SHIMADZU

Application News

MALDI-TOF Mass Spectrometry No.B05

Phosphorylation Analysis by MALDI-TOF MS (1) Measurement Mode and Matrix Selection for Phosphopeptide Detection

Phosphorylation is one type of protein post-translational modification (PTM) that plays a significant role in important biological functions. Mass spectrometry is recently being applied in phosphorylation interrogation, and various results have been reported. In almost all cases, however, the instrument configuration used has been based on electrospray ionization (ESI). Here we introduce a method useful for the detection of phosphopeptides by MALDI technique.

Measurement of peptides is typically conducted in the reflectron mode to achieve higher resolution and accuracy. However, the signal of the phosphopeptide of interest is difficult to detect in this mode due to a phenomenon called neutral loss, in which the phosphate group detaches from the phosphopeptide. Generally, this type of unstable compound is first measured using the linear mode. Referring to Fig. 1, only the signal corresponding to the phosphopeptide was observed in the linear mode. Furthermore, when DHB was used as the matrix, a spectrum in which neutral loss was relatively subdued was obtained even in the reflectron mode.

On the other hand, neutral loss can be used to determine the presence or absence of phosphorylation. If neutral loss can be found through comparison of measurements in the reflectron mode and linear mode, or with DHB and CHCA, there is a high possibility that the peptide in question is phosphorylated.

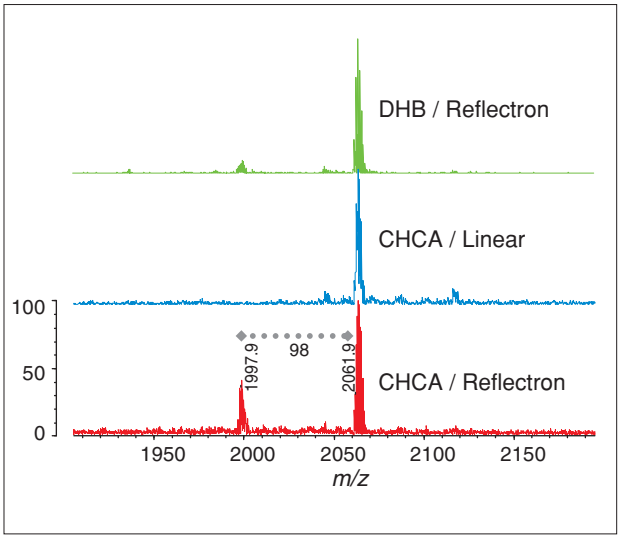

Fig. 1 Effect of Measurement Mode and Matrix

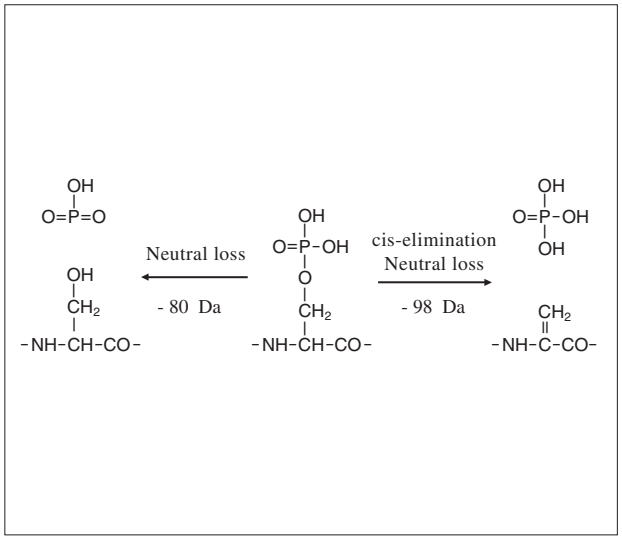

Fig. 2 Neutral Loss of Phospho Group

＜Analytical Conditions＞

Instrument：AXIMA-Performance

Matrix ：10 mg/mL (50 % acetonitrile, 0.1 % TFA)

Sample ：β casein-derived monophosphopeptide (SIGMA)

Another problem to be addressed in phosphopeptide analysis is the dramatically reduced detection sensitivity of the peptide due to phosphorylation modification. This commonly makes it difficult to observe phosphopeptides in protein enzyme digests. Approaches that are employed to resolve this problem include, among others, the addition of phosphoric acid to the matrix1) and increasing the concentration of TFA (trifluoroacetic acid) to maintain acidity. Fig. 3 shows the effect of phosphoric acid addition when DHB is used as the matrix. A sample consisting of a mixture of four types of protein digests was measured in the reflectron mode.

