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EVALUATION OF LC-ESI/LC-MALDI IN THE ANALYSIS OF PHOTOSYSTEM I AND II COMPLEX OF BARLEY

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OVERVIEW

- **Here we describe the analysis of the Photosystem I and II complex from barley (*hordeum vulgare)* by mass spectrometry**
- **Increased confidence in protein identification, and amino acid sequence coverage, is obtained by combining two ionization techniques, MALDI and ESI**
- **MALDI and ESI were used on the on the same quadrupole orthogonal TOF mass spectrometer**

INTRODUCTION

In this poster we present a combined strategy using LC-ESI MS/MS and LC-MALDI MS/MS for the sequence level analysis of the photosystem I and II complex from barley. The protein complex was extracted, tryptically digested and the sample analyzed using nanoscale HPLC followed by ESI MS/MS on a Q-Tof mass spectrometer. A second analysis was performed where the eluent from a nanoscale HPLC separation was fractionated onto a MALDI target plate, with the fractions subsequently analyzed on a MALDI Q-Tof mass spectrometer. Novel software for precursor ion selection was employed for the MALDI experiments.
Both ESI and MALDI experiments identify similar numbers of proteins with comparable sequence coverage. However, the results show that unique tryptic peptides are identified by MALDI and ESI techniques and combining the data from both approaches results in an increased total sequence coverage and a greater number of proteins identified.

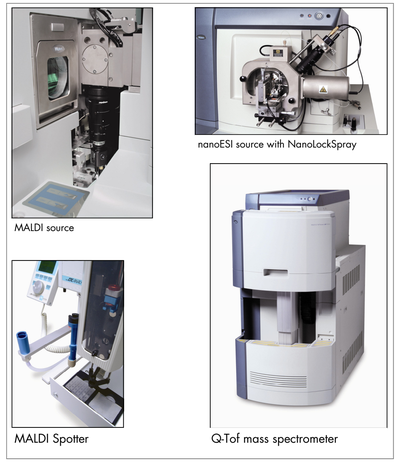

Figure 1. Overview of MALDI and nano electrospray sources on the Q-Tof mass spectrometer, used to compare ionization techniques

METHODS

Sample preparation and UPLC conditions

A nanoACQUITY UPLC™ system (Waters, Milford, MA) was used for the chromatographic separation, both for the on-line (ESI) and off-line MALDI experiments, with identical LC conditions.
The Photosystem I and II tryptic digest was diluted 1:10 in 0.1% formic acid, and 4 µL of the diluted sample loaded.
Trapping column: Symmetry C18 (5µm)180 µm x 20 mm column (Waters, Milford, MA)
Analytical column: BEH C18 (1.7µm) 75 µm x 100 mm column (Waters, Milford, MA), flow rate of300 nL/min
For off-line sample preparation, the eluent from the analytical column was deposited onto a MALDI target plate using a CTC-MALDI spotting robot (CTC, Basel, CH). The eluent was mixed in a Y connector with α-cyano-4-hydroxycinnamic acid (CHCA) matrix solution (2 mg/mL in 1:1 MeCN:0.1% TFA), with a spotting time of 40 seconds per spot.
The on-line and off-line experiments were performed in triplicate.

Mass Spectrometry

Both ESI and MALDI experiments were performed on the same Q-Tof Premier mass spectrometer (Waters, Milford, MA); Figure 1. The total turn-around time for switching between modes was three hours.All data, both ESI and MALDI, was acquired using a data directed analysis (DDA) strategy.
In the case of the on-line ESI analysis, MS information was evaluated 'on-the- fly' during a chromatographic run and the instrument switched into the MS/MS mode, selecting the 3 most intense precursor ions, when multiply charged ions breached a threshold of 15 counts/ sec in the MS mode.
In the MALDI experiment it is possible to interrogate the same sample spot multiple times, and thus the MS data can be acquired from all sample spots associated with an LC run and subsequently MS/MS data from selected spots can be acquired, based upon the MS information obtained, Figure 2. Mass accuracy was ensured using [Glu]-fibrinopeptide B, spotted on a separate position on the target plate.

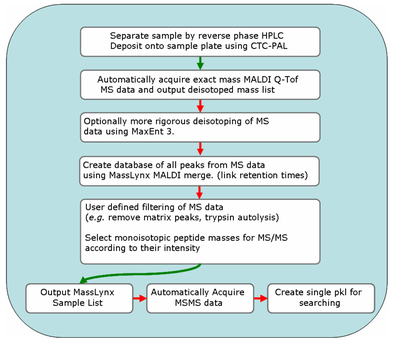

Figure 2. Flow diagram illustrating the data processing scheme used for the LC- MALDI experiments

RESULTS AND DISCUSSION

A typical example of a MALDI MS/MS databank search results, obtained from the photosystem sample, is shown below in Figure 3.

