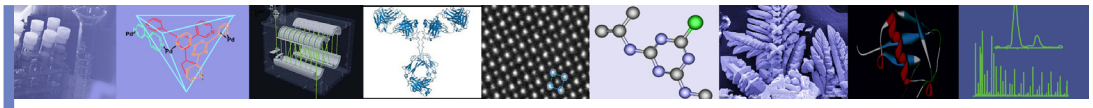

Applications Note

SpiralTOF-TOF

Distinguishing Lysine and Glutamine in a Peptide

Introduction:

Lysine and glutamine are not easily distinguished by the most common approaches to peptide sequencing which involve mass spectrometers with low to moderate resolving power and low-energy collision-induced dissociation (CID). Lysine ($C_6H_{14}N_2O_2$ with a mass of 146.1055 u) and glutamine ($C_5H_{10}N_2O_3$ with a mass of 146.0691) differ by only 0.036 u. In this study, we demonstrate the measurement of a mixture of Substance P and a synthesized peptide (3-Gln) with glutamine substituted for lysine in the Susbstance P sequence. Because the mass difference between Substance P and 3-Gln is 0.036 u, a resolving power of greater than 37,000 is required to separate each peptide. Additionally, we show that the TOF-TOF mode can be used to distinguish lysine and glutamine in these peptides by comparing the peak area ratio between a ions and d ions in the high-energy CID mass spectra.

Experimental:

Substance P standard was obtained from Sigma-Aldrich. The 3-Gln sample was synthesized and then provided by Hayashi Kasei.

1. Substance P, Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Leu-Met (Sigma-Aldrich)
2. 3-Gln, Arg-Pro-Gln-Pro-Gln-Gln-Phe-Phe-Leu-Met (Hayashi Kasei)

The peptide standard samples were dissolved in water containing 0.1% trifluoroacetic acid (TFA) at a concentration of 10 pmol/µL. α-Cyano-4-hydroxycinnamic acid (CHCA) was used as the matrix and was dissolved in 1:1 water/acetonitrile containing 0.1% TFA. Next, the peptide standard solution and CHCA solution were mixed together 1:1 by volume. Afterwards, 0.5 µL of this mixture was placed on the MALDI target plate (2.5 pmol/spot). Finally, the dried sample was measured using the SpiralTOF and TOF-TOF option available on the JMS-S3000 SpiralTOF MS system.

Results:

(1) SpiralTOF measurement

The MALDI mass spectra are shown in Figure 1, and expanded mass spectra are shown in Figure 2 for the monoisotopic ion of the protonated molecules $[M + H]^+$. The mass resolving power was approximately 60,000 for each of the $[M + H]^+$ peaks in both substance P and 3-Gln mass spectra. Excellent mass accuracy (less than 10 ppm) was obtained with external calibration in all mass spectra. Additionally, we demonstrated that the high resolving power of the SpiralTOF could separate both components in the mixture.

(2) TOF-TOF measurement

The product-ion mass spectra are shown in Figure 3. In both product-ion spectra, the characteristic a ions and d ions were observed in the high-energy CID. We cannot distinguish lysine and glutamine by mass accuracy and mass resolution in the product-ion mass spectra measured in TOF-TOF mode. However, we can identify lysine and glutamine by examining the a3/d3 ion intensities ratio [1]. The d3 ion intensity is higher than a3 ion for glutamine due to the stable ejected radical ($\bullet CH_2–CO–NH_2$). Conversely, the d3 ion intensity is lower than a3 ion for lysine due to the unstable ejected radical ($\bullet CH_2–CH_2–CH_2–NH_2$) [2]. This information clearly identifies the presence of lysine in Substance P and glutamine in 3-Gln, respectively, as the third amino acid in each peptide sequence.

