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SEAMLESS PSD USING A HIGH RESOLUTION CURVED FIELD REFLECTRON TOF MASS SPECTROMETER

AXIMA

APPLICATION NOTE

SEAMLESS PSD USING A HIGH RESOLUTION CURVED FIELD REFLECTRON TOF MASS SPECTROMETER

INTRODUCTION

Matrix-assisted laser desorption ionisation 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 the high resolution/high sensitivity AXIMA-CFR instrument[4]. The performance of the sPSD characteristics of this instrument is described in this application note.

METHOD

The curved-field reflectron MALDI TOF mass spectrometer (AXIMA–CFR) was used, which comprises: UV nitrogen laser (337nm), 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, and a high resolution ion gate for parent ion selection for sPSD analyses. Samples were prepared on a microplate format target.

Proteins were resuspended in buffer (0.1M ammonium bicarbonate), reduced with DTT, alkylated with iodoacetamide and digested with trypsin (1:10 molar ratio trypsin to protein) for 2½ hours at 37°C. The digestion mixture was diluted five-fold and used without further clean-up.

All results shown were obtained using the dried droplet sample (0.5µl, equivalent to approximately 5 pmols of undigested protein) loading technique with α-cyano-4-hydroxy cinnamic acid matrix (0.5µl) saturated in 1:1 acetonitrile / 0.1% TFA.

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

RESULTS

The spectra shown in Figure 1 illustrate the high sensitivity sPSD that was achieved with the AXIMA-CFR. Analyses of both 50 and 5 fmol of angiotensin II yield sPSD spectra with good signal-to-noise ratio and interpretable ions. Information within this data would be sufficient to perform a protein database search to identify the protein of origin. H corresponded to the histidine immonium ion.

The spectra obtained using the AXIMA-CFR retained isotopic resolution even in sPSD analyses, as demonstrated in Figure 2. The fragment ions of luteinising hormone releasing hormone (LHRH) in the insets in Figure 2: 523 Da, 743 Da and 1013 Da, showed 700, 910 and 1920 resolution respectively.

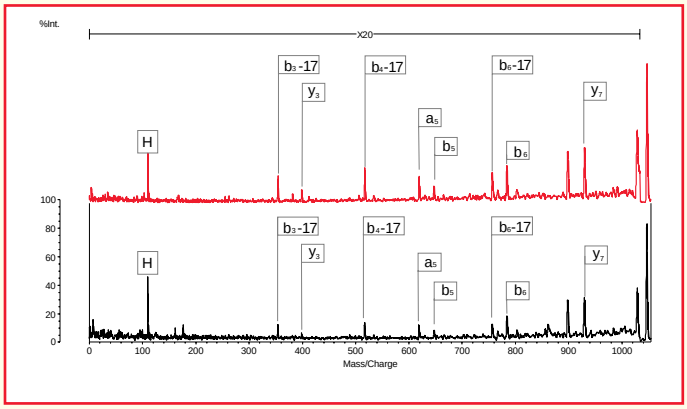

Figure 1. sPSD of Angiotensin II-50 fmol (red spectrum) and 5 fmol (black spectrum)

