ANALYSING NATURE WITHOUT COSTING THE EARTH

KRATOS ANALYTICAL
A SHIMADZU GROUP COMPANY

NESTED-PSD™ FOR INTERPRETATION OF PEPTIDE SEQUENCES

KOMPACT

APPLICATION NOTE

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INTRODUCTION

Matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI) has been used to characterize peptides in a number of ways. Originally, linear time of flight was used to obtain the molecular weight of the intact peptide as only the parent mass was observed[1,2]. Recently, in source decay has been described as a method of sequencing peptides from fragments detected in linear mode under controlled conditions (prolonged extraction delay), but relies on pure samples for interpretable results[3]. Reflectron time of flight was initially used to improve resolution, and therefore accuracy, of parent ions. But the reflectron also exposed the incidence of fragmentation or post source decay (PSD) which occurs during linear time of flight[4]. PSD was observed to be comparable to fragmentation observed by MS/MS methods (for example triple quadrupole systems), in that peptide sequence information could be determined. However, conventional reflectron systems do not focus all the fragment ions to the detector at the same time. It is necessary to step the reflectron voltage in order to focus fragments of different molecular weight, thereby creating a number of mass windows to observe the full PSD spectrum. The curved field reflectron (CFR) is unique in focusing all of the fragments to the reflectron detector in a single spectrum so that generating seamless PSD (sPSD) data is as quick as generating parent spectra (Figure 1)[5]. The CFR therefore offers a rapid method for obtaining structural information about peptides and the possibility for developing novel sequencing strategies, such as Nested-PSD™ [6].

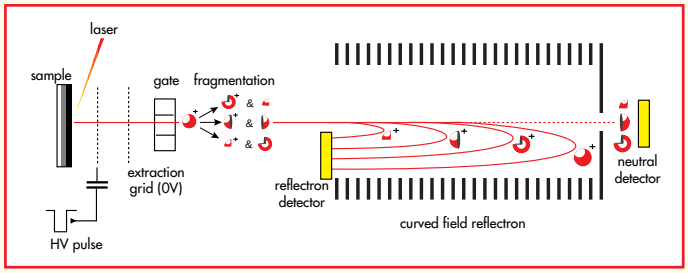

Figure 1. Curved field reflectron

Interpretation of PSD spectra can be slow as fragmentation of the peptide backbone results in a number of possible decay products (for example - a, a-17, b, b-17 and c ions for the N-terminal fragments, Figure 2). A strategy has been developed to assist sequencing from sPSD data. Enzymic (carboxypeptidases and aminopeptidases) methods have been developed for limited cleavage of peptides, preferably removal of a single amino acid (Figure 3). Thereafter, the whole peptide and truncated peptide (lacking a single amino acid) are analyzed by sPSD and treated with Nested-PSD™ software. In theory, those signals that are common to the two spectra are associated with the unmodified terminus (illustrated with human angiotensin I in Figure 4). Those signals that are different are associated with the modified terminus. Therefore, from a complex spectrum, signals can be separated to allow simpler interpretation of the peptide sequence.

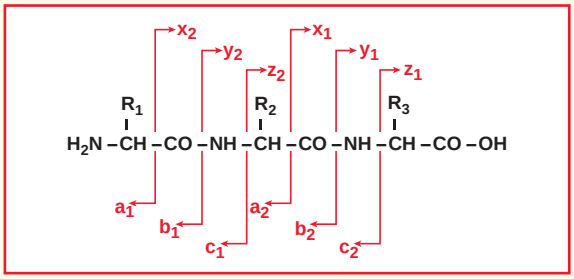

Figure 2. Some of the fragmentation sites in peptides

To enhance analysis of Nested-PSD™ spectra, unique automation software tools have been created to generate the parent peptide spectrum, set the ion gate for selection of parents and generate sPSD data. In addition, software has been developed to compare Nested-PSD™ spectra, separate common signals from unique signals and determine the peptide sequence from the filtered data.