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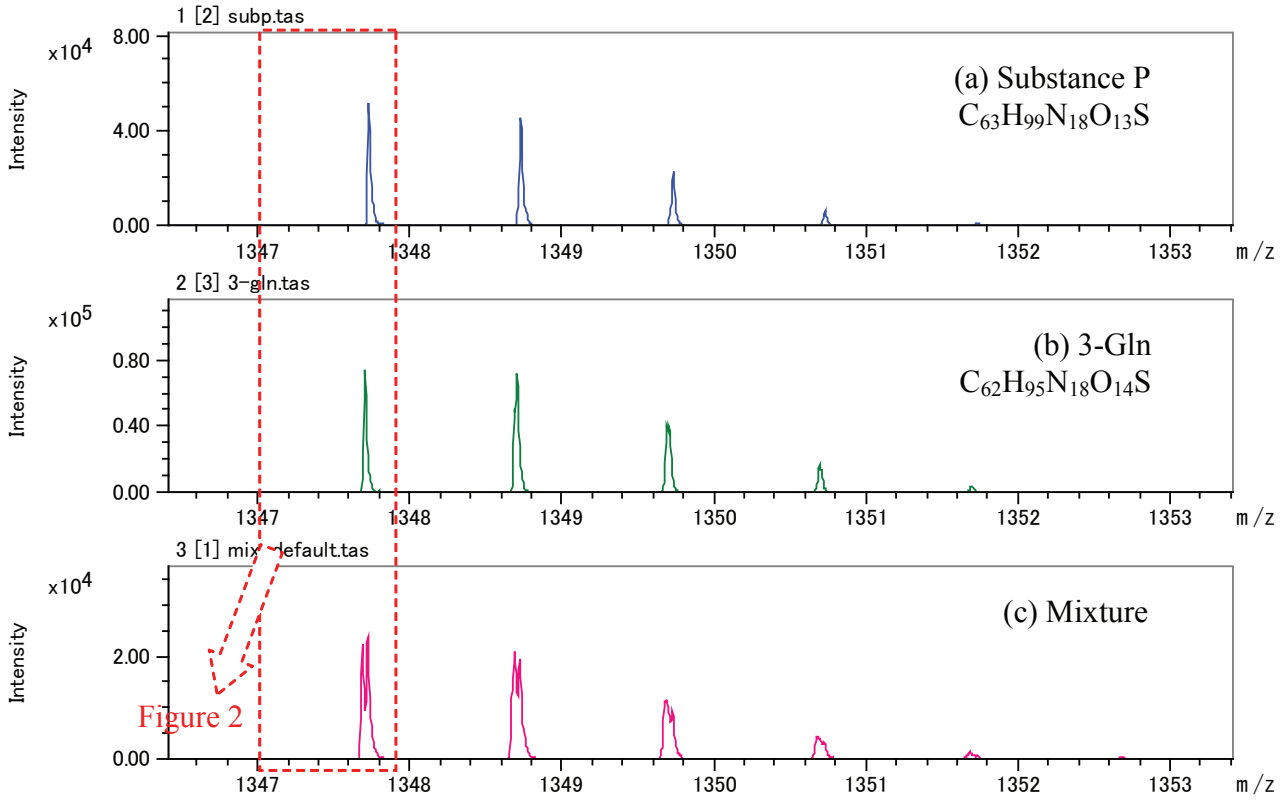

Figure 1. MALDI mass spectra of peptides: (a) Substance P, (b) 3-Gln, (c) Mixture

