Genomics and Proteomics
(BIOT 3014)
Unit 4:
PART-I
PROTEIN SEQUENCING:EDMAN DEGRADATION METHOD

Dr. Satarudra Prakash Singh
Department of Biotechnology
Mahatma Gandhi Central University, Motihari

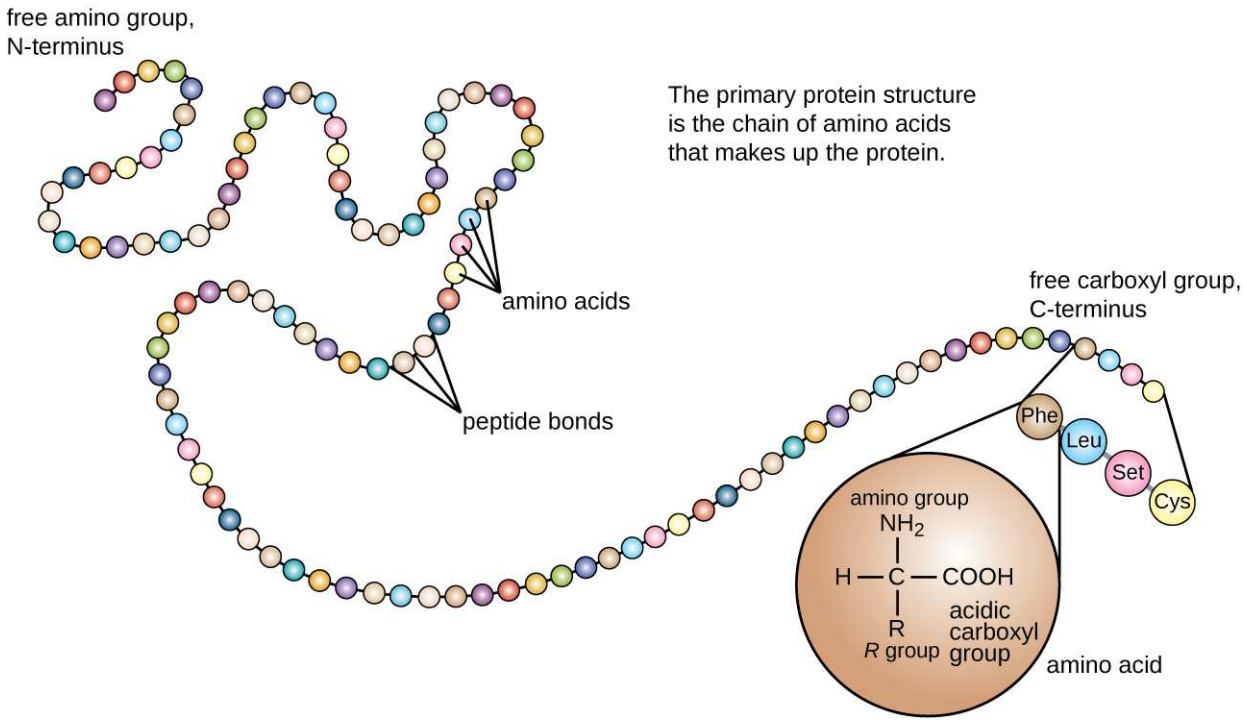
Protein sequencing

- Proteins in mixtures are first separated by 1D or 2D gel electrophoresis and then blotted onto a Polyvinylidene difluoride (PVDF) membrane.

- Further, the proteins of interest detected by staining (e.g., Coomassie blue) is cut out from PVDF membrane and loaded into the Edman sequencer developed by Pehr Edman.

- It can label and cleave the peptide from N-terminus without disrupting the peptide bonds formed between other amino acid residues of a protein.