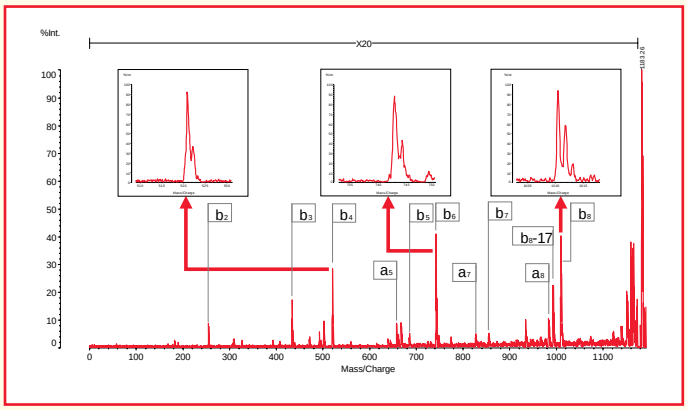

Figure 2. Isotopic resolution of the sPSD fragment ions for LHRH

The peptide mass map of myoglobin obtained by MALDI analysis is shown in Figure 3. It was found that 22 of the peaks present in the spectrum corresponded to identifiable tryptic digestion products of myoglobin as listed in Table 1. Sequence coverage for the tryptic mass map was calculated to be 99.3% with only a single amino acid not being covered (Figure 7). This is probably due to the immediate sequence surrounding the missed amino acid being KKKG and hence even in the case of partial digestion this amino acid would only be covered by peptides of sequence K; KK or KKK all of which lie within the matrix region and would not be easily distinguishable.

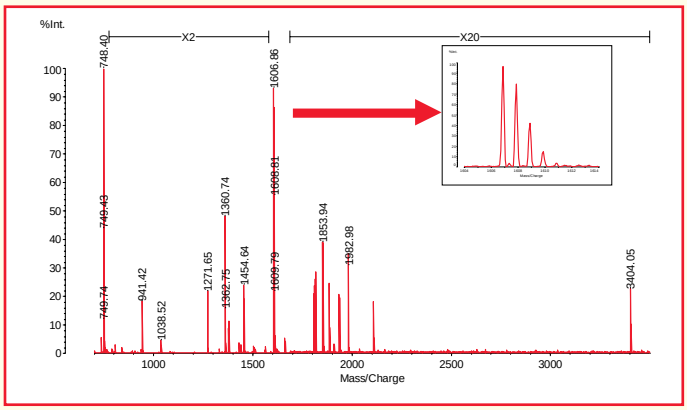

Figure 3. Peptide mass map of myoglobin tryptic digest

Protein	MW	Residues	Sequence	sPSD	Ion Types	Deduced aa	% coverage by sPSD*
myoglobin	631.34	140-145	NDIAAK	N			
myoglobin	684.37	43-47	FDKFK	N			
myoglobin	708.32	51-56	TEAEMK	N			
myoglobin	735.49	97-102	HKIPIK	N			
myoglobin	748.44	134-139	ALELFR	Y	a, b, y	ALELFR from y-ions ALELFR from b-ions ALELFR from a-ions	100
myoglobin	790.43	57-63	ASEDLKK	N			
myoglobin	941.47	146-153	YKELGFQG	Y	a, a-17, b, b-17, y	YKELGFQG from y-ions YKELGFQG from b-ions YKELGFQG from a-ions	87.5
