SHIMADZU

Application News

MALDI-TOF Mass Spectrometry No.B25

A Simple and Highly Successful C-terminal Sequence Analysis of Proteins by Mass Spectrometry

Protein identification via Peptide Mass Fingerprinting (PMF) is conducted by enzymatically digesting the protein and analyzing the resulting digest using mass spectrometry. A database search is then applied to the list of peaks obtained from this analysis. However, assignment of the N and C terminal sequences is not always easily accomplished with a database search because 1) the protein N and C terminals are often changed due to processing and post-translational modification, and 2) a portion of the protein sequence may not be detectable by the mass spectrometer due to the ion suppression effect.
The protein N-terminal amino acid sequence can be determined using a protein sequencer (PPSQ-31A/33A) or a protein N-terminal sequencing kit (ORFinder-NB™). However, in the case of the C-terminal, there has been a need for a technique importance of protein terminal amino acid sequence analysis is becoming more important than ever.
Here we introduce an example of mass spectrometric analysis of a sample consisting of selectively collected protein C-termini, demonstrating a newly developed, successful method of amino acid sequencing**.

* Japan Pharmaceutical Affairs Bureau Notification No. 571 (May 1, 2001)
** Characterization testing and standard testing of polymer pharmaceuticals and biological pharmaceuticals focusing on antibody drugs conducted by Shimadzu Techno-Research

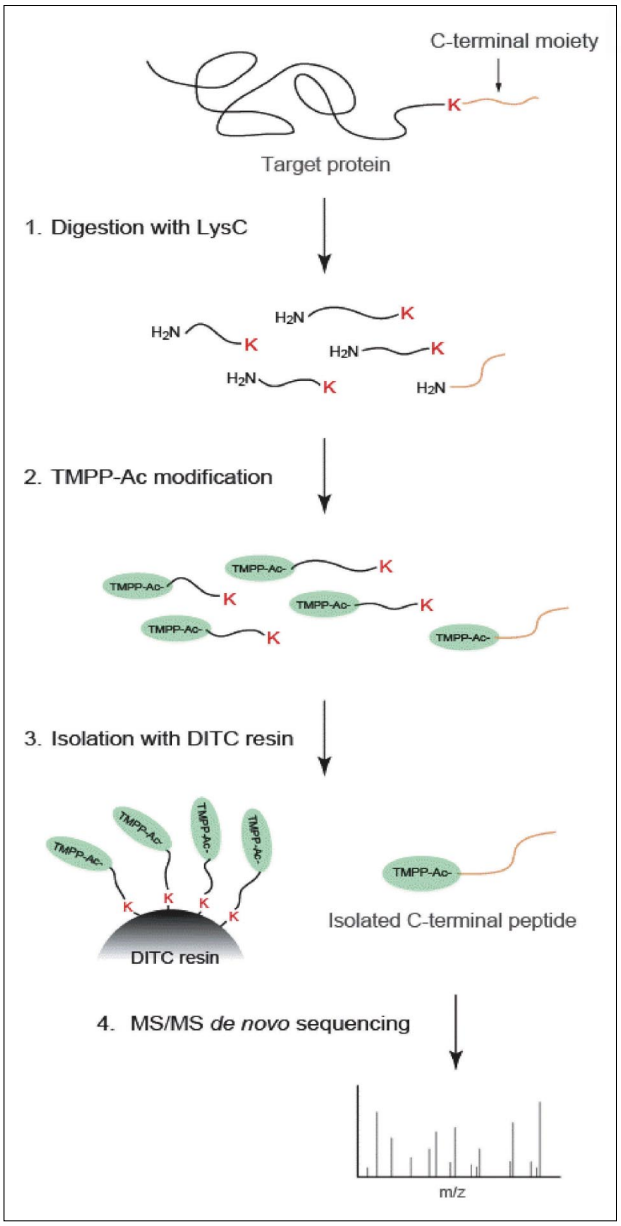

Fig. 1 Schematic Overview of C-terminal Sequence Analysis of Proteins by Mass Spectrometry

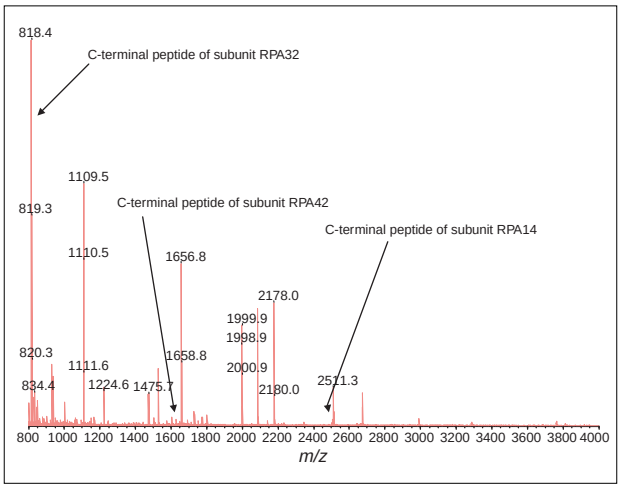

Fig. 2 MALDI-TOF Mass Spectrum after TMPP Modification of LysC Digest from PfuRPA Protein Complex

