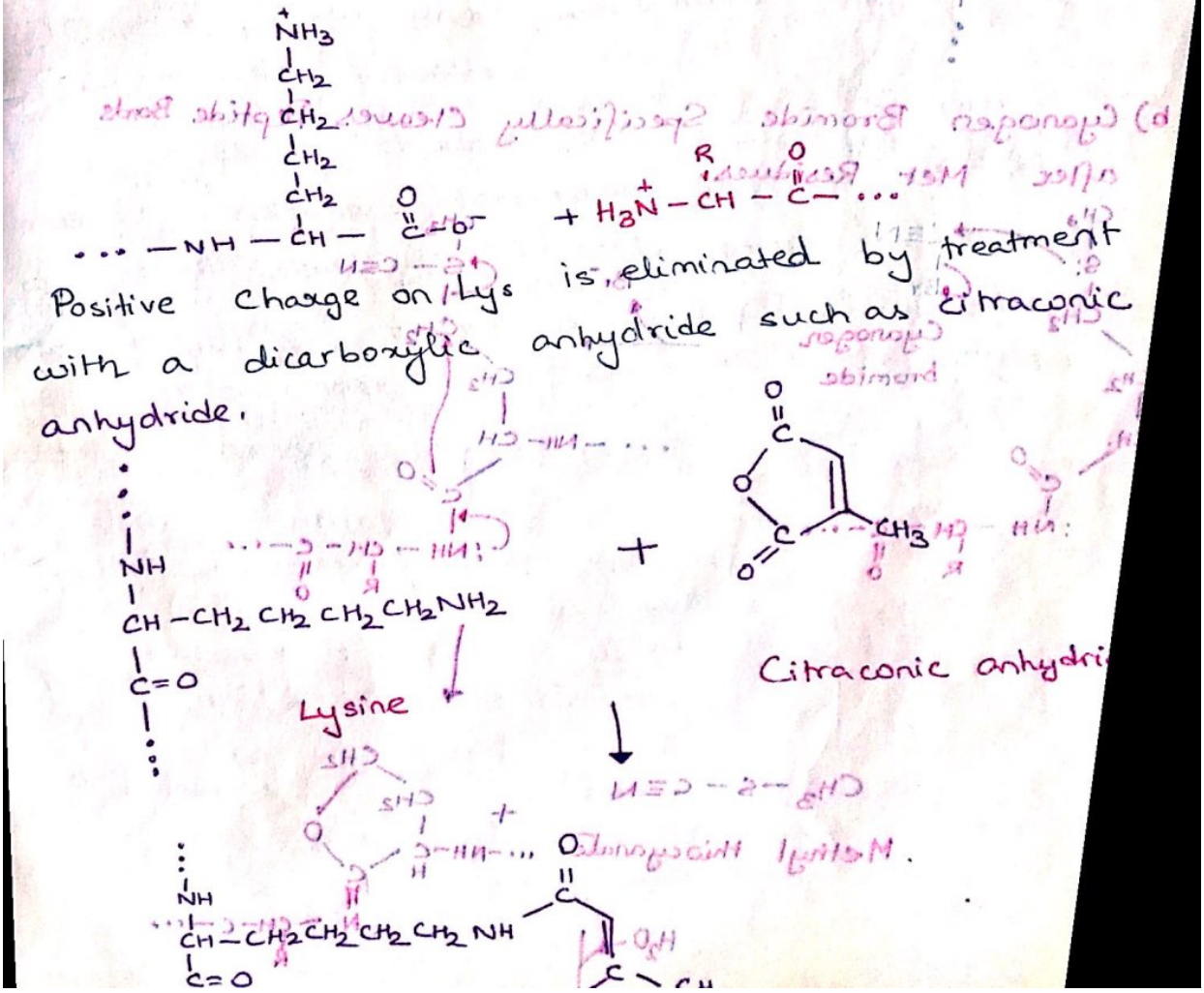
Enzyme	Source	Specificity	Comments
Trypsin	Bovine pancreas	R_{n-1} = +vely charged residues: Arg, Lys, $R_n \neq$ Pro	Highly specific
Chymotrypsin	Bovine pancreas	R_{n-1} = bulky hydrophobic residues: Phe, Trp, Tyr, $R_n \neq$ Pro	Cleaves more slowly for R_{n-1} = Asn, His, Met, Leu
Elastase	Bovine pancreas	R_{n-1} = Small neutral residues: Ala, Gly, Ser, Val; $R_n \neq$ Pro	
Thermolysin	Bacillus thermoproteolytieus	R_n = Ile, Met, Phe, Trp, Tyr, Val; $R_{n-1} \neq$ Pro	Occasionally cleaves at R_n = Ala, Asp, His, Thr, heat stable