PROTEIN SEQUENCING: EDMAN DEGRADATION METHOD



CYCLIC EDMAN DEGRADATION

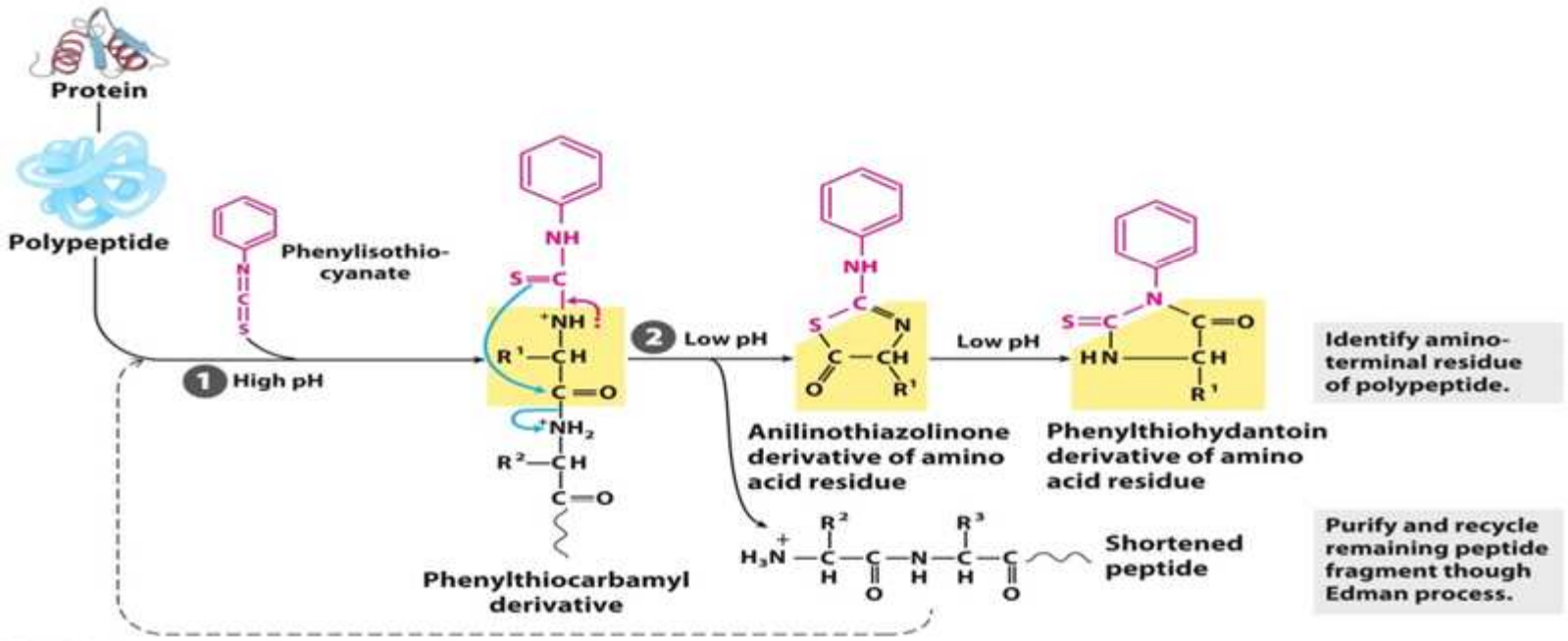


Figure 3-27
Lehninger Principles of Biochemistry, Sixth Edition

CYCLIC EDMAN DEGRADATION

- In cyclic degradation of peptides, a reaction of phenylisothiocyanate (PITC) with the free amino group of the N-terminal residue is performed where one amino acid is removed at a time and identified as a phenylthiohydantoin derivative (PTH-amino acid).

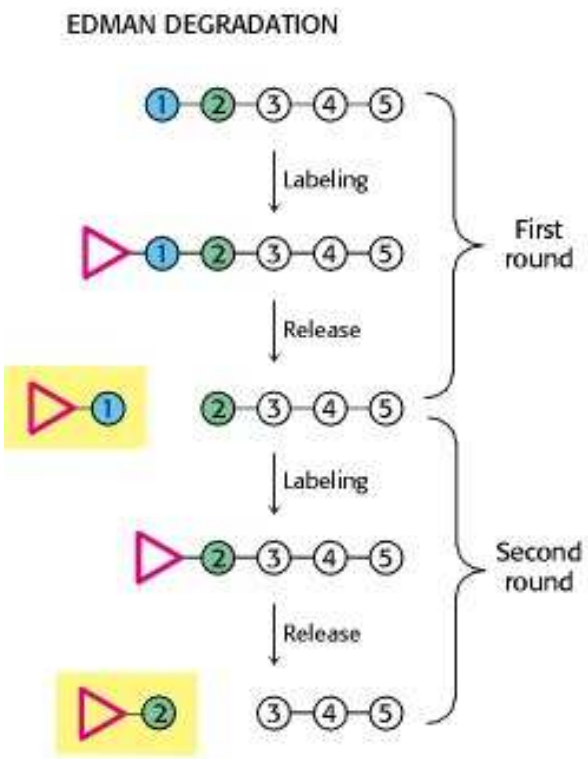
- Initially, the reaction is performed under mildly alkaline conditions to give a phenylthiocarbamoyl derivative (PTC-peptide).