myoglobin	1086.56	48-56	HLKKTEAEMK	N			
myoglobin	1271.66	32-42	LFTGHPETLEK	Y	a, b, b-17, y	LFTGHPETLEK from y-ions LFTGHPETLEK from b-ions LFTGHPETLEK from b-17-ions LFTGHPETLEK from a-ions	81.8
myoglobin	1360.76	134-145	ALELFRNDIAAK	Y	a, b, b-17, y	ALELFRNDIAAK from b-ions	8.3
myoglobin	1378.84	64-77	HGTVVLTALGGILK	Y	a, b, b-17, y	HGTVVLTALGGILK from y-ions HGTVVLTALGGILK from b-ions HGTVVLTALGGILK from a-ions	42.8
myoglobin	1502.67	119-133	HPGDFGADAQGAMTK	N			
myoglobin	1506.94	63-77	KHGTVVLTALGGILK	N			
myoglobin	1506.94	64-78	HGTVVLTALGGILKK	N			
myoglobin	1606.86	17-31	VEADIAGHGQEVLIR	Y	a, a-17, b, b-17, y	VEADIAGHGQEVLIR from y-ions VEADIAGHGQEVLIR from b-ions VEADIAGHGQEVLIR from a-ions VEADIAGHGQEVLIR from a-17-ions	100
myoglobin	1661.85	32-45	LFTGHPETLEKFDK	N			
myoglobin	1815.9	1-16	GLSDGEWQQVLNVWGK	Y	a, b, b-17, y	GLSDGEWQQVLNVWGK from y-ions GLSDGEWQQVLNVWGK from b-ions	12.5
myoglobin	1853.96	80-96	GHHEAELKPLAQSHATK	Y	a, b, b-17, y	GHHEAELKPLAQSHATK from y-ions GHHEAELKPLAQSHATK from y-ions	29.4
myoglobin	1885.02	103-118	YLEFISDAIIHVLHSK	Y	a, b, y	YLEFISDAIIHVLHSK from y-ions YLEFISDAIIHVLHSK from b-ions	25
myoglobin	1982.06	79-96	KGHHEAELKPLAQSHATK	N			
myoglobin	2111.16	78-96	KKGHHEAELKPLAQSHATK	Y	a, b, b-17, y	KKGHHEAELKPLAQSHATK from b-ions	10.5
myoglobin	3404.74	1-13	GLSDGEWQQVLNVWGKVEADIAGHGQEVLIR	N			

