Complementarities of MALDI and ESI for 2DLC MS/MS: Expanding proteome coverage of complex samples with multiple dimensions of analysis

Introduction

In proteomics, use of mass spectrometry plays a key role. However, given the number of peptides coming from digestion of complex proteins mixtures and given the dynamic range of their concentration, it is impossible to identify all proteins of a very complex sample without any prior fractionation. The on-line coupling of LC-MS in ESI mode (RPLC-ESI) is the most frequent set-up. Using an automate, it is also possible to collect the peptides eluted from nano flow-rate LC for efficient implementation of the off-line coupling of LC-MS in MALDI mode (RPLC-MALDI). Combined analysis of a given sample with both ESI and MALDI improves the sequence coverage of proteins of interest. In this work we have compared the results obtained combining these 2 ionization modes in order to established some workflow for a better proteome coverage of the samples.

Methods

Samples:

LCP = tryptic digest of 6 proteins mix from 11 kDa to 135 kDa (Cytochrome C, Lysozyme, Alcohol dehydrogenase, Bovine serum albumin, Serotransferrin and ß-Galactosidase, Dionex, Netherlands); *Escherichia coli* = tryptic digest of 40 fmol/µL of E.coli (different injection volume); Sample= tryptic digest of human *stratum corneum*

Liquid Chromatography:

1D RPLC : on Ultimate 3000 (Dionex).
Trap-column: C18 PepMap100, 3 µm, 300 µm i.d., length 5 mm (Dionex) equilibrated at 20 µL/min with solvent A.
Analytical column: C18 PepMap100, 3 µm, 100 Å, 75 µm i.d., length 15 cm (Dionex).
Linear gradient: from 0 to 50% of B in 35min, flow rate of 220nL/min.
To eliminate polymeric contaminants a purification cartridge (C18 PepMap100, 5µm, 100 Å, 1 mm i.d., longueur 15 mm, Dionex) is inserted between the loading pump and the injection valve. After sample injection, this cartridge is flushed with 40µL/min of solvent B during 25min. (see poster ThPI 225 for details on contaminants removal)

2D LC : on Dual Ultimate 3000 (Dionex)
First dimension: Strong Cation Exchange separation
Second dimension : As described above. (see poster ThPI 241 for details on 2DLC setup)

Solvents : solvent A: H20/AcN/FA, 98/2/0,1, v/v/v, solvent B: H20/AcN/FA

Microfraction collector

MALDI target preparation was realized using a Probot™ microfraction collector (Dionex) starting 15 min after the injection of the sample. For each run, 240 spots (1 spot every 10 seconds) are collected with a coaxial matrix (αcyanohydroxycinnamic acid, CHCA, 50% AcN) at flow rate 442 nl/min.

Mass spectrometry

MALDI-TOF/TOF

Tandem mass spectrometer 4800 MALDI-TOF/TOF Analyzer (Applera, Applied Biosystems Inc.), in automatic switching mode between MS and MS/MS; MS in positive reflectron mode on a mass range 700-4000 Da, intensity of the laser just above the desorption threshold; MS/MS generated on the top 7 precursors with minimum S/N=40. Peptides are automatically selected in the spot where the signal is most intense for MS/MS analyses in positive mode (2kV-TIS200 with an intensity of the laser approximately of 150 % superior to that in MS mode).

nanoESI LTQ-FT (ThermoFisher Scientific)

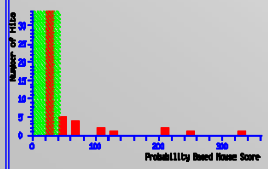
Automatic high dynamic mode : alternate acquisitions in FTMS full scan survey mode (m/z range 400-2000), 3 FTMS SIM scan mode for exact mass and charge state determination of peptide and 3 LIT MS/MS mode for sequencing

vMALDI-LTQ-Orbitrap (ThermoFisher Scientific) Please refer to posters TPBB MALDI/ Tandem MS 67

nanoESI QTof2 (Micromass, Manchester, UK).

Data acquisition was realized in automatic mode switching between the survey acquisition in MS and fragmentation acquisition in MSMS on the 4 most intense ions detected in the former survey scan.

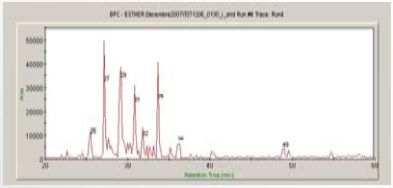
Results



Protein Name	Accession Number	Number of peptides	Protein Score
Serotransferrin precursor	Q29443	8	330
Serum albumin precursor	P02769	7	247
Cytochrome C	P00015	3	208
Lysozyme C	P00698	3	111
Beta-Galactosidase	P00722	2	74
Alcohol Deshydrogenase	P00330	2	64

Protein identified

Analysis of 3 fmol of LCP by nanoLCMALDI TOF/TOF



tryptic digest chromatogramm

Our experimental set-up for LC-MALDI was first validated on standard mixtures as shown here. Furthermore, it gives a more extensive analysis of peptides: several global analyses in MS and MS/MS can be successively realized on the same LC separation with different parameters on the opposite of electrospray analysis where the sample is definitively injected and is not any more available afterwards. It is also interesting to observe the differences between both methods of ionization.