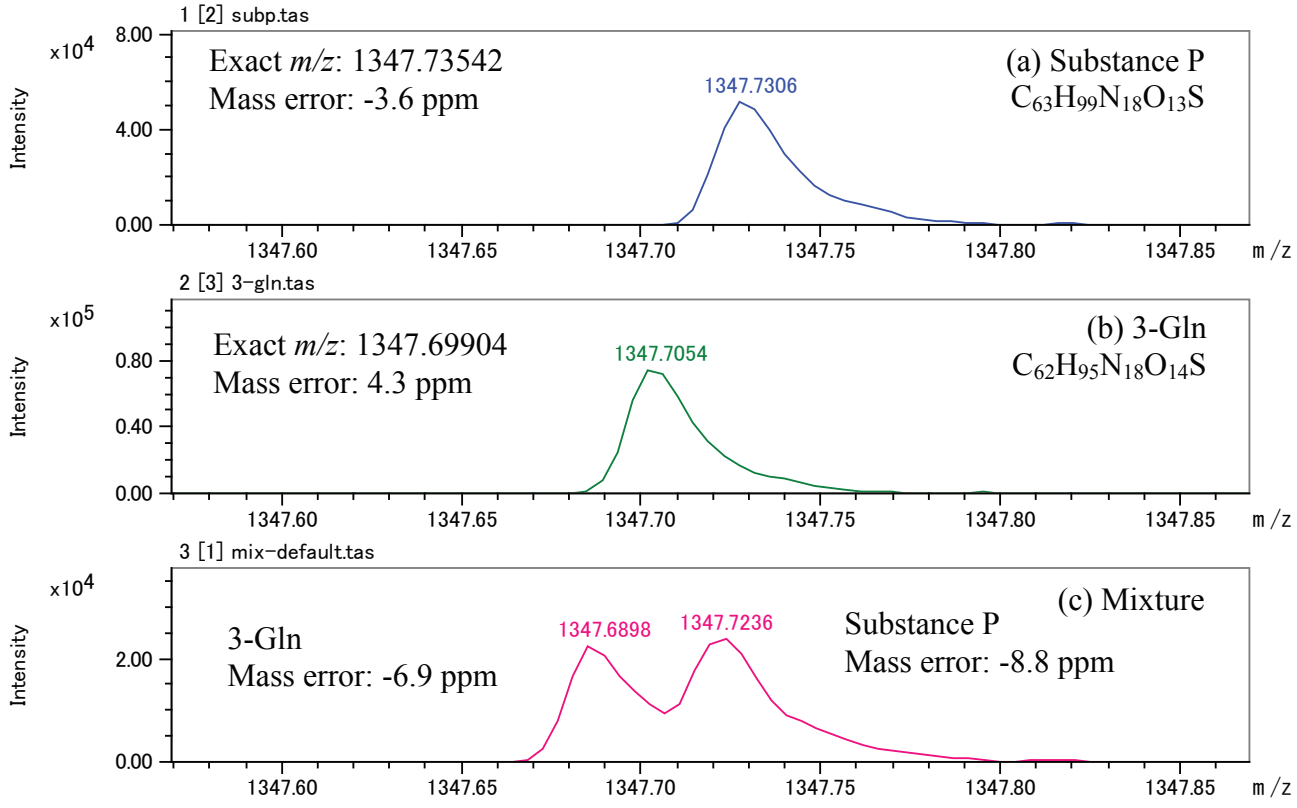

Figure 2. Expanded MALDI mass spectra showing the [M + H]+ monoisotopic ions for (a) Substance P, (b) 3-Gln, and the (c) mixture