Enzyme	Source	Specificity	Comments
Pepsin	Bovine gastric mucosa	R_n = Leu, Phe, Trp, Tyr; $R_{n-1} \neq$ Pro	Also others; quite nonspecific pH optimum = 2
Endopeptidase V8	Staphylococcus aureus	R_{n-1} = Glu	

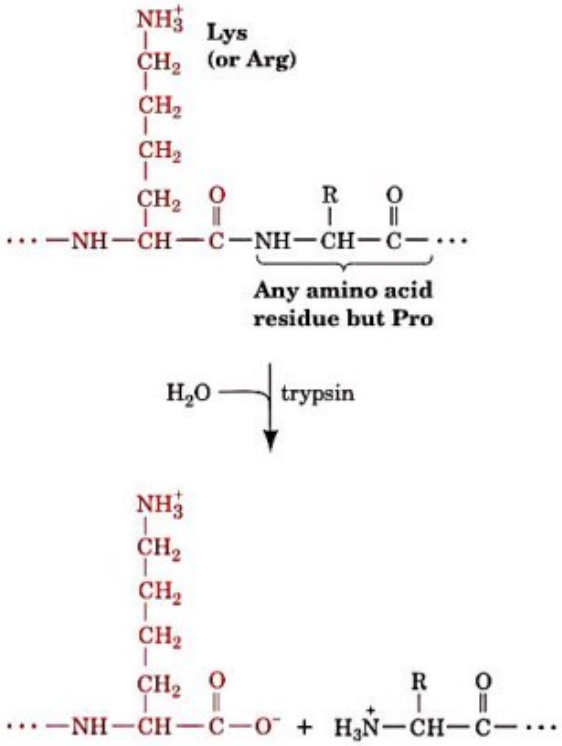
2) Trypsin Specifically Cleaves Peptide Bonds After +vely Charged Residues.



Positive charge on Lys is eliminated by treatment with a dicarboxylic anhydride such as citraconic anhydride.



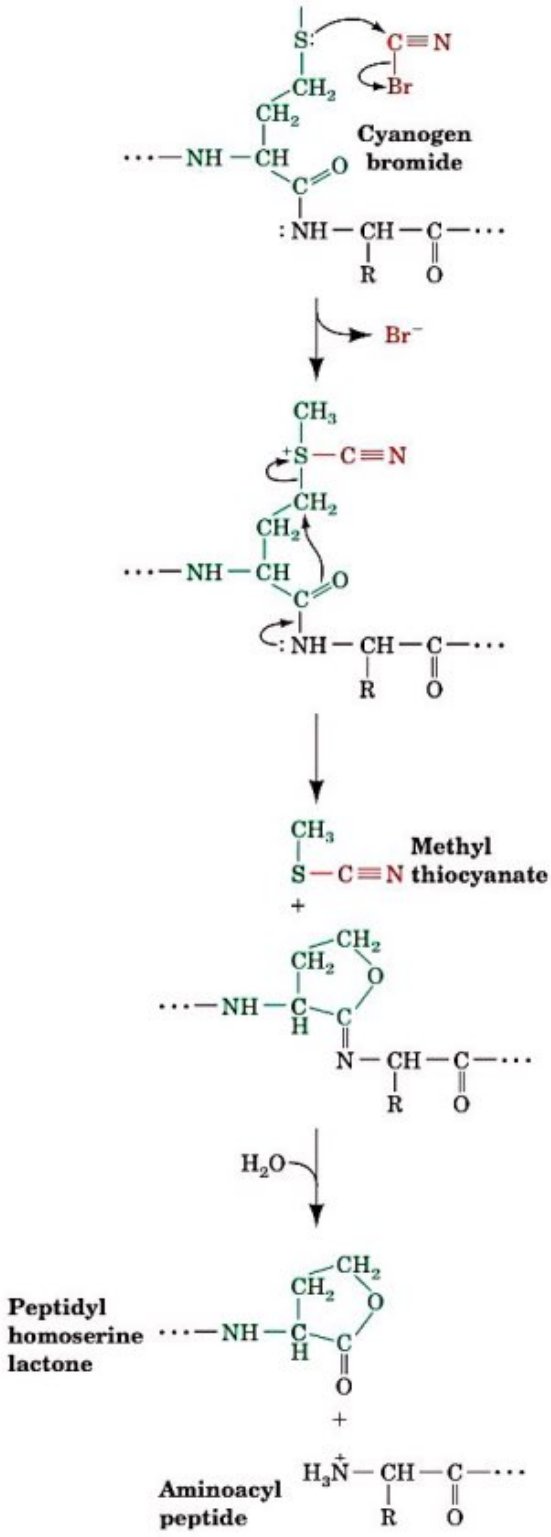
most valuable member of the arsenal of peptidases used to fragment polypeptides. It cleaves peptide bonds on the C-side (toward the carboxyl terminus) of the positively charged residues Arg and Lys if the next residue is not Pro:



