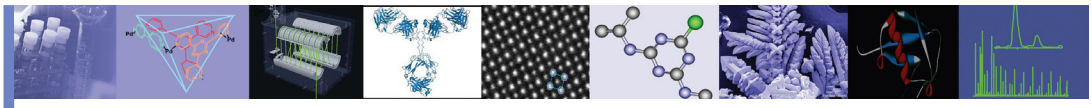

Applications Note

SpiralTOF-TOF

Analysis of Phosphopeptide Using TOF-TOF

Introduction:
Phosphorylation is a type of post-translational modification of proteins that is used for the intracellular signal transduction in a wide range of living species. For this reason, it is very important to determine where the protein (amino acid) is phosphorylated. In this work, we measure a monophosphopeptide (FQ pS EEQQQTEDELQDK) that was obtained from the tryptic digestion of β-casein (Bovine) using the TOF-TOF option that is available for the JEOL SpiralTOF™ system.

Experimental:
The monophosphopeptide sample was dissolved in water containing 0.1% trifluoroacetic acid at a concentration of 10 pmol/µL. The matrix for this analysis was made by mixing 150 µL of CHCA (Methanol) at a concentration 30 mg/mL with 35 mg of 3-Aminoquinoline. Next, the monophosphopeptide sample solution and matrix 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 sample spot was measured using the TOF-TOF option available on the JMS-S3000 SpiralTOF MS system.

Results & discussion:
The MALDI-TOF mass spectrum of the monophosphopeptide is shown in Figure 1. The peak corresponding to the protonated molecule for this compound was observed at *m/z* 2061.8. The monoisotopic peak was then selected for high-energy CID TOF-TOF analysis. The resulting product-ion mass spectrum for this protonated molecule is shown in Figure 2. Because Lysine is present at the C-terminal for basic amino acids, we were able to observe the y-ion series generated by cleavage of the main-chain peptide bonds. Additionally, the w-ion series generated by elimination of the side-chains from the corresponding y-ions were observed.

Generally, the identification of the phosphorylation position using low-energy CID is considered very difficult because the phosphate group is lost before the cleavage of the main-chain peptide bond [1]. In contrast, high-energy CID does provide information about the location of phosphorylated amino acids because the y- and w- ion series were observed without the loss of the phosphate group (Figure 2) [2]. In Figure 2, the mass difference of the y13-ion and the y14-ion is 167u, which shows the position of the phosphorylated serine.

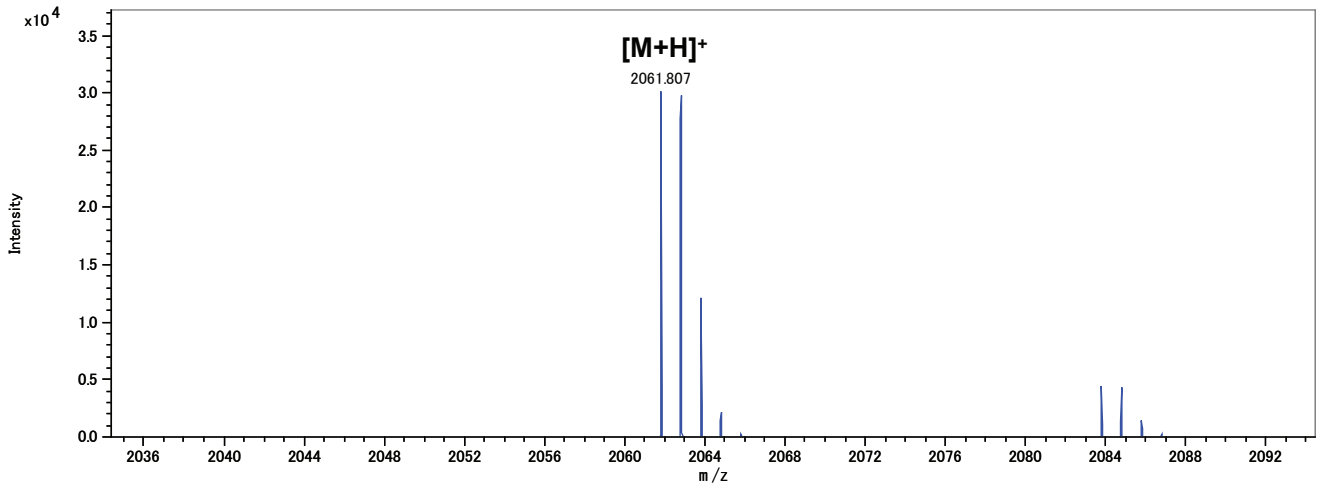

Figure 1. Mass spectrum of the monophosphopeptide.

