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Application News

MALDI-TOF Mass Spectrometry No.B16

Protein Sequencing Using In-Source Decay

The MALDI-TOF mass spectrometer can detect ions that are formed due to fragmentation within the ion source (ISD, In-Source Decay). When ISD occurs in analysis of proteins, primarily it is the c-type ion (Fig. 1) of the N-terminus that is detected.

Up to now, 2,5-dihydroxybenzoic acid (DHB) has been used widely as a matrix for inducing ISD, but recently, 1,5-diaminonaphtalene (DAN) has been reported to be a more effective matrix in this regard (see References). Amino acid sequence information is easily acquired by detection of ISD ions using DAN, and highly homologous proteins can be investigated using homology searches.

Fig. 2 shows a mass spectrum (linear mode) of bovine serum albumin (BSA) obtained using the DAN matrix. Assignment of c9 – c41 (excluding c34 and c39) was achieved for the amino acid sequence of the BSA N-terminus. The peak assignment (Leu/Ile and Lys/Gln could not be distinguished) operation was conveniently facilitated using the Database Viewer function provided in the AXIMA software.

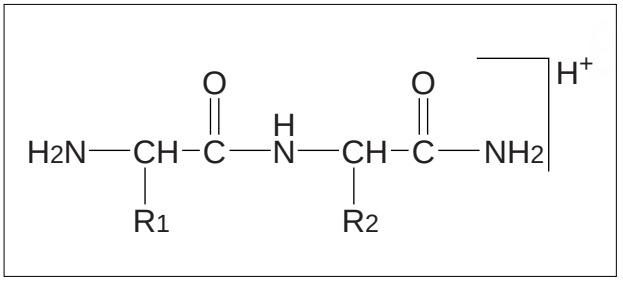
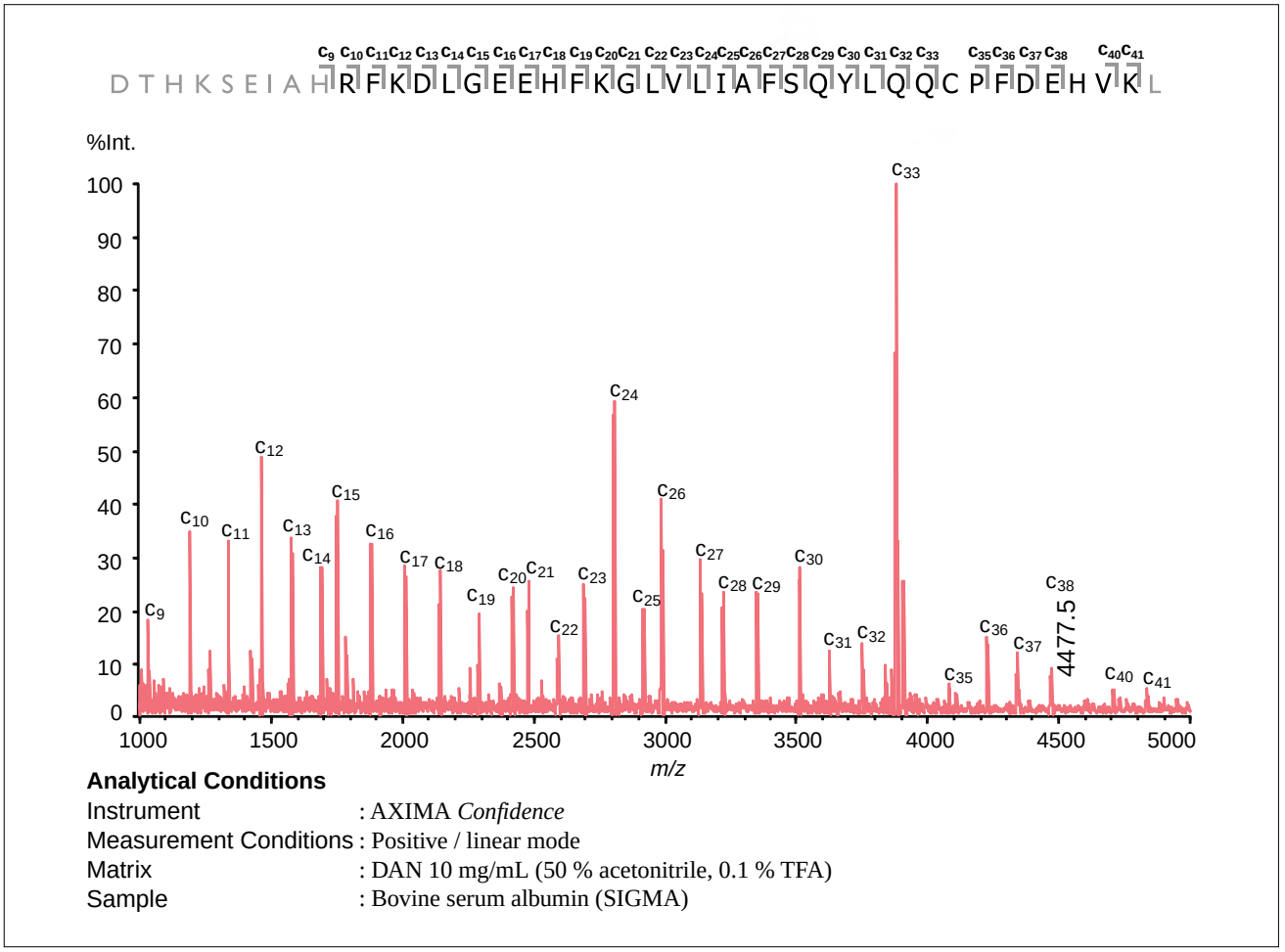


