AXIMA

Characterization of *Salmonella typhimurium* membrane proteins separated by 2D gels

The AXIMA-CFR high performance MALDI-TOF with curved field reflectron, was used to analyze a membrane fraction of *S. typhimurium* proteins resolved by 2D gel electrophoresis.

The rapid acquisition of high quality seamless PSD data allowed unequivocal identification and characterization of co-migrating proteins which upon tryptic digestion had generated peptides of a similar nominal mass.

SHIMADZU BIOTECH

bringing analysis to life

Characterization of *Salmonella typhimurium* Membrane Proteins Separated by 2D Gels

Introduction

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI)[1] is now widely used in the field of proteomics. Key requirements in this area are: high sensitivity, high resolution and mass accuracy and rapid analysis for high throughput. Both precursor and product ion (in the form of PSD) data can be used for the positive identification of proteins. Until now, with conventional reflectron designs[2], the acquisition of post-source decay spectra (PSD) has been quite time consuming requiring multiple analyses at varied reflectron voltages and stitching together of the resulting spectra. The incorporation of the curved-field reflectron[3] into MALDI TOF instruments significantly increased the speed and sensitivity of the PSD analysis by enabling the acquisition of a fully focused PSD spectrum in a single analysis: seamless PSD (sPSD). All the advantages of the curved-field reflectron have now been integrated into a high resolution/high sensitivity MALDI TOF instrument[4], the AXIMA-CFR.

Proteomics provides a powerful tool for exploring protein levels at the whole cell level. It can be used to determine the response of bacteria to their environment and as such, provides a fingerprint of the status of the cell at a given point in time. This strategy can be used to examine membrane preparations of bacteria for the presence of surface proteins and transport proteins. The AXIMA-CFR was used to analyze a membrane fraction of *Salmonella typhimurium* proteins which had been resolved by 2D gel electrophoresis (Figure 1).

Method

Protein spots were treated by in-gel digestion using trypsin, the peptides extracted and then desalted prior to MALDI sample preparation. The curved-field reflectron MALDI TOF mass spectrometer (AXIMA-CFR) was used, which comprises: UV nitrogen laser (337 nm), near ultra high vacuum system with novel laser beam focusing mechanism for increased ion extraction efficiency and increased sensitivity, an integrated 1 GHz transient recorder, optimized ion optics for high resolution and accuracy, a high resolution ion gate for parent ion selection for sPSD analyses, and a microplate-type target.

All the MALDI analyses, for each sample, were performed on a single 0.5 µl aliquot using the dried droplet sample loading technique with α-cyano-4-hydroxy cinnamic acid matrix saturated in 1:1 acetonitrile/0.1% TFA.

Database searches were performed using the Mascot™ search engine (Matrix Science).

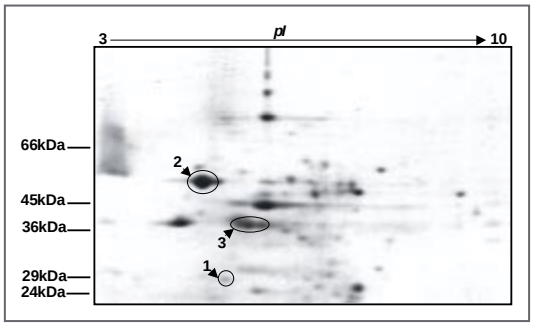

Figure 1. 2D Gel of the membrane fraction of Salmonella typhimurium *proteins. Spots identified by MS analysis: 1. OMP D (outer membrane protein D); 2. FliC (phase-I Flagellin); 3. OMP A and EF-Tu (Outer Membrane Protein A precursor and Elongation Factor-Tu)*

Results

The performance of the instrument in relation to in-gel digest of 2D gel separated proteins is illustrated with an example from *Salmonella typhimurium* (protein spot 3). The following observations were made:-

1 A 40 kDa protein (Coomassie Blue stained) digested with trypsin generated a complex series of signals in the mass range 800 to 3500 Da (Figure 2). Isotopic resolution was obtained across the whole mass range at a single pulsed extraction value. This enabled the monoisotopic peak from each signal to be used in the Mascot™ peptide mass fingerprint search engine and resulted in the primary identity of the protein to be outer membrane protein A (OMP A) from *Salmonella typhimurium* (Table 1).

