

# Application News

MALDI-TOF Mass Spectrometry

No.B06

## Phosphorylation Analysis by MALDI-TOF MS (2) Phosphopeptide Sequence Analysis Using Seamless PSD

Recently, mass spectrometry is being applied to the analysis of phosphopeptide and phosphorylation sites in proteins. Phosphorylation sites are determined using various types of MS/MS (techniques used for detecting fragment ions derived from ions of interest). In almost all cases, however, the instrument configuration used has been based on electrospray ionization (ESI). Here we introduce the phosphorylation site analysis technique based on seamless PSD (sPSD) by MALDI. In the CID method (Collision-Induced Dissociation) used in MALDI-MS/MS amino acid sequence analysis, phosphorylation site information is difficult to obtain due to the marked desorption of the fragile phosphate group caused by collision with the inert gas. However, when sPSD is used in the analysis, the obtained spectra clearly indicate some degree of suppressed phosphate desorption, which is attributed to the absence of inert gas.

Fig.1 shows a sPSD spectrum of a doubly-phosphorylated peptide obtained using DHB as the matrix. From the fragment ion assignment results of Fig. 1, it is clear that the y-ion which is the C-terminal fragment is formed preferentially. In addition, phosphate group desorption from fragment ions was observed in lower intensity (y7-98 in the figure), and the sPSD spectrum was sufficient for deciphering the entire sequence from the masses of the phosphorylated amino acid residues.

It is known that this type of effect is especially amplified when DHB is used<sup>1)</sup>, but conventional PSD has not been very practical due to insufficient fragment ion detection sensitivity. With sPSD, the superior fragment ion generation efficiency is a feature that makes it more attractive than conventional PSD. Therefore, this practical level of sensitivity makes MS/MS measurement of such phosphopeptides possible.

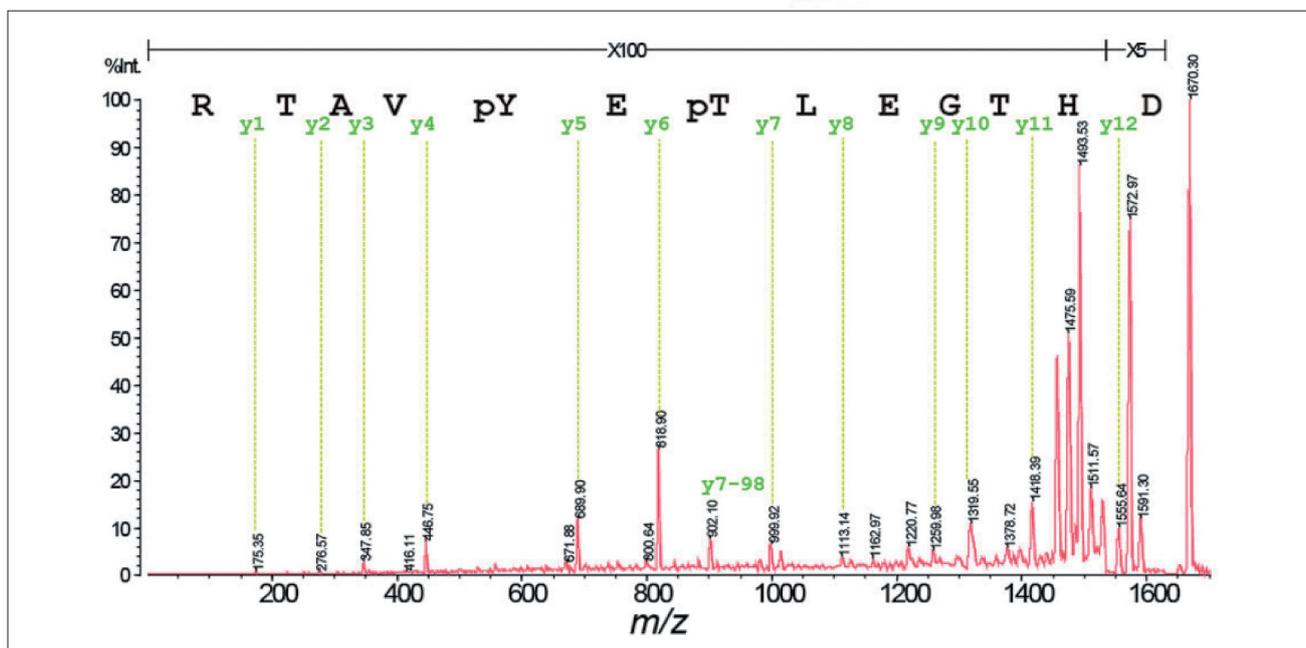


Fig. 1 sPSD Spectrum of MAP Kinase Fragment 177-189 (a doubly-phosphorylated peptide)

### < Analytical Conditions >

Instrument : AXIMA-Performance

Matrix : 2, 5-DHB (dihydroxybenzoic acid) 10 mg/mL (50 % acetonitrile, 0.1 % TFA)

Sample : MAP Kinase fragment 177-189 (doubly-phosphorylated peptide) (Invitrogen)

([M + H]<sup>+</sup> = 1669.7; DHTGELpTEpYVATR)

If a MALDI enrichment plate ( $\mu$ Focus plate, Hudson Surface Technology) is used, even higher sensitivity measurement becomes possible. This plate is designed with a hydrophobic area and a hydrophilic well (diameter 0.6 mm), so that when an aqueous solvent sample is deposited, the sample is concentrated in the hydrophilic well. Since the well diameter of a standard plate is 3 mm, the sample concentration per unit area is 10-fold, thereby improving sensitivity.