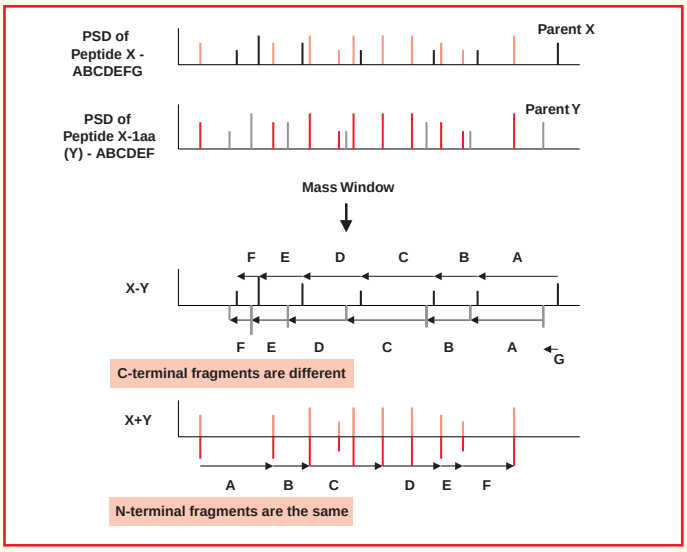

Figure 3. Nested-PSD™ - based on removal of a single amino acid from the C-terminal end of putative peptide - ABCDEFG

human angiotensin I [1-10] DRVYIHPFHL			human angiotensin I [1-9] DRVYIHPFH		
Average Masses $(M+H)^+$					
Singly charged fragments			Singly charged fragments		
n	b	y	n	b	y
0	—	19.02	0	—	19.02
1	116.10	132.18	1	116.10	156.16
2	272.28	269.32	2	272.28	303.34
3	371.42	416.50	3	371.42	400.46
4	534.59	513.62	4	534.59	537.60
5	647.75	650.76	5	647.75	650.76
6	784.90	763.92	6	784.90	813.94
7	882.01	927.10	7	882.01	913.07
8	1029.19	1026.23	8	1029.19	1069.26
9	1166.33	1182.42	9	1166.33	1184.35
10	1279.49	1297.51			

Figure 4. Theoretical b and y ions for human angiotensin I

METHODS AND MATERIALS

Sample Preparation - Carboxypeptidase Y Digest

The peptide sample (10 µl, e.g. human angiotensin I at 10 pmol/µl) was placed into an eppendorf tube and 1 µl of carboxypeptidase Y (0.2 mg/ml in 10% methanol/90% water) added. The mixture was vortexed, centrifuged and incubated at 27°C. Aliquots (0.5 µl) were taken at time intervals (e.g. 0.5, 2, 5 and 10 minutes) for MALDI analysis. Each aliquot was placed onto a separate sample position on the KOMPACT target, 0.5 µl of α-cyano-hydroxycinnamic acid added, allowed to dry and the sample analyzed by MALDI. As the carboxypeptidase Y (Roche Molecular Biochemicals) was provided as a solid in the presence of buffer (50 mM sodium citrate, pH 6.0 on resuspension), no additional buffer was required.

MALDI Analysis

MALDI analysis was performed in the Kratos KOMPACT Discovery, with both linear (0.7 m drift length) and curved field reflectron time of flight (1.8 m drift length). The instrument employs pulsed extraction as standard and ion gate (resolution of +/-12.5 Da at 1000 Da) for parent ion selection prior to seamless PSD. Data were generated for the digest time points using automation software to:

- generate parent ion spectra - obtain masses for the whole peptide and digest products
- select parent ions for sPSD
- generate sPSD spectra for the parent ions corresponding to whole peptide and with a single amino acid removed.

Nested-PSD™ software was then used to interpret the two sPSD spectra and determine the peptide sequence.

RESULTS

The concept of Nested-PSD™ is illustrated here for C-terminal truncation (of human angiotensin I), using carboxypeptidase Y, but applies equally to N-terminal modification using an aminopeptidase. Chemical methods such as manual Edman degradation or partial acid hydrolysis to remove a single terminal amino acid, can also be used.

