

Application News

No. B73

MALDI-TOF Mass Spectrometry

Analysis of Phosphoproteins Using a Benchtop MALDI-TOF Mass Spectrometer

MALDI-TOF mass spectrometers are a mass spectrometer type that is used in a wide range of fields likewise LCMS, in terms of high through-put and high sensitivity. These instruments have recently being utilized more and more for simple molecular weight measurement and profiling of synthesized products and high-molecular compounds. This is because instruments of this type have several advantageous features: singly-charged ions are generated so molecular weights can be recognized easily, the mass range is wide, and there are many solvent options because the sample is dried before measurement.

On the other hand, due to changes in social conditions in these several years, government offices, universities, and private enterprises strongly request the reduction of costs for both introduction and running of instruments used for such applications. The benchtop "MALDI-8020" MALDI-TOF mass spectrometer is a new instrument that can sufficiently meet such market needs. The noteworthy point of this instrument is that it has a shorter flight tube, which is the key feature of its small size, while retaining the performance equal to or higher than that of a conventional model.

Development of instruments in recent years is moving toward higher specifications. In the case of MALDI-TOF mass spectrometers, reflectron mode has attracted attention because of its high resolving power and structure analysis function by MS/MS. The usability of linear mode, on the other hand, has been known for a long time; however, there have been no major technical progressions in linear mode recently. The linear mode is, for example, effective in detecting phosphorylation that is a typical post-translational modification of proteins, and in confirming molecular weights of unstable compounds.

This article introduces an example of measuring digests of phosphoprotein and analyzing phosphate modifications using the benchtop MALDI-TOF mass spectrometer "MALDI-8020".

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■ Benchtop MALDI-TOF MS: MALDI-8020

The MALDI-8020 (Fig. 1) is a compact-design, minimal-space linear mode-only MALDI-TOF mass spectrometer. Its performance in linear mode (positive ion) is comparable to the same mode of a conventional MALDI-TOF mass spectrometer. Equipped with a 200 Hz solid-state laser and a load-lock chamber mechanism that enables a target plate change while maintaining the degree of vacuum at the measuring position, the instrument ensures rapid measurements.

The flight tube of this instrument is shortened to 0.85 m long; however, the mass resolving power is as good as that of a conventional model. The instrument has a practical resolving power that enables isotope separation even near m/z 4000.

■ Usability of Linear Mode

The linear mode is effective in measuring high molecular weight compounds and compounds having unstable functional groups. Ionized compounds may be decayed due to their excess internal energy within the free flight space by the MALDI method. Since the velocities of fragment ions generated in this process are the same as that of the original compound, they are recognized as the same molecular weight in the linear mode. On the other hand, in the reflectron mode, such fragments are kinetic-energy dependently separated into masses, which are observed as individual masses. Therefore, mass resolving power and accuracy can be obtained in reflectron mode but there are also disadvantages: when functional groups are desorbed from components, their signals may become too strong, or in an extreme case, molecular ions may not be detected at all.

As shown in Fig. 2 of phosphorylated peptides may fragment easily during the flight. For such compounds, linear mode is suitable for measurement.



Fig. 1 Benchtop MALDI-TOF MS: MALDI-8020

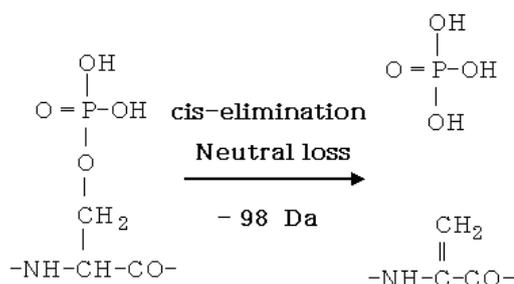


Fig. 2 Ser Phosphate Modification and Neutral Loss

■ Phosphoprotein Sequence Analysis Example

Bovine α -casein, a phosphoprotein, was digested by trypsin, and approx. 1 pmol (converted into protein) was spotted on a thin layer of CHCA matrix and then dried. This digest was analyzed in linear mode using the MALDI-8020. The result is shown in Fig. 3. By conducting a Mascot search, bovine α -casein was identified from the mass spectrum of the detected peptides in the range of m/z 900 to 2500. In addition, as the advantage of linear mode, multiple phosphorylated peptides were detected and by conducting a database search we were able to obtain information about the site of modification (Fig. 4). These results indicate that this instrument has a mass resolving power sufficient for digested peptide analysis. However, no matter how excellent the MALDI-8020 is as a linear mode-only instrument, it is not suitable for large-scale, extensive analyses such as proteome analysis. On the other hand, we consider that useful information can be obtained rapidly by using this instrument such as for identifying isolated and purified proteins by using non-omics techniques or for analyzing post-translational modifications.

■ Conclusion

We performed a sequence analysis using the benchtop MALDI-TOF mass spectrometer "MALDI-8020", and obtained data comparable to that of a large-sized MALDI-TOF mass spectrometer, proving its applicability to phosphoprotein identification and phosphorylated spot analysis.