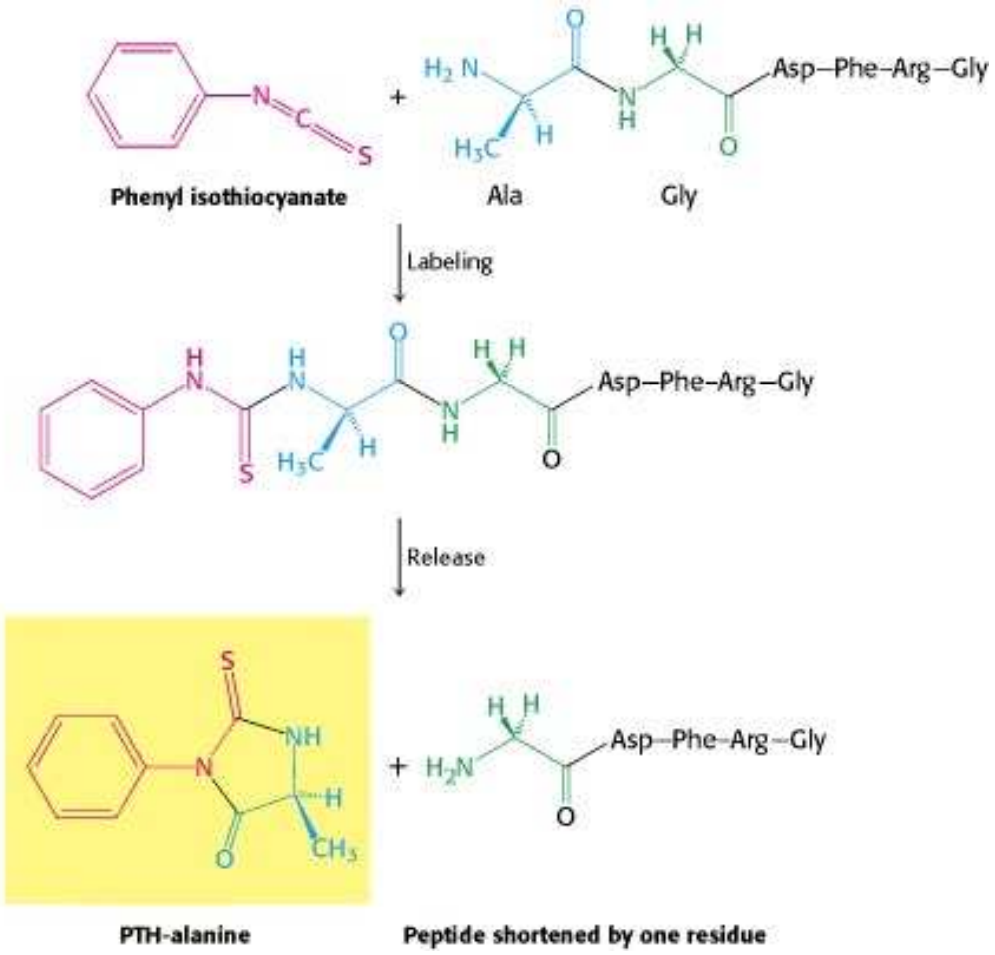
- Then, under acidic conditions, the thiocarbonyl sulfur of the derivative attacks the carbonyl carbon of the N-terminal amino acid.

- The first amino acid is cleaved as anilinothiazolinone derivative (ATZ-amino acid) and the remaining part of the peptide can be isolated and subjected to the degradation of next cycle.

- The thiazolone derivative is more stable than phenylthiocarbamyl derivative.

CYCLIC EDMAN DEGRADATION





CYCLIC EDMAN DEGRADATION

- The ATZ amino acid is then removed by extraction with ethyl acerate solvent (ester of ethanol and acetic acid) and converted to a PTH-amino acid where chromatography technique can be used to identify the PTH residue generated by each cycle.