	Accession	Mass	Score	Description
1.	gi|1279140	37566	66	OUTER MEMBRANE PROTEIN A PRECURSOR (OUTER MEMBRANE PROTEIN
2.	gi|1279143	37710	57	OUTER MEMBRANE PROTEIN A PRECURSOR (OUTER MEMBRANE PROTEIN
3.	gi|7444571	44130	52	probable integrase - Rhodobacter capsulatus prophage cp4-57
4.	gi|1279137	26128	48	OUTER MEMBRANE PROTEIN A (OUTER MEMBRANE PROTEIN II)
5.	gi|2507935	26124	48	outer membrane protein A - Escherichia fergusonii (ATCC 354
6.	gi|1279139	37176	47	OUTER MEMBRANE PROTEIN A PRECURSOR (OUTER MEMBRANE PROTEIN
7.	gi|7388818	37168	44	(AF134269) outer membrane protein A [Escherichia coli]
8.	gi|7364451	60797	43	(AB000974) ATP-dependent Clp protease subunit ClpAlike [Ent
9.	gi|2828868	16207	43	pseptin - Moraxella bovis
10.	gi|2947721	42150	42	Chain A, Elongation Factor Complex Ts-TuEF-Ts From Escheric
11.	gi|133284	37673	41	DNCA PROTEIN
12.	gi|4455921	42155	41	Chain A, Intact Elongation Factor From E.Coli
13.	gi|1352132	43256	41	ELONGATION FACTOR TU (EF-TU)
14.	gi|1352283	43286	41	ELONGATION FACTOR TU (EF-TU) (P-43)
15.	gi|296738	43224	41	translation elongation factor EF-Tu.A - Salmonella typhimur
16.	gi|2388396	43256	41	translation elongation factor EF-Tu.A - Escherichia coli
17.	gi|1169925	107097	41	MINOR TRICHOIC ACID2 BIOSYNTHESIS PROTEIN GGAB
18.	gi|6268727	37348	41	(AL139078) possible sugar nucleotide epimerase/dehydratase
19.	gi|6225188	21940	41	GTP-BINDING PROTEIN CGRA
20.	gi|6256284	107371	40	(AF136191) BsiIM (Bacillus sp. NRS-606)

1. gi|1279140 Mass: 37566 Score: 67
OUTER MEMBRANE PROTEIN A PRECURSOR (OUTER MEMBRANE PROTEIN 3IE) (OU

Observed	Mr(expt)	Mr(calc)	Delta	Start	End	Miss	Peptide
872.09	871.07	871.51	-0.44	223 -	229	1	RVEIRVK
915.20	914.19	914.52	-0.33	95 -	103	0	AQVVQLTAK
1063.10	1062.10	1062.54	-0.36	223 -	231	0	RVQLPHPRK
1187.27	1186.26	1186.59	-0.33	322 -	332	0	AALIDCLASDR
1233.26	1232.26	1232.63	-0.38	118 -	128	1	LGGNVNRADTK
1264.36	1263.36	1263.65	-0.30	256 -	267	0	DGSVVVLCFTDR
1361.15	1360.15	1360.65	-0.50	268 -	280	0	IGSDATVKQLSER
1470.93	1469.92	1469.70	0.22	82 -	94	1	HFTNSGDWINGATK
1608.30	1607.30	1607.81	-0.43	104 -	117	0	LGTDITDDLDVTTR
2302.30	2301.29	2302.20	-0.91	195 -	217	0	FGSQSAAFVVAPAPAPAPAPVQTK

2. gi|1279143 Mass: 37710 Score: 57
OUTER MEMBRANE PROTEIN A PRECURSOR (OUTER MEMBRANE PROTEIN II)