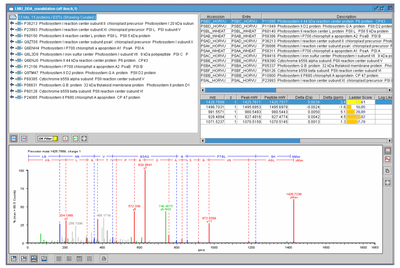

Figure 3. Example of databank search results from one LC-MALDI experiment

Comparison between MALDI and ESI data

The total number of proteins identified from photosystem I and II of barley or closely related species was fifteen. The proteins identified are listed in Table 1. The % sequence coverage is relatively low for the proteins identified, due to the nature of the proteins contained within the photosystem complex. These are known to contain significant transmembrane domains which are not readily amenable to digestion and may hinder the operation of trypsin. The transmembrane domains in the sequence of the protein PSBD are highlighted in Figure 4. This protein was identified in both the MALDI and ESI MS/MS experiments.

MTIALGRVPKEENDLFDTMDDWLRRDRFVFVGWSG**LLLFPCAYFALGGW**
FTGTTFVTSWYTHGLASSYLEGCNFLTAAVSTPANSLAHSLLLLWGPEAQ
GDFTRWCQL**GGLWTFVALHGAFALIGFMLRQ**FELARSVQLRP**YNAISFSG**
PIAVFVSVFLIYPLGQSGWFFAPSFGVAAIFRFILFFQGFHN**WTLNPFHMM**
GVAGVLGAALLCAIHGATVENTLFEDGDGANTFRAFNPTQAEETYSMVTA
NRFWSQIFGVAFSNK**RWLHFFMLFVPVTGLWMSAIG**VVGLALNLRAYDFV
SQEIRAAEDPEFETFYTKNILLNEGIRAWMAAQDQPHENLIFPEEVLPRGNA
L

Figure 4. Illustration of the extensive transmembrane domains found in many Photosystem proteins, here highlighted in the sequence of Photosystem II D2 protein, PSBD_HORVU

There is great similarity in the results obtained from the MALDI and ESI techniques, this can clearly be seen in Figure 5. MALDI and ESI experiments were replicated three times. The average MALDI experiment identified 11 proteins using 18 peptide MS/MS spectra and the average ESI experiment identified 11 proteins using 17 peptide MS/MS spectra. From these figures it is clear that each protein identification is underpinned by a relatively low number of MS/MS spectra. For this reason any additional information that may be gleaned, through the use of a complementary technique, such as using a different ionization mode, greatly strengthens the identification and level of confidence obtained.

dB entry	Description	M. Wt	% Coverage		
			MALDI	ESI	Combined
PSAA_WHEAT	P58311 Photosystem I P700 chlorophyll a apoprotein A1 PsaA PSI A	83.1 kDa	1.1%	1.1%	1.1%
PSAB_WHEAT	P58386 Photosystem I P700 chlorophyll a apoprotein A2 PsaB PSI B	82.6 kDa	2.7%	1.6%	2.7%
PSAC_HORVU	P69416 Photosystem I iron sulfur center Photosystem I subunit VII 9 kDa polypeptide PSI C F	8.8 kDa	11.3%	11.3%	11.3%
PSAD_HORVU	P36213 Photosystem I reaction center subunit II chloroplast precursor Photosystem I 20 kDa subun	21.9 kDa	9.3%	18.5%	18.5%
PSAE_HORVU	P13194 Photosystem I reaction center subunit IV chloroplast precursor PSI E Photosystem I 10 8	15.6 kDa	0.0%	21.8%	21.8%
PSAF_HORVU	P13192 Photosystem I reaction center subunit III chloroplast precursor Light harvesting complex	24.8 kDa	6.3%	6.3%	12.7%
PSAK_HORVU	P36886 Photosystem I reaction center subunit psaK chloroplast precursor Photosystem I subunit X	13.7 kDa	10.1%	10.1%	10.1%
PSAL_HORVU	P23993 Photosystem I reaction center subunit XI chloroplast precursor PSI L PSI subunit V	22.2 kDa	4.7%	0.0%	4.7%
PSBA_HORVU	P05337 Photosystem Q B protein 32 kDa thylakoid membrane protein Photosystem II protein D1	38.9 kDa	3.1%	12.2%	12.2%
PSBB_HORVU	P10900 Photosystem II P680 chlorophyll A apoprotein CP 47 protein	56.1 kDa	2.2%	2.6%	4.7%
PSBC_HORVU	P11095 Photosystem II 44 kDa reaction center protein P6 protein CP43	52.1 kDa	11.6%	11.2%	13.1%
PSBD_HORVU	P11849 Photosystem II D2 protein Photosystem Q A protein PSII D2 protein	39.6 kDa	9.1%	9.1%	9.1%
PSBE_HORVU	P69390 Cytochrome b559 alpha subunit PSII reaction center subunit V	9.3 kDa	12.2%	0.0%	12.2%
PSBF_HORVU	P60126 Cytochrome b559 beta subunit PSII reaction center subunit VI	4.3 kDa	21.1%	21.1%	21.1%
PSBL_WHEAT	P60140 Photosystem II reaction center L protein PSII L PSII 5 kDa protein	4.3 kDa	37.8%	37.8%	37.8%