When conducting measurement with DHB alone, phosphopeptides can be observed if they exist in large quantities, but when they are few in number, they can hardly be observed. However, by using a matrix consisting of DHB spiked with phosphoric acid, phosphopeptides were observed in the vicinity of *m/z* 3000.

Thus, by changing the composition of measurement mode and matrix, even mixtures of phosphopeptides can be detected with greater sensitivity. MS/MS and other types of structural analyses are based on this fundamental step.

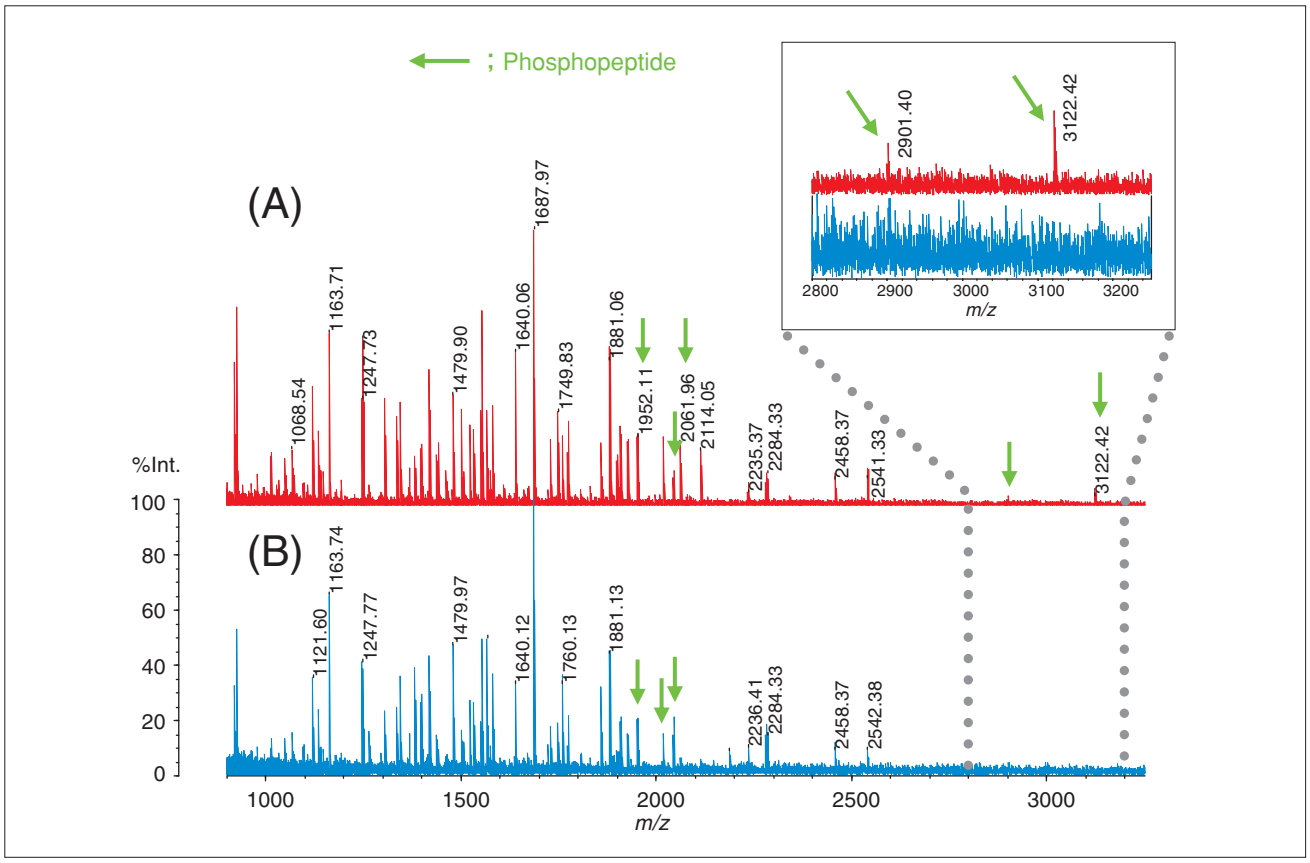

Fig. 3 Effect of Phosphoric Acid Addition
(A) Phosphoric acid addition (0.5%), (B) No addition

＜Analytical Conditions＞

Instrument ： AXIMA-Performance

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

Sample ：Tryptic digest mixture （BSA, α-casein, β-casein, ovalbumin）

[References]
1) Anal Chem., 76 (17), 5109-17, (2004)

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