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ENHANCED IDENTIFICATION ON 2D GEL ISOLATED PROTEINS FROM *E. coli* USING PSD MX™ - PART 2

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OVERVIEW

• This application note compares the use of MALDI MS and PSD MX™ data acquired from a Waters® MALDI micro MX™ mass spectrometer, for the characterization of 2D-gel isolated proteins originating from *E.coli*.
• PSD MX enabled protein identification when there were insufficient numbers of tryptic peptides available for unambiguous identification using Peptide Mass Fingerprinting (MALDI MS).
• PSD MX provided additional confidence in protein assignment due to the specificity of MS/MS information.

INTRODUCTION

Peptide Mass Fingerprinting (PMF) is widely accepted and successful technique for protein identification. In some circumstances, however, it will not result in an unambiguous identification. Typical reasons for this are where the protein sequence is not present in a database; if a mixture of proteins with a wide dynamic range is present; or if the number of tryptic peptides produced by the proteolytic digestion is low.
In such cases, limited fragment ion information can provide sufficient information to overcome the limitations of PMF, resulting in unambiguous protein identification.
Here we show a novel form of Post Source Decay (PSD) analysis, PSD MX, allowing fragment ions from multiple precursor ions to be analyzed in parallel (1). This novel approach simplifies the PSD experiment since; no precursor ion selection is necessary, it is highly automatable, reduces sample consumption and increases the number of peptides analyzed. The advantage of performing a PSD MX, or MS/MS analysis on a group of peptides, is that additional sequence specificity is conveniently provided from the components in a single experiment.

EXPERIMENTAL RESULTS & DISCUSSION

Experimental details are as previously described(2).
Here we show three examples, where the use of PSD MX either greatly enhances the confidence of a protein identification made by PMF or where the use of PSD MX unambiguously identifies proteins, not found by PMF.

Example 1. Insufficient tryptic peptides for PMF

PMF analysis may give ambiguous results when the enzymatic process produces insufficient tryptic peptides. An example is shown in Figure 1. PMF database searching of this information resulted in an ambiguous result, with only four peptides putatively identified. Therefore, a confident protein identification could not be made.

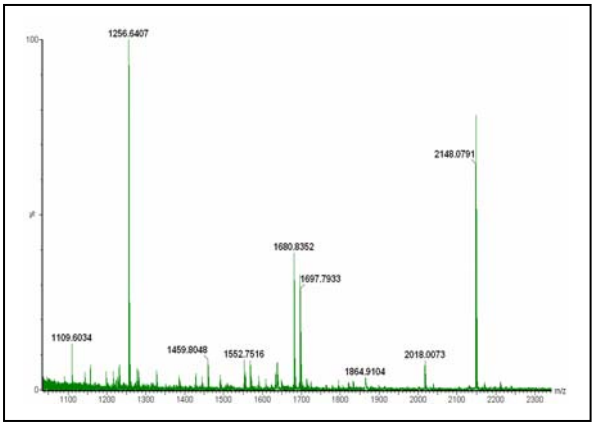

Figure 1. MALDI MS spectrum of the 2D gel spot. Databank searching of the resulting PMF information resulted in an ambiguous result.

However, a PSD MX experiment provided additional fragment ion information and through subsequent use of this information in a databank search, it was possible to unambiguously identify Arginine-binding periplasmic protein 2 (ARTJ_ECOLI).

Figure 2 shows the three peptides that gave fragment ion information which led to the protein identification.
Databank searching of the PSD spectra from each peptide led to positive identification of the parent protein. When the PMF and PSD MX data were combined, the MOWSE score increased further to 125; with only three peptides matched in total (the minimum MOWSE score for a 95% probable identification is 65).

Example 2. PSD MX and protein mixtures

The benefit of using PSD MX over the PMF approach is clearly demonstrated in the following example. Analysis of the 2D gel spot contained in position D, 4 by MALDI PMF identified two proteins, SERC_ECOLI (MOWSE PMF score of 110) and TYRA_ECOLI (MOWSE PMF score of 74).