Carboxypeptidase digestion can offer extensive sequence information in its own right, but the number of amino acids determined is very much dependent on the sequence, i.e. different amino acids are cleaved at different kinetic rates. In addition, carboxypeptidases are unstable and activity is reduced rapidly once the reaction has started, which again can limit the amount of sequence information determined.

For Nested-PSD™, only one amino acid needed to be cleaved and this was relatively successful, occurring within 1 to 2 minutes (Figure 5). The original peptide and truncated peptide were then analyzed using curved field reflectron time of flight to generate seamless PSD (Figure 6). N-terminal fragments of human angiotensin I remained the same, whereas the C-terminal fragments were changed by the removal of a single C-terminal amino acid.

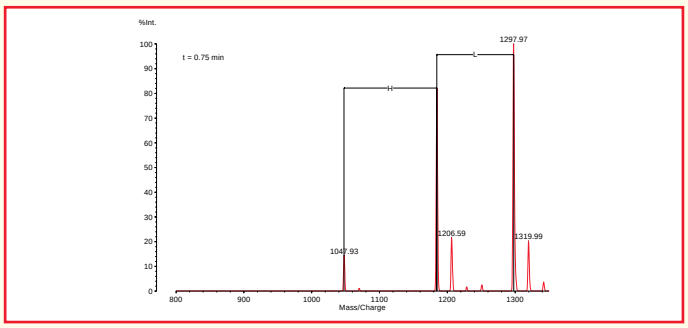

Figure 5. Carboxypeptidase digest of human angiotensin I - DRVYIHPFHL.

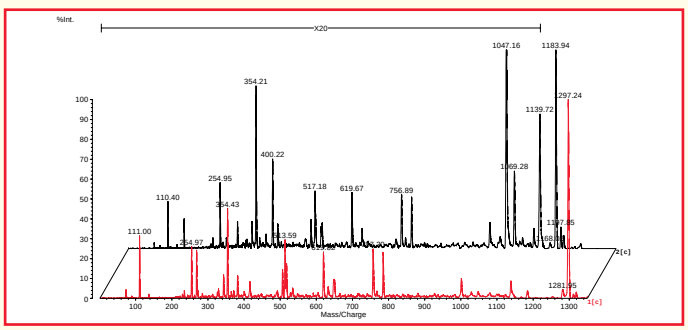

Figure 6. Nested-PSD™ of carboxypeptidase products - human angiotensin I

Nested-PSD™ software (Figure 7) was then used to analyze the two spectra. In the first instance the mass signals were separated into three groups:

1) those that are common to both spectra
2) those that are unique to the whole peptide
3) those that are unique to the truncated peptide.

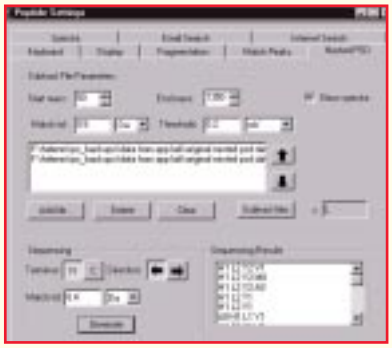

Figure 7. Nested-PSD™ software

The data were reprocessed into a simplified form as three conceptual spectra, each mass signal represented as a line (examples shown in Figures 8 and 9). The Nested-PSD™ software was then used to define the sequence in each of the three cases. The user can select any of the signals within the spectrum to start the sequence calculation. The results are presented as a list of putative sequences (in single letter code) any of which can be overlaid on the spectra (Figure 7). A number after each amino acid informs the user of other signals in the spectrum that qualify the identity of that amino acid. This enables the putative sequences to be listed in order of most likely fit, as the accumulated score corroborates the evidence within the spectrum.

