

## CONCEPT: PEPTIDE SEQUENCING: PARTIAL HYDROLYSIS

### Partial Hydrolysis with Endopeptidases

- **Recall:** Before sequencing, peptides are \_\_\_\_\_ hydrolyzed into \_\_\_\_\_ peptide chains (fragments).
  - Dilute \_\_\_\_\_: hydrolyzes at \_\_\_\_\_ amino acids.
  - **Endopeptidases:** \_\_\_\_\_ that selectively hydrolyze peptide bonds at \_\_\_\_\_ amino acids.
    - Hydrolyze bonds at \_\_\_\_\_ group of following \_\_\_\_\_-terminal amino acids.

Enzyme Catalyzed Hydrolysis	
Enzyme	Location of Bond Cleavage
Trypsin	_____ & _____
Chymotrypsin	_____, _____, _____
Pepsin	Phe, Trp, Tyr _____, _____, _____

**MEMORY TOOL 1:** \_\_\_\_\_ing and \_\_\_\_\_ing will \_\_\_\_\_ you up.

**MEMORY TOOL 2:** \_\_\_\_\_owder is very **aromatic**.

**MEMORY TOOL 3:** **Pepsin** breaks down **aromatic** \_\_\_\_\_ntils in \_\_\_\_\_ stomach.

**NOTE:** Enzymes will not cleave if \_\_\_\_\_ at cleavage site.

**EXAMPLE:** Label locations of hydrolysis in the following peptide with corresponding enzymes.

(T = Trypsin, C = Chymotrypsin, P = Pepsin.)



**PRACTICE:** How many peptide chains does the following peptide produce after being treated with chymotrypsin?

Thr-Lys-Cys-Tyr-His-Asp-Trp-Ile-Val-Phe

- a) 1      b) 2      c) 3      d) 4

**PRACTICE:** The following peptide is treated with pepsin and trypsin. Show where the enzymes will cleave the peptide.

Lys-Gly-Glu-Pro-Tyr-Gln-Asn-Ala-Ser-Leu-Gln-Phe-Ser-Cys-Trp

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### Peptide Sequencing using Fragments

- After fragments are sequenced, they are fitted together to determine the \_\_\_\_\_ peptide sequence.

**EXAMPLE:** Trypsin and Chymotrypsin were used to partially hydrolyze a peptide. Propose a sequence for the peptide from the following peptide fragments produced.

**Trypsin:** Ala-Trp-Gly-Cys-Tyr-Arg

Phe-Lys

Leu-Val

**Chymotrypsin:** Phe-Lys-Ala-Trp

Arg-Leu-Val

Gly-Cys-Tyr

**PRACTICE:** Acid-catalyzed partial hydrolysis of an unknown peptide produced the following peptide chains. Propose a sequence for the peptide.

Gly-Ile-Gly-Cys

Ile-Trp-Gly

Cys-Asn-Pro

Gly-Cys-Asn

Pro-Phe-Leu

Trp-Gly-Ile-Gly

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### Partial Hydrolysis with Exopeptidases

- **Exopeptidases:** enzymes that hydrolyze peptide bonds at the \_\_\_\_-terminal ends of a peptide chain.
  - **Carboxypeptidase A:** cleaves off C-terminal amino acids; **EXCEPTIONS:** \_\_\_\_ & \_\_\_\_.
  - **Carboxypeptidase B:** cleaves off C-terminal Arg and Lys \_\_\_\_\_.

**EXAMPLE:** Peptide chain is reacted with carboxypeptidase A, no reaction was observed. Next it was reacted with carboxypeptidase B, unknown amino acid was released. Reaction of peptide chain with trypsin and pepsin produced the following fragments. What is the sequence of the peptide chain?

**Trypsin:** Phe-Arg

Val-Ile-Asp-Gln-Lys

Lys-Gln-Lys

**Pepsin:** Gln-Lys

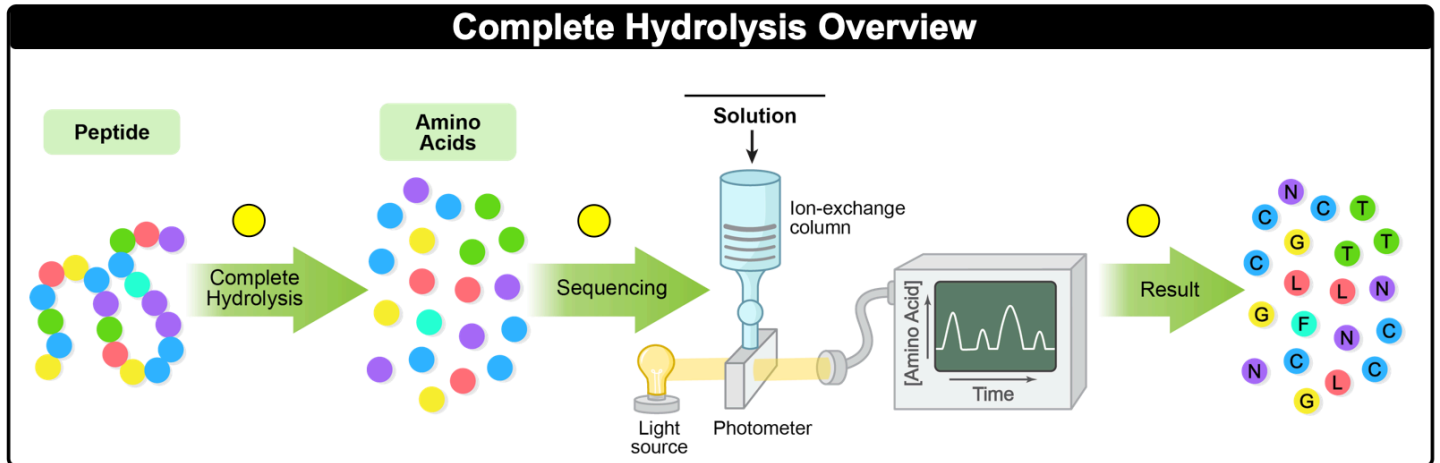
Arg-Val-Ile-Asp

Lys-Gln-Lys-Phe

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### Complete Hydrolysis

- Performed to confirm \_\_\_\_\_ of amino acids and their relative \_\_\_\_\_.
- A** Peptide is hydrolyzed by boiling in \_\_\_\_\_.
- B** Amino acid solution is passed through ion-exchange chromatography.
- C** Does \_\_\_\_\_ provide the sequence of amino acids but serves as a reference for \_\_\_\_\_ hydrolysis.



**EXAMPLE:** You are tasked with proposing a peptide sequence from following results of hydrolysis.

Complete Hydrolysis: Ala, Pro, Phe, Arg, Tyr, Trp, Ile, Lys, Cys, Glu

Treatment with carboxypeptidase A: Tyr

Treatment with trypsin:                    Ile-Trp-Lys                    Cys-Phe-Glu-Pro-Arg                    Ala-Tyr

Treatment with chymotrypsin:            Lys-Cys-Phe                    Glu-Pro-Arg-Ala-Tyr                    Ile-Trp