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INCREASED SEQUENCE COVERAGE OF PHOTOSYSTEM I AND II-COMPLEX OF BARLEY USING COMBINED ESI/MALDI ANALYSIS

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OVERVIEW

- Mass spectral analysis of photosystem I and II complexof barley (*hordeum vulgare)*
- Increased sequence coverage and confidence in protein identification from a complex sample by combining two mass spectrometric ionization sources.
- Demonstration of MALDI and nanoESI on the same quadrupole orthogonal Tof geometry.
- Discussion of the differences between ESI and MALDI results.

INTRODUCTION

In this poster we show a strategy using LC-ESI Q-Tof MS/MS and LC-MALDI Q-Tof MS/MS for the sequence level analysis of the components of Photosystem I and II complex of barley. The protein complex was extracted and tryptically digested. The sample was analyzed using nanoscale HPLC followed by nano-electrospray MS/MS on a Q-Tof mass spectrometer. A second analysis was performed where the eluent from a nanoscale HPLC separation was collected in fractions on a MALDI target plate. The MALDI fractions were analyzed on a Q-Tof mass spectrometer operating in MALDI mode. Novel software for precursor ion selection was employed for the MALDI experiments.
Early results show that both ESI and MALDI experiments identify similar numbers of proteins with similar sequence coverage. Examination of the results show that some tryptic peptides are only identified by either MALDI or ESI. When MALDI and ESI data are combined the total sequence coverage and the number of proteins identified is increased. A detailed breakdown of these results will be provided.

METHODS

The experimental set-up is shown schematically in **Figure 1**.

LC Conditions

Sample: tryptic digest of Photosystem complex of Barley (diluted 1:1)
Experiment: fast loading on trap column, followed by desalting and separation on analytical column.
LC Pump: nanoACQUITY UPLC™ System (Waters, Milford, MA)
Trapping column: Waters Symmetry® C18, 5 µm, 180 µm x 20 mm, nanoACQUITY column (Waters, Milford, MA)
Analytical column: Waters BEH C18, 1.7 µm, 75 µm x 100 mm, nanoACQUITY column (Waters, Milford, MA)
Flow rate: 250 nL/min (direct flow)
Gradient: 3 - 40 % MeCN over 30 minutes

LC-MALDI spotting: MALDI Spotter/ Fraction Collector (CTC, Basel, CH)
Matrix addition: CHCA 2 mg/mL (in 1:1 0.1% TFA:MeCN) teed into eluent flow at1700 nL/min
Spotting duration: 30 seconds per spot

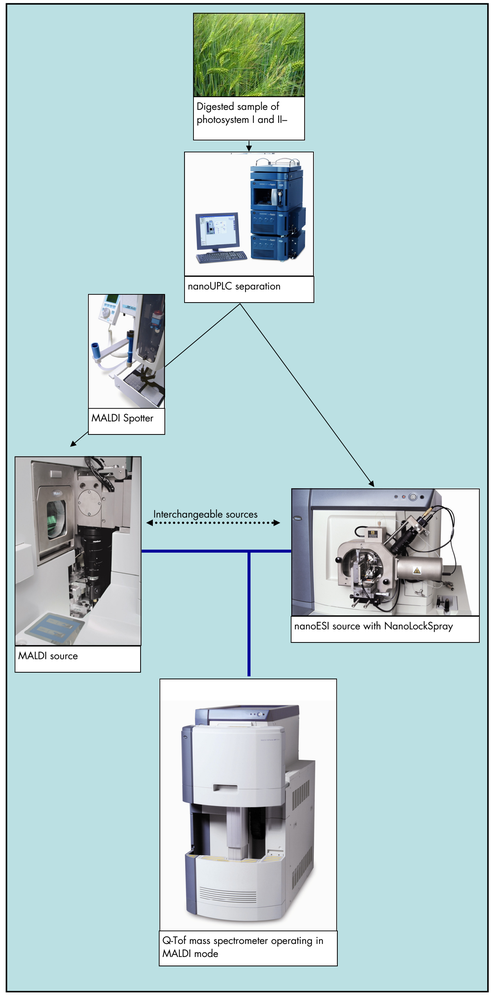

Figure 1: *Experimental set-up and work-flow*

MS Conditions

All MS and MS/MS data were acquired on a Q-Tof™ Premier mass spectrometer (Waters, Manchester, UK). The mass spectrometer was operated in nano electrospray mode and MALDI ionization mode. The nano ESI experiment was a data directed analysis (DDA) experiment where the instrument switched from MS to MS/MS mode when multiply charged species were detected. Exact mass accuracy (better than 3 ppm RMS) was maintained by sampling a reference spray.
For the LC-MALDI experiment a modified DDA approach was used, MS data from the whole LC run was acquired. These data were automatically deisotoped and a list of all MS/MS candidates was generated. MS/MS data were then acquired from the same target plate.

RESULTS AND DISCUSSION

The protein identifications obtained using MALDI and ESI ionization are summarized in **Table 1**. It should be noted that identifications with a Mascot score of less than 36 are below the 95% confidence limit of the databank search. Four hits with lower scores have been included on the basis of manual validation. It can be seen that although 11 proteins were identified using both techniques, further proteins were identified by only MALDI or ESI. **Figure 2** illustrates the differences in the MALDI and ESI results on the protein and peptide level.
When a protein is identified using both techniques the identification is frequently based on different peptides. This means that a dual ionization approach increases sequence coverage (*c.f.* **Figure 3**).

