

# Differentiation of isobaric residues in SPITC-derivatized tryptic peptides using MS/MS technique in a novel 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 reported these differentiations elsewhere<sup>(1)</sup> by using a commercial CFR, the disadvantage of which was unit-mass resolution in MS/MS,

while it had unique features, namely, a simultaneous detection of all fragment ions generated by both high-energy CID (HE-CID) and PSD, and a high reproducibility of the fragment ions intensities. Recently, we modified the previous ion optics to obtain a high resolution in MS/MS with a new equipment for fragment ion focusing placed just after CID cell. Here we report our study of differentiation of these residues by using this novel ion optics based on the CFR.

## Methods

All experiments were carried out with a prototype MALDI-TOF/MS, in which a novel ion optics was adopted, and AXIMA-Performance (Shimadzu Biotech/Kratos). HE-CID MS/MS in two instruments were conducted with a helium as collision gas. All MS/MS experiments were carried out with a laboratory collision energy of 20keV in positive ion mode. Axial spatial distribution focusing (ASDF) cell was placed between CID cell and the CFR. A pulsed static

electric field was applied to the ASDF cell at the moment when fragment ions fly in to the cell, which results in a high resolution in MS/MS spectrum. Tryptic digests of BSA were subjected to HE-CID MS/MS to perform Ile/Leu differentiation. Synthesized peptides including  $\alpha$ - and  $\beta$ Asp, which were originated from human  $\alpha$ -crystallin, were applied to PSD. Guanidination and sulfonation of the peptides were carried out by following a previous report<sup>(2)</sup>.

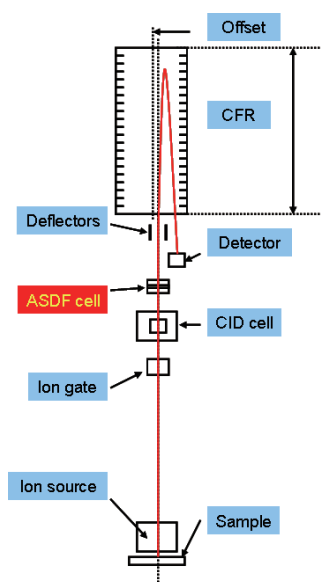


Fig. 1 Description of the ion optics

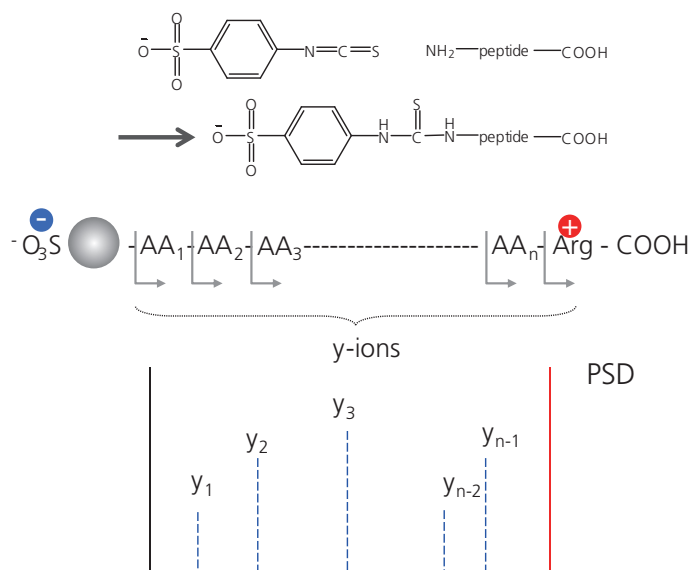


Fig. 2 SPITC derivatization for *de novo* sequencing

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## Result 1: Differentiation of Ile/Leu by HE-CID

