

Application News

MALDI-TOF Mass Spectrometry

No.B45

Differentiating β Asp Residue by PSD in a Curved Field Reflectron (1)

Aspartic acid in proteins is known to form a 5-membered ring when it undergoes isomerization (isoaspartate; isoAsp or β Asp) due to ultraviolet irradiation or aging, etc.^[1] As this isomerization means the formation of a bond between the C=O group of an aspartic acid side chain and the NH group of a neighboring residue, it is thought that this imparts instability to the main chain of the protein, eventually leading to modification of the protein structure and cohesion between proteins. In fact, it has been reported that α crystallin including isoaspartate exists in the crystalline lens of cataract patients. The detection and quantitation of isoaspartate is mainly

conducted by protein sequencing and HPLC, but due to the difficulty in separating the isomers and the very small amounts present, analysis is generally difficult. MALDI-TOFMS is an effective method of analyzing trace level analytes, but because the masses of isomer residues are identical, isoaspartate detection analysis is not possible using simple molecular weight measurement. Here, we report the results of detection of the characteristic fragment ion of β Asp and its differentiation from ordinary Asp using TOF post source decay (PSD) analysis with Shimadzu's original Curved Field Reflectron technology^[5].

* This report represents part of the results obtained in joint research with Dr. Fujii of the Kyoto University Research Reactor Institute.

Fig. 1 shows the structures of the Asp isomers. The characteristic feature of β Asp is the reversed main and side chain structure. Fig. 2 shows the conditions that were used for PSD measurement by MALDI-TOFMS. For the sample, an isomerized synthetic peptide from an Asp site in an α crystallin partial amino acid array was used (Fig. 2). It is known that Asp included in this partial sequence is susceptible to isomerization due to aging, etc.

The PSD spectra of the synthetic T6 peptide are shown on the following page. The spectra of these different isomerized Asp included in the array are

extremely similar, but it is clear that the intensities of fragmentation ions y_7 and y_8 before and after the Asp residue are extremely different. In addition, the characteristic y_{8-46} from the peptide including β Asp was detected notwithstanding its weak intensity. PSD measurement can be conducted very easily by selecting the precursor and setting the laser power to a value higher than that used in typical MS. Thus, it was shown that β Asp, which has been so difficult to distinguish by conventional methods, can easily be identified using PSD.

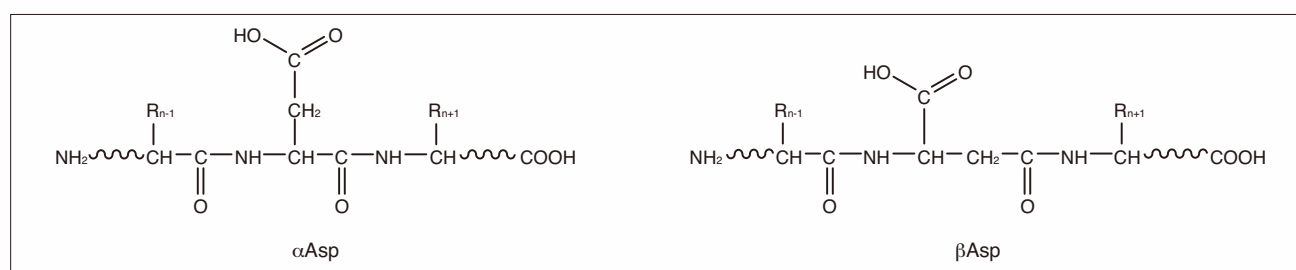


Fig. 1 Chemical Structures of α - and β Asp Residues

[Sample] T6 peptide
(Partial array of human α crystallin)
Concentration: 1 mg/mL
Sequence: TVLDSGISEVR (1175.6)

4th Asp residue was replaced with following isomers.
L α : TVL (α D) SGISEVR
L β : TVL (β D) SGISEVR

[Measurement]
Instrument : AXIMA-Performance™
Instrument conditions : Reflectron/positive
Matrix : α -CHCA 5 mg/mL 50 % acetonitrile (0.1 % TFA)
Mass calibration : The following external standardswere used for mass calibration.
Angiotensin II : m/z 1046.54
ACTH18-39 : m/z 2465.20