Observed	Mr(expt)	Mr(calc)	Delta	Start	End	Miss	Peptide
872.09	871.07	871.51	-0.44	354 -	360	1	RVEIRVK
915.20	914.19	914.52	-0.33	95 -	103	0	AQVVQLTAK
1027.27	1026.26	1026.58	-0.32	294 -	303	1	GIPAPKIEAD
1063.10	1062.10	1062.54	-0.36	224 -	232	0	RVQLPHPRK
1187.27	1186.26	1186.59	-0.33	313 -	323	0	AALIDCLASDR
1233.26	1232.26	1232.63	-0.38	118 -	128	1	LGGNVNRADTK
1641.39	1640.38	1639.81	0.57	104 -	117	0	LGVDITDDLDVTTR
1803.41	1802.40	1802.98	-0.58	283 -	299	1	AQVVVDLTLSNEDIRAK
2672.02	2671.02	2670.29	0.73	257 -	281	1	DGSVVVLGTTDRIGSEAYNQGLSER

13. gi|119212 Mass: 43256 Score: 42
ELONGATION FACTOR TU (EF-TU)

Observed	Mr(expt)	Mr(calc)	Delta	Start	End	Miss	Peptide
927.25	926.24	926.49	-0.25	119 -	124	0	EKILLGR
1027.27	1026.26	1026.58	-0.32	271 -	280	0	AGEMVSVLLK
1214.29	1213.29	1213.62	-0.34	305 -	314	0	FESNVTILSK
1218.34	1217.33	1217.66	-0.33	178 -	188	0	ALEGDAEWEAK
1233.26	1232.26	1232.61	-0.35	326 -	334	0	GTEPQFTVR
1617.19	1616.18	1615.73	0.45	250 -	263	1	EIGHSICTCGVEMKR
1803.41	1802.40	1802.88	-0.48	60 -	75	0	GITINTAHVEYDTPTR
1964.19	1963.17	1963.96	-0.78	156 -	172	0	ELLEQIGFNPERDTPIVR

Table 1. Mascot™ database search results

2 Fourteen sPSD spectra were generated for twelve of the signals from a single loading of the desalted sample to the target. Selected sPSD spectra were used to confirm the identity of the protein using the Mascot™ MS/MS ion search engine. The results from these searches (Figures 3-7) indicated the presence of a second protein in the 2D gel spot - Elongation Factor-Tu (EF-Tu). A review of the parent mass searches indicated that EF-Tu was also present in the search results though at a lower score. A complete analysis of the parent and sPSD spectra is summarized in Table 2 indicating that there is strong evidence for the presence of both proteins in the same 2D gel spot. Assignment of all the signals from the parent spectrum to the sequences of the two proteins indicated that 60% sequence coverage was obtained for OMP A and 30% for EF-Tu.

3 sPSD analysis and database searching of the 1641 Da signal (Figure 5) corresponded to a peptide, LGYPITDDLDVYTR, found in a variant of OMP A that was different from the one identified from the parent mass fingerprint. The published sequence of this variant had only one amino acid difference (F114 <-> V114) from the form identified by the peptide mass fingerprint. The equivalent peptide, LGYPITDDLDFYTR, was also observed in the parent spectrum, though not subjected to sPSD, which indicated that both forms of OMP A were present in the 2D gel protein spot.

4 sPSD analysis of the 1617 Da signal was useful in identifying one of two OMP A peptides of similar mass. sPSD fragmentation confirmed that the signal corresponded to OMP A 250-263 (theoretical mass MH^+ = 1616.74) and not OMP A 46-59 (theoretical mass MH^+ = 1617.79).

5 Further, sPSD analysis of the 1233 Da peak was useful in identifying one of two OMP A peptides of similar mass. sPSD fragmentation confirmed that the signal corresponded to OMP A 326-334 (theoretical mass MH^+ = 1233.62) and not EF-Tu 118-128 (theoretical mass MH^+ = 1233.64).