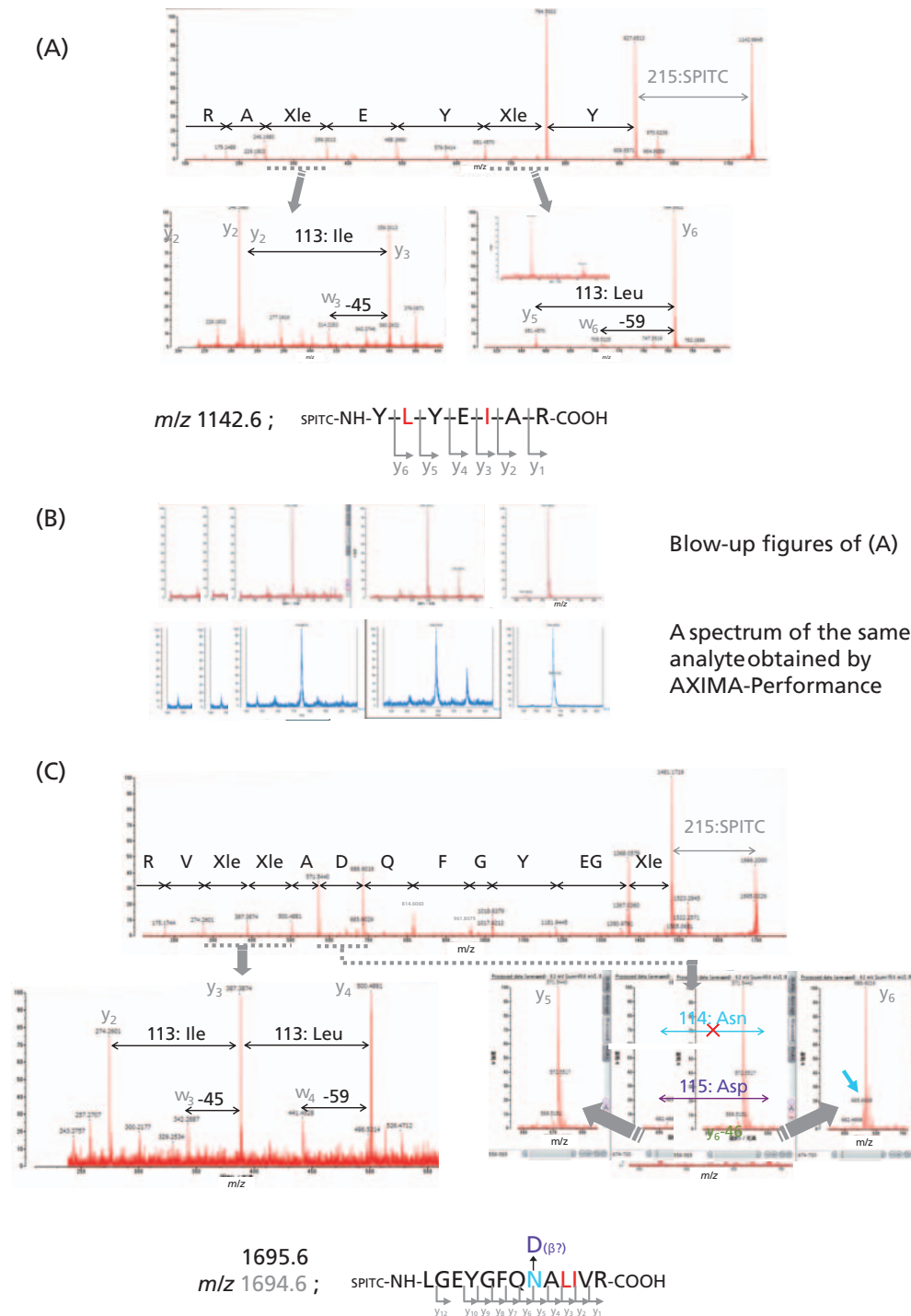


Fig.3 HE-CID spectra of derivatized peptides taken by the prototype. MS/MS of  $m/z$  1142.6 (A), a comparison of MS/MS spectra taken by the prototype and by AXIMA-Performance (B), and MS/MS of  $m/z$  1695.6 (C). In (C), note that deamidation possibly occurs on Asn residue, which is indicated with a mass difference, 115. Isomerization of the residue is also speculated with a specific  $y_6-46$ .