However, subsequent searching of the PSD MX data identified an additional protein as the top scoring hit, ALF_ECOLI.
Present in figure 3 is the MS spectrum of sample D,4, with matching peptides from the 3 proteins highlighted

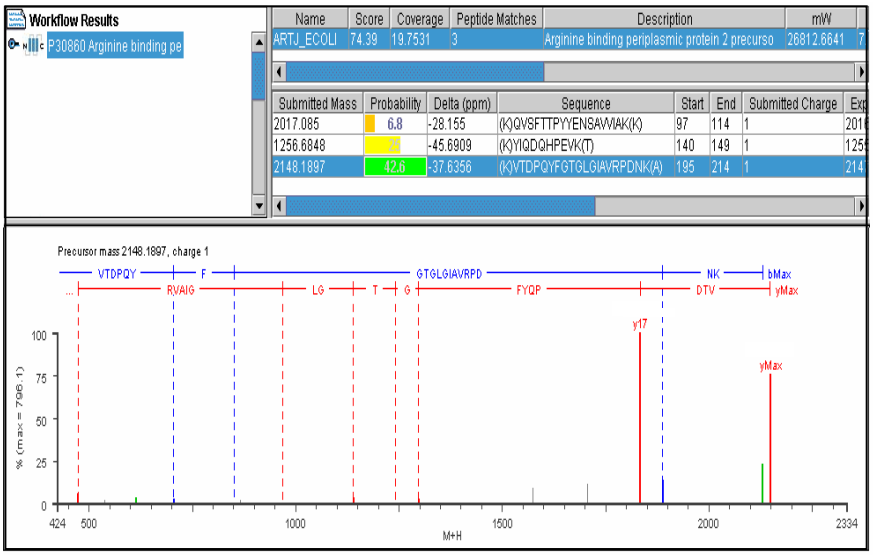

Figure 2. Results from the PSD MX fragment ion database search. Highlighted is the PSD MX spectrum for m/z 2148.2.

SERC_ECOLI protein was identified by 14 peptides, which are of relatively low intensity. Similarly, the protein TYRA_ECOLI was identified by eight peptides, which are also low in intensity.
The protein ALF_ECOLI was identified using PSD MX information from four peptides present in the mixture. This number of peptides is below the minimum (5) usually required for an identification from a PMF database search. The four peptides assigned to ALF_ECOLI yielded PSD fragments and due to the specificity of this structural information, the protein ALF_ECOLI could be confidently assigned.

In addition, PSD MX was used to correct the assignment of one peptide in this mixture. Initially, using MS data only, the peptide at m/z 1877.9535 was matched to TYRA_ECOLI. However, following the databank search of the PSD MX data, the ion was identified as a tryptic peptide from ALF_ECOLI (IFDFVKPGVITGDDVQK) with 9 fragment ions matched to the sequence (Figure 4).