6 Even low intensity signals such as 1803 Da generated useful sPSD data (Figure 6) for the structural confirmation and correct protein identity.

7 Some signals (e.g. 1438 Da) did not correspond to peptides from any of the three proteins identified. These signals may represent post transitional modifications and will be subject to further investigation by sPSD.

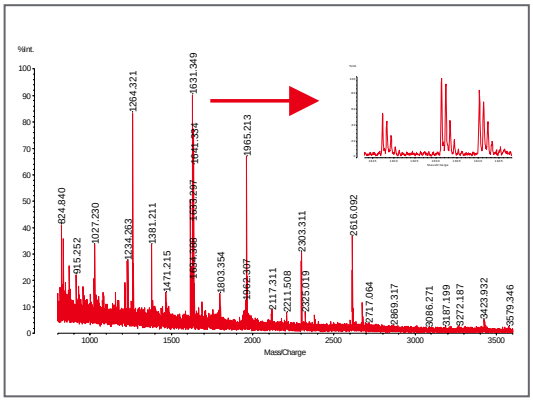

Figure 2. Parent spectrum indicating signals from Outer Membrane Protein A and Elongation Factor-Tu

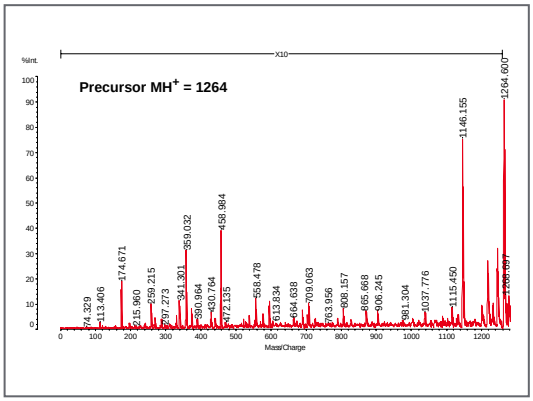

Figure 3. sPSD of 1264.6 Da precursor supporting the identity of Outer Membrane Protein A

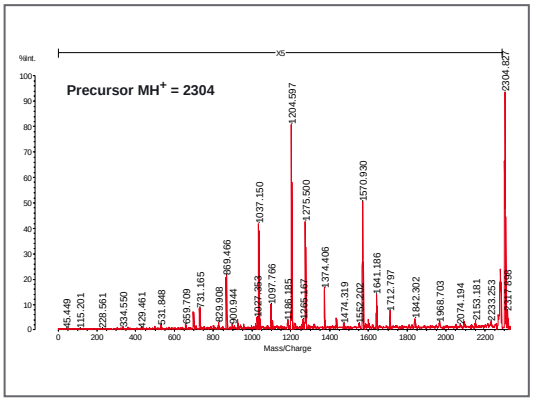

Figure 4. sPSD of 2304.8 Da precursor supporting the identity of Outer Membrane Protein A

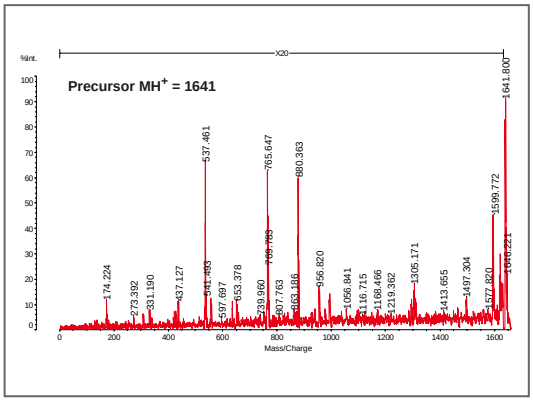

Figure 5. sPSD of 1641.8 Da precursor supporting the identity of Outer Membrane Protein A variant

