

Complementary Analysis of Human CSF Proteins by Nano LC-MALDI and ESI/MS/MS

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Overview

Purpose: To evaluate the benefit of combining Electrospray and MALDI ionization techniques for characterization of the human cerebral spinal fluid (CSF) proteome

Methods: Human CSF was fractionated on an anion exchange column using a pH gradient and then digested. Digested fractions were analyzed by reversed-phase nano LC with a post column flow split equally into two for 1) online (ESI) and 2) offline sample spotting for MALDI/MS/MS

Results: Superior sequence coverage and the highest number of identified peptides and proteins for the analysis of CSF was found by combining data from both ESI-MS/MS and MALDI-MS/MS experiments rather than the use of either technology alone. 577 total proteins were identified – there was only 35% overlap between the list of proteins found by ESI and MALDI, indicating that these techniques are very complementary.

Introduction

Electrospray and MALDI ionization are both commonly used to generate biomolecular ions for mass spectrometry analysis. Findings from previous studies have shown that both ionization techniques can be complementary and, when combined, can greatly improve proteome coverage and overall protein identification in complex protein mixtures(1).

In this study, we utilized the combination of a nano LC-ESI/MS/MS with concurrent LC-MALDI/MS/MS on the same mass spectrometer (LTQ linear ion trap) to evaluate the individual and complementary values of both ionization techniques for the characterization of simple standard protein digests and complex protein mixtures such as human cerebrospinal fluid (CSF) and serum.

Methods

Protein samples:

Six protein mixture digest (LC Packings)

Reduced and alkylated human serum enzymatic digest

Human CSF separated into 6 fractions using a strong anion exchange spin column with a pH step gradient (ProteinChip® Serum Fractionation kit) followed by enzymatic digest

SDS-PAGE:

CSF fractions (~20 µL) were diluted two-fold with gel sample loading buffer, containing DTT, heated for 10 min at 70°C, and loaded on 4-12% NuPAGE® Bis Tris gel. The gel was run and stained with SilverQuest™ silver stain according to the manufacturer's instructions.

Chromatographic Conditions for reversed phase nano LC with a post column flow split :

HPLC system: Finnigan Surveyor MS pump with a flow splitter
 Column: C18 column, 150 µm ID x 15 cm (MicroTech Scientific)
 Injection volume: 2-10 µL
 Flow Rate: 2 µL /min, post split to 1:1
 Mobile Phase: A: Water, 0.1% formic acid
 B: Acetonitrile, 0.1% formic acid

Gradient: 0 - 85% B in 20 min for 6 protein digest
 0 - 40% B in 90 min, 40-85% B in 20 min for serum and CSF digests

Sample collection: Probot™, 300-500 nL/spot

Mass Spectrometric Conditions for nano ESI/MS/MS:

Mass Spectrometer: Finnigan™ ProteomeX LTQ™
 Spray Voltage: 1.8 kV
 Capillary T: 160 °C
 MS: 460 -1600 amu
 MS/MS: Triggered automatically by data dependent scan with dynamic exclusion On (repeat count: 2)

Mass Spectrometric Conditions for MALDI:

Mass Spectrometer: Finnigan vMALDI LTQ
 Matrices: 1 µL of DHB (50 mg/ml) or CHCA (2.5 mg/ml)
 MS: 900-4000 amu
 MS/MS: Top 25 triggered automatically by data-dependent scan with dynamic exclusion and maximum acquisition time of 5-10 min/spot.

3. Database search

BioWorks 3.2 (SEQUENT®) database search algorithm was used to identify proteins with following filters : Xcorr vs Charge State 1.5-1; 2-2; 2.5-3; minimum two different peptides per protein. Swiss-Prot and Human protein sequence databases were used.

Results

To optimize separation and mass spectrometry conditions for LC-MS/MS, analysis of the simple protein mixture digest (six protein mix in equal concentrations) by LC/ESI-MALDI/MS/MS with post-column split flow was performed. As shown in Table 1, maximum sequence coverage as expected was achieved by combining ESI-MS/MS results with MALDI data.