Table 1. Summary of photosystem I and II proteins identified by both ionization techniques

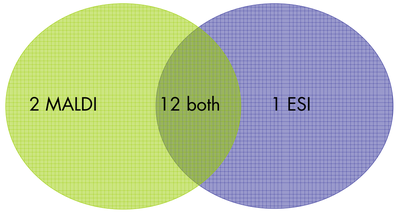

Figure 5. Summary of the photosystem proteins identified by MALDI and ESI. In total 15 proteins were identified with the vast majority being identified by both techniques.

Comparison of ESI and MALDI MS/MS data

An example of a common peptide identification made between MALDI and ESI is shown in Figure 6. The two MS/MS spectra exhibit different fragment ions and different fragment ion intensities, as might be expected.

The observed differences are primarily due to the charge state of the precursor ion selected for MS/MS. MALDI yields predominantly singly charged peptides, whereas ESI tends to produce multiply charged ions. In this example a strong contiguous series of Y'' type ions is observed in the ESI spectrum, typical of the fragments observed from a doubly charged precursor ion. The dominant peak in the MALDI spectrum is a Y'' ion peak associated with cleavage next to an acidic amino acid (Asp), which is typical for the singly charged precursor.

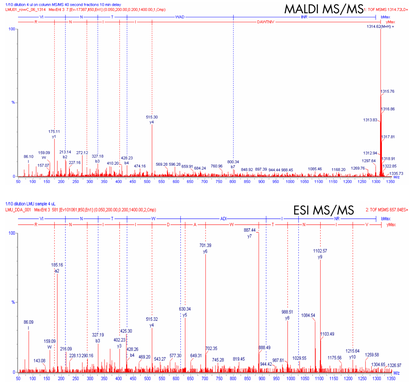

Figure 6 MS/MS spectra for VINTWADIINR from PSBA_HORVU. Top spectrum from the MALDI experiment, Bottom spectrum from the ESI experiment.

Amino acid composition

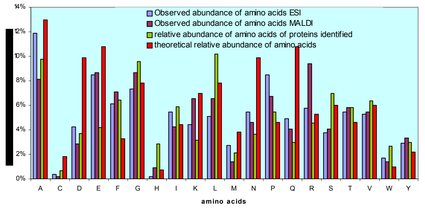

Figure 7 "Average" amino acid composition of the tryptic peptides identified by MALDI and ESI compared to the theoretical abundance of the amino acids in the photosystem proteins identified.

Peptide length

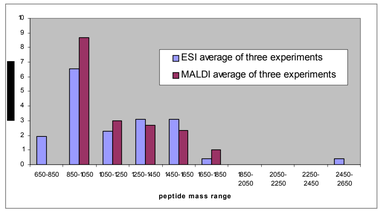

Figure 8. Relationship between peptide size and detection by ESI and MALDI. In general the two techniques are similar, although the ESI technique identifies a large peptide that is missed by the MALDI approach. Acquisition in the MALDI experiment did not extend below m/z 800 and therefore no peaks were detected in the lowest mass segment.

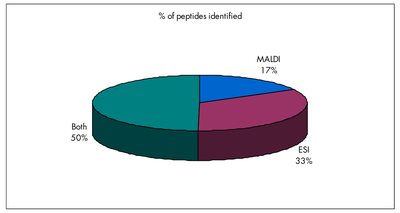

Figure 9. Percentage of the amino acid sequence identified by the ESI and MALDI ionization technique, determined from the matching tryptic peptides.

CONCLUSION

- **Comparison of MALDI and ESI LC-MS/MS was performed on a single MS platform.**
- **In total 15 proteins from the Photosystem I and II complex were identified**
- **Slightly higher amino acid sequence coverage was obtained using ESI LC-MS/MS**
- **Using a combination of ESI and MALDI allowed the number of proteins and sequence coverage to be maximized**
- **MS/MS spectra from common peptides identified by MALDI and ESI exhibit different fragmentation patterns, owing primarily to the precursor ion charge state**

FUTURE WORK

- **Different MALDI matrices will be tested to increase sequence coverage**
- **The use of surfactants will be investigated to improve digestion efficiency**

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