

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

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

## 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  $\alpha$ - / $\beta$ Asp are performed successfully.
- Switching PSD to HE-CID rapidly is valuable for de novo sequencing.

## Introduction

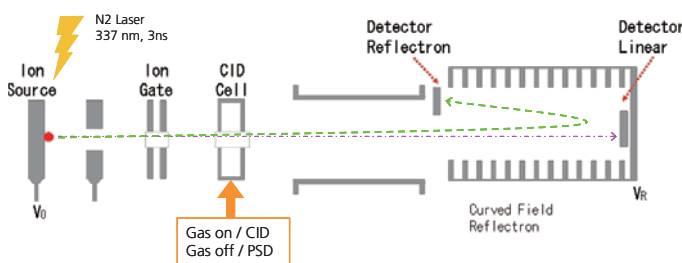
Fixing a strong negative charge at N-terminus of tryptic peptide is a quite effective chemical derivatization for *de novo* sequencing by using post-source decay (PSD) on MALDI-TOFMS. However, whereas the chemical derivatization causes interpretable y-ions mainly, one

cannot differentiate isobaric amino acid residues, for instance, Ile/Leu and  $\alpha$ - / $\beta$ Asp. We report our study of differentiation of these residues by using PSD and a high energy CID.

## 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<sup>(1),(2)</sup>

- 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  $\beta$ -casein  
Synthesized peptides including  $\alpha$ - and  $\beta$ Asp, originated from human  $\alpha$ -crystallin

### Derivatization of 4-sulphophenyl isothiocyanate (SPITC) on a ZipTip<sup>(3)</sup>.

Guanidination: 3 hours at 37°C  
Sulfonation: 2 hours at 50°C

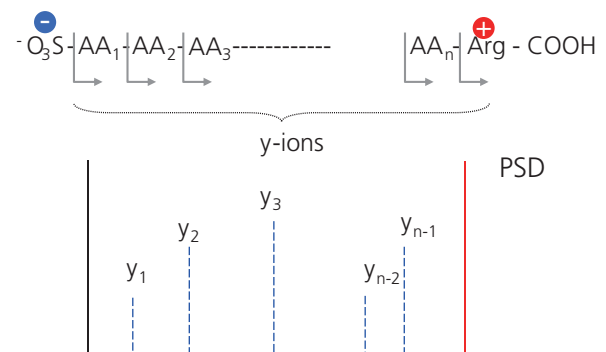
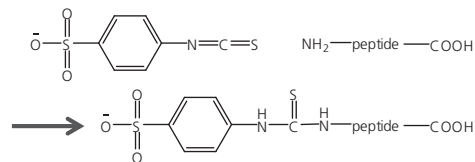