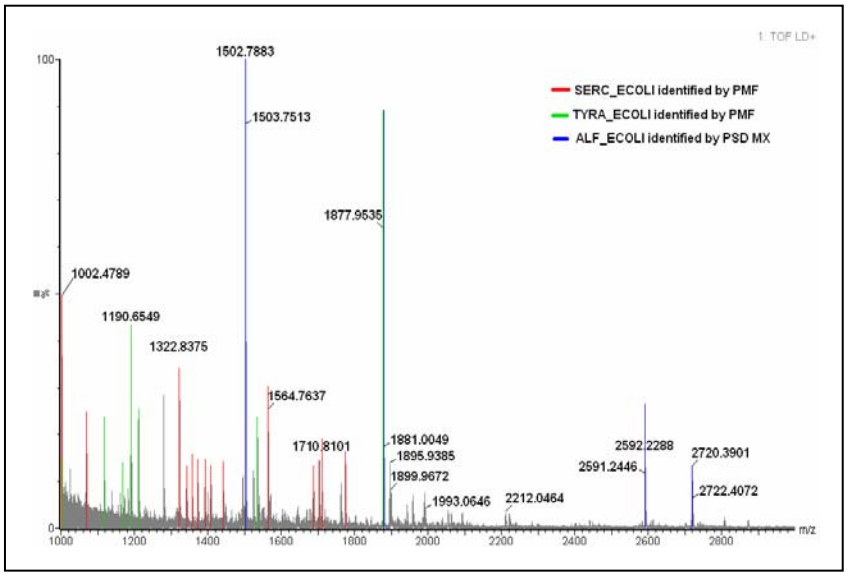

Figure 3. MS spectrum of sample D,4. Peaks highlighted in red have been matched to SERC_ECOLI with PMF. Peaks highlighted in green have been matched to TYRA_ECOLI with PMF. Peaks highlighted in blue have been matched to ALF_ECOLI with PSD MX.

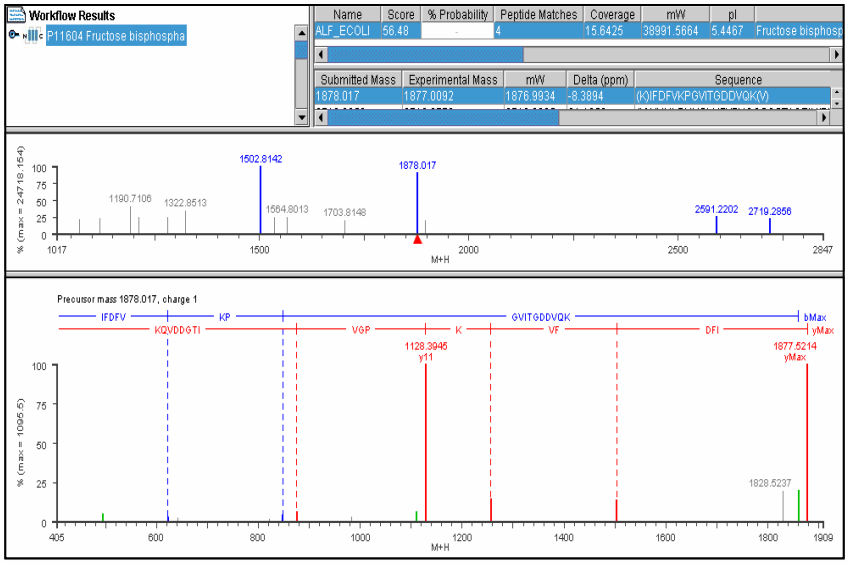

Figure 4. Results of the fragment only PSD MX database search. Highlighted is the PSD MX spectrum of m/z 1878.0.

Example 3. PSD MX provides added confidence in protein assignment

The 2D gel spot located at position F5, gave a highly confident score of 120 when analyzed by PMF. If the PSD MX information is utilized then the score increased to 277, leading to more confident protein identification. In this case, the score obtained from each PSD MX spectra, using the four most intense ions, were individually high enough to identify the protein confidently (figure 5).