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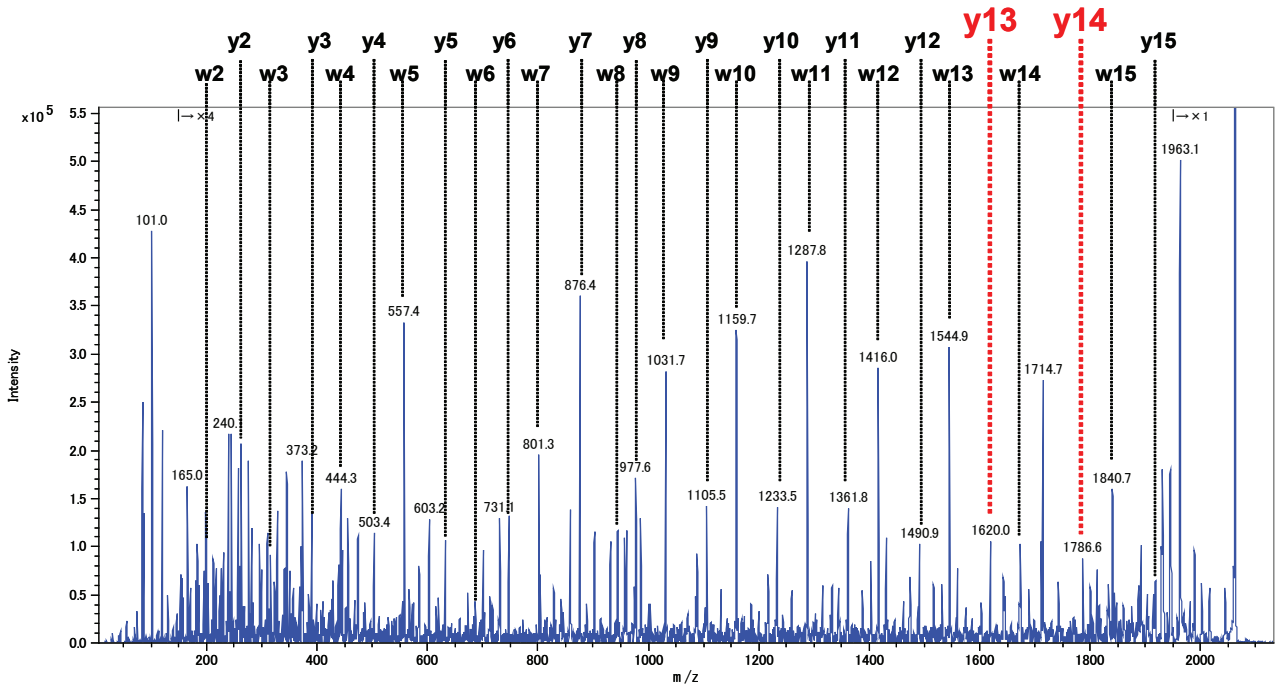

Figure 2. High-energy CID product ion spectrum of the monophosphopeptide.

Conclusions:

As this work shows, we can identify the location of phosphorylated amino acids in a peptide by using the high-energy CID TOF-TOF option available for the JMS-S3000 SpiralTOF system. Furthermore, this data shows the possibility of using this instrument for the primary structural analysis of peptides, particularly for de novo sequencing.

References:

[1] A. Stensballe, O.N. Jensen, J.V. Olsen, K.F. Haselmann, R.A. Zubarev, Rapid Communications in Mass Spectrometry 14 (2000) 1793.

[2] S. Shimma, H. Nagao, A.E. Giannakopulos, S. Hayakawa, K. Awazu, M. Toyoda, Journal of Mass Spectrometry 43 (2008) 535.

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