Fig.2 SPITC derivatization for de novo sequencing

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## Results

### Differentiation of Ile/Leu

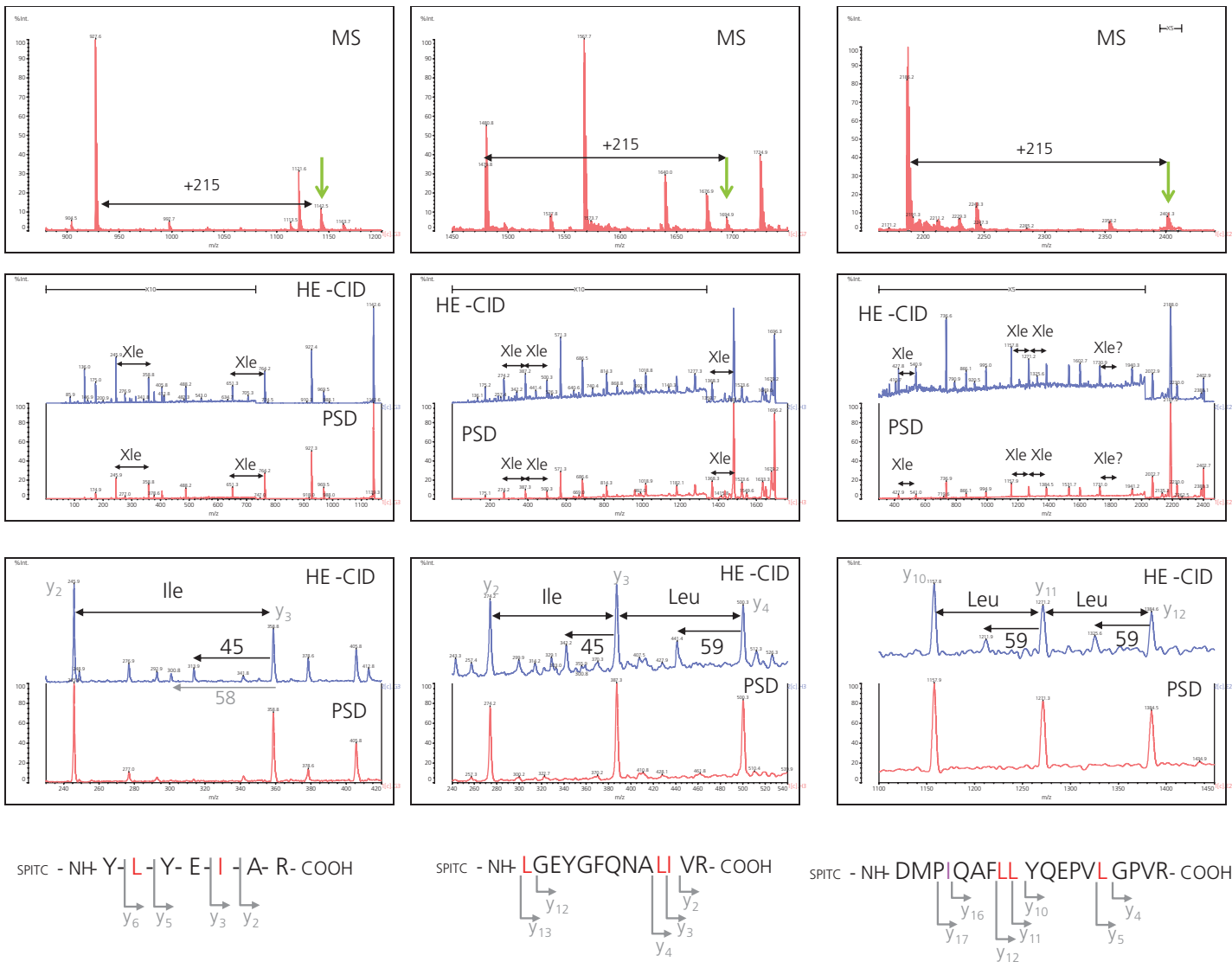


Fig. 3 Analysis of tryptic peptide of BSA #1

Fig. 4 Analysis of tryptic peptide of BSA #2

Fig. 5 Analysis of tryptic peptide of  $\beta$ -casein

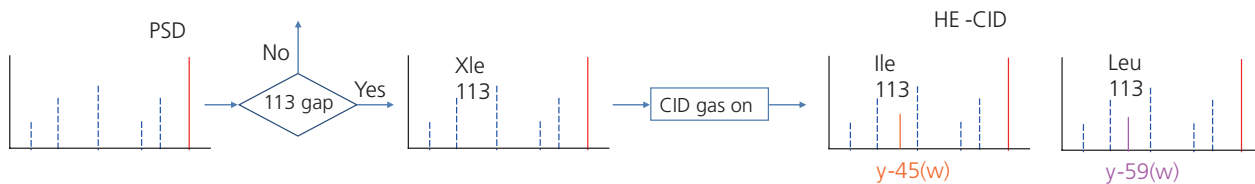


Fig. 6 A possible flow, where de novo sequencing incorporates the differentiation.