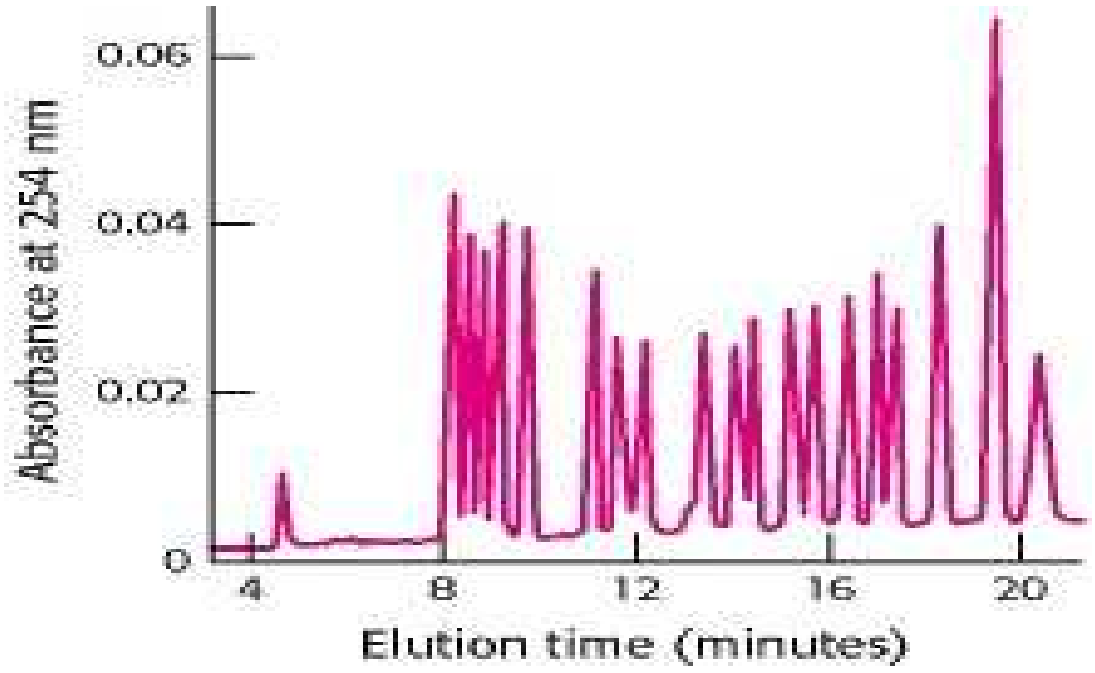
- In case, the released amino acids are identical with respect to molecular weight (for example, isoleucine and leucine have a molecular mass of 113 Da) they can be identified by different retention time.

- One cycle of the Edman degradation (cleavage of an amino acid from a peptide + identification) is carried out in less than 1 hour.

- By repeated degradations, the amino acid sequence of about 50 residues in a protein can be identified.

Separation of PTH-Amino Acids

In HPLC profile, a mixture of PTH-amino acids is clearly resolved into its components. An unknown amino acid can be identified by its elution position relative to the known ones.