The other endopeptidases listed in Table 7-2 exhibit broader side chain specificities than trypsin and often yield a series of peptide fragments with overlapping sequences. However, through **limited proteolysis,** that is, by adjusting reaction conditions and limiting reaction times, these less specific endopeptidases can yield useful peptide fragments. This is because the complex native structure of a protein (subunit) buries many otherwise enzymatically susceptible peptide bonds beneath the surface of the protein molecule. With proper conditions and reaction times, only those peptide bonds in the native protein that are initially accessible to the peptidase will be hydrolyzed. Limited proteolysis is often employed to generate peptide fragments of useful size from subunits that have too many or too few Arg and Lys residues to do so with trypsin (although if too many are present, limited proteolysis with trypsin may also yield useful fragments).

b. Cyanogen Bromide Specifically Cleaves Peptide Bonds after Met Residues

Several chemical reagents promote peptide bond cleavage at specific residues. The most useful of these, **cyanogen bromide** (CNBr), causes specific and quantitative cleavage on the C-side of Met residues to form a **peptidyl homoserine lactone:**



The reaction is performed in an acidic solvent (0.1*M* HCl or 70% formic acid) that denatures most proteins so that cleavage normally occurs at all Met residues.

Questions with Answers- Proteins & Enzymes

A. A peptide with 12 amino acids has the following amino acid composition:
2 Met, 1 Tyr, 1 Trp, 2 Glu, 1 Lys, 1 Arg, 1 Thr, 1 Asn, 1 Ile, 1 Cys

Reaction of the intact peptide with fluorodinitrobenzene followed by acid hydrolysis creates a derivative of Ile.
A specific cleavage of the intact peptide produces fragments with the following sequences:

Glu-Cys-Asn-Met-Lys
Met-Glu-Thr-Arg-Trp
Ile-Tyr (Questions 1-5)

1._____ Which reagent was used for the specific cleavage?
a) chymotrypsin
b) trypsin
c) V8 protease
d) cyanogen bromide

2._____ Which amino acids would be released when the intact peptide was treated first with V8 protease followed by treatment with cyanogen bromide?
a) Glu and Met
b) Glu and Lys
c) Met and Lys
d) Glu, Met, and Lys

3._____ Which treatment would result in the release of Lys and Arg from the intact peptide?
a) trypsin
b) trypsin followed by dansyl chloride
c) trypsin followed by carboxypeptidase
d) trypsin followed by mild acid

4._____ If this intact peptide is sequenced using the Edman degradation, which step will be part of the procedure?
a) The Edman reagent will react with all 12 amino acids simultaneously.
b) Lithium borohydride will react with an α-carboxyl group.
c) Phenylisothiocyanate will react with an α-amino group.
d) Strong acid will be used to cleave off one modified amino acid.

