ASMS 2004

THE USE OF EXACT MASS AND HIGH-RESOLUTION PRECURSOR ION SELECTION ON A Q-TOF MASS SPECTROMETER FOR *DE NOVO* PEPTIDE SEQUENCING

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OVERVIEW

- This poster demonstrates the MS/MS capabilities of a hybrid quadrupole-orthogonal acceleration time-of-flight mass spectrometer equipped with a MALDI source.
- Precursor ions separated by 3 Da in the mass spectrum were selected individually using the quadrupole. The selected ions were fragmented in the collision cell and the fragments mass analysed using the orthogonal TOF analyzer.
- The exact mass nature of the MS/MS spectra allows confident de novo sequencing.
- Two modified peptides, that were not identified by a peptide mass fingerprint, were successfully characterized using exact mass MS/MS.

INTRODUCTION

Tryptic digestion of a single large protein or complex multi-protein mixture often produces peptides that are similar in mass. If no chromatographic separation of these species is performed, then the isotopic peak envelopes from peptides that are separated by only a few Daltons will overlap. In MS/MS product ion experiments it is important that the precursor ion window be set to transmit only those ions of interest and not isotopes from other peptide species. This will provide the most specific information. If the precursor ion transmission window of MS1 is not sufficiently stringent, then more than one ion will be transmitted and the acquired MS/MS spectrum will contain fragment ions from the other peptide species transmitted, making the interpretation more problematic. Here we show the advantage of isolating two peptides separated in mass by 3 Da at m/z 1530. In this study a quadrupole mass filter is used as MS1, with the ions subsequently fragmented using low-energy CID and the fragment ions accurately mass measured using an orthogonal acceleration TOF analyser. Peptide sequence information was obtained from the MS/MS data using a *de novo* sequence algorithm that exploited the specificity afforded by exact mass measurement.

EXPERIMENTAL

- All data were generated using a Q-Tof Ultima MALDI mass spectrometer (Waters, Manchester UK).
- MALDI matrix, α-cyano-4-hydroxycinnamic acid, and the tryptic digest of Glycogen phosphorylase B were obtained from Waters, Milford, MA.
- The matrix concentration used was 2 mg/mL (in 1:1 acetonitrile:0.1% TFA) and the digest concentration used was 500 fmol/µL (in 0.1% TFA).
- Equal volumes of matrix and sample solution were mixed and 2 µL of the resultant mixture was spotted on a MALDI target plate.
- Peptides mass fingerprinting data were acquired in automated mode and databank searched using ProteinLynx Global SERVER 2.1 (Waters, Manchester UK).
- All MS/MS data were acquired manually. The resolution settings were adjusted, as required, to set the desired transmission window for the precursor ion to the hexapole collision cell.
- Fragment ions were produced by raising the collision energy on the hexapole gas cell to the appropriate value to induce optimal fragmentation.

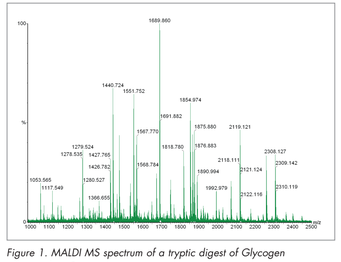

Figure 1. MALDI MS spectrum of a tryptic digest of Glycogen phosporylase B.

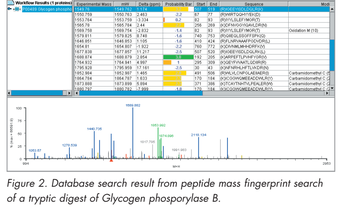

Figure 2. Database search result from peptide mass fingerprint search of a tryptic digest of Glycogen phosporylase B.

RESULTS AND DISCUSSION

MS results peptide mass fingerprint search

Figure 1 shows the MALDI mass spectrum for a Glycogen phosphorylase B digest. This spectrum was automatically deconvoluted and the monoisotopic masses obtained were submitted for a databank search using the ProteinLynx Global SERVER 2.1 search engine. The protein was confidently identified (see Figure 2). However, despite the majority of the peptide ions matching to the theoretical tryptic digest sequence of the protein, not all peptides could readily be assigned to Glycogen phosphorylase B including two peptides of similar mass (1531 Da and 1534 Da). To establish the identity of these peptides they were selected for further investigation by MS/MS.

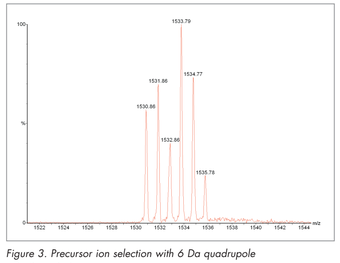

Figure 3. Precursor ion selection with 6 Da quadrupole selection window.

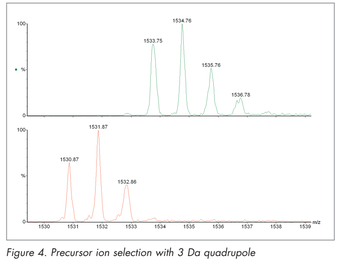

Figure 4. Precursor ion selection with 3 Da quadrupole selection window.

Precursor ion selection

To establish the optimal conditions for the MS/MS product ion acquisition of the two unknown peptide components, two different resolution settings on the quadrupole, MS1, were tested. The two resolution settings allowed either a 6 Da window to be transmitted to the gas cell, or a 3 Da transmission window. The effect of the quadrupole resolution settings on the selected precursor ion transmission is presented in Figures 3 and 4 respectively. An MS/MS spectrum was subsequently obtained using the 6 Da transmission setting (Figure 5), whilst two MS/MS spectra were recorded for the 3 Da setting (Figures 6 and 7). The spectrum shown in Figure 5, obtained when both peptides are transmitted to the gas cell is extremely complex and it was not possible to obtain any significant sequence information from this. The complexity of the two spectra obtained with a 3 Da transmission window is significantly reduced, and the analysis and interpretation of these MS/MS spectra is discussed in detail below.