Fig. 1 Structure of c-Type Ion



Analytical Conditions

Instrument	: AXIMA *Confidence*
Measurement Conditions	: Positive / linear mode
Matrix	: DAN 10 mg/mL (50 % acetonitrile, 0.1 % TFA)
Sample	: Bovine serum albumin (SIGMA)

Fig. 2 Mass Spectrum of BSA and Assignment of ISD Ions

It is possible to conduct an MS/MS ion search of an ISD spectrum in the same manner as when analyzing the MS/MS spectrum of a peptide. In Fig. 2 a specific c-ion (c38 *m/z* 4477.5) was selected as a "pseudo" precursor based on an assumption of the type of MS/MS spectrum that would be obtained. An MS/MS ion search (MASCOT) was conducted (Fig. 3) using C-terminus amidation as a variable modification and by selecting FTMS-ECD for the instrument type which includes the c-type ions in the database search. Considering the mass error of the ISD ions obtained in linear mode, the search results were obtained using a relatively high mass tolerance setting. Serum albumin precursor (bovine) was identified as the protein with the highest correlation (score of 248). Assignment was achieved for the N-terminus c-ion residues 10 – 37, except for c34.

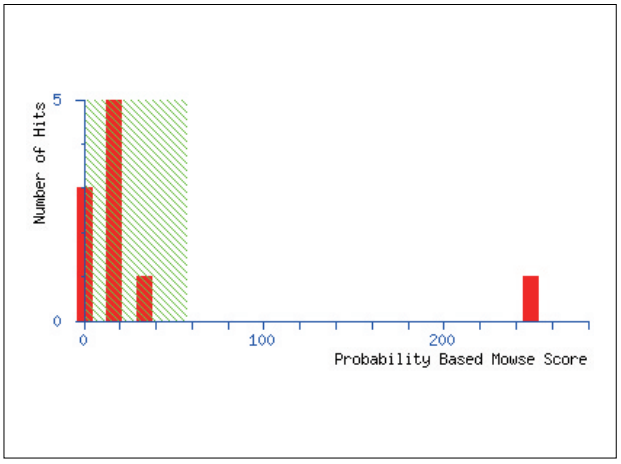


Fig. 3 MS/MS Ion Search Result

#	c	Seq.	y	z+1	z+2	#
1	133.1253	D				38
2	234.2292	T	4362.8974	4346.8748	4347.8827	37
3	371.3685	H	4261.7935	4245.7709	4246.7788	36
4	499.5408	K	4124.6542	4108.6316	4109.6395	35
5	586.6181	S	3996.4819	3980.4593	3981.4672	34
6	715.7321	E	3909.4046	3893.3820	3894.3899	33
7	828.8897	I	3780.2906	3764.2680	3765.2759	32
8	899.9676	A	3667.1330	3651.1104	3652.1183	31
9	1037.1069	H	3596.0551	3580.0325	3581.0404	30
10	1193.2926	R	3458.9158	3442.8932	3443.9011	29
11	1340.4665	F	3302.7301	3286.7075	3287.7154	28
12	1468.6388	K	3155.5562	3139.5336	3140.5415	27
13	1583.7262	D	3027.3839	3011.3613	3012.3692	26
14	1696.8838	L	2912.2965	2896.2739	2897.2818	25
15	1753.9351	G	2799.1389	2783.1163	2784.1242	24
16	1883.0491	E	2742.0876	2726.0650	2727.0729	23
17	2012.1631	E	2612.9736	2596.9510	2597.9589	22
18	2149.3024	H	2483.8596	2467.8370	2468.8449	21
19	2296.4763	F	2346.7203	2330.6977	2331.7056	20
20	2424.6486	K	2199.5464	2183.5238	2184.5317	19
21	2481.6999	G	2071.3741	2055.3515	2056.3594	18
22	2594.8575	L	2014.3228	1998.3002	1999.3081	17
23	2693.9886	V	1901.1652	1885.1426	1886.1505	16
24	2807.1462	L	1802.0341	1786.0115	1787.0194	15
25	2920.3038	I	1688.8765	1672.8539	1673.8618	14
26	2991.3817	A	1575.7189	1559.6963	1560.7042	13
27	3138.5556	F	1504.6410	1488.6184	1489.6263	12
28	3225.6329	S	1357.4671	1341.4445	1342.4524	11
29	3353.7621	Q	1270.3898	1254.3672	1255.3751	10
30	3516.9354	Y	1142.2606	1126.2380	1127.2459	9
31	3630.0930	L	979.0873	963.0647	964.0726	8
32	3758.2222	Q	865.9297	849.9071	850.9150	7
33	3886.3514	Q	737.8005	721.7779	722.7858	6
34	3989.4943	C	609.6713	593.6487	594.6566	5
35	4086.6095	P	506.5284	490.5058	491.5137	4
36	4233.7834	F	409.4132	393.3906	394.3985	3
37	4348.8708	D	262.2393	246.2167	247.2246	2
38		E	147.1519	131.1293	132.1372	1

[References]
Demevre, K. et al, Anal. Chem., 79, 8678-85,(2007)
Fukuyama, Y.et al, J. Mass Spectrum., 41, 191-201,(2006)

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