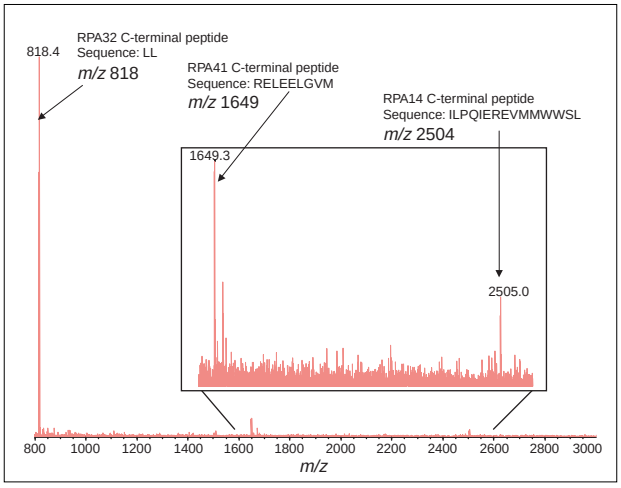

Fig. 3 MALD-TOF Mass Spectrum after Isolation of Three C-terminal Peptides using DITC Resin

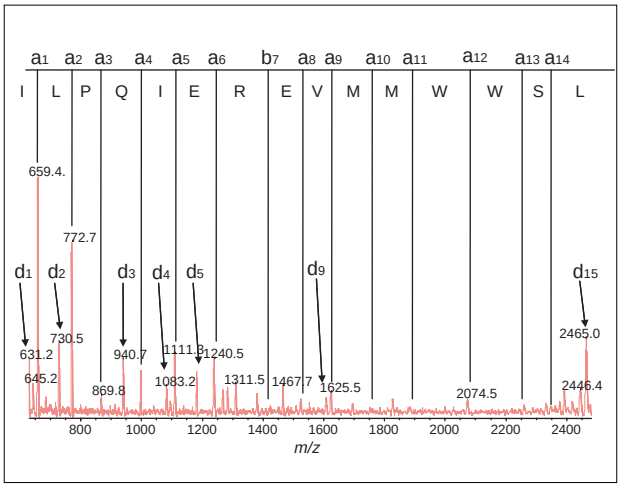

Fig. 4 MS/MS Spectrum of Isolated C-terminal Peptide from RPA14

The operational flow of a protein C-terminal amino acid sequence analysis is shown in Fig. 1. In step 1, when the target protein is digested using lysyl endopeptidase (LysC), all of the digest peptide carboxy-terminal amino acids, except for those derived from the protein C-terminal, are converted to lysine (except when the protein C-terminal amino acid is lysine). In step 2, the LysC digest is reacted with (succinimidyloxycarbonylmethyl) tris (2,4,6-trimethoxyphenyl) phosphonium bromide (TMPP-Ac-OSu) so that the α amino groups are selectively TMPP-Ac-modified. In step 3, TMPP-Ac-modified the LysC digest is added to p-phenylenediisothiocyanate-resin (DITC resin or glass), so that all of the peptides that have lysine as an ε amino group side chain - in the sequence are trapped by the DITC resin. - Protein-C terminal-derived digest peptides without a free amino group are not trapped by the DITC resin. In step 4, when isolated protein C-terminal peptides are measured by MS/MS, only fragment ions containing the strongly positively charged TMPP are observed in the MS/MS spectrum. Assignment of the protein C-terminal amino acid sequence is possible by comparing the mass difference between these fragment ions and the terminal amino acid sequence predicted from the genetic sequence.

Fig. 2 shows a MALDI-MS spectrum obtained following TMPP-Ac modification of a LysC digest of the recombinant protein complex PfuRPA consisting of 3 types of subunits. Each of the subunits, RPA14, RPA32, and RPA42 derived from the digest peptides, are observed in the MS spectrum, but the C-terminal peptides associated with RPA14 and RPA32 can hardly be seen. Next, Fig. 3 shows an MS spectrum following isolation of each of the subunit C-terminal peptides by reaction of the PfuRPA TMPP-Ac modified LysC digest with DITC resin. All the peptides derived from the internal sequence are trapped by the DITC resin, and only the peaks originating from each of the subunit C-terminal peptides are detected and observed in the MS spectrum. MS/MS measurement was conducted on all 3 of these peaks derived from the C-terminal peptides, and by comparing the terminal amino acid sequence predicted from the genetic sequence with the mass differences between the detected fragmentation ions, we were able to assign the C-terminal sequence for each subunit. Shown here as an example are the results of MS/MS measurement of the *m/z* 2504 peak using the high-energy CID method, a feature of the AXIMA Performance (Fig. 4).

[References]
1) Kuyama, H., Shima, K., et al., Proteomics 2008, 8, 1539-1550.

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