Applications Note

SpiralTOF™

High Resolution and High Mass Accuracy MALDI-ISD Measurements

Introduction

Matrix assisted laser desorption/ionization (MALDI) combined with in-source decay (ISD) is a useful tool for doing top-down sequencing of intact proteins. This technique can provide enough information to determine both N- and C-terminal sequences. In this work, we measured the ISD fragment ions generated for several peptides using the JEOL SpiralTOF MALDI-MS system.

Experimental

ACTH18-39 and oxidized Insulin B chain peptide samples were separately dissolved in 0.1% trifluoroacetic acid aqueous with the concentration fixed at 10 pmol/µL. 1,5-diaminonaphtalene (DAN), which can provide good S/N for ISD fragment ions [1], was used as the MALDI matrix. The DAN matrix was dissolved to 0.1% trifluoroacetic acid aqueous/50% acetonitrile with the matrix concentration fixed at 10 mg/mL. Subsequently, the matrix and sample solutions were mixed 1/1 (v/v), and then 1 µL of each solution was deposited and dried on the MALDI target plate. Afterwards, each sample was analyzed in triplicate on the JEOL JMS-3000 SpiralTOF MALDI-MS system.

Results

As a starting point, MALDI imaging was used to visualize the location of the sample ion distributions within the DAN crystal matrix. A comparison between an optical microscope image and the MALDI image for the oxidized Insulin B chain y10 fragment ion (*m/z* 1215.6) are shown in Figure 1. These results clearly showed that the ISD fragment ions were concentrated along the edges of the DAN matrix crystals. As a result, the MALDI laser irradiation for each sample spot was focused on the crystal edges in order to obtain sufficient sensitivity for the ISD measurements.

Afterwards, the ISD mass spectra were measured for each peptide, and the results are shown in Figure 2. The ACTH18-39 peptide produced ISD spectra that mostly consisted of the c-ion series. However, for the oxidized Insulin B chain, y-ion series dominated the ISD spectra. It should be noted here that when the basic amino acids are located on the N-terminal side, c-ion series are the dominant ions generated during ISD. However, when the basic amino acids are located on the C-terminal side, the y-ion series are the dominant ions generated during ISD [2]. These results highlight the difference in location for the basic amino acids for each of the peptide chains tested.

Next, each ISD fragment ion series was evaluated for mass accuracy using an external calibration. These analyses showed an average RMS of 4.0 ppm for the ACTH18-39 c ion series and an average RMS of 4.7 ppm for the y-ion series produced from the oxidized Insulin B chain, as shown in Tables 1 and 2, respectively.