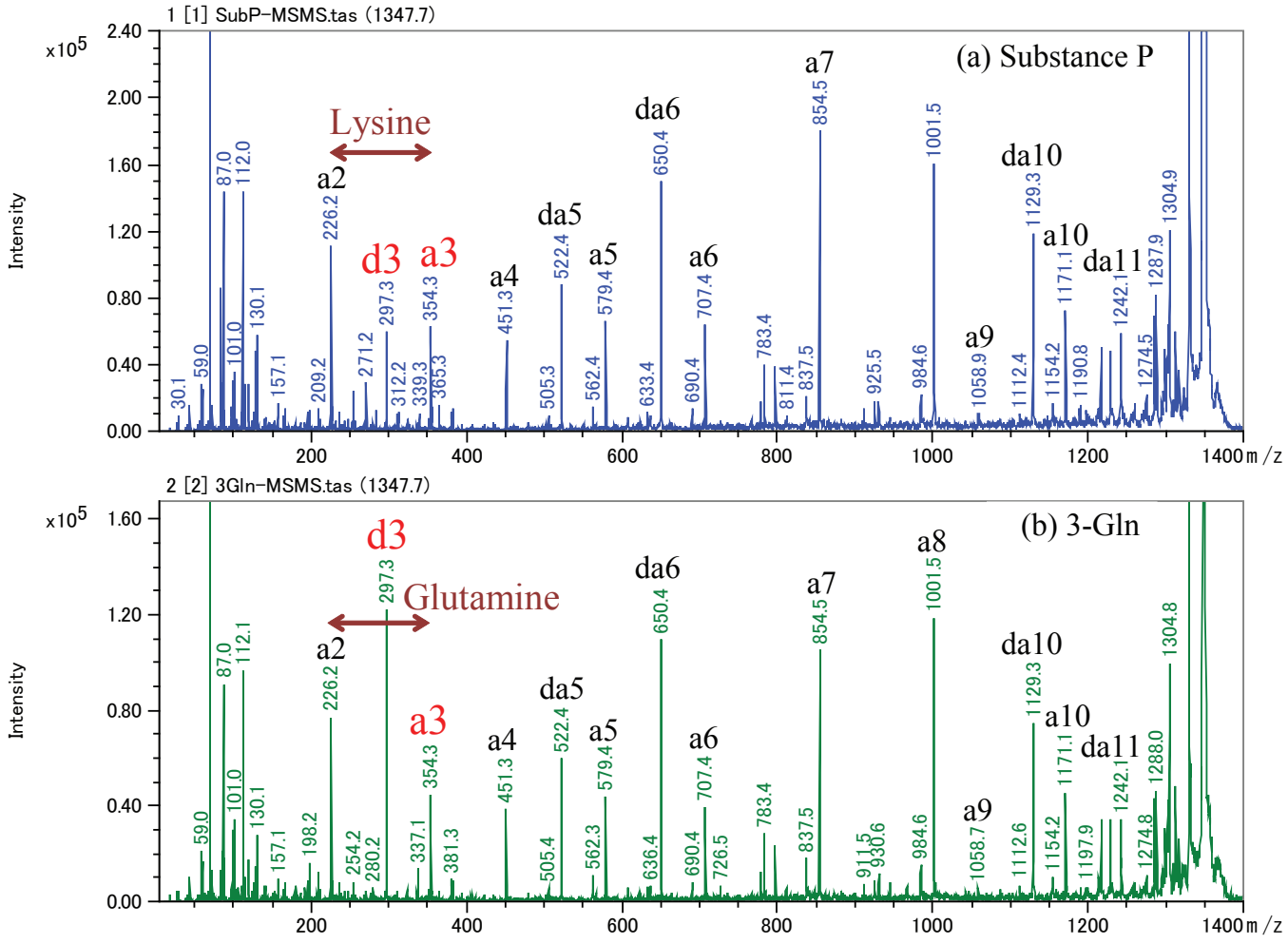

Figure 3. TOF-TOF product ion spectra for (a) Substance P and (b) 3-Gln

Conclusion:

In this study we demonstrated that the JEOL SpiralTOF MALDI mass spectrometer can distinguish lysine and glutamine in a peptide.

SpiralTOF mode provides

- Ultra high resolving power sufficient to separate isobaric ions (0.036 u in this study)
- Excellent mass accuracy with external calibration (less than 10 ppm)

TOF-TOF mode provides

- High-energy (20 kV) CID
- Lysine and glutamine can be distinguished by examining the a3/d3 peak area ratio differences

Acknowledgement:

These data were obtained through collaborative research between Mass Spectrometry Group, Department of Physics, Graduate School of Science, Osaka University and JEOL Ltd.

Reference:

[1] R.S. Johnson, S.A. Martin, K. Biemann, I*nternational Journal of Mass Spectrometry and Ion Processes* 86 (1988) 137.

[2] M. Toyoda, A. E. Giannakopulos, A. W. Colburn, O. J. Derrick, *Review of Scientific Instuments* , 78 (2007) 074101.

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