ANALYSING NATURE WITHOUT COSTING THE EARTH

KRATOS ANALYTICAL
A SHIMADZU GROUP COMPANY

NESTED-PSD™ OF PROTEIN TRYPTIC DIGESTIONS USING A HIGH RESOLUTION CURVED-FIELD REFLECTRON MALDI-TOF

AXIMA

APPLICATION NOTE

NESTED-PSD™ OF PROTEIN TRYPTIC DIGESTIONS USING A HIGH RESOLUTION CURVED-FIELD REFLECTRON MALDI TOF

INTRODUCTION

Matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI)[1] is an established technique for protein identification in the field of proteomics. Peptide mass mapping using enzymatic treatment of a protein and MALDI MS analysis results in a unique spectrum characteristic of the original protein. This fingerprint can be used to identify the protein through database searching. However, with the expansion of proteomics and the ever-increasing number of proteins present in web-based or commercially available databases, the likelihood of identifying the protein unambiguously from a peptide mass map alone is rapidly decreasing. The information obtained from PSD fragmentation of selected peptides can significantly improve confidence in the protein identity acquired from a database search. In addition, it can be used to aid sequence elucidation.

The incorporation of the curved-field reflectron into MALDI TOF instruments significantly increases the speed and sensitivity of PSD analysis by enabling the acquisition of a fully focussed PSD spectrum in a single analysis: seamless PSD (sPSD). The Axima-CFR integrates all of the advantages of the curved-field reflectron into a high resolution/high sensitivity MALDI TOF instrument.

A number of groups have focused on strategies that enhance PSD analysis, in particular, chemical modifications of the peptide to promote fragmentation and dedicated software tools to enable peptide sequence interpretation. The Nested-PSD™ software application[2] has been designed by Kratos Analytical to aid in peptide sequencing by comparing signals present in the sPSD spectra of a particular peptide and the same peptide treated briefly with carboxypeptidase (CPY) or aminopeptidase (AMP) to remove one amino acid[3]. In theory, signals present in both sPSD spectra are associated with the unmodified terminus, whereas signals unique to the individual spectra are associated with the modified terminus, thus aiding in the interpretation of the data (Figure 1).

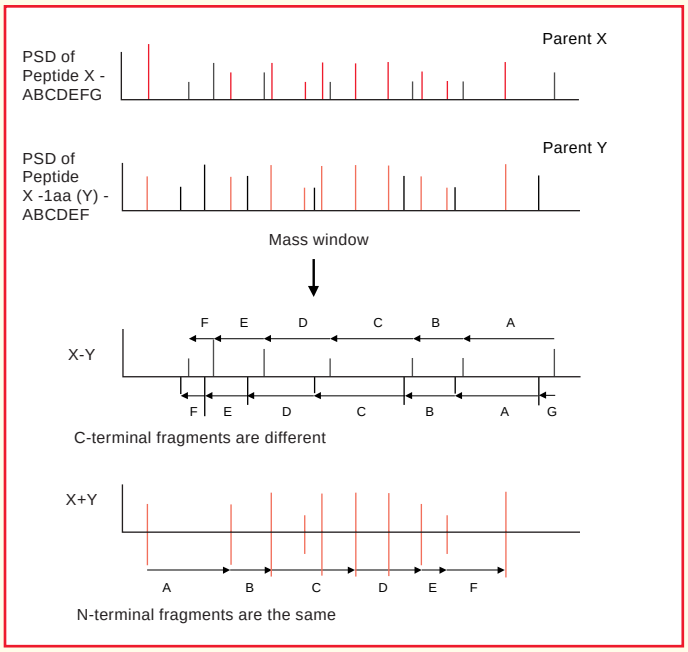

Figure 1. Nested-PSD - based on removal of a single amino acid from the C-terminal end of putative peptide - ABCDEFG

Unique software tools have been created to automatically acquire the peptide mass map, set the ion gate for selection of parents and generate seamless PSD (sPSD) data on the Axima-CFR. Once produced, the spectra are processed by the Nested-PSD™ application, which separates common signals from those that are unique and determines the peptide sequence from the filtered data.

METHOD

The curved-field reflectron MALDI TOF mass spectrometer (Axima–CFR) was used, comprising of 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, a high resolution ion gate for parent ion selection for sPSD analyses, and a microtitre-type target.

Myoglobin was resuspended in buffer (0.1M ammonium bicarbonate) and digested with trypsin (1:10 molar ratio trypsin to protein) for 3½ hours at 37°C. The digestion mixture was diluted two-fold and used without further clean-up.

