CONCEPT: EDMAN DEGRADATION

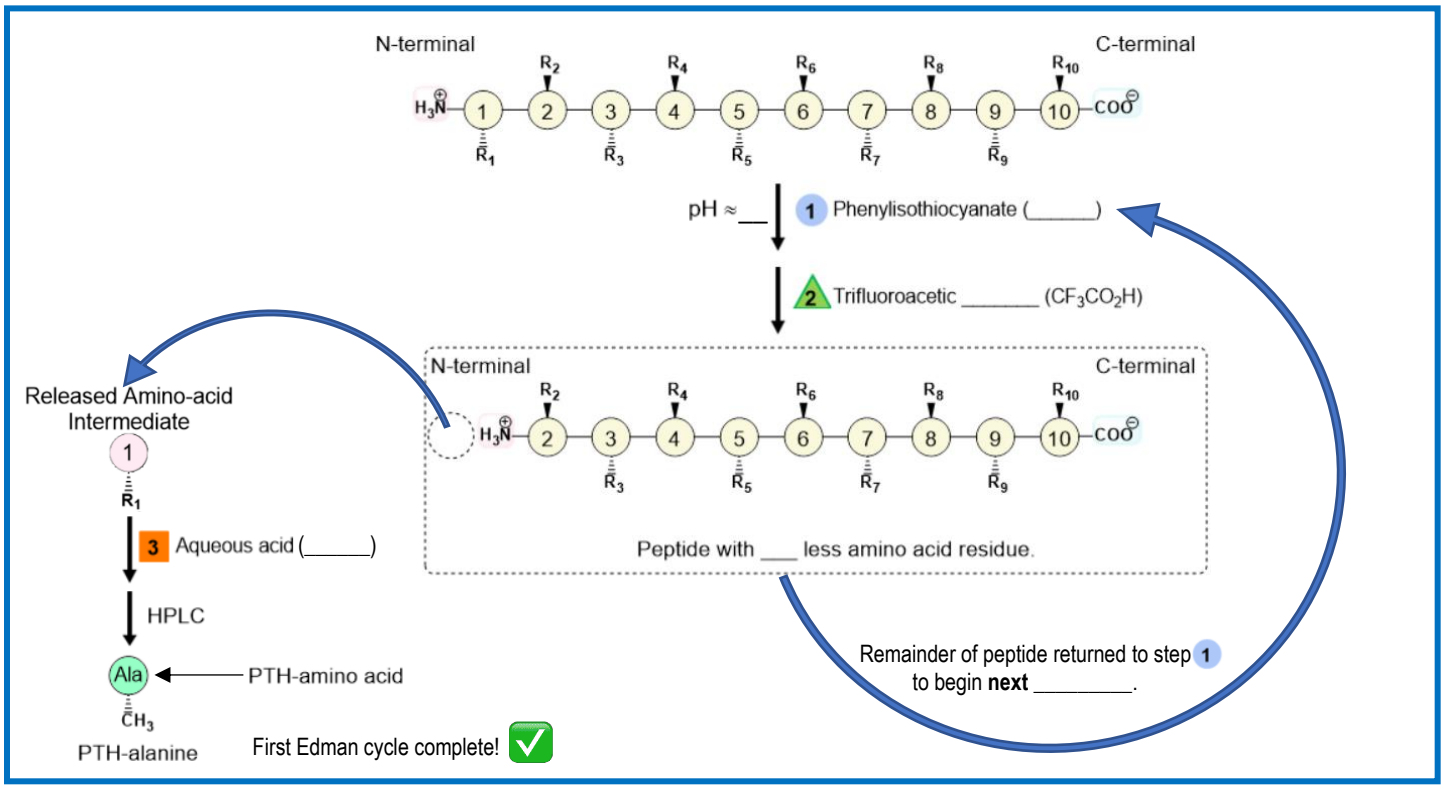
●*Edman* _____ *Procedure*: protein sequencing technique developed by Pehr _____ in the 1960's:

- □ Used on one _____ *small* peptide chain at a time.
- □ *Cycle* of 3 reactions removes one ___-terminal amino acid residue at a time & identifies it upon removal.
- □ Peptide sequenced from the ____-terminal towards the ____-terminal end.