5._____ If this peptide is normally part of a multimeric protein composed of four identical subunits, what procedure might be needed prior to performing the Edman degradation?
a) The four subunits should be separated and sequenced individually.
b) Two specific cleavages should be done to create two sets of fragments.
c) Peptide bonds should be broken using hydrazine.
d) Disulfide bonds should be reduced with mercaptoethanol.

B. A peptide has the following amino acid composition:
2 Met, 2 Phe, 2 Glu, 1 Arg, 1 Lys, 1 Val, 1 Leu, 1 Gly, 1 Ser

Reaction of the intact peptide with dansyl chloride followed by acid hydrolysis creates a derivative of Met.

A specific cleavage of the intact peptide produces fragments with the following sequences:

Fragment A: Glu-Gly-Lys-Phe
Fragment B: Met-Ser-Leu-Arg
Fragment C: Met-Val-Glu-Phe (Questions 6-10)

6._____ Which reagent was used for the specific cleavage?
a) cyanogen bromide
b) V8 protease
c) chymotrypsin
d) trypsin

7._____ Which reagent would break only one peptide bond in the intact peptide?
a) cyanogen bromide
b) V8 protease
c) chymotrypsin
d) trypsin

8._____ Which amino acid would be released if the intact peptide was treated with a combination of trypsin and chymotrypsin?
a) Lys
b) Phe
c) Glu
d) Met

9._____ What information do these result give about the sequence of the peptide?
a) The sequence is: Met-Val-Glu-Phe-Glu-Gly-Lys-Phe-Met-Ser-Leu-Arg
b) The sequence is: Met-Ser-Leu-Arg-Met-Val-Glu-Phe-Glu-Gly-Lys-Phe
c) The sequence is: Met-Val-Glu-Phe-Met-Ser-Leu-Arg-Glu-Gly-Lys-Phe
d) The sequence is: Met-Ser-Leu-Arg-Glu-Gly-Lys-Phe-Met Val-Glu-Phe

10._____ This peptide is one polypeptide chain of a multimeric protein that contains two non-identical subunits. What problem might be seen when analyzing the primary structure of the protein if the subunits were not separated?
a) Fluorodinitrobenzene might react with two different amino acids.
b) Carboxypeptidase might not react with the C-terminals.
c) Mercaptoethanol might not reduce disulfide bonds.
d) Lithium borohydride might cleave peptide bonds randomly.

C. Protein A is an α-keratin while Protein B is a transport protein. (Questions 11-15)

11._____ Which characteristic could be shared by Protein A and Protein B?

a) Both could be fibrous proteins containing multiple polypeptide chains.
b) Both could be globular proteins with similar primary structures.
c) Both could contain disulfide bridges linking methionine residues.
d) Both could contain hydrogen bonds between peptide bond atoms.

12._____ When comparing Protein A to a β-keratin

a) the α-keratin has a parallel structure while the β-keratin has an antiparallel structure.
b) the α-keratin has a compact structure while the β-keratin has a more extended structure.
c) both have similar secondary structures that are low-energy states for the proteins.
d) both contain hydroxyproline which functions as a prosthetic group.

13._____ When comparing Protein B to collagen,

a) both are stabilized by van der Waals interactions.
b) both are stabilized by extensive regions of left-handed coils.
c) both contain regions of random secondary structures.
d) both contain α-helices as well as β-pleated sheets.

14._____ Which interaction is likely to occur in Protein B?

a) A hydrophobic interaction could form between the R-groups of Val and Leu.
b) A hydrogen bond could form between the R-groups of Ser and Phe.
c) A salt bridge could form between the R-groups of Arg and His.
d) An ionic bond could form between the R-groups of Gln and Trp.

15._____ When comparing Protein A to Protein B,

a) both could be denatured by using heat to break amide bonds.
b) both could have primary structures that form with the help of chaperones.
c) both have conformations stabilized by numerous non-covalent bonds.
d) both contain the same proportions of hydrophilic and hydrophobic R-groups.