Creative Proteomics

PROTEIN SEQUENCING

1 METHODS FOR PROTEIN SEQUENCING

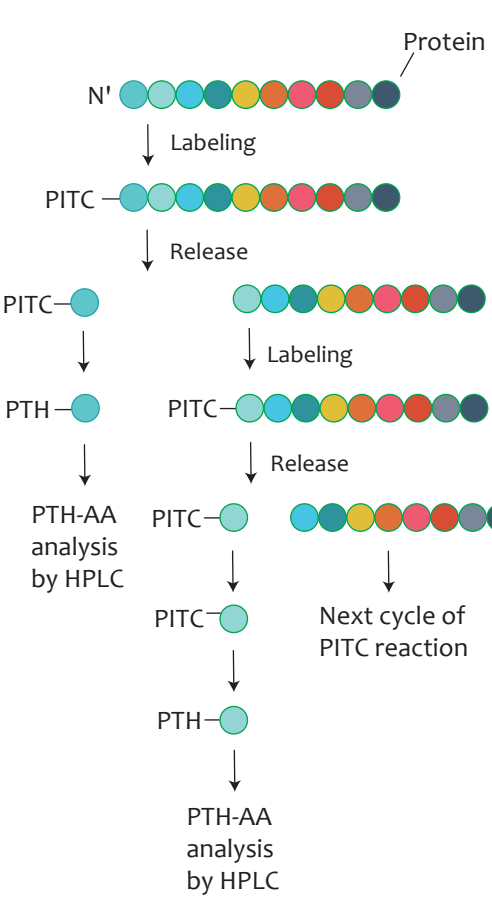
Edman Degradation is one of the N-terminal amino acid sequence analysis methods for peptide chains/proteins sequencing. The protein is reacted with PITC under weakly basic conditions, and then treated with an acid to free the amino terminal residue of the peptide chain in the form of PTH-AA for subsequent analysis.

Peptide Mapping analysis is an effective method for rapidly localizing protein sequences and is a commonly used strategy in protein identification.The method uses mass spectrometry for peptide analysis, and compares the obtained spectra with a protein database to obtain amino acid information.

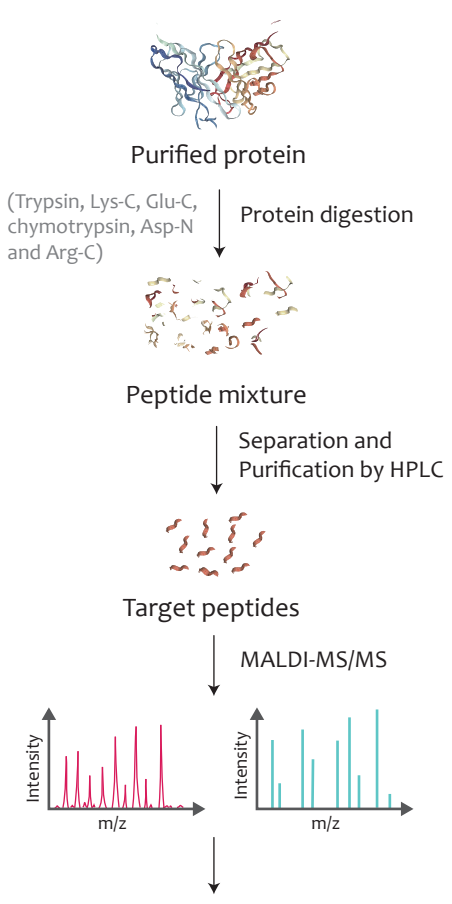
***De Novo* Protein Sequencing** is a method based on the enzymatically cleaved peptides that exhibit regular fragmentation in mass spectrometry to obtain amino acid information from the mass differences in regular mass spectral peak.

