

Determination of N-terminal amino acid and C-terminal amino acid of degraded proteins

From the aspect of quality control of biopharmaceuticals, it is important to clarify impurities originated from main protein components. Here, we introduce a method to specify N-terminal and C-terminal amino acids of proteins degraded from main protein components.

◆ Analysis flow : Determination of N- and C-terminal amino acids of degraded proteins

Step 1 : Receive information about samples.

Amino acid sequence

If there is no information, we identify degraded proteins by searching protein sequence databases based on the results of molecular weight measurement and N-terminal sequencing.

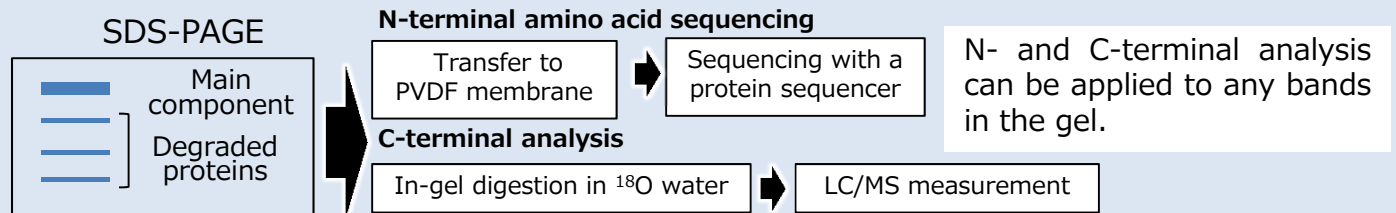
Approximate position of C-terminal

If there is no information, we:

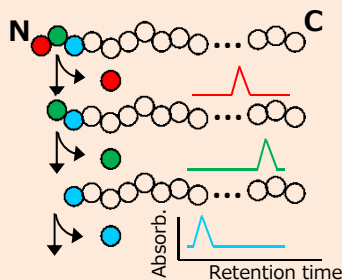
- 1) estimate the approximate positions of C-terminals from their sequences and molecular weights
- 2) choose a digestive enzyme to generate an appropriate C-terminal peptide

Step 2 : Measure the molecular weights of the degraded proteins with LC/MS (if necessary)

Step 3 : Separate the degraded proteins by SDS-PAGE (or LC), and analyze the N-terminal and C-terminal amino acids using a protein sequencer and LC/MS.



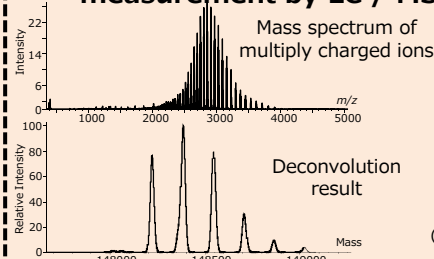
◆ N-terminal amino acid sequencing



PPSQ-33A
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Sequential determination of N-terminal amino acid is carried out by Edman degradation.

◆ Molecular weight measurement by LC / MS

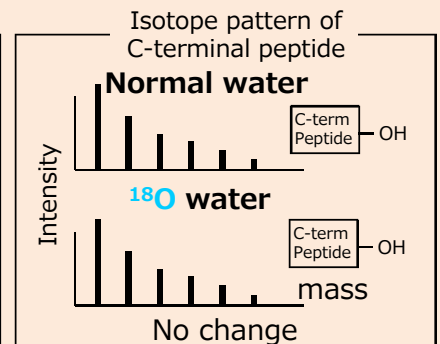
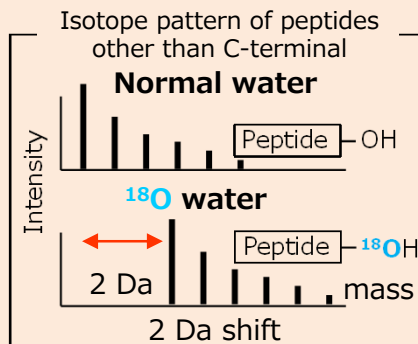
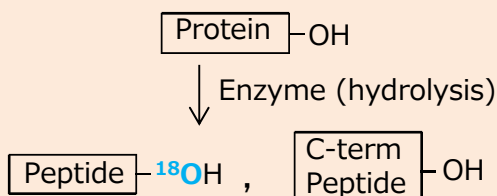


Orbitrap Fusion Lumos
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Accurate molecular weight of protein is measured by intact protein analysis.

◆ C-terminal analysis

Enzymatic digestion in ^{18}O water



When digesting (hydrolyzing) protein in ^{18}O water, ^{18}O is introduced into each internal peptide, while ^{18}O is not introduced into C-terminal peptide. Based on the 2 Da mass shift with ^{18}O , C-terminal peptide is identified, which clarifies degraded position in the sequence of main component.

Please feel free to contact us for details

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