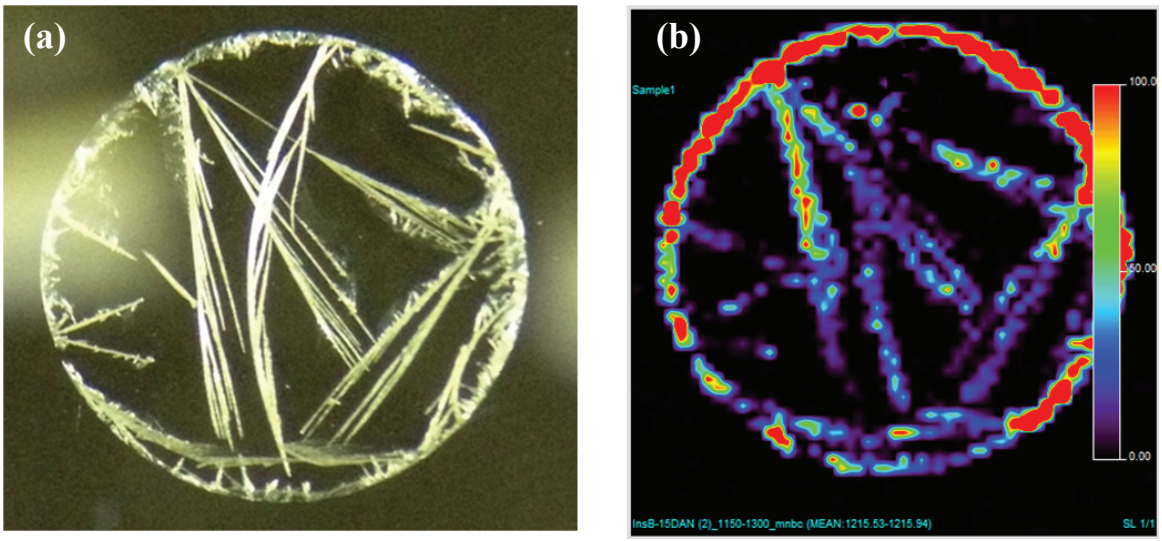

Figure 1. (a) Optical microscope image of DAN crystal, (b) MALDI imaging of *m/z* 1215.6 (y10 ISD fragment ion of oxidized Insulin B chain (width 3.0mm x height 3.0mm, interval 0.05mm).

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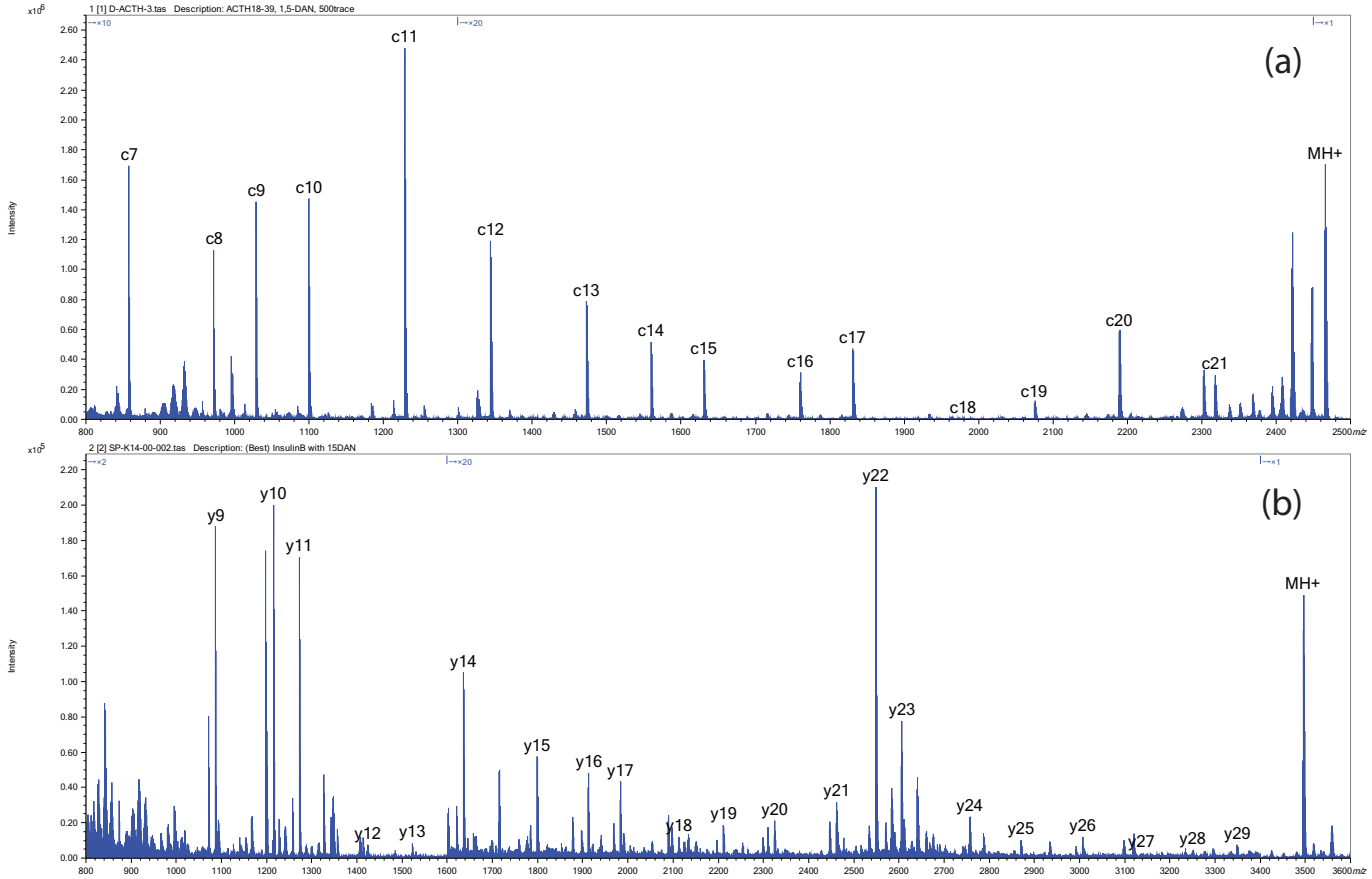

Figure 2. ISD mass spectra for (a) ACTH18-39, (b) oxidized Insulin B chain.