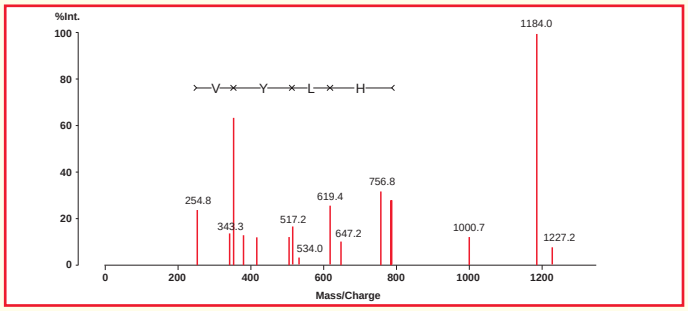

Figure 8. Common ions spectrum: possible N-terminal ions of angiotensin I

If the conceptual spectrum of common signals (a, b an c related ions) from carboxypeptidase digestion of angiotensin I was used for interpretation, then N-terminal sequence information was obtained (Figure 8). If the conceptual spectra of unique signals (x, y and z ions) are used for interpretation, then C-terminal sequence information is obtained (Figure 9).

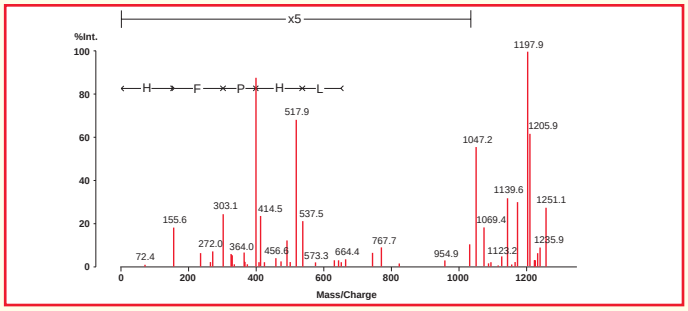

Figure 9. Unique ions spectrum - truncated peptide: possible C-terminal sequence of angiotensin I

The angiotensin I data indicated an overlap in these two sequence interpretations which further helped to confirm the sequence. The C–terminal amino acid was readily obtained from the parent ion spectrum (Figure 5). The full sequence results are summarized in Figure 10. Nested-PSD™ has now been applied to a number of examples some of which are shown in Table 1.

Human Angiotensin I:	DRVYIHPFHL
C-terminal amino acid:	DRVYIHPFHL
Sequence from Similarities:	DRVYIHPFHL
Sequence from Differences:	DRVYIHPFHL
Overall sequence deduced by Nested-PSD™ analysis:	DRVYIHPFHL

Figure 10. Summary of sequence information obtained by Nested-PSD™

Peptide	Peptide sequence	Molecular weight, MH^+	Cleavage method
human angiotensin I (synthetic)	DRVYIHPFHL	1296.5	carboxypeptidase Y
goosefish angiotensin I (synthetic)	NRVYVHPFHL	1282.5	carboxypeptidase Y
synthetic	LVPLPKIKNSTFT	1458.8	carboxypeptidase Y
synthetic	YPDEIEYIFKPS	1501.7	carboxypeptidase Y
synthetic	AEFHRWSSYMVHWK	1865.1	carboxypeptidase Y
luteinizing hormone release hormone analogue (synthetic)	pyro-QFWSYALRPG-amide	1207.4	acid hydrolysis

Table 1. Peptides analyzed by Nested-PSD™

CONCLUSIONS

A simple method has been developed to enhance sequence interpretation of what has traditionally been a complex analysis - post source decay fragmentation. In order to achieve this a number of important features have been taken into consideration:

1) methods for rapid removal of single N- or C-terminal amino acid
2) easily generated seamless PSD using the curved field reflectron
3) Nested-PSD™ software tools for the processing of sPSD data.

To date most of the examples of peptides analyzed by Nested-PSD™ are well characterized so that the concept of Nested-PSD™ can be established. The real potential of Nested-PSD™ will be in proteomics, where MALDI can be used to extend protein characterization beyond protein identification, by peptide mass fingerprinting, and into novel protein sequencing and structural characterization of post-translational modifications.

The success of this approach is dependent on the quality of fragmentation obtained, which can be sequence dependent. New methods to improve fragmentation, which complement seamless PSD, are under investigation, so that peptide analysis will become sequence independent.

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