TABLE 1. Number of peptides identified in the digest of a six protein mixture from a 20 min. gradient HPLC run

Protein	# Unique Peptides Identified		
	ESI+MALDI	ESI	MALDI
Bovine sero-transferrin	37	33	28
BSA	40	35	30
Bovine cytochrome-C	9	7	7
E.coli beta-galactosidase	31	23	15
Chicken lysozyme	11	6	7
Yeast ADH 1	11	10	5

The total number of identified peptides was greater with nano ESI/MS/MS analysis, likely due to differences in acquisition parameters such as the mass range, 460-1600 m/z for ESI compared with 900-4000 m/z for MALDI. Given identical scan rates for the 2 instruments, this means fewer MS/MS experiments were actually performed during the MALDI analysis.

This was confirmed with the vMALDI analysis of whole human serum digest with different acquisition times per spot. As shown in Figure 1, when data were collected for 10 min per spot, 61 peptides were identified by MALDI as compared with 54 peptides from ESI in human serum albumin, and only 35 if each spot was analyzed only for 4 min. On the other hand, if acquisition time per spot was only 4-5 min, total number of identified proteins was decreased just by 5 % (data not shown), but total experimental time was reduced two-fold. Thus, from the practical standpoint all other experiments were run using acquisition times of 5 min per spot.

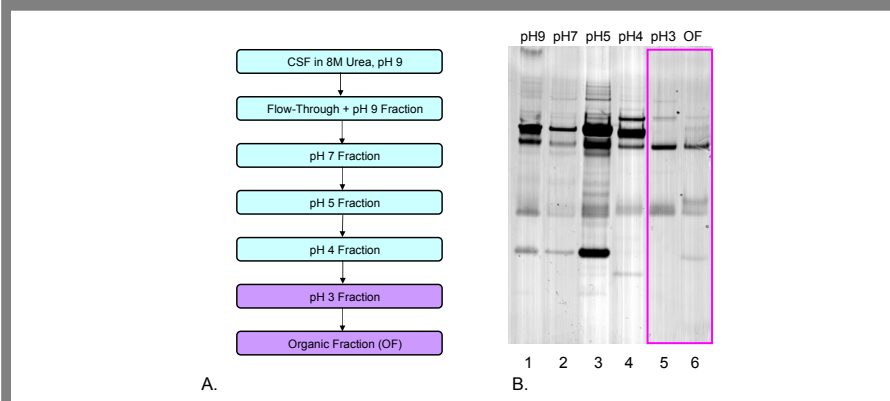
FIGURE 1. Combined human serum albumin sequence coverage for LC/ESI/MS/MS + MALDI/MS/MS from serum digest

DAHSEK VAHRFKDLGE ENFKALVLA FAQYLQCPF EDHVKLVNEV TEFAKTCVAD ESAENCCKSL HTLFGDKLCT VATLRETYGE MADCCAKQEP ERNECFLOHK DDNPNLRLV PEVDVMCTA FHDNEETFLK KYLYEJARRH PYFYAPELLF FAKRYKAAFT ECCQAADKAA CLLPKLDLDR DEGKASSAKQ RLKCSASLQKF GERAFKAWAV ARLSQRFPKA EFAEVSRLVT DLTKVHTECC HGDLLCADD RADLAKYCE NQDSISSKLLK ECCEKPLLEK SHCIAEAVEND EMPADLPPLA ADFVESKDVVC KNYAEAKDVF LGMFLYEYAR RHPDYSVLL LRLAKTYETT LEKCCAAADP HECYAKVDE FKPLVEEPPON LIQKNCLEFE QLGEYKQNA LLVRYTKVVP QVSTPTLVEV SRNLGKVGSK CKKHPEAKRM PCAEDYLSVV LNQLCVLHEK TPVSDRVTKC CTESLVNRRP CFSALEVDET YVPKFEAET FTFHADICTL SEKERQIKKQ TALVELVKHK PKATKEQLKA VMDDFAAFVE KCCKADDDCT CFAEEGKLVV AASQAALG

LC/ESI+MALDI/MS/MS- 86.6%
 ESI-72.6% (54 peptides)
 MALDI-81.9%(10 min/spot, 61 peptides); 53% (4 min/spot, 35 peptides)

The optimized protocol for LC-ESI-MALDI/MS/MS was used to identify proteins in CSF which was separated into 6 fractions on a micro-spin anion exchange column using pH gradient elution (Figure 2A). Each fraction was analyzed by 1 D SDS-PAGE for the presence of highly abundant proteins such as albumin and antibodies (Figure 2B). Based on gel data fractions 5 (eluted at pH 3) and 6 (eluted with 33.3% isopropanol/16.7% acetonitrile/0.1% trifluoroacetic acid) were selected. Prior to mass spec analysis, fractions were digested and then reduced but not alkylated.