In recent years, MALDI-TOF mass spectrometers have evolved into high specification instruments and have become large-sized and complicated. As a result, the instruments are typically over-specified for most routine applications requiring linear mode analysis. Size and initial/running costs also pose a high hurdle for introducing the instrument.

The MALDI-8020, the world's smallest commercially available MALDI-TOF mass spectrometer, satisfies the needs of protein analysis in linear mode, and its future dissemination in biochemistry and molecular biology is expected.

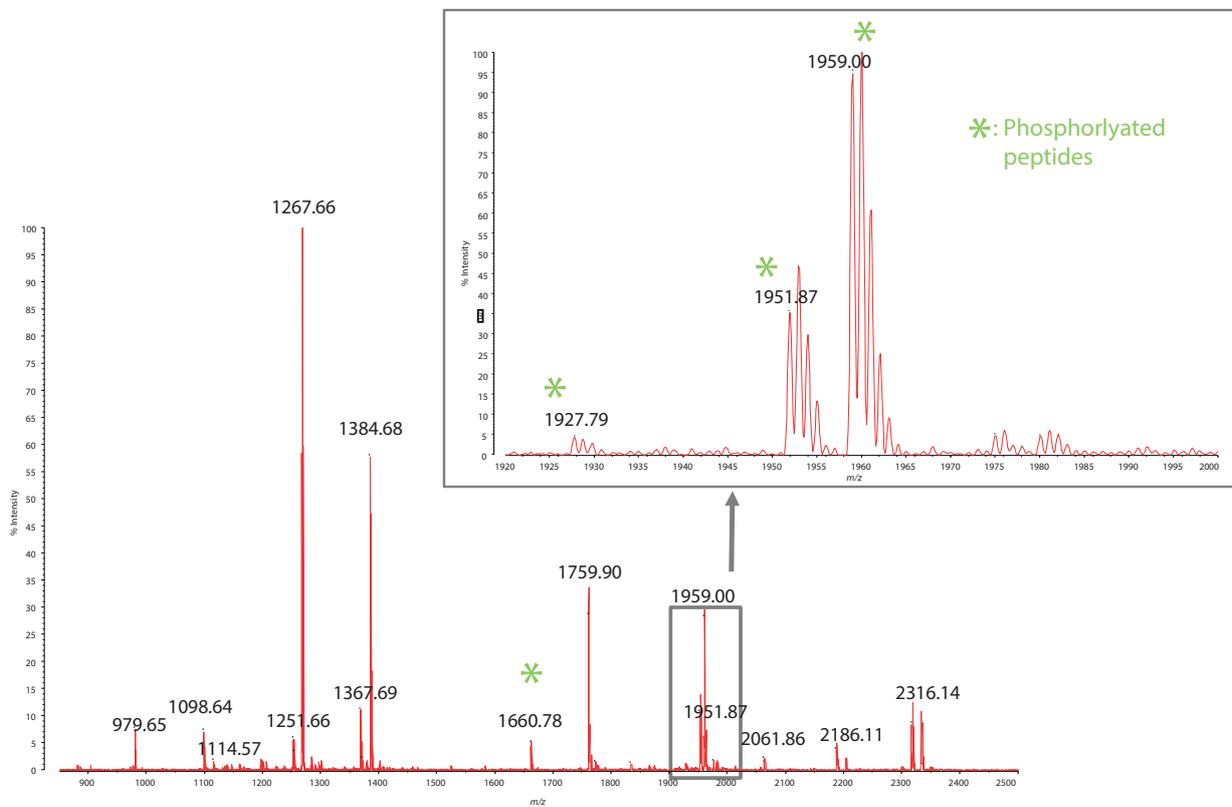


Fig. 3 Mass Spectrum of Bovine α -casein Digested by Trypsin

Start - End	Observed	Mr (expt)	Mr (calc)	Delta M	Peptide
2 - 18	1959.0000	1957.9927	1958.1515	-0.1588	M.KLLILTCLVAVALARPK.H + Phospho (ST)
23 - 37	1759.9000	1758.8927	1758.9377	-0.0449	K.HQGLPQEVLNENLLR.F
38 - 49	1384.6800	1383.6727	1383.7227	-0.0500	R.FFVAPFPEVFGK.E
58 - 73	1927.7900	1926.7827	1926.6843	0.0985	K.DIGSESTEDQAMEDIK.Q + 2 Phospho (ST)
106 - 115	1267.6600	1266.6527	1266.6972	-0.0445	R.YLGYLEQLLR.L
119 - 134	1951.8700	1950.8627	1950.9452	-0.0824	K.YRVPQLEIVPNSAER.L + Phospho (ST)
121 - 134	1660.7800	1659.7727	1659.7869	-0.0142	K.VPQLEIVPNSAER.L + Phospho (ST)
148 - 166	2316.1400	2315.1327	2315.1296	0.0031	K.EPMIGVQELAYFPPELFR.Q

Fig. 4 PMF Result

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