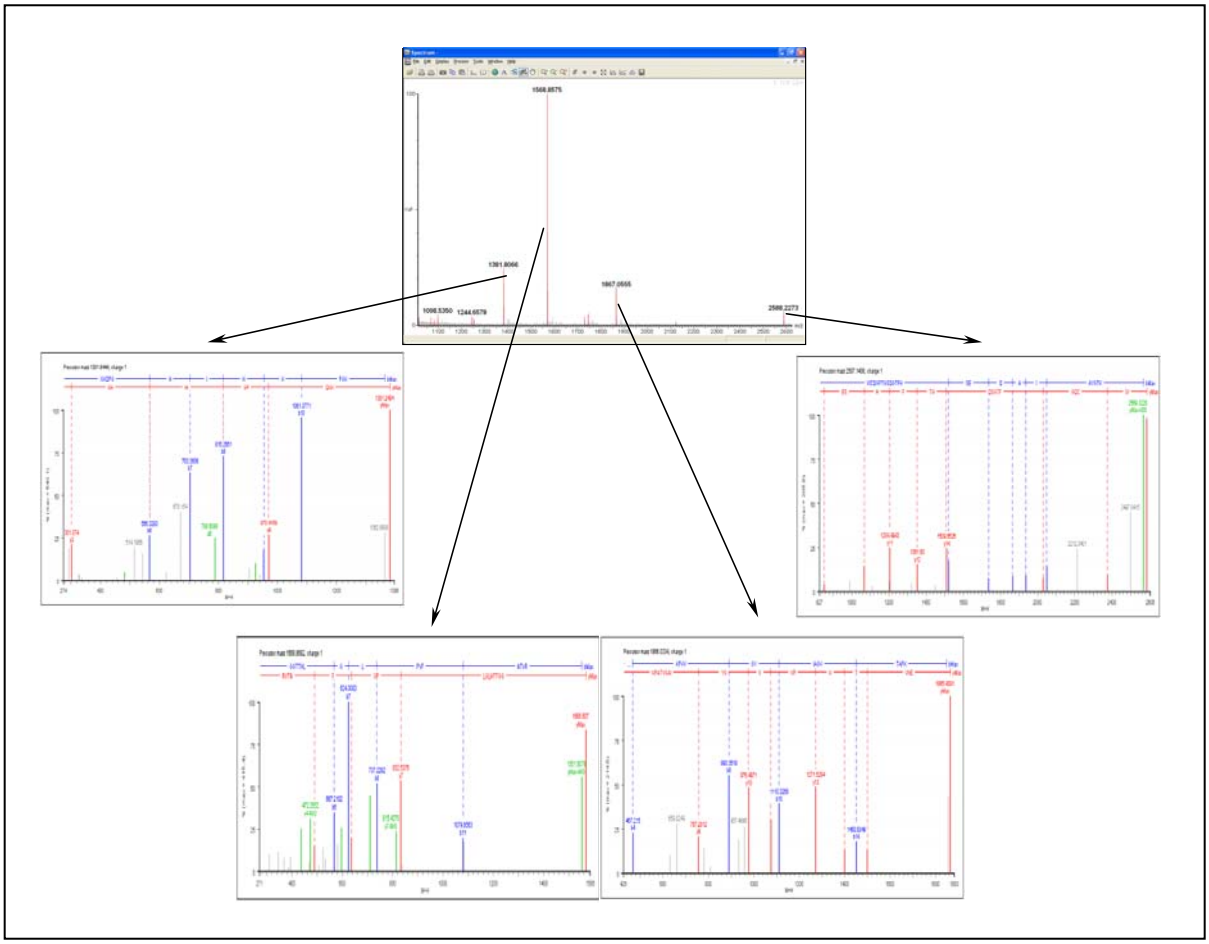

Figure 5. Automated display obtained from ProteinLynx™ Global SERVER following the PSD MX database search. Highlighted are PSD MX spectra from four peptides from sample F,5 which gave excellent fragmentation (sequence) information.

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CONCLUSIONS

• In this application note three example were highlighted where the use of PSD MX enhanced protein identification of 2D gel isolated proteins.
• Example 1 showed that the use of PSD MX derived fragment ion information, led to identification of a protein that could not be identified by PMF (as too few peaks were observed).
• Example 2 showed that PSD MX identified a protein, not identified using PMF, even when part of a three protein mixture.
• Example 3 showed that combining PMF and PSD MX (fragmentation) information together can significantly increase the confidence score.

REFERENCES

(1) D. Kenny, J. Brown, M. Snel, *Technical note*: Multiplexed Post Source Decay (PSD MX) A novel technique explained. (720000948EN).
(2) E.Claude, M. Snel, *Application note*: Enhanced identification of 2D gel isolated proteins from ecoli using PSD MX. (720001155EN).

Waters MALDI micro MX Mass Spectrometer, MassLynx™ software and ProteinLynx Global SERVER bioinformatics.

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