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## Result 2: Differentiation of $\alpha$ - and $\beta$ Asp(1) by PSD

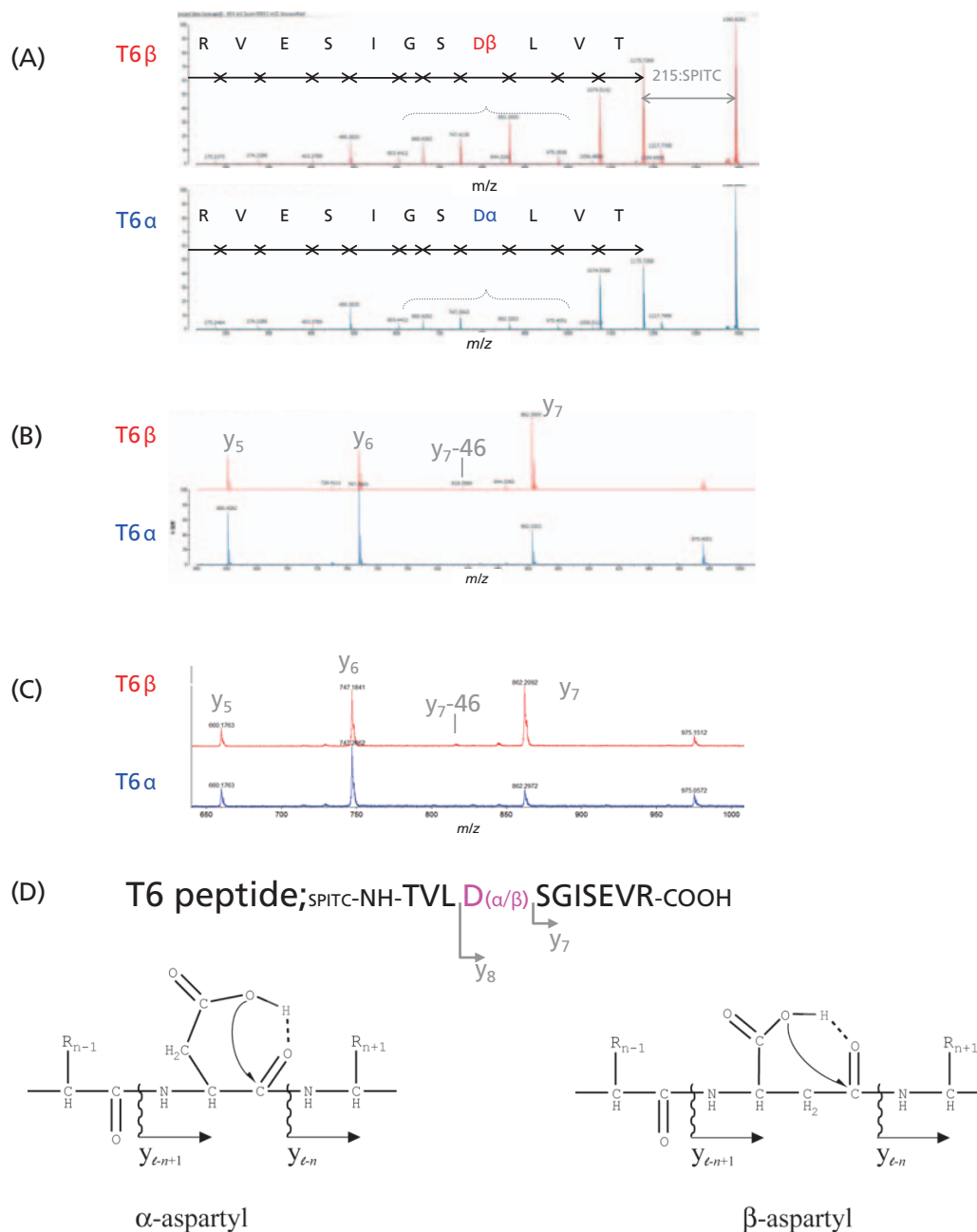


Fig. 4 PSD spectra of derivatized peptides taken by the prototype (A), blow-up figures of the PSD (B), the spectra obtained by AXIMA-Performance (C), and a proposed fragmentation of Asp isomers (D).

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## Conclusions

- A prototype instrument succeed unique features of the current CFR, and enabled a precise analysis of the isobaric residues with a newly developed MS/MS.
- Ile and Leu in SPITC-derivatized peptides were differentiated successfully by using HE-CID of the prototype instrument.
- Obtaining spectrum in both PSD and HE-CID is preferable to analyze a complete amino acid sequence.
- As well as the current instrument, the reported specific y-ion ratios depending on Asp isomers were still observed by using the prototype instrument.
- An accidental deamidation happened in the process of the SPITC derivatization was identified doubtlessly by using a high resolution of the MS/MS.

## References

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## Related information;

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