Table 1. Summary of tryptic peptides observed in the peptide mass map of myoglobin highlighting those analysed by sPSD and indicating the sequence coverage by sPSD

Following this, 10 of the 22 tryptic peptides were analyzed by sPSD to obtain amino acid sequence information (Figures 4, 5 and 6). The coverage of each individual peptide is shown in Table 1. The coverage calculations are based on interpretable amino acid differences (two consecutive ions of the same series). The percentage of the total myoglobin sequence covered by sPSD analysis was 36.6% (Figure 7). Most importantly, all of the above analyses were carried out using a single 0.5µl MALDI sample spot.

A number of other proteins were also evaluated to assess the sequence coverage by the tryptic mass map and sPSD analysis obtained from a single MALDI sample spot (Table 2). In all cases, sequence coverage by sPSD analyses was found to be extremely good for positive identification by database searching.

Similar analyses were carried out for an in-gel digestion of a protein separated by 2D SDS-PAGE (Figure 8).

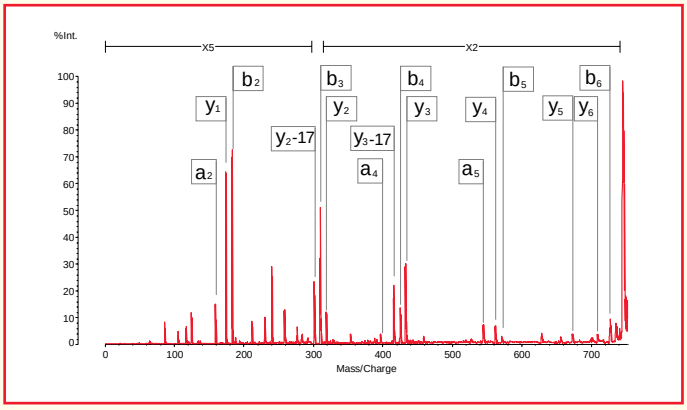

Figure 4. sPSD of MH+ 748 from myoglobin digest

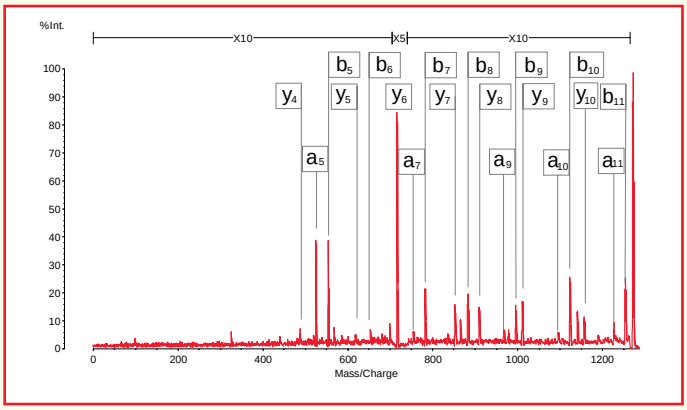

Figure 5. sPSD of MH+ 1272 from myoglobin digest

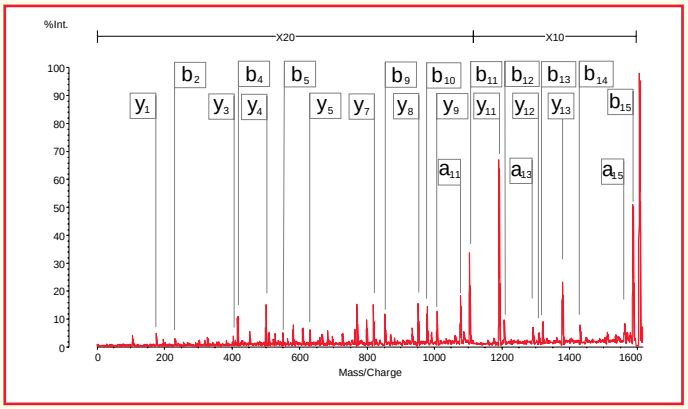

Figure 6. sPSD of MH+ 1607 from myoglobin digest

From tryptic mass map:

GLSDGEWQQVLNVWGKVEADIAGHGQEVLIRLFTGHPETLEKFDKFKHLK
TEAEMKASEDLKKHGTVVLTALGGILKKKGHHEAELKPLAQSHATKHKIPIK
YLEFISDAIIHVLHSKHPGNFGADAQGAMTKALELFRNDIAAKYKELGFQG

From sPSD sequence analysis:

GLSDGEWQQVLNVWGKVEADIAGHGQEVLIRLFTGHPETLEKFDKFKHLK
TEAEMKASEDLKKHGTVVLTALGGILKKKGHHEAELKPLAQSHATKHKIPIK
YLEFISDAIIHVLHSKHPGNFGADAQGAMTKALELFRNDIAAKYKELGFQG

Figure 7. Sequence coverage of myoglobin from the peptide mass map and deduced from sPSD

Protein	Percentage sequence coverage from...		Number of sPSD analyses from a sample single spot
	...Tryptic mass map	...sPSD analysis	
Lysozyme	85.3	41.1	8
α-lactalbumin	56.9	46.3	8
Cytochrome C	57.7	22.1	9
Bovine Serum Albumin	40.4	13.9	11

Table 2. Summary of the sequence coverage observed in the tryptic peptide mass maps and sPSD analyses