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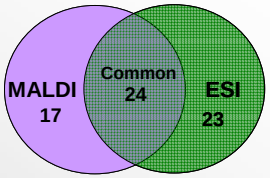
Analysis of complementarity between ESI and MALDI after 1D LC separation

To observe the complementarity between both types of ionization we realized two comparable studies with mass spectrometers of equivalent range. A first comparative study was realized between LC-MALDI-TOF/TOF and LC-ESI-QTOF, and a second between LC-MALDI-LIT-Orbitrap and the LC-ESI-LIT-FTICR. Sample used: tryptic digest of *E.coli*.

Complementarity of proteome

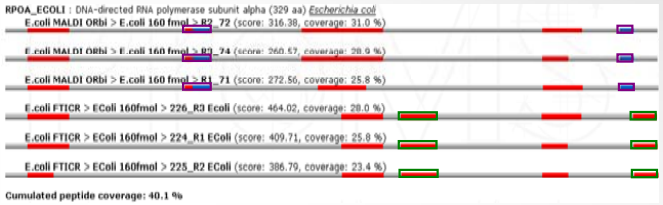
To study the complementarities of analysis, proteins identified in both analyses are selected. The associated identified peptides are listed together with the percentage of sequence coverage within one single analysis and within both combined analyses.



LC-MALDI TOF/TOF vs LC-ESI-LIT-FTICR

Peptide complementarity

LC-MALDI-LIT-Orbitrap vs LC-ESI-LIT-FTICR

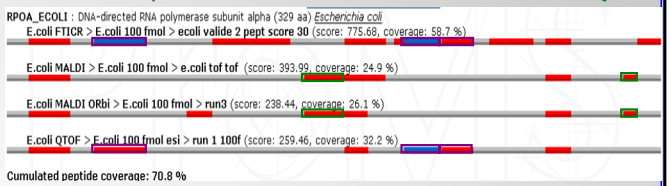


→ Specific peptides in the ionization MALDI

→ Specific peptides in the ionization ESI

In spite of the different dynamic ranges of the mass spectrometers it is interesting to combine the various results to observe the specificity of peptides according to the ionization used. The schematic representation shows the specificity of peptides that appear only with one ionization mode.

LC-MALDI-LIT-Orbitrap vs LC-ESI-LIT-FTICR vs LC-MALDI-TOF/TOF vs LC-ESI-QTOF

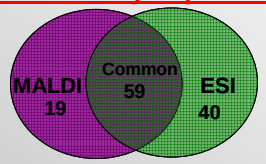


In these examples we selected the DNA-directed RNA polymerase subunit alpha which is common in both method of analysis. MyProMs® (Proteome Analysis using Mass Spectrometry with MYSQL Database) software, developed at Institut Curie (Paris, France), allows to visualized the results with a schematic representation of the sequence coverage for each protein and for the two ionization modes. It underlines the complementarity between ESI and MALDI and calculates the increase of sequence coverage.

Analysis of complementarity between ionization ESI and MALDI after 2D LC separation

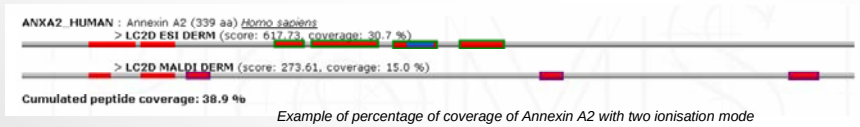
2D-LC is a powerful tool to increase the proteome coverage. It was applied using a off-line mode for the characterization of a tryptic digest of human *Stratum corneum*. After the first dimension a total of 22 fractions was collected and divided in two aliquots. The first one was analyzed in LC-ESI-LIT-FTICR and the other one was analyzed in LC –MALDI-TOF/TOF. The MALDI target spotting was optimized for up to 2880 spots (12 fractions), so 2 plates were necessary for the whole analysis.

Complementarity of proteome



2D-LC-MALDI-TOF/TOF vs 2D-LC-ESI-LIT-FTICR

2D-LC-MALDI-TOF/TOF vs 2D-LC-LIT-FTICR



Example of percentage of coverage of Annexin A2 with two ionisation mode

→ Specific peptides in the ionization MALDI

→ Specific peptides in the ionization ESI

ABI MALDI –PLATE 2880 spots

The complementarity of ESI and MALDI is still verified with 2D-LC separation, even if we have noticed that the higher the dynamic range and sensitivity, the more common peptides are observed in both ESI and MALDI. These results show the importance of LC-MALDI coupling for the improvement of proteome coverage.

LCMS is important for the identification of complex samples. However one single ionization mode is not sufficient for an extensive characterization of the sample. One alternative ionization mode contributes to a better coverage of the sample (the proteome coverage). Furthermore the use of both ionization modes allows to increase significantly the protein sequence coverage.

2D-LC is very powerful. The same complementarity is observed. For optimal peak capacity, the selection of precursors for MS/MS analyses must be done considering all the runs in once, to avoid redundancy between 1st dimension fractions and to optimize S/N of MS/MS spectra.

Poullet P., Carpentier S., Barillot E., Proteomics 2007;7,2553 Hu,R. J. Nol, H. Li, A Makarov, M. Hardman and R.G Cooks Int. J Mass Spectrom, 2005;40,430

Wanda M. Bodnar, R Kevin Blackburn, Jo M Krise, J Am Sco Mass Spectrom, 2003;14,971-979

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