CPY and AMP digestion of the tryptic peptide mixture was performed by adding 0.5µl to a 2µl aliquot of tryptic digestion mixture. Time points (0.5µl) were taken at t=0; t=1 min; t=2 min; t=5 min and t=10 min.

All of the MALDI analyses, for each sample, were performed on a single 0.5µl 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).

RESULTS

The use of Nested-PSD™ is illustrated using a tryptic digestion of myoglobin (Figure 2). The peptide mass map obtained from the digest was submitted to a web-based protein database (NCBI) using the Mascot™ database search engine (www.matrixscience.com). The search results are shown in Figure 3: Myoglobin and a number of its variants are listed as potential matches.

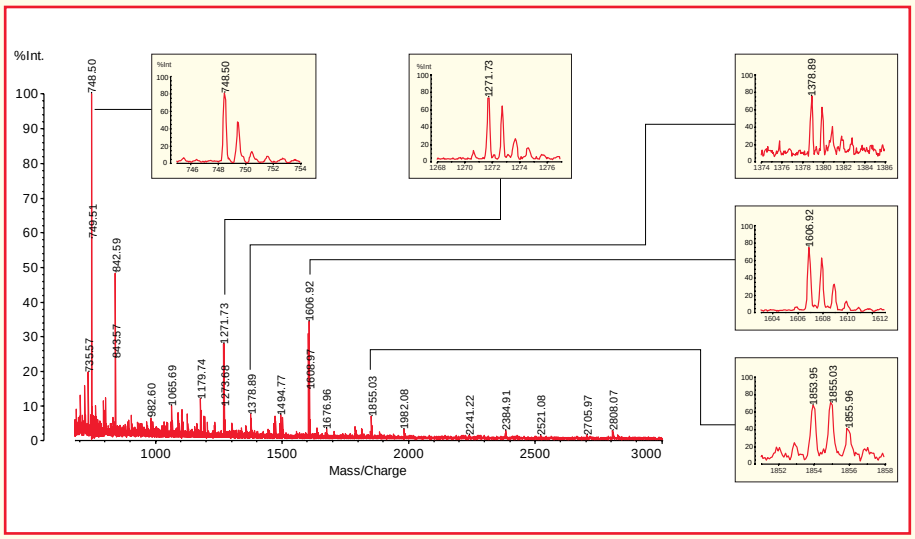

Figure 2. Peptide mass map of myoglobin digested with trypsin

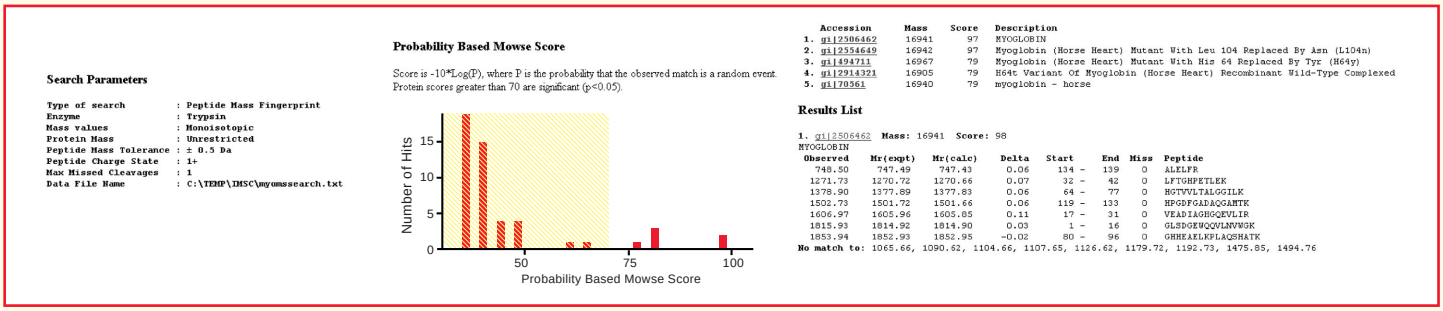

Figure 3. Peptide mass fingerprint match for the myoglobin tryptic digestion products using Mascot™

A portion of this myoglobin tryptic digest was treated with aminopeptidase M (AMP) and aliquots taken at given timepoints to follow the reaction. The spectra in Figure 4 illustrate the progression of the N-terminal cleavage of the tryptic peptide T2 at mass 1607 Da (sequence VEADIAGHGQEVLIR); the loss of the N- terminal valine (99 Da) is clearly observed (1508 Da). A number of N- terminal losses were also observed for other tryptic peptides present in the myoglobin digest.