Protein	Mascot Score	% coverage combined	% coverage ESI	% coverage MALDI
PSBC_HORVU (P11095)	563	16.9%	14.6%	13.5%
PSAD_HORVU (P36213)	387	49.3%	32.7%	42.0%
PSBD_HORVU (P11849)	347	13.9%	13.9%	9.1%
PSAF_HORVU (P13192)	310	29.8%	16.6%	17.4%
PSBA_HORVU (P05337)	281	15.9%	6.2%	12.7%
PSBB_HORVU (P10900)	230	19.9%	12.4%	9.1%
PSAK_HORVU (P36886)	149	16.0%	15.3%	16.0%
PSAH_HORVU (P20143)	148	16.8%	15.4%	16.8%
PSAE_HORVU (P13194)	147	35.4%	35.4%	0.0%
PSAG_HORVU (Q00327)	120	23.1%	5.6%	23.1%
PSAB_WHEAT (P58386)	111	2.6%	2.6%	1.0%
CB24_ARATH (P27521)	97	6.8%	6.8%	0.0%
PSAA_WHEAT (P58311)	79	4.4%	3.2%	1.2%
PSBH_HORVU (P12363)	43	43.1%	0.0%	43.1%
PSAC_HORVU (P69416)	30	22.5%	0.0%	22.5%
ATPB_HORVU (P00828)	24	2.4%	0.0%	2.4%
PSBF_HORVU (P60126)	24	21.1%	0.0%	21.1%
PSBL_HORVU (P60143)	16	36.8%	36.8%	0.0%

Table 1: *Databank search results and % sequence coverage by ionization technique. Scores are combined scores from the LC-MALDI MS/MS and LC-nanoESI MS/MS experiments.*

The differences between ESI and MALDI MS/MS data are shown in **Figure 4**. The different fragmentation observed is due to the fact that ions generated by MALDI generally singly charged, whereas ESI generated ions tend to be multiply charged.
From the data obtained in this experiment whether a peptide is identified from the MALDI or ESI experiment is influenced by the length of the peptide (see **Figure 5**). Longer peptides are more likely to be identified from the ESI experiment, whereas shorter peptides will be more readily detected in the MALDI experiment.

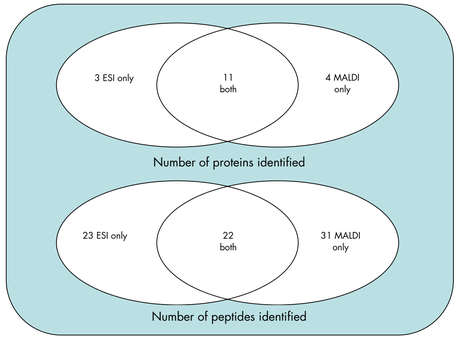

Figure 2: *Comparison of proteins and peptides identified using MALDI and electrospray ionization*

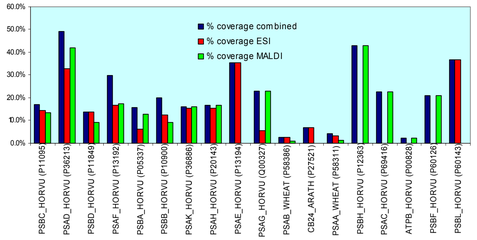

Figure 3: *Sequence coverage of proteins identified*

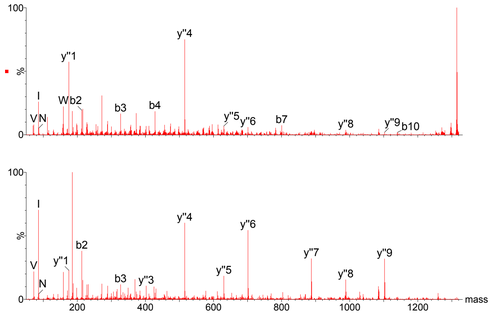

Figure 4: *MS/MS data of VINTWADIINR. Top spectrum from MALDI experiment, Bottom spectrum from ESI experiment*

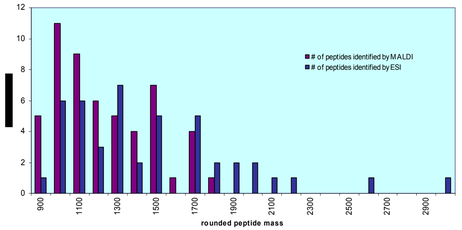

Figure 5: *Effect of tryptic peptide length on detection by ESI/MALDI*

An initial evaluation of the effect of peptide composition on the likelihood of peptide identification was made (see **Figure 6**). Both MALDI and ESI ionization display a bias to peptides containing glutamic acid residues as well as lysine and arginine, whereas leucine and serine containing peptides are under represented. The overrepresentation of arginine and lysine is clearly due to the protease used in the digestion of the proteins. Further work is required to fully investigate the other observations.

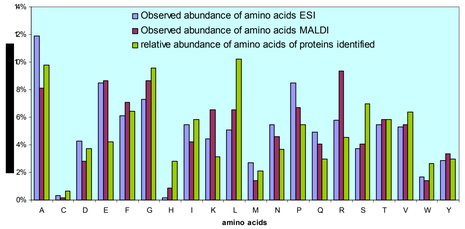

Figure 6: *Average amino acid composition of peptides identified by MALDI and ESI compared to the theoretical abundance of amino acids in the proteins identified.*

CONCLUSIONS

- The combination of MALDI and ESI MS/MS produced an increase in the number of different peptides identifiable from a tryptic digest of photosystem I and II-complex from barley.
- The sequence coverage of identified proteins is increased through the complimentary nature of the data obtained from the two techniques.
- A clear correlation between peptide length and ionization technique was observed.

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