Fig. 2 Synthetic Peptides and Experimental Conditions

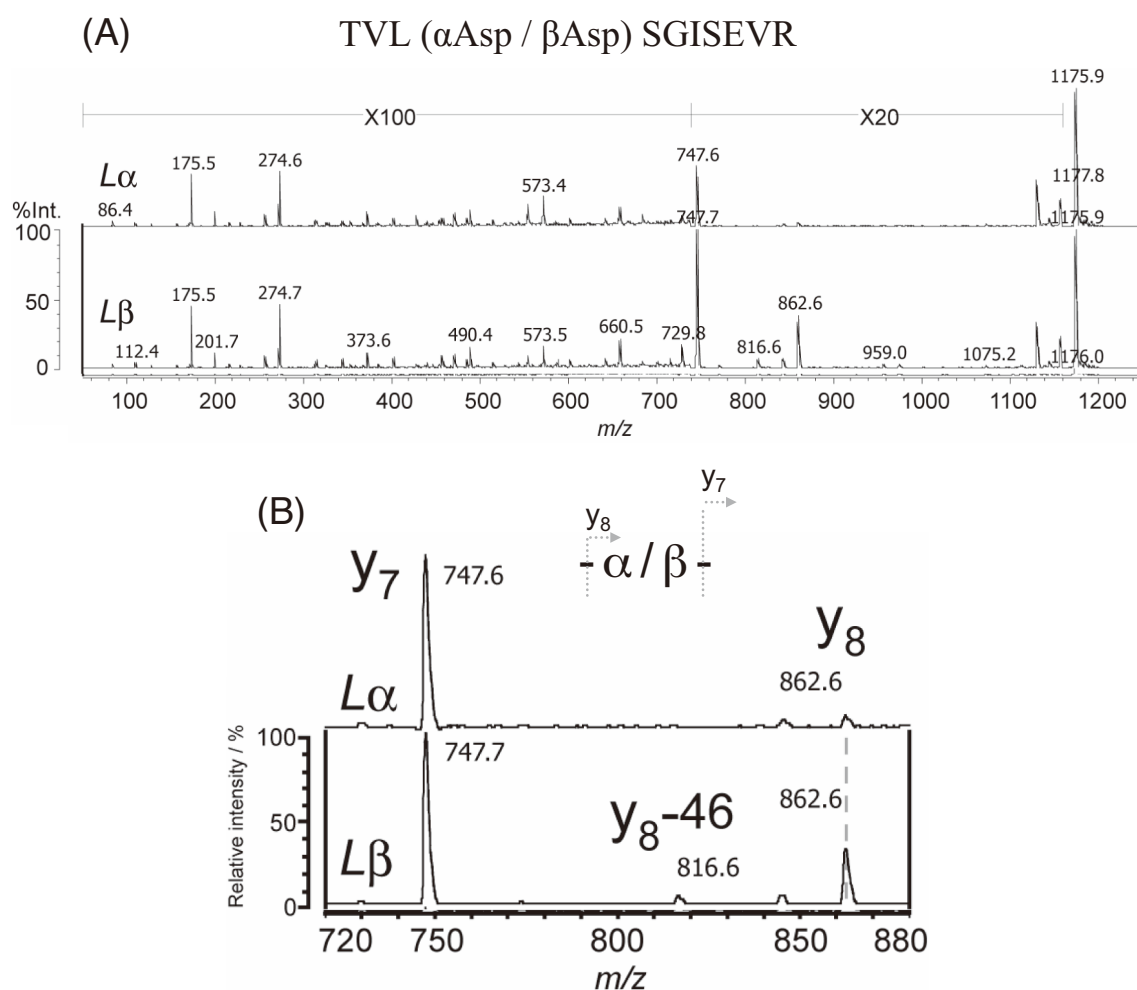


Fig. 3 PSD Spectra of T6 Peptides; (A), and Enlarged View; (B)

[References]

- [1] Biochem Biophys Res Commun., 294, 1047-1051 (2002) [2] Rapid Commun. Mass Spectrom., 14, 2092-2102 (2000)
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 [5] Anal. Chem., 82, 6384-6394 (2010)