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NOVEL ENHANCEMENTS TO POST SOURCE DECAY MALDI-TOF ANALYSIS

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OVERVIEW

Aim

To demonstrate that Post Source Decay (PSD) fragment ions from multiple precursors may be recorded simultaneously and correctly matched to their corresponding precursor ion.

Method

A novel, axial geometry, MALDI-TOF mass spectrometer has been designed which removes the necessity for an ion gate in PSD experiments. This allows fragment ion data to be acquired more quickly and use less sample than in comparison with conventional PSD methods.

Results

The validity of this new multiplexed PSD technique has been demonstrated by simultaneously acquiring fragment ion information from all of the peptides in a tryptic digest of alcohol-dehydrogenase (ADH) and correctly identifying 10 of these peptides when database searched.

INTRODUCTION

- Post source decay MALDI-TOF analysis is an established technique capable of providing complementary structural information.
- A conventional PSD experiment requires each precursor ion of interest to be isolated using an ion gate so that all fragment ions may be associated with their correct precursor. This serial nature of data acquisition consequently consumes both time and sample.
- By removing the ion gate it is possible to simultaneously record fragment ions from multiple precursors and hence obtain parallel data acquisition.
- To determine which fragment ion originated from which precursor ion, two spectra for each PSD segment are acquired, the second at a slightly different reflectron voltage. It is possible to determine the precursor ion mass for each fragment ion by measuring the resultant shift in time-of-flight.

EXPERIMENTAL

- All PSD experiments were performed using a new benchtop MALDI-TOF mass spectrometer (Waters MALDI micro MX) incorporating the new multiplexed technique. This new instrument is shown schematically in Figure 1.
- For ADH and standard peptides, the analyte solution was mixed 1:1 by volume with alpha-cyano-4-hydroxycinnamic acid matrix (concentration 1mg/ml) and 1µL of the resultant mixture was spotted onto a target plate. (For the concentration of the analyte solutions, refer to the relevant area of the poster).

Conventional PSD Experiment

- **Time 1** Ions of three different mass are formed and then accelerated out of the ion source.
- **Time 2** The ion gate is opened to allow a specific precursor mass (red) to be transmitted whereas the lighter precursor ions (green) have been deflected.
- **Time 3** The ion gate is closed again and therefore deflects the heavier precursor ions (blue). Whilst inside the field free region between the source and the reflectron some of the selected (red) precursor ions undergo metastable decay.
- **Time 4** The precursor ions penetrate deep into the reflectron so that they are temporally focussed when they reach the detector. Fragment ions do not penetrate as deeply and consequently are not as well focussed. This is overcome by acquiring several spectra (known as segments) at reduced reflectron voltages. The well focussed regions of each segment are then stitched together to form a single fragment ion spectrum.

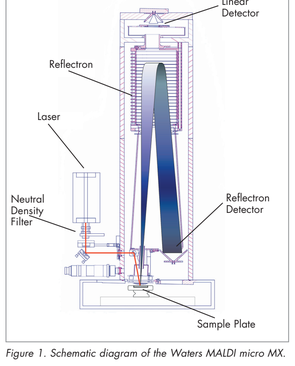


Figure 1. Schematic diagram of the Waters MALDI micro MX.

Multiplexed PSD Experiment

- **Time 1** Ions of three different mass are again formed and then accelerated out of the ion source.
- **Time 2** The ion gate is now absent and so all three precursor masses are transmitted towards the reflectron.
- **Time 3** Fragmentation occurs for all three precursors whilst in the field free region.
- **Time 4** Fragments from all of the precursors are reflected and hence simultaneously detected. The requirement for obtaining several segments and stitching the focussed regions to form a single spectrum is still present in this scheme.

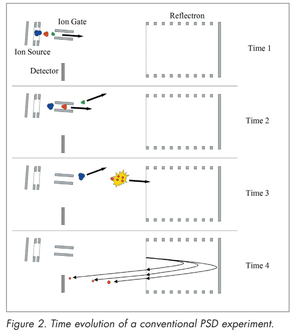


Figure 2. Time evolution of a conventional PSD experiment.

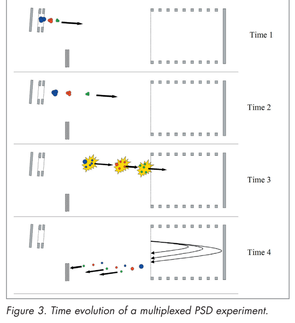


Figure 3. Time evolution of a multiplexed PSD experiment.

THEORY

Overview

- Assigning fragment ions to their corresponding precursor ion may be achieved by acquiring two spectra at slightly different reflectron voltages for each PSD segment.
- The first spectrum for each segment is acquired at the same reflectron voltage as for conventional PSD and is named the *Major* spectrum. The second spectrum is acquired at a reflectron voltage 4% lower and is called the *Minor* spectrum.
- The precursor of each fragment ion may be determined by measuring the shift in TOF between the two spectra for each fragment. This concept is shown schematically in Figure 4 where the red and blue peaks represent fragment ions from two different precursors.