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## Differentiation of $\alpha$ - and $\beta$ Asp<sup>(4)</sup>

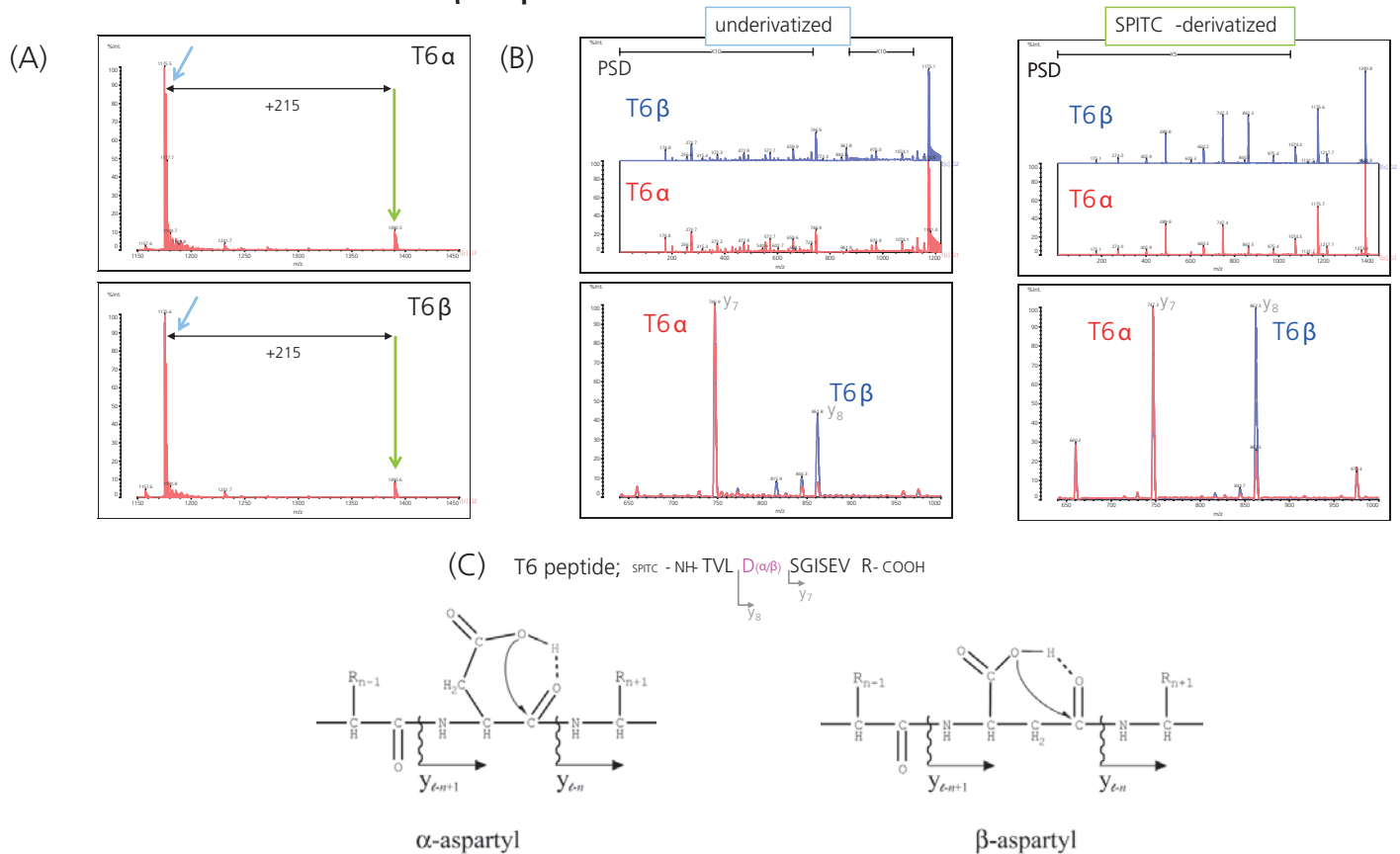


Fig. 7 Effect of the derivatization on a differentiation of Asp isomers. MS spectra after the derivatization (A), PSD spectra (B), and an amino acid sequence of T6 peptide and a proposed fragmentation of Asp isomers (C).

## 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 underivatized counterpart.
- Notably, the specific y-ion ratios of Asp isomers were still retained after the derivatization, 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.



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