MS/MS Measurement of Phosphorylated Peptide using AXIMA-TOF²

The AXIMA-TOF² features high-sensitivity MS/MS measurement based on its highly efficient fragment ion formation. This feature of the AXIMA-TOF² is explained below using the analysis of a phosphorylated peptide as an example.

H_2N-FQpSEEQQQTEDELQDK-COOH

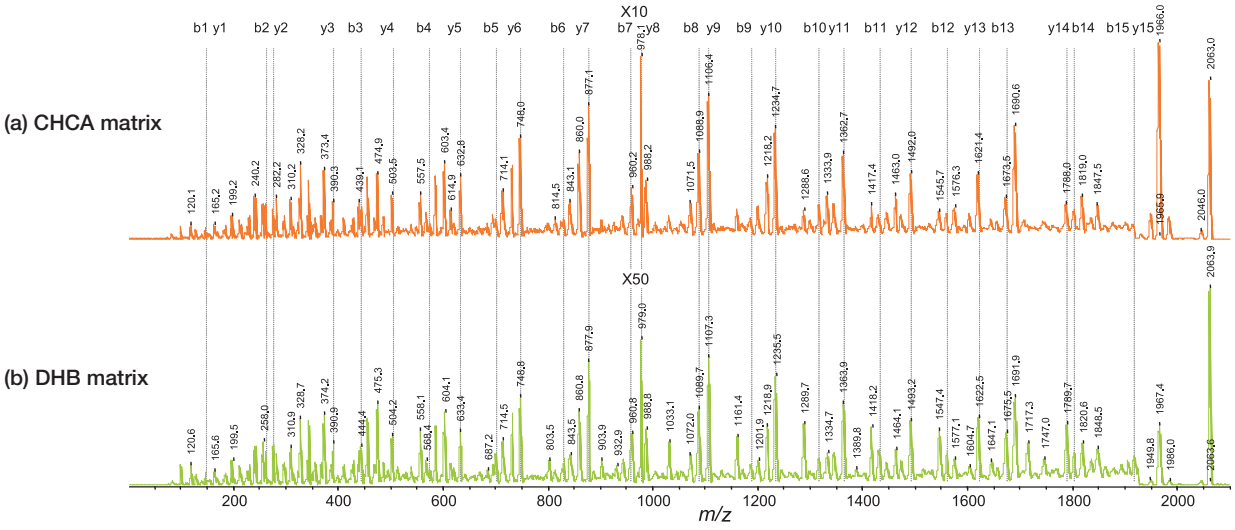

Fig. 1 MS/MS Measurement of Monophosphorylated Peptide (500 fmol)

In MS/MS measurement of phosphorylated peptides, the phosphate group easily detaches (-98 Da), and it is normal for the resulting fragment ions, that impart sequence information, to display weak intensity. However, Fig. 1 (a) shows that those ions are formed with extremely high efficiency. Moreover, if DHB, known as the "Cold Matrix (with low fragmentation efficiency)", is used, an MS/MS spectrum of the same quality as that with CHCA is obtained (Fig. 1(b)).

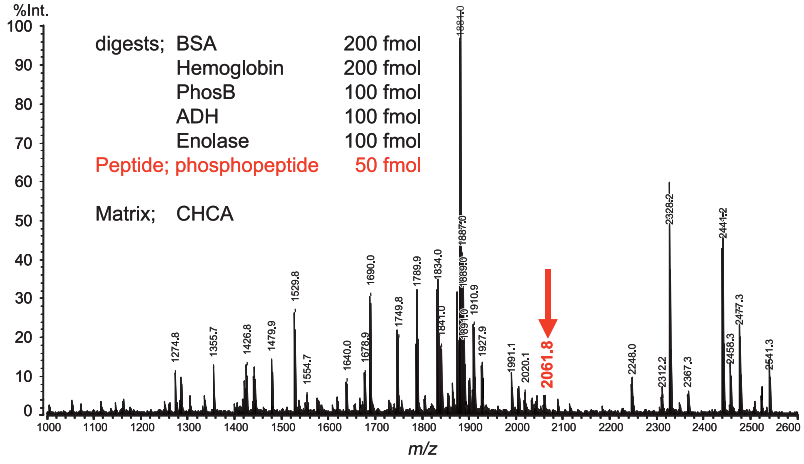

Fig. 2 MS Measurement of Protein Digests and Phosphorylated Peptide Mixture

In Fig. 2, an intentionally complex mixture was prepared to demonstrate the performance of the AXIMA-TOF² using a typical sample. Utilizing the high precursor ion selectivity of the AXIMA-TOF², we performed MS/MS measurement of the phosphorylated peptide (indicated with red arrow) in the mixture.

