Differentiation of isobaric residues in SPITC-derivatized tryptic peptides using MS/MS technique in a Curved Field Reflectron.

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Overview

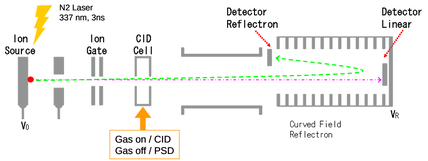
- Specific fragment ions obtained by CFR can discriminate isobaric residues in SPITC-derivatized peptides without impairing interpretable sequence information.
- Differentiations of Ile/Leu and α- /βAsp are performed successfully.
- Switching PSD to HE-CID rapidly is valuable for *de novo* sequencing.

1: Introduction

Fixing a strong negative charge at N-terminus of tryptic peptide is a quite effective chemical derivertization for *de novo* sequencing by using post-source decay (PSD) on MALDI-TOFMS. However, whereas the chemical derivertization causes interpretable y-ions mainly, one cannot differentiate isobaric amino acid residues, for instance, Ile/Leu and α- /βAsp. We report our study of differentiation of these residues by using PSD and a high energy CID.

2: Methods

- *MALDI-TOFMS*
  Instrument: AXIMA-Performance (Shimadzu Biotech/Kratos)
  Measurement: PSD and high-energy CID-MS/MS in positive ion mode.
  Collision gas: helium
  Collision energy: 20 keV (laboratory frame of reference).

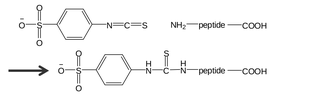


Curved Field Reflectron[1),2)]
- Mix-mode of PSD and CID.
  All fragment ions generated in both PSD and CID are detected simultaneously.
- No precursor suppression system.
- No stitch, seamless PSD.
- Only 30 sec. to switch PSD to CID.
- High energy collision at 20 keV.
- No deceleration, no re-acceleration.
- Easy operation.

Fig.1 Description of CFR

- *Samples*
  Tryptic digests of BSA and β-casein
  Synthesized peptides including α- and βAsp, originated from human α-crystallin

- *Derivatization of 4-sulphophenyl isothiocyanate(SPITC) on a ZipTip*[3)].
  Guanidination: 3 hours at 37 ℃
  Sulfonation: 2 hours at 50 ℃



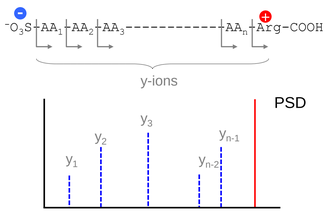


Fig.2 SPITC derivatization for *de novo* sequencing

3: Results

3-1: Differentiation of Ile/Leu

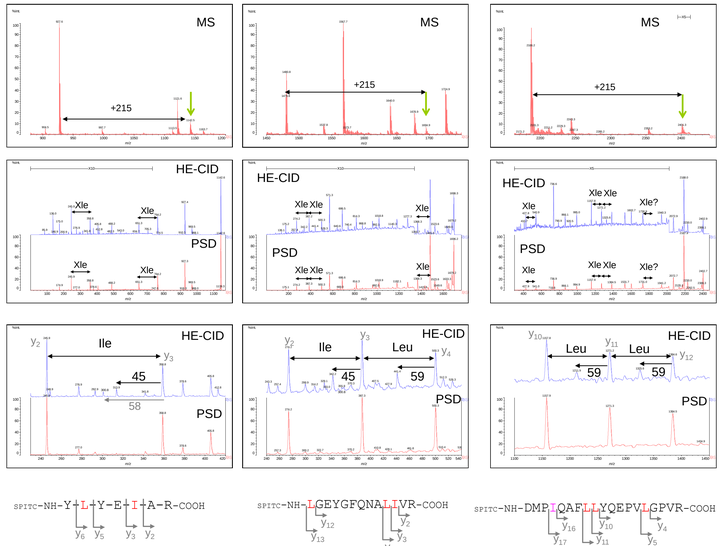


Fig.3 Analysis of tryptic peptide of BSA #1

Fig.4 Analysis of tryptic peptide of BSA #2

Fig.5 Analysis of tryptic peptide of β-casein

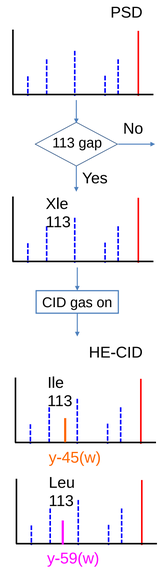


Fig.6 A possible flow, where *de novo* sequencing incorporates the differentiation.

3-2: Differentiation of α- and βAsp[4)]

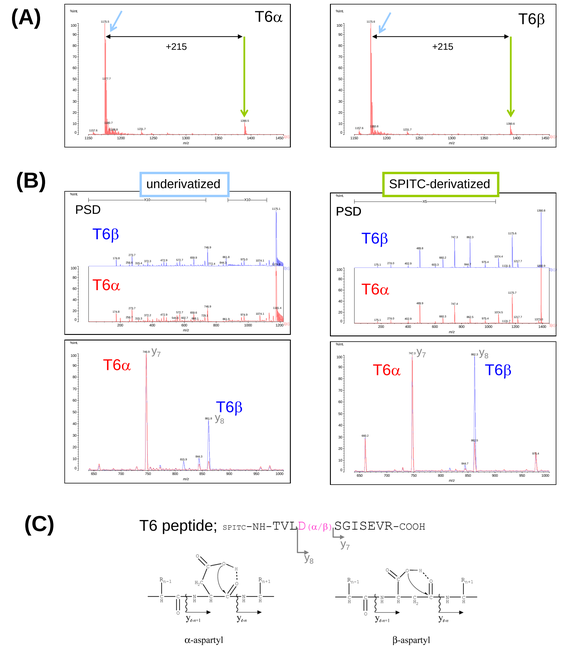


Fig.7 Effect of the derivatization on a differentiation of Asp isomers. MS spectra after the derivatization (A), PSD spectra (B), and an amino acid sequence of T6 peptide and a proposed fragmentation of Asp isomers (C).

4: Conclusions

- ✓Ile and Leu in SPITC-derivatized peptides were differentiated successfully by high energy CID-MS/MS in a CFR.
- ✓y-59 and y-45 between two y-ions, a mass difference of which is 113, were specific side chain fragmentations that indicate Leu and Ile respectively.
- ✓Since PSD of the derivatized-peptide is still useful to read out an amino acid sequence easily, taking spectrum in both PSD and HE-CID could be more preferable to obtain a complete amino acid sequence.
- ✓The SPITC derivatization induced more cleavable N-terminal side of the Asp residue than the one in a underivertized counterpart.
- ✓Notably, the specific y-ion ratios of Asp isomers were still retained after the derivertization, which is thought to be useful to perform further quantitative analysis.

References;

(1)Cordero MM, et al; Rapid Commun Mass Spectrom., 1995, 9, pp1356-61.
(2)Cornish TJ, et al; Rapid Commun Mass Spectrom., 1993, 7, pp1037-40.
(3)Chen, P., et al; Rapid Commun. Mass Spectrom., 2004, 18, pp191-198.
(4)Yamazaki, Y.,et al; Anal. Chem., 2010, 82 (15), pp 6384–6394.