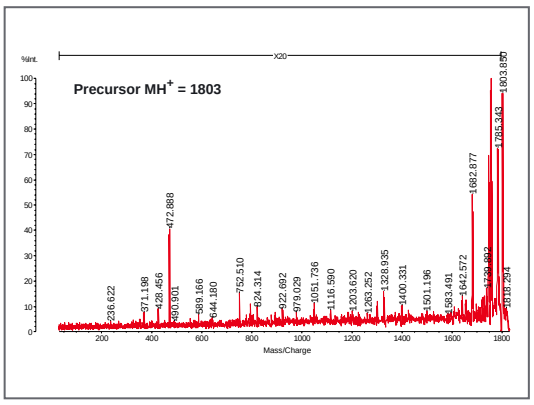

Figure 6. sPSD of 1803.8 Da precursor supporting the identity of Elongation Factor-Tu

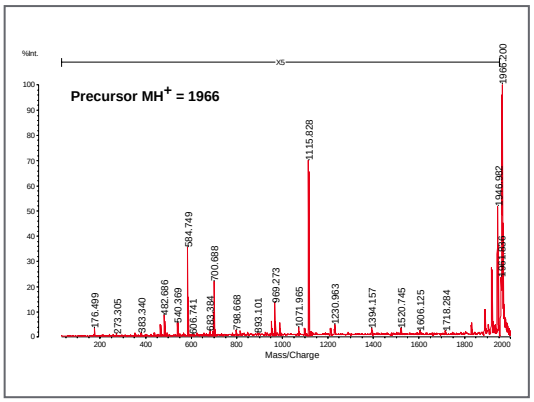

Figure 7. sPSD of 1966.2 Da precursor supporting the identity of Elongation Factor-Tu

	OMP A variant 1	OMP A variant 2	EF-Tu
Parent spectrum			
number of peptides	**10**	**9**	**8**
database position[1]	**1**	**2**	**14**
sPSD[2] - parent peak Da			
1027			**B**
1233			**B**
1264	**A**		
1381		**B**	
1438	**?**	**?**	**?**
1471	**A**		
1617			**B**
1641		**A**	
1903			**A**
1965			**A**
2303		**B**	
2616		**B**	

[1]protein identified with Mascot™ peptide mass fingerprinting search engine
[2]A = protein identified with Mascot™ MS/MS ion search engine
B = protein identity confirmed from the theoretical fragment comparison

Table 2. Summary of proteins identified from seamless PSD spectra

Conclusions

The integration of the curved-field reflectron into a high performance MALDI TOF mass spectrometer has enabled:

- Very high sensitivity: sPSD analysis : ≤50 fmol
- Good sequence coverage for database searching
- High speed analysis: 60 to 90 sec per sPSD analysis
- Typical 8 to 10 seamless PSD spectra can be obtained from a single 0.5 µl loading of a digested Coomassie Blue stained protein and 3 to 4 from a silver stained protein
- The data obtained from sPSD provided critical support for the identification and structural characterization of proteins from 2D gels which frequently contained more than one entity

Acknowledgements

We would like thank N. Sienkiewicz[2], A. D. Cronshaw[1,2], F. B. Ward[2] and M. P. Gallagher[2] (The Welmet Facility[1], Institute of Cell and Molecular Biology[2], Biology Division, University of Edinburgh, Mayfield Road, Edinburgh EH9 3JR) for preparing the *Salmonella typhimurium* proteins by 2D gel electrophoresis and in situ trypsin digestion.

REFERENCES

1. Tanaka, K. et al; Rapid Comm. Mass Spectrom., 2, (1988), 151-3

2. Mamyrin, B.A.; USSR patent no 198,034, (1966)

3. Cotter, R.J. et al; Rapid Comm. Mass Spectrom., 8, (1994), 781-5

4. Bowdler, A. et al; ASMS Conf. Mass Spectrom. Allied Topics (1999)

SHIMADZU BIOTECH
bringing analysis to life

© January 2002 Shimadzu Biotech

info@shimadzu-biotech.net

E & OE Ref: MO167 Issue 2