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Application News

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MALDI-TOF Mass Spectrometry

Protein Identification from Two-dimensional Gel Electrophoresis Based on Peptide Mass Fingerprinting (PMF) Using a Benchtop MALDI-TOF Mass Spectrometer

At present, shotgun proteomics techniques using liquid chromatography mass spectrometry are utilized mainly as high-throughput methods for identifying many different proteins in cellular cytoplasm. However, these techniques are not necessarily effective for identifying all proteins. In particular, when handling proteins separated by means of two-dimensional electrophoresis etc., the protein spots detected on the electrophoresis gel must be linked to the results of protein identification. For such analyses, there may be many cases where using MALDI-TOF mass spectrometry is more efficient than using liquid chromatography mass spectrometry after enzyme-treating the protein spots separated from the gel.

This article introduces an example of protein identification using two-dimensional electrophoresis and a benchtop MALDI-TOF mass spectrometer.

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■ Two-dimensional Electrophoresis of Serum Protein

0.5 µL of human serum was diluted in 100 µL of water. After adding methanol/chloroform/water (3:1:4) solution, the total solution was centrifuged to extract proteins. Then, the extracted proteins were cleaned with methanol and left to dry. A buffer solution for two-dimensional electrophoresis (Swelling Buffer, SHARP) was mixed with reducing agents and ampholytes, and added to the proteins to perform two-dimensional electrophoresis using Auto2D (SHARP). The electrophoresis for the first and second dimensions was performed for a little less than two hours.

The gel resulting from electrophoresis was stained with CBB (InstantBlue, Expedeon Ltd.) to detect protein spots (Fig. 1).

■ Gel Separation, Enzyme Treatment and Salt Removal

Some spots were separated from the obtained two-dimensional electrophoresis gel, destained with 25 mM ammonium bicarbonate/50% acetonitrile, and reduced in 10 mM DTT (dithiothreitol)/50 mM ammonium bicarbonate followed by alkylation in 55 mM IAA (iodoacetamide)/50 mM ammonium bicarbonate. Next, the spots were cleaned sequentially with 50 mM ammonium bicarbonate, 25 mM ammonium bicarbonate/50 % acetonitrile, and acetronitrile to remove the excess volume of DTT and IAA. After removing the excess reagents, the gel was dried with a centrifugal evaporator, and then added an appropriate volume of 20 µg/mL Lys-C (Mass spec grade, Promega) to make the gel swollen on ice. The swollen gel was immersed in 50 mM ammonium bicarbonate and enzyme-treated at 37 °C overnight.

40 µL of 50 % acetonitrile/0.1 % TFA (trifluoroacetic acid) was added to the enzyme-treated gel specimen, and the solution was collected after shaking for 30 minutes. In addition, 40 µL of 75 % acetonitrile/0.1 % TFA was added to the gel specimen and the solution was collected after shaking for 30 minutes. The collected solutions were mixed and dried with a centrifugal evaporator. The protein digest contained in the collected solutions was re-dissolved in 10 µL of 0.1 % TFA, and salt was removed with ZipTip µC18 (Merk Millipore).

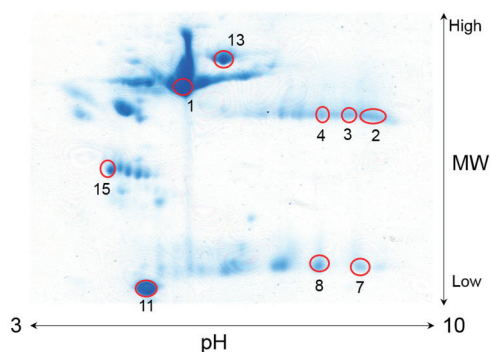**Fig. 1 Two-dimensional Electrophoresis Gel Image of Human Serum Protein**
Red Circles: Separated Spots

■ Mass Spectrometry

Following salt removal, the sample solution was loaded on the MALDI target plate, and 0.5 µL of matrix solution was added for mass spectrometry. The matrix solution was prepared by dissolving CHCA (α-cyano-4-hydroxycinnamic acid) in 50 % acetonitrile/0.05 % TFA at a concentration of 5 mg/mL. As for mass spectrometry, a benchtop MALDI-TOF mass spectrometer "MALDI-8020" (Fig. 2) was used.

Fig. 2 Appearance of Benchtop MALDI-TOF MS "MALDI-8020"

When the protein digest extracted from randomly selected spots was analyzed by mass spectrometry, the clear mass spectrum shown in Fig. 3 was obtained. A mass list was taken from the obtained mass spectrum, and Mascot PMF search was conducted. Proteins shown in Table 1 were identified.

As demonstrated above, a system combining two-dimensional electrophoresis and a benchtop positive linear mode-only MALDI-TOF mass spectrometer "MALDI-8020" can be used for analyses which require protein spot information obtained by electrophoresis and protein identification obtained by mass spectrometry to be linked.
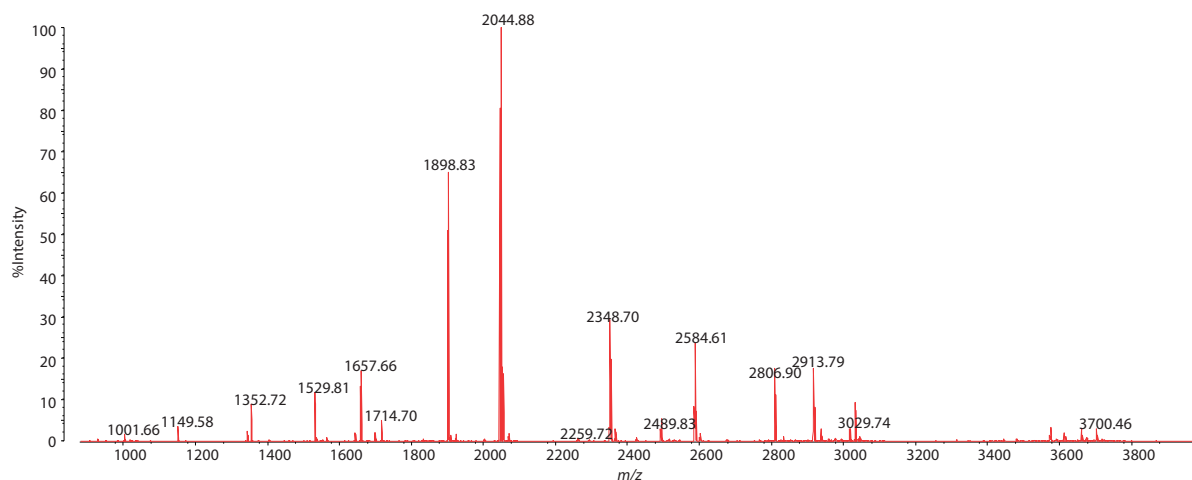
Fig. 3 Mass Spectrum of Spot No. 1
Measurement mode: Positive Linear

Table 1 Identified Proteins in a Randomly Selected Spot
(Parentheses: the items with an insufficient identification score)

Spot	Score	Description of protein
1	116	Serum albumin, human
2	90	Ig gamma-1 chain C region
3	68	Ig gamma-1 chain C region
4	68	Ig gamma-1 chain C region
7	60	Ig kappa chain C region
	59	Ig rambda-2 chain C region
8	60	Ig kappa chain C region
11	(50)	(Apolipoprotein A-1)
12	99	Alpha-1-antitrypsin
13	193	Serotransferrin
15	73	Complement C3
22	(47)	(Vitamin-D binding protein)

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