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Application News

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MALDI-TOF Mass Spectrometry

Protein Sequence Analysis by In-Source Decay Using a Benchtop MALDI-TOF Mass Spectrometer

MALDI-TOF mass spectrometers are frequently used for molecular weight measurement and identification of proteins. To identify proteins using a mass spectrometer, generally tryptic digestion is required for preparation; however, if the protein is isolated and purified, by detecting the ions produced by fragmentation within an ion source (ISD, In-Source Decay), sequence analysis of intact proteins can be performed with no need to digest the protein with trypsin.

When measuring molecular weights of proteins using MALDI, reagents (matrices) comprising sinapinic acid are normally used; however, when performing sequence analysis by ISD, 1,5-diaminonaphthalene (DAN) is used as a matrix. By conducting a database search using the fragment ions produced by ISD, e.g. using Mascot, proteins can be identified. In addition, by conducting a homology search for amino acid sequence information obtained from the masses of fragment ions, it is possible to infer the proteins which were not matched against the protein database.

This article introduces an example of measuring molecular weights of proteins and performing sequence analysis of proteins by ISD using a benchtop MALDI-TOF mass spectrometer "MALDI-8020".

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Benchtop MALDI-TOF MS: MALDI-8020

The MALDI-8020 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.

Measurement Example of Protein Molecular Weight

250 fmol (approx. 17 ng) bovine serum albumin (BSA) was mixed with sinapinic acid (10 mg/mL, 50 % acetonitrile/0.1 % trifluoroacetic acid solution) and analyzed in linear mode using MALDI-8020. The result is shown in Fig. 2. The peak of the BSA's singly-charged protonated molecule is detected at *m/z* 66430 with S/N > 100.

As demonstrated by this measurement example, the specifications of linear mode, including sensitivity, mass accuracy and resolving power, compare favorably with those of the same mode of a conventional MALDI-TOF mass spectrometer.

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

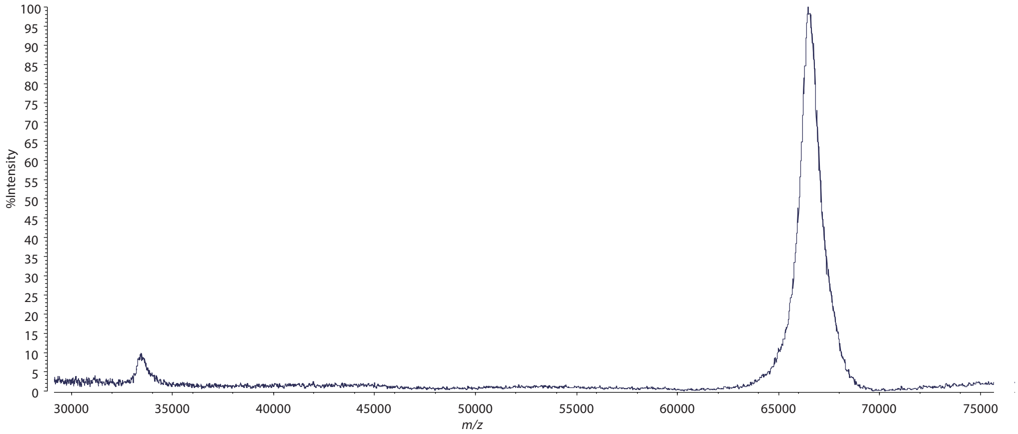


Fig. 2 **Mass Spectrum of a Trace Amount of Protein (Bovine Serum Albumin)**

Example Sequence Analysis of Proteins by ISD

5 pmol BSA was mixed with DAN (10 mg/mL, 50 % acetonitrile/0.1 % trifluoroacetic acid solution), and analyzed in linear mode using MALDI-8020. The result is shown in Fig. 3. The c-series ions, c9 to c44 (except c34, c39 and c42) which are N-terminal fragment ions of BSA produced by ISD, were detected.