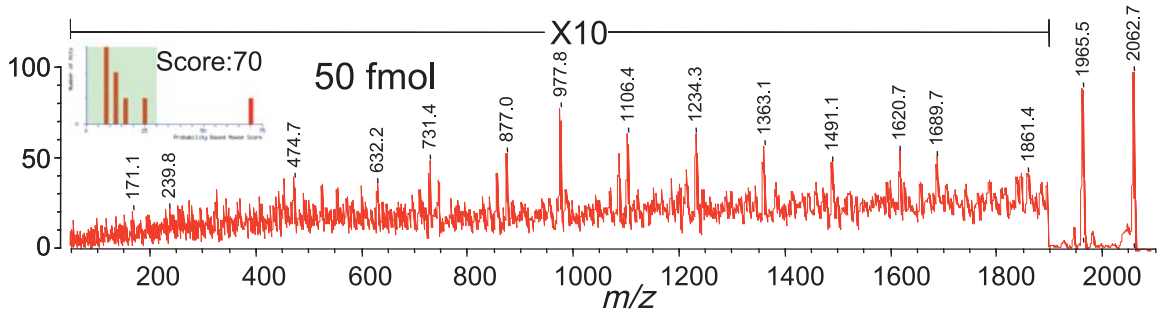

Fig. 3 MS/MS Measurement of Phosphorylated Peptide in Mixture

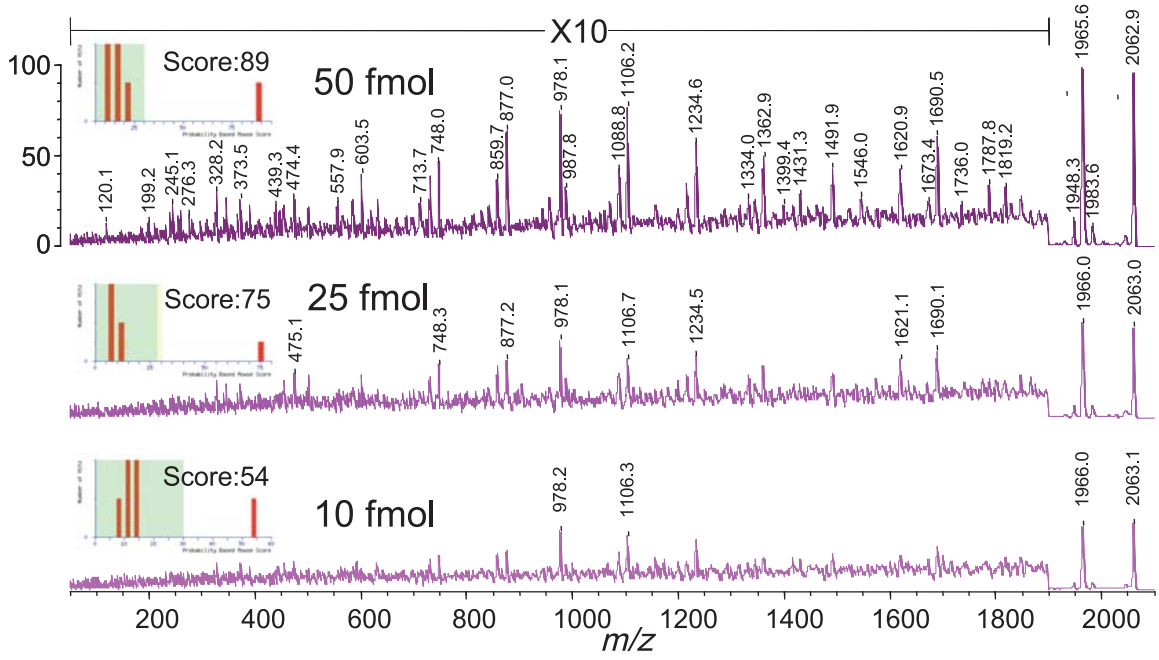

Fig. 4 MS/MS Measurement of Phosphorylated Peptide (isolated)

As shown in Fig. 3, an MS/MS spectrum was obtained that allows identification of the phosphorylated peptide in the mixture, even at 50 fmol level. It is generally acknowledged that the sensitivity of MS/MS measurement of a peptide in a mixture is markedly lower than that obtained when measuring an isolated, purified peptide.

Therefore, we compared the spectrum of Fig. 3 with the corresponding spectrum of the isolated peptide shown in Fig. 4. Comparison of the 50 fmol spectra in these figures showed that, indeed, the MS/MS spectrum of the peptide in the mixture was somewhat inferior to that of the isolated peptide, but the extent of the difference was unexpectedly small.

In this way, it is shown that the AXIMA-TOF^2 is extremely effective in measurement of peptides in complex mixtures, an inherent quality expected of a MALDI-TOF, and also it is applicable for measurement of difficult samples such as those containing phosphorylated peptides.