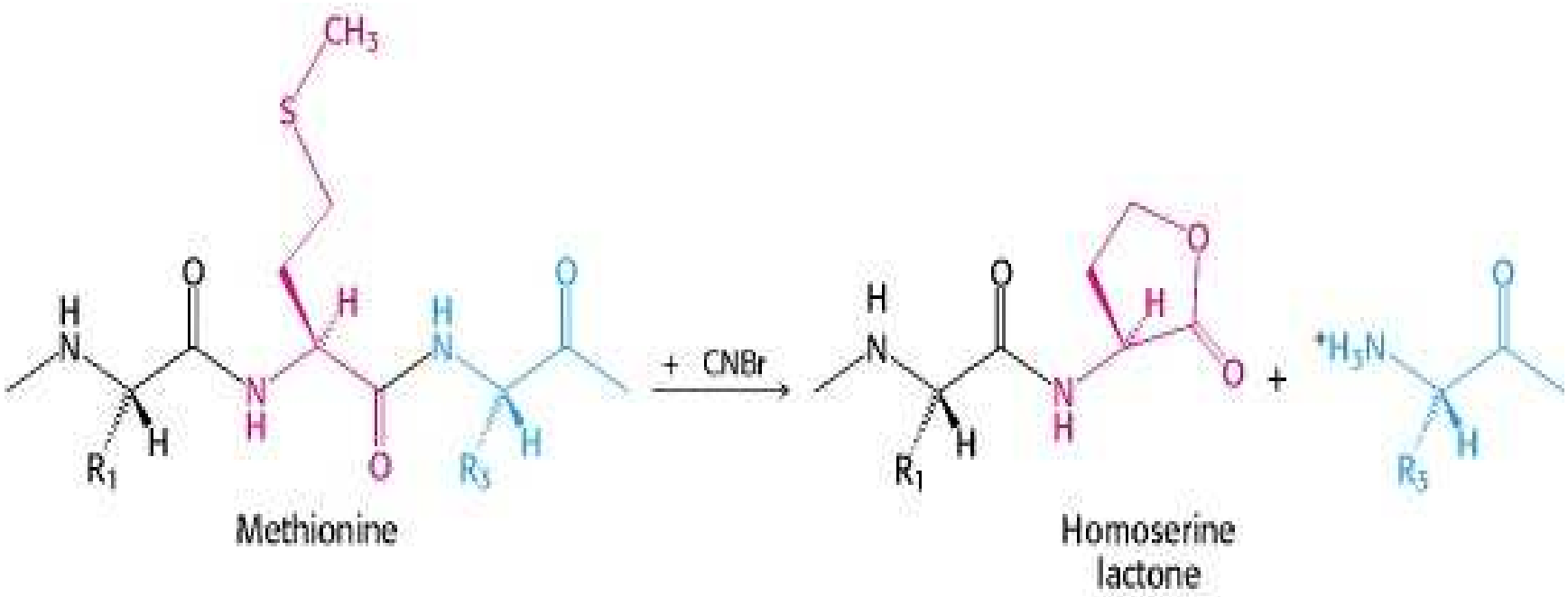
Applications

- Edman sequencer can analyze picomole (pmol) quantities of peptides/proteins sample directly eluted from a single band of an SDS-PAGE.
- With the development of mass spectrometry (MS), the use of Edman degradation sequencing began to decrease.
- However, it is still used to verify the N-terminal boundary of recombinant proteins or determining the N-terminus of protease-resistant domains, specially when the protein or domain is > 40 to 80 kDa or cannot be easily purified.
- It also can be used to identify the new N-terminal and proteolytic cleavage site in the protein fragments.
- In addition, for novel proteins/peptides where MS database searching is not possible, Edman degradation method can be used for amino acid sequence analysis.

Limitations

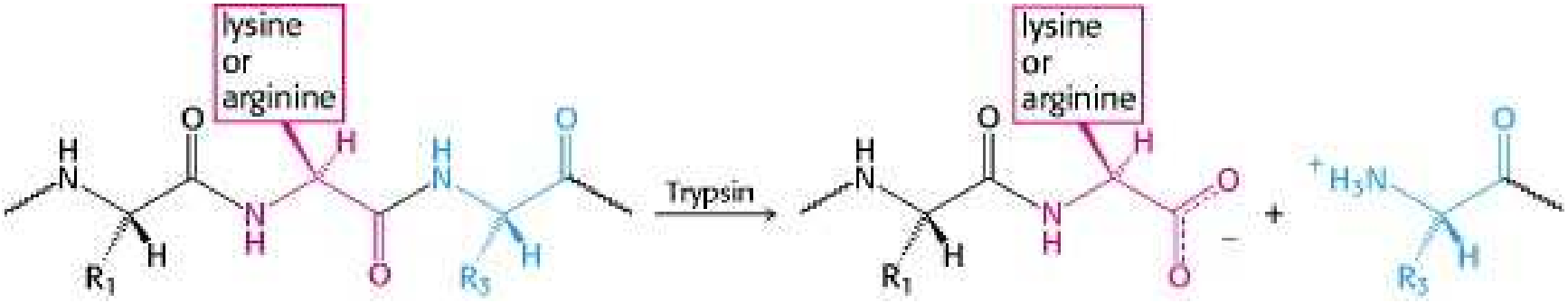
- It will not be used when the N-terminus of peptide has been chemically modified, such as acetylation.
- As the PITC cannot reactive with non-α-amino acid, Edman sequencing will stop if any non-α-amino acid is encountered like isoaspartic acid.
- The larger proteins cannot be sequenced by the Edman sequencing method.
- However, larger proteins can be specifically cleaved by chemical (e.g., cyanogen bromide) or enzymatic (e.g., trypsin)methods into small peptides to facilitate the analysis.