Ion species	Calc. m/z	Mass error (ppm)		
		Data 1	Data 2	Data 3
c7	857.5356	−3.73	13.18	2.92
c8	971.5785	−1.96	12.87	3.50
c9	1028.6000	−0.49	11.76	3.01
c10	1099.6371	−0.36	11.55	2.46
c11	1228.6797	1.06	10.09	2.28
c12	1343.7066	−0.52	7.59	0.52
c13	1472.7492	0.95	6.52	−0.88
c14	1559.7812	0.26	4.62	−1.15
c15	1630.8184	−0.06	3.07	−1.59
c16	1759.8610	−1.88	1.99	−2.84
c17	1830.8981	−0.66	0.44	−4.64
c18	1977.9665	6.77	−3.08	−6.32
c19	2075.0192	−1.11	−0.82	−5.73
c20	2188.1033	−5.12	−4.30	−8.91
c21	2317.1459	−4.19	−3.88	−8.03
Abs. Average		**1.94**	**6.38**	**3.65**

Table 1. Accurate mass measurement results for c-ion series of ACTH18-39.

Ion species	Calc. m/z	Mass error (ppm)		
		Data 1	Data 2	Data 3
y9	1086.5731	1.76	0.10	−0.08
y10	1215.6157	0.76	−0.48	−1.22
y11	1272.6372	−0.98	−0.04	−1.06
y12	1423.6311	−11.51	−11.51	−9.12
y13	1522.6995	−9.25	−4.98	−7.81
y14	1635.7836	−4.19	−5.41	−3.82
y15	1798.8469	−4.55	−6.27	−7.49
y16	1911.9310	−8.29	−4.89	−9.81
y17	1982.9681	−3.56	−6.33	−5.33
y18	2112.0107	2.30	−6.37	−3.29
y19	2211.0791	−4.42	−8.94	−5.69
y20	2324.1631	−3.97	−5.31	−8.92
y21	2461.2220	−1.15	−1.15	0.96
y22	2548.2541	−2.77	−4.42	−3.44
y23	2605.2755	−3.82	−4.81	−2.28
y24	2756.2695	−5.68	−5.43	−2.96
y25	2869.3535	−4.47	−8.76	−2.87
y26	3006.4124	−5.47	−0.55	−4.40
y27	3134.4710	−6.13	−13.47	16.65
y28	3248.5139	−1.40	−16.76	−13.22
y29	3347.5824	3.33	−6.83	4.04
Abs. Average		**4.37**	**5.10**	**4.55**

Table 2. Accurate mass measurement results for y-ion series of oxidized Insulin B chain.

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Conclusion

In this work, we showed a brief study in which the ISD measurements for standard peptides were measured by using the SpiralTOF. It should be noted that ISD measurements generally require a relatively high laser power to generate ISD fragment ions which in turn detrimentally impacts the mass resolving power of the instrument. As a result, it is generally very difficult to achieve high mass resolving power using a high laser power. However, the Spiral trajectory ion optics provides a 17m flight path that overcame this problem to produce resolving powers of 20,000-35,000 for each of the measured ions. Furthermore, as shown with these peptide sequences, the system provided excellent mass accuracy even when an external calibration was used for the data.

Reference

[1] Issey Osaka, Mami Sakai, Mitsuo Takayama, 5-Amino-1-naphthol, a novel 1,5-naphthalene derivative matrix suitable for matrix-assisted laser desorption/ionization in-source decay of phosphorylated peptides, Rapid Communications in Mass Spectrometry, Volume 27, Issue 1, pages 103–108, 15 January 2013.

[2] Mitsuo Takayama, The Characteristics of In-source Decay in Mass Spectrometric Degradation Methods _Hydrogen-Attachment Dissociation (HAD)_, J. Mass Spectrom. Soc. Jpn., Vol. 50, No. 6, 2002.

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