2 THE PROGRESS OF THESE THREE METHODS

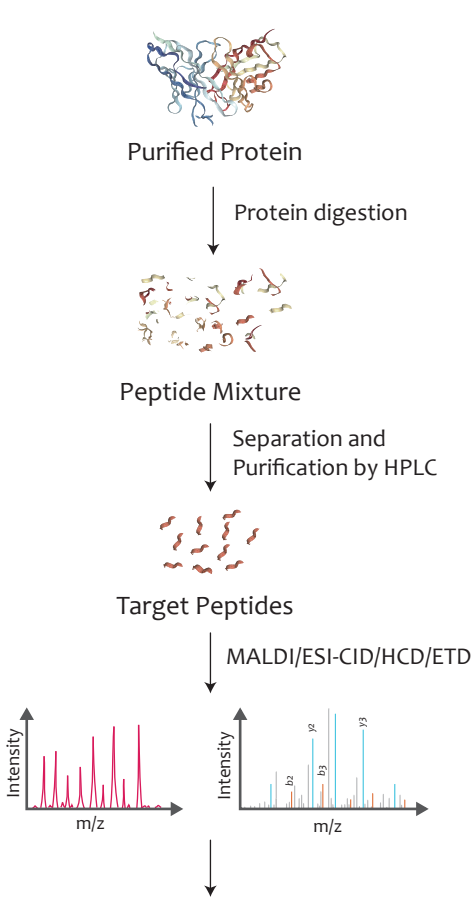
Edman Degradation



Peptide Mapping



De Novo Sequencing



3 CHARACTERISTICS

Edman Degradation

(1) Identify the exact N-terminal amino acid.
(2) The released amino acids are identified and quantifed by chromatography.
(3) Enable N- terminal sequencing of proteins in mixtures.

(1) It will not work if the N-terminus has been chemically modified.
(2) Sequencing wil stop if a non-α-amino acid is encountered.
(3) Larger proteins cannot be sequenced by the Edman sequencing.
(4) Edman degradation is generally not useful to determine the positions of disulide bridges.

Peptide Mapping

(1) High specific enzyme digestion produces peptide fragments ranging in size from 400 < m/z < 5000, corresponding to ~4–45 amino acid residues with sufficient specificity, consistent with the scanning range of common mass analyzers.
(2) Combination of specific and non-specific protein enzymes to achieve 100% coverage of any protein (polypeptide).

(1) Only peptide mass are measured, contamination from other protein can be interference to the accuracy of protein analysis.
(2) Only protein from the database can be identified.

De Novo Sequencing

(1) Does not require a reference database to deduce full-length or partial tag-based peptide sequences directly from experimental tandem mass spectrometry spectra.
(2) Identify novel peptides, unsequenced organisms and antibody drugs.

Sample contamination, isotope peak interference, loss of important b-ion peak or y-ion peak in most maps caused by incomplete dissociation of peptide segments, loss of information at n-terminal and c-terminal as well as various noise interference will lead to decreased accuracy of ab initio sequencing method and difficult to obtain correct peptide sequence information.

4 APPLICATIONS

Method:

- This study presents an improvement of ADC peptide mapping protocol to characterize the drug-loaded peptides by LC-MS analysis. All the steps of this protocol including enzymatic digestion were improved to maintain the hydrophobic drug-loaded peptides in solution by the addition of solvents.

Highlight:

- This is the first time that the payload positional isomers on the heavy chain of an ADC conjugated on native cysteines, were characterized by peptide mapping LC MS analysis.
- This method can provide important structural information about the locations of conjugation sites on cysteines residues of heterogeneous ADCs.

Janin-Bussat, Marie-Claire, et al. "Characterization of antibody drug conjugate positional isomers at cysteine residues by peptide mapping LC–MS analysis." *Journal of Chromatography B* 981 (2015): 9-13.

Method:

- This study combines standard proteogenomics with peptide de novo sequencing to refine annotation of the well-studied model fungus Sordaria macrospora.

Highlight:

- 104 so-far hidden proteins and annotation changes in 575 genes, including 389 splice site refinements were detected.
- This approach provides peptide-level evidence for 113 single-amino-acid variations and 15 C-terminal protein elongations originating from A-to-I RNA editing.

Blank-Landeshammer, B., et al. "Combination of Proteogenomics with Peptide De Novo Sequencing Identifies New Genes and Hidden Posttranscriptional Modifications." *mBio* 10.5 (2019): e02367-19.

Contact Us

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