Cleavage by Cyanogen Bromide (CNBr):
CNBr cleaves polypeptides on the carboxyl side of methionine residues.



Cleavage by Trypsin:

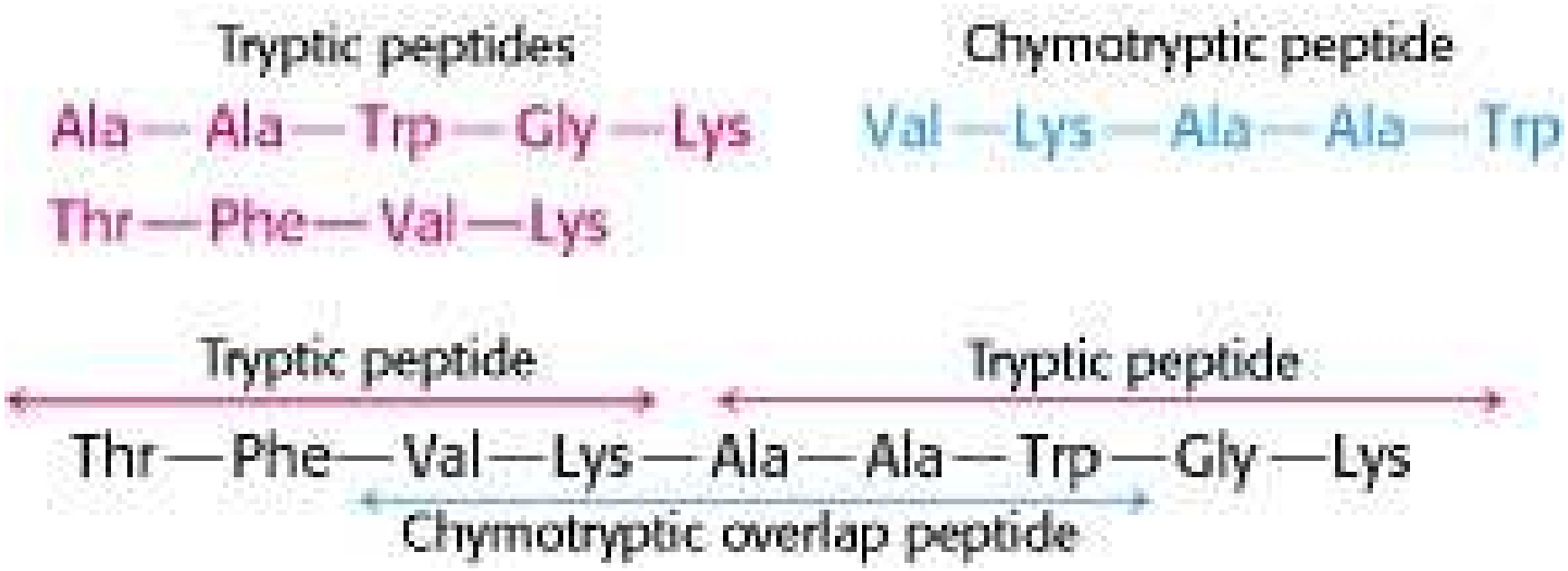
Trypsin hydrolyzes polypeptides on the carboxyl side of arginine and lysine residues.



Ordering of the peptides to obtain the full amino acid sequence of the original protein

- A second enzyme (e.g., chymotrypsin) is used to split the polypeptide chain at different linkages.
- The chymotrypsin cleaves preferentially on the carboxyl side of aromatic and some other bulky non-polar residues.
- These chymotryptic peptides overlap two or more tryptic peptides and this information can be used to develop the order of the peptides in a protein sequence.

Ordering of the peptides to obtain the full amino acid sequence of the original protein



References

- https://www.creative-proteomics.com/blog/index.php/protein-sequencing-of-edman-degradation/
- Smith J B. Peptide sequencing by Edman degradation. eLS, 2001.
- Berg JM, Tymoczko JL, Stryer L. Biochemistry. 5th edition. New York: W H Freeman; 2002.
- Reim D F, Speicher D W. N-Terminal Sequence Analysis of Proteins and Peptides. Current protocols in protein science, 2001: 11.10. 1-11.10. 38.
- https://www.ncbi.nlm.nih.gov/books/NBK22571/

Thank you.

Email: sprakashsingh@mgcub.ac.in