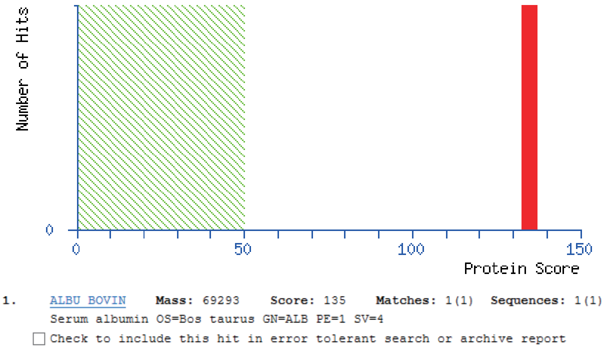
Furthermore, from the mass spectrum shown in Fig. 3, by supposing it to be an MS/MS spectrum in which certain c-ions are detected as precursor ions, it is possible to conduct a database search for protein identification in the same way as an MS/MS spectrum of peptides digested by trypsin. As for the fragment ion information shown in Fig. 3, we set the c33 ion (*m/z* 3886) as a pseudo-precursor ion, selected MALDI-ISD as the instrument type, and conducted a database search of MS/MS ions using Mascot (Matrix Science). As the result of the database search, bovine serum albumin is suggested as the top candidate (Fig. 4).

Conclusion

These examples demonstrate that the benchtop MALDI-TOF mass spectrometer "MALDI-8020" can be used for molecular weight measurement and protein sequence analysis by ISD at the same level as larger-sized MALDI-TOF mass spectrometers.

In recent years, development of MALDI-TOF mass spectrometers has led to larger instruments with high specifications and, due to their size and initial/running costs, there has been a barrier to introducing the instruments for some more routine applications.

The MALDI-8020, the world's smallest class of commercially available MALDI-TOF mass spectrometer, satisfies the needs of protein analysis in linear mode, and its future dissemination and wider use are expected.



1. ALBU_BOVIN Mass: 69293 Score: 135 Matches: 1(1) Sequences: 1(1)
Serum albumin OS=Bos taurus GN=ALB PE=1 SV=4
☐ Check to include this hit in error tolerant search or archive report

Fig. 4 MS/MS Ion Search Result

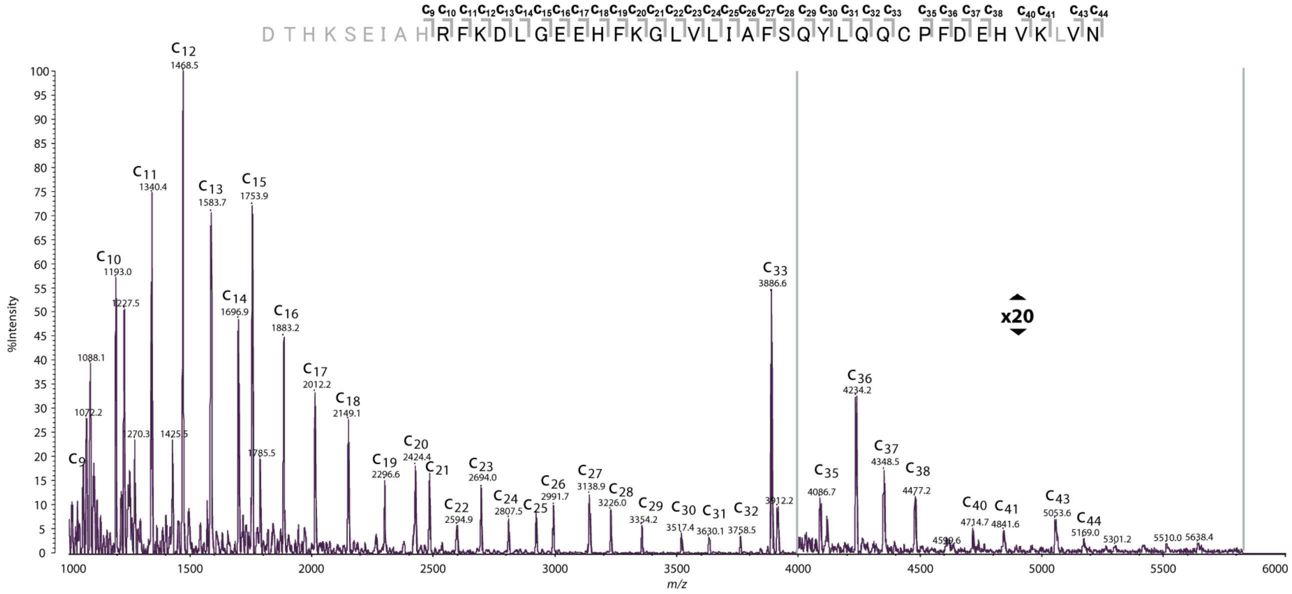


Fig. 3 Sequence Analysis of Bovine Serum Albumin by ISD

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MALDI-8020

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