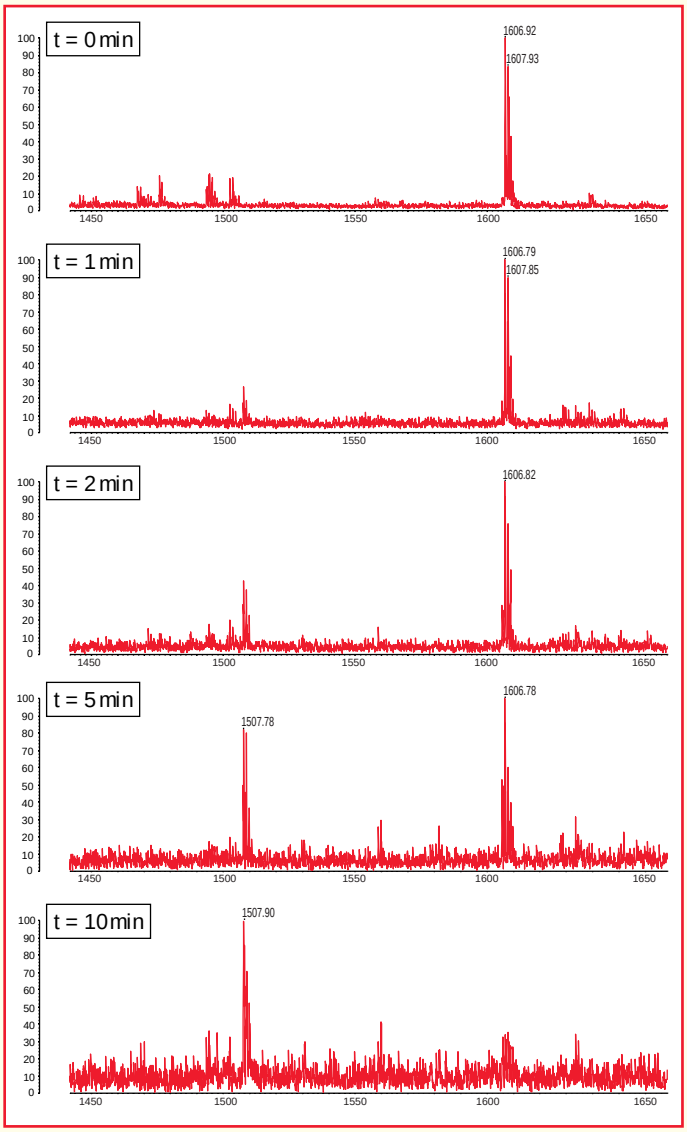

Figure 4. Aminopeptidase digestion of myoglobin tryptic digestion mixture (timepoints: 0, 1, 2, 5 and 10 min)

Nested-PSD™ interpretation software is illustrated here using the sPSD spectrum acquired for the original tryptic peptide T2 at 1607 Da and that for the first N-terminal truncation of peptide T2 : 1508 Da (Figures 5 and 6). Simple parameters such as mass range, match tolerance between the sPSD spectra and minimum intensity threshold are set (Figure 7), and the sPSD spectra subtracted from one another. Three conceptual spectra are produced: that resulting for the similarities between the sPSD spectra; that arising from ions uniquely present in the sPSD spectrum from 1607 and that arising from ions that are uniquely present in the sPSD spectrum from 1508 (Figure 8).

The Nested-PSD™ interpretation software was used to deduce the amino acid sequence of peptide T2.

The sequence deduced from the spectrum of common ions was associated with the C-terminus as these ions were unaffected by the AMP digestion. A series of 7 consecutive amino acids (EADL/IAGH) was deduced from the spectrum of common ions - y-ions (Figure 8). The spectrum of unique ions from the truncated T2 fragment showed the presence of LR and GQ and correspond to the N-terminal ion series: a or b-ions.

The sequence information was submitted to the Mascot™ database search engine and myoglobin was unequivocally identified (Figure 9).