●Peptide is sequentially treated with:

1. *Phenyl isothiocyanate (*_____ or Edman Reagent*).*
2. *Trifluoroacetic acid (*_____*).*
3. Released N-terminal amino acid derivative is treated with *aqueous acid* (_____) prior to being identified.

EXAMPLE: Edman Degradation.



●_____*-amino acid* is the _____ product that is analyzed to identify the N-terminal amino acid.

- □ The entire Edman Degradation process is repeated as a _____ until the full peptide is sequenced.

PRACTICE: The peptide Leu—Cys—Arg—Ser—Gln is subject to Edman degradation. Products of the 1st cycle include:

a) PTH—Leu, PTH—Cys, PTH—Arg, PTH—Ser, and PTH—Gln

b) PTH—Leu—Cys—Arg—Ser—Gln

c) PTH—Gln and Leu—Cys—Arg—Ser

d) PTH—Leu and Cys—Arg—Ser—Gln

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PRACTICE: 1-1) Suppose you isolated a nonapeptide (9 amino acid residues) from a patient's blood. Reaction of the nonapeptide with FDNB followed by acid hydrolysis produces a DNP-product with a sulfhydryl R-group, indicating that:

a) C-terminal residue of the nonapeptide is Cysteine.
b) N-terminal residue of the nonapeptide is Cysteine.
c) N-terminal residue of the nonapeptide is Tyrosine.
d) C-terminal residue of the nonapeptide is Threonine
e) C-terminal residue of the nonapeptide is Tyrosine or Glycine.

PRACTICE: 1-2) Treatment of the nonapeptide from the previous problem (1-1) with CNBr produces a tetrapeptide containing the N-terminal amino acid and a pentapeptide. After one round of Edman degradation on the pentapeptide, a product is produced that contained a nonpolar, aliphatic R group, meaning that the pentapeptide has:

a) N-terminal I.
b) N-terminal S.
c) C-terminal E.
d) N-terminal H.
e) N-terminal M.

PRACTICE: 1-3) The second and third rounds of Edman degradation on the same pentapeptide from the problem above (1-2) produced products with aliphatic alcohol groups, meaning that the pentapeptide had:

a) S and H.
b) I and Y.
c) M and C.
d) T and S.
e) M and Y.

PRACTICE: 1-4) Hydrazinolysis of the same pentapeptide from the problems above produced modified amino acids & a free α-amino acid with an aromatic-alcohol group. Combining the info from these problems, the pentapeptide is most likely:

a) STIRY.
b) HTSMY.
c) TISMY.
d) ISTRY.
e) ISMRY.

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PRACTICE: 1-5) Deduce the entire sequence of the original nonapeptide using the following hint and the information from the previous four practice problems (1-1 through 1-4). *Hint*: The sequence of the nonapeptide using the one-letter amino acid codes reveals a relevant academic subject.

PRACTICE: A group of peptides that influence nerve transmission in certain parts of the brain has been isolated from normal brain tissue. These peptides are known as opioids, because they bind to specific receptors that also bind opiate drugs, such as morphine and naloxone. Opioids thus mimic some of the properties of opiates. Some researchers consider these peptides to be the brain's own painkillers. Using the information below, determine the amino acid sequence of the pentapeptide opioid leucine enkephalin. Explain how your structure is consistent with each piece of information below.

● Complete hydrolysis by 6 M HCl at 110 ˚C followed by amino acid analysis indicated the presence of Gly, Leu, Phe, and Tyr, in a 2:1:1:1 molar ratio.

● Treatment of the peptide with 1-fluoro-2,4-dinitrobenzene (FDNB) followed by complete hydrolysis and chromatography indicated the presence of the 2,4-dinitrophenyl (DNP) derivative of tyrosine.

● Complete digestion of the peptide with chymotrypsin followed by chromatography yielded free tyrosine and leucine, plus a tripeptide containing Phe and Gly in a 1:2 ratio.