Precursor Assignment

In conventional PSD, the time of flight (TOF) of a PSD fragment ion is given as,

$$TOF = \sqrt{m_p}\,a + \frac{m_f}{\sqrt{m_p}}\,b$$

where mp is the mass of the precursor ion, m_f is the mass of the fragment ion and where a, b and b' are coefficients obtained via calibration on known PSD masses. In multiplexed PSD two TOFs are measured, one each for the Major and Minor spectra, which allows both the precursor and fragment mass to be solved from the resulting pair of simultaneous equations,

$$TOF_{major} = m_p\,a + \frac{m_f}{m_p}\,b \qquad TOF_{minor} = m_p\,a + \frac{m_f}{m_p}\,b'$$

$$m_p = \left(\frac{TOF}{a} - \frac{b}{a}\cdot\frac{\Delta TOF}{\Delta b}\right)^2$$

$$m_f = \frac{\Delta TOF}{a\cdot\Delta b}\left(TOF - \frac{b\cdot\Delta TOF}{\Delta b}\right)$$

where $\Delta TOF = TOF_{major} - TOF_{minor}$ *and* $\Delta b = b - b'$.

A conventional precursor ion spectrum may be referred to and candidate precursor ion masses from the PSD acquisition may be matched to those in this more accurate precursor ion spectrum. (Using the more accurate values for the m_p in the above equation, improvements may then be made to the accuracy of the value mf).

Fragment Ion Matching

The main difficulty in automating the multiplexed process is the identification of the same fragment ion in both the Major and Minor spectra. This is overcome by using the conventional precursor mass spectrum to provide a list of likely precursor ion masses. A list of precursor ion masses are used to calculate a tentative fragment ion mass for each fragment peak in each mass spectrum. Therefore, for each fragment peak there will be as many calculated candidate fragment ion masses as there are candidate precursor ion masses.

It is only necessary to compare the list of candidate fragment ion masses for the two spectra and to look for matches within a specified mass window compatible with the expected accuracy of mass measurement. In this way the process of assigning the same peak within each spectrum is automated. A secondary benefit of the process is the reduction of noise within the resulting spectra as noise or interference peaks must appear in both the Major and Minor spectra, and be shifted by the correct amount to be associated with a particular precursor.

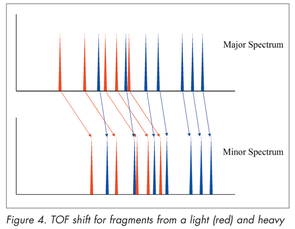


Figure 4. TOF shift for fragments from a light (red) and heavy (blue) precursor.

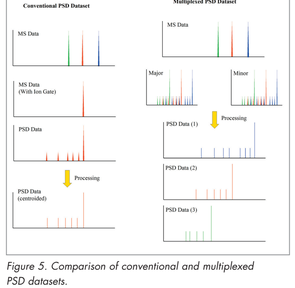


Figure 5. Comparison of conventional and multiplexed PSD datasets.

RESULTS

- Figure 5 displays a schematic representation of the datasets obtained from a conventional and a multiplexed PSD experiment.
- In the conventional experiment, a normal MS spectrum is recorded and used to determine a precursor ion to be isolated using an ion gate for subsequent PSD analysis. PSD data is then acquired, processed and stitched together too give a single fragment ion spectrum.
- In the multiplexed PSD experiment a normal MS spectrum is again recorded. Major and Minor PSD spectra may then be acquired immediately. Subsequent computer processing deconvolutes the fragment ion spectra and produces stitched centroided spectra for all of the precursors.
- Figure 6 is an example of a deconvoluted spectra resulting from the processing of multiplexed PSD data from a sample containing ACTH.
- To demonstrate the multiplexed PSD technique on a more complex sample, 6 segments of multiplexed data were obtained from 50fmol of a tryptic digest of ADH.

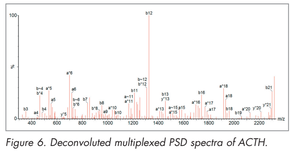


Figure 6. Deconvoluted multiplexed PSD spectra of ACTH.

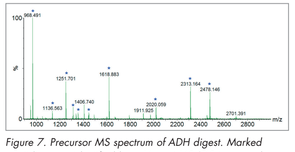
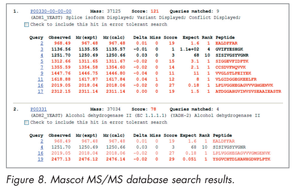


Figure 7. Precursor MS spectrum of ADH digest. Marked peptides were identified by a Mascot MS/MS ion search.