Fig. 2 shows the enrichment effect achieved using the  $\mu$ Focus plate. When conducting evaluation using a monophosphopeptide, MS measurement is possible at 750 attomol, and at 1 femtomol or greater, phosphopeptide sequence analysis is shown to be possible by MS/MS (sPSD).

These results suggest that the combination of sPSD and the DHB matrix is a suitable system for analysis of unstable phosphopeptides.

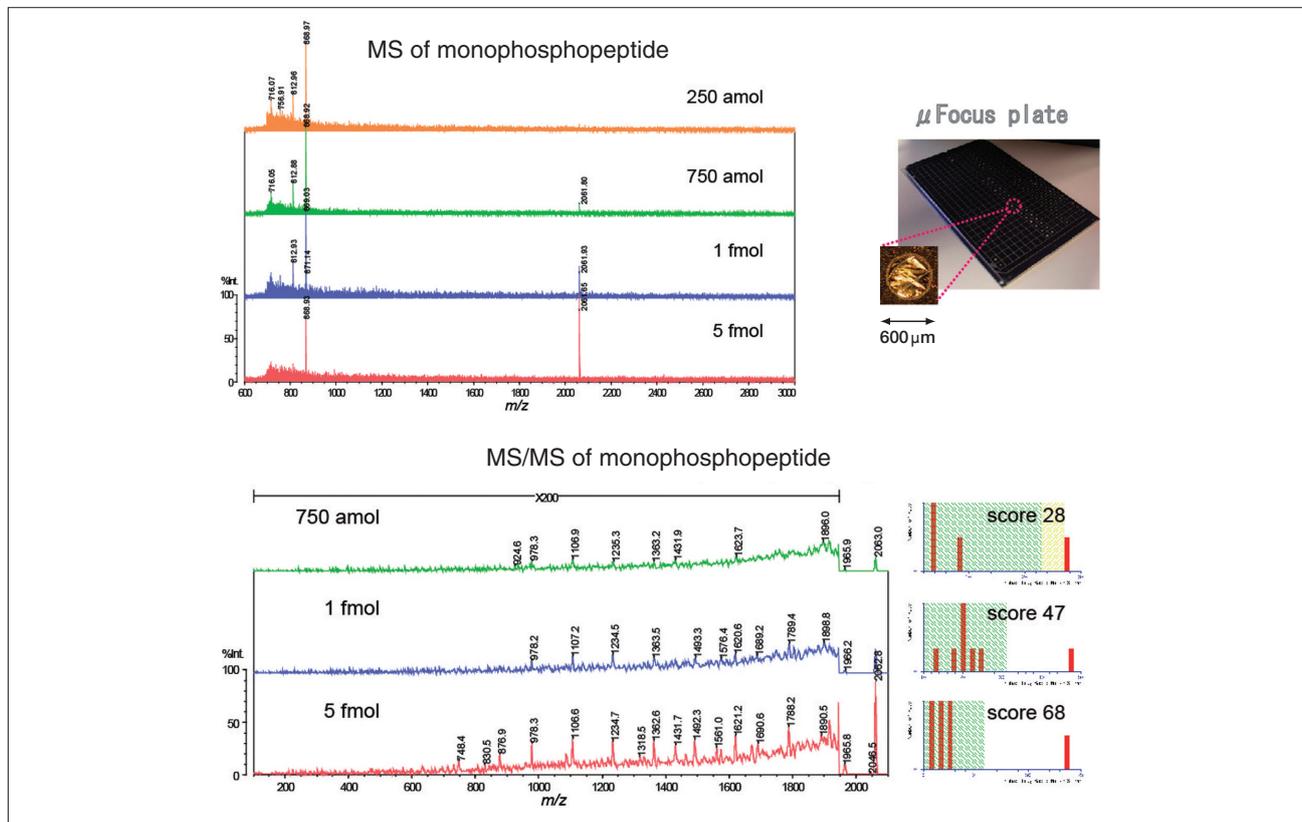


Fig. 2 Detection Sensitivity of a Phosphopeptide Using Enrichment Plate ( $\mu$  Focus plate)

#### <Analytical Conditions>

Instrument : AXIMA-Performance

Matrix : 2, 5-DHB (dihydroxybenzoic acid)  
10 mg/mL (50 % acetonitrile, 0.1 % TFA)

Sample :  $\beta$  casein-derived monophosphopeptide (SIGMA)

#### ■ MALDI-TOF MS Enrichment Plate $\mu$ Focus MALDI Plate

- Features 10 times the sensitivity of conventional plate, improved hydrophobic/hydrophilic membrane
- processing technology
- Accommodates 384 samples
- Excellent cost performance
- Plate is disposable, reducing contamination concerns.

#### Ordering Guide

P/N	Product Description	Qty
HMFP-5M	$\mu$ Focus MALDI plate Magnetic Holder for Shimadzu	1 pc
MFP001BD10	$\mu$ Focus MALDI plate 600um Shimadzu (384 well)	10 pc
MFP002BD10	$\mu$ Focus MALDI plate 1000um Shimadzu (384 well)	10 pc



AXIMA-Performance



HMFP-5M



MFP001BD10

#### [References]

1) Int J Mass Spectrum Ion Process, 169/170, pp231 (1997)

#### NOTES:

\*This Application News has been produced and edited using information that was available when the data was acquired for each article. This Application News is subject to revision without prior notice.



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