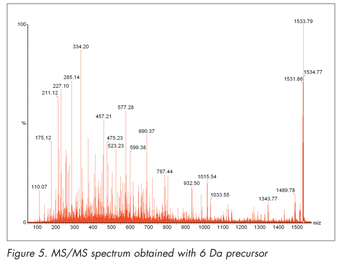

Figure 5. MS/MS spectrum obtained with 6 Da precursor selection window.

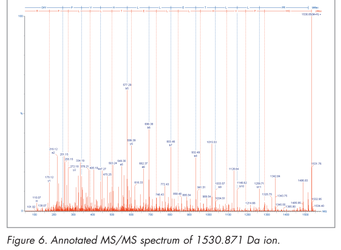

Figure 6. Annotated MS/MS spectrum of 1530.871 Da ion.

MS/MS results from m/z 1530.9

The MS/MS spectrum obtained from the fragmentation of the peptide with a mass of 1530.871 Da was deconvoluted using MaxEnt3 software. The processed MS/MS spectrum was submitted to the *de novo* sequencing software MassSeq™ (Waters, Manchester). Initially a mass standard deviation of 25 mDa was chosen. This parameter is a measure of the mass accuracy of the MS/MS data. With a value of 25 mDa two possible sequences were proposed, (Table 1). When the tolerance was narrowed down to a more reasonable 5 mDa, only one possible sequence was suggested. The theoretical mass of this peptide differed by 4.6 ppm from the experimentally determined mass. The putative sequence, GWPVHLLETLLPR, was compared to the Swiss-Prot entry for Glycogen phosporylase B. The sequence is completely homologous apart from the N-terminal residue where the amino acid glycine is inserted, to the C-terminal side of the arginine present in the databank entry, (see Figure 8).

MassSeq mass standard deviation (mDa)	Sequence	Delta (mDa)	Delta (ppm)
5	GWPVHLLETLLPR	7	4.6
25	KDPVHLLETLLPR	28	19.5
	GWPVHLLETLLPR	7	4.6

Table 1. Comparison of the amino acid sequences generated by the MassSeq algorithm with different minimum mass standard deviation settings.

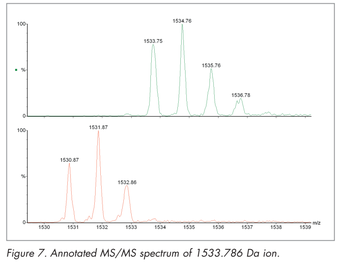

Figure 7. Annotated MS/MS spectrum of 1533.786 Da ion.

–ALERWPVHLLETLLPRHL Phosphorylase B Sequence from Swiss Prot (P00489)
GWPVHLLETLLPR Experimentally determined sequence from peptide (1530.871 Da)

Figure 8. Comparison of the experimentally determined amino acid sequence with the published Swiss-Prot sequence.

MS/MS results from m/z 1533.8

The MS/MS spectrum of the second peptide at 1533.786 Da (Figure 7) proved more complicated to sequence. The results from the MassSeq *de novo* sequencing, did not return a confident, complete sequence for the entire peptide, however it did have 100% confidence in a significant sub-sequence, PEFTLPV. Subsequent BLAST searching of this sequence resulted in a confident match to glycogen phosphorylase B, with the sequence being homologous across a variety of species. Further investigation suggested that the peptide could correspond to the sequence ARPEFTLPVHFYG(R). The theoretical monoisotopic mass of this peptide would be 1533.785 Da, which differs from the experimentally measured mass by 1 mDa (0.7 ppm). This gives additional confirmation that the proposed sequence is indeed correct. In addition, matching of the theoretical fragment ions to the MS/MS data shows that many of the low mass ions (m/z 100– 600) match to internal acyl fragment ions (Figure 9), which are common in singly charged peptide MS/MS spectra. Internal acyl ions are not considered as primary fragmentation routes by the MassSeq algorithm, however, these ions are very useful in helping to corroborate the sequence. It can, therefore, be concluded that this second peptide is also from glycogen phosphorylase B but was not identified by peptide mass fingerprinting as the peptide obserevd is not the result of a specific tryptic cleavage. Instead the experimentally observed peptide is produced by loss of the C-terminal arginine residue from the theoretical tryptic peptide.

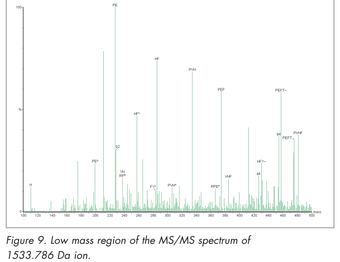

Figure 9. Low mass region of the MS/MS spectrum of 1533.786 Da ion.

CONCLUSIONS

- Use of a quadrupole mass filter allows the separate transmission of ions close in mass for subsequent MS/MS studies, (example shown is 3 Da at m/z 1530).
- This allows the acquisition of two separate MS/MS spectra to be obtained, from the individual peptide species.
- The ion at m/z 1530.871 is modified through the insertion of an additional glycine at the N-terminal of the peptide sequence. The peptide could not be identified from the peptide mass fingerprint due to this modification.
- The ion at m/z 1533.786 is also not a predicted tryptic peptide, but is formed due to loss of the C-terminal arginine
- Exact mass measurement of both precursor and fragment ions greatly enhances the confidence with which assignments could be made.

May 2004 720000893EN LLPDF