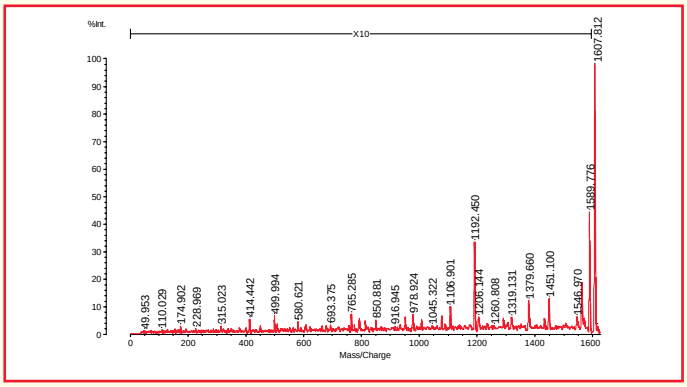

Figure 5. Seamless PSD data for tryptic peptide: 1607 Da

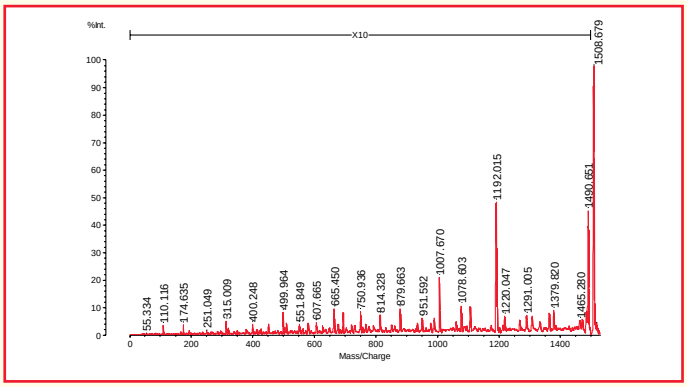

Figure 6. Seamless PSD data for AMP treated tryptic peptide: 1508 Da

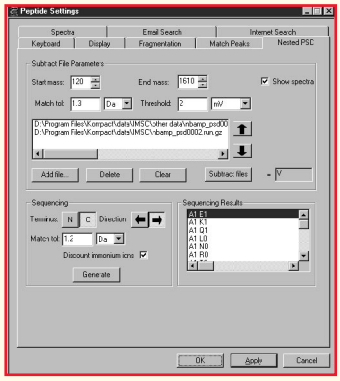

Figure 7. Nested-PSD™ interface

CONCLUSIONS

A simple method has been developed that enhances sequence interpretation of what has traditionally been a complex analysis - Post-Source Decay. In order to achieve this a number of important features have been taken into consideration:

- methods for removal of single N or C-terminal amino acid from peptides within a complex mixture such as a tryptic digest
- the ease with which seamless PSD can be generated on the AXIMA-CFR - made possible with the use of the unique curved-field reflectron
- Nested-PSD™ software tool for the processing of seamless PSD data

The combination of these features enabled 80% (VEADI/LAGHGQEVI/LR) of the peptide sequence of T_2 to be deduced and hence allowed a single database match to be found.

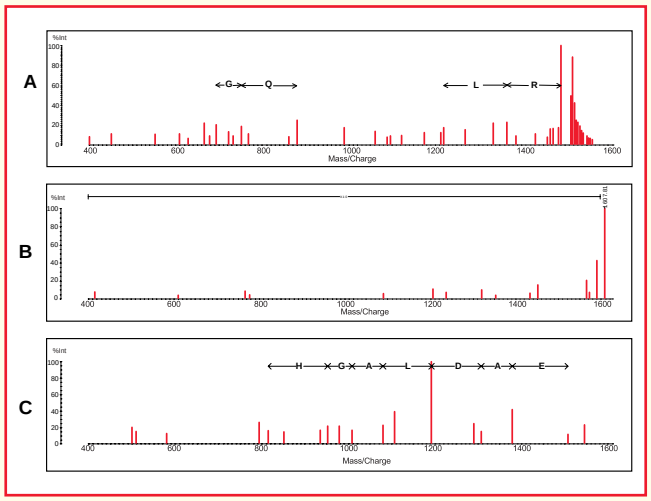

Figure 8. Conceptual spectra resulting from the Nested-PSD™ analysis of the PSD spectra from the 1607 and 1508 ions. A: Unique ions from the 1508 ion; B: Unique ions from the 1607 ion and C: Ions common to both 1607 and 1508

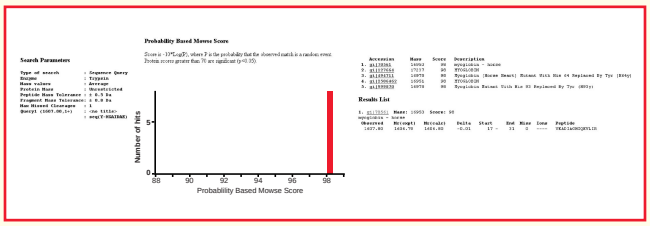

Figure 9. Database match for the sequence deduced from Nested-PSD™ of the 1607 Da myoglobin tryptic fragment using Mascot™

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