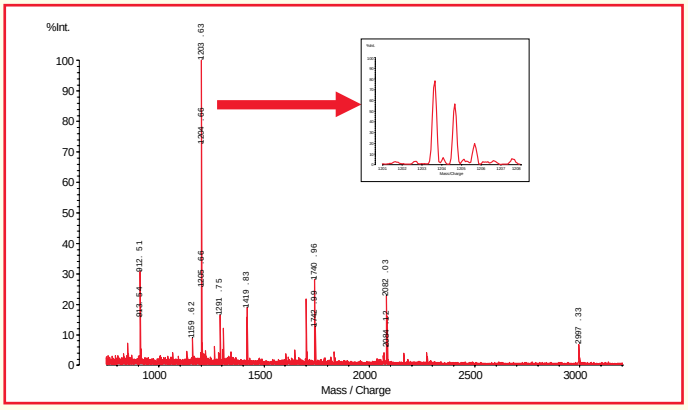

Figure 8. Peptide mass map of an *in-gel* tryptic digest of laminin

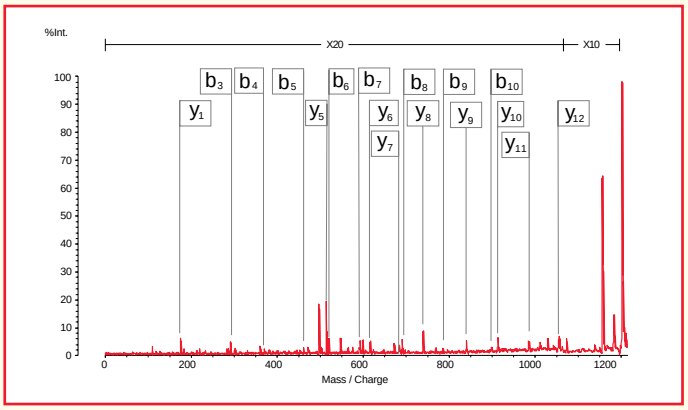

Figure 9. sPSD of MH+ 1204 from laminin

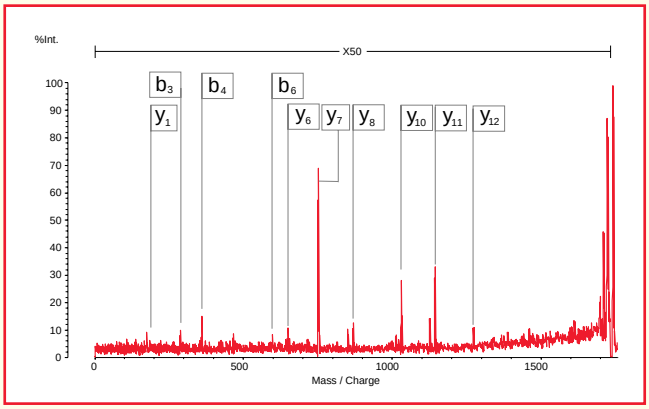

Figure 10. sPSD of MH^+ 1741 from laminin

As the identity of the protein was not known the peptide mass map obtained was submitted to a protein database search engine (Mascot™). 10 of the 16 masses submitted matched laminin. sPSD analyses of a number of the tryptic peptides were performed as illustrated in Figures 9 and 10.

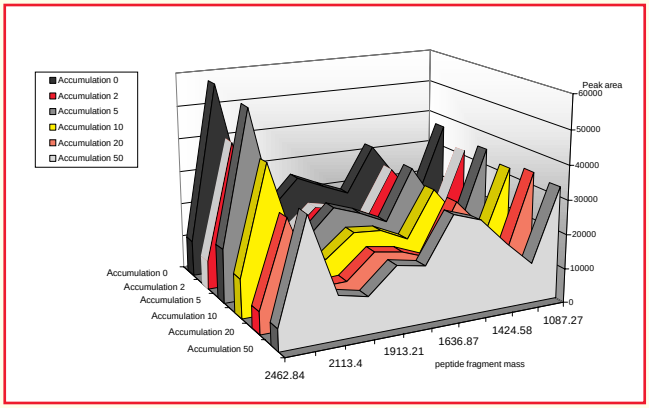

Figure 11. Comparison of peak area for selected oxidized insulin B chain fragment ions versus fragment mass and laser accumulation

Another key parameter in high throughput applications such as proteomics is the speed of the analysis. A study was carried out to investigate methods that would enable increase in analysis speed without compromise of the sPSD data obtained. The AXIMA-CFR has been equipped with accumulation hardware which allows the laser to be fired at a higher repetition rate (10 Hz). This is achieved by accumulating the signals at the data acquisition and processing hardware rather than at the external PC (Launchpad software). Figure 11 illustrates the sPSD data obtained for oxidized insulin B chain (3496 Da). The peak area for selected sPSD fragment ions was calculated and plotted against mass and accumulation rate. The analysis time measured for each of these acquisitions decreased from 59 to 34 seconds, reaching a plateau at 16 seconds for analyses with accumulation rates ≥ 5. The quality of the sPSD data, as represented in Figure 11, was found to be almost equivalent in each of the analyses: hence accumulation rates of 5 or 10 were considered the optimum acquisition conditions for high throughput.

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
- Very high resolution: isotopic resolution for sPSD fragment ions
- Good sequence coverage for database searching
- Large numbers of sPSD spectra to be acquired from a single 0.5µl sample spot
- High speed analysis: 16 sec per sPSD analysis (total 100 laser shots)

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