- Figure 7 displays part of the precursor MS spectra for this experiment and Figure 8 summarises the peptides identified by Mascot (Matrix Science) when the multiplexed PSD data was database searched.
- The top hit from the database search was ADH1_YEAST, the correct protein. A total of 9 of the tryptic peptides from this protein were correctly identified, 7 of them with a top ranking score. The second hit from the database was ADH2_YEAST, which has four matched peptides. ADH2 has significant homology with ADH1, but the identification of the 2477 Da peptide from its PSD data indicates that the original digest contained a mixure of both proteins.
- In obtaining this data, a total of 12 segments of a single PSD spectrum were recorded (6 Major and 6 Minor segments), five times less than the 60 segments (6 segments for each of 10 separate PSD spectra) that would have been required to obtain the same data using conventional PSD techniques.



1. BIO320-DD-DD-DD Mass: 37125 Score: 181 Queries matched: 9
   ADH1_YEAST: Splice isoform Displayed: Variant Displayed: Conflict Displayed:
   Check to include this hit in error tolerant search

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
2	948.45	947.48	947.48	0.00	0	19	1.6	1	RALGFYER
3	1136.56	1135.55	1135.57	-0.01	0	1	1.1e+02	4	GVTFTEIEHAE
5	1251.70	1250.69	1250.66	0.03	0	3	68	10	RIEIYGGYVGNR
7	1352.66	1351.65	1351.67	-0.02	0	16	5.1	1	IGGHMVQIGVL
8	1406.74	1405.73	1405.73	-0.00	0	15	8.3	1	VGSMGHIRSFE
9	1447.76	1446.75	1446.80	-0.04	0	11	11	1	YVSLGTLPEIYK
11	1618.88	1617.87	1617.84	0.04	1	12	8	1	YLGIGGGGHGEELFR
14	2028.05	2028.04	2028.04	-0.02	0	27	0.18	1	LFLVGGEGALGGVVVGAEGMGFK
17	2312.15	2311.14	2311.14	0.00	0	19	1.5	1	ATGGAGHGYIHVGYEAEAAIEAATER

2. BIO321 Mass: 37034 Score: 78 Queries matched: 6
   ADH2_YEAST: Alcohol dehydrogenase II SEC 1.1.1.1: (NAD+-2) Alcohol dehydrogenase II
   Check to include this hit in error tolerant search

Query	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect	Rank	Peptide
4	947.45	947.48	947.48	0.00	0	19	1.6	1	RALGFYER
6	1251.70	1250.69	1250.66	0.03	0	3	68	10	RIEIYGGYVGNR
16	2015.05	2014.04	2014.06	-0.02	0	21	0.16	1	LRGVHGGSAGIVFVVGMGSRVY
19	2477.12	2476.12	2476.14	-0.02	0	29	0.051	1	YSGVGKYSLLAAMHGGRMVFLFTK

Figure 8. Mascot MS/MS database search results.

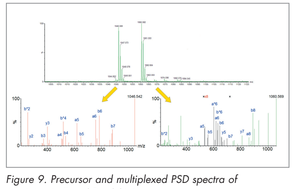


Figure 9. Precursor and multiplexed PSD spectra of Angiotensin II and Bradykinin.

- A requirement of multiplexed PSD is the accurate assignment of fragment ions to their corresponding precursor.
- To illustrate how fragments may still be correctly assigned to their precursor ion for two peptide seperated by only 14 Da, 250fmol of Angiotensin II (M+H 1046.542) and Bradykinin (M+H 1060.569), were spotted onto a target plate and 6 Major and 6 Minor segments of multiplexed PSD data were acquired.
- Figure 9 displays part of a precursor MS spectrum of the two peptide standards in addition to their deconvoluted and stitched fragment ion spectra.
- Despite the proximity of the two precursor ions, there is little interference between the two deconvoluted fragment ion spectra.
- In addition, this data allowed the unambiguous identification of both peptides when database seached.

Future work

Further refinements to the fragment matching algorithm, with a view to increasing the number of fragment ion peaks that can be confidently assigned to a precursor ion, are to be investigated. Possible refinements include the comparison of peak intensity and widths (shapes) and the use of a third spectrum (equivalent to a second Minor spectrum) which can be used to confirm, or otherwise, the results from the first two spectra.

CONCLUSION

- A new parallel PSD technique is presented in which there is no requirement for an ion gate, the use of which can reduce the transmission and resolution of an ion beam.
- Using multiplexed PSD enables complementary fragment ion information to be recorded faster, and using less sample than with conventional PSD.
- Precursor ion identification is currently comparable with the resolution of an ion-gate (50-100).
- It is expected that further refinement of the peak matching algorithm will increase this to 150 or greater.

Other ASMS posters describing the application of multiplexed PSD:

1. Parallel PSD analysis of complex protein mixture WPY 475.
2. Analysis of protein phosphorylation using a novel "parallel PSD" approach on a MALDI mass spectrometer ThPN 283.

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