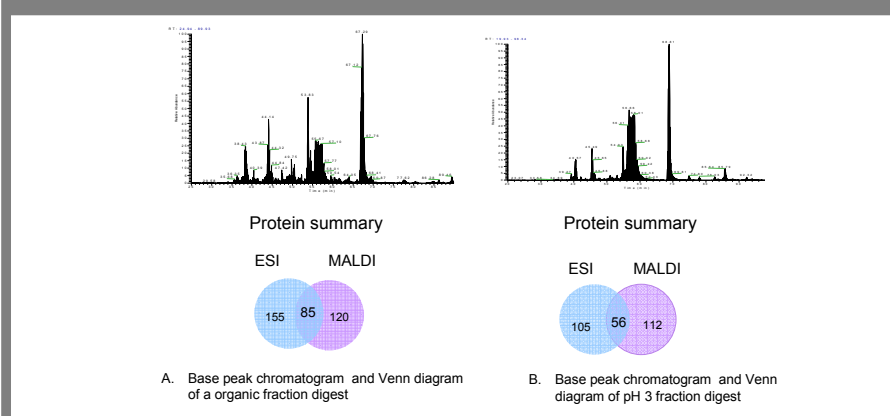
FIGURE 2. CSF fractionation on Q-HyperD® F strong anion exchange spin column (A) and SDS-PAGE of fractions (B)



Sample collection for MALDI analysis was based on the ESI-LTQ base peak chromatograms (Figure 3). Fractions were collected between 30 and 95 min.

A total of 273 and 360 proteins were identified from the complimentary LC-ESI-MALDI/MS/MS-LTQ analysis using Swiss Prot database and search filters as specified in "Methods" in fraction 5 and 6, respectively. Some overlap in proteins identified by both ESI and MALDI techniques was observed as shown by the Venn diagrams (Figure 3) which is in accordance to data obtained on CSF and serum digests on ESI/vMALDI/LTQ by Tegeler et al(3).

FIGURE 3. Analysis of CSF fraction digests by LC/ESI-MALDI/MS/MS



Detailed analysis of data from CSF fraction digests indicates that the majority of peptides were identified by both techniques in the case of high to moderately expressed proteins (Table 2) which is the same as for the simple protein mixture (Table 1). However, in case of low abundance proteins such as Q14573 and Q92608 for example, combining the two ionization techniques increased the protein sequence coverage by 20% even though the same mass analyzer (LTQ) and same chromatographic conditions were applied. This result is a clear indication of the benefit of using both techniques in order to achieve the highest confidence in protein identification and the greatest depth of analysis in a complex sample.

TABLE 2. Examples of complementarity of LC/ESI/MS/MS and LC-MALDI/MS/MS analysis of the CSF proteome fraction #5 (see Figure 2)

Protein	Sequence coverage, % (# identified peptides)		
	ESI	MALDI	ESI+MALDI
Transthyretin (P02766)	49 (5)	53(7)	53 (7)
Hemopexin (P02790)	34(11)	20(5)	37(14)
Fibrinogen (P02671)	5(3)	2(2)	8 (5)
Angiotensinogen (P01019)	30(9)	16(4)	30(9)
Inositol receptor (Q14573)	0.4(1)	6(9)	6.4(9)
Dedicator of cytokinesis protein 2 (Q92608)	1.3(2)	1(2)	2(4)
Total # of proteins	240	205	360

Conclusions

Concurrent analysis of CSF fractions by ESI and MALDI resulted in large numbers of proteins identified by both techniques. Combination of results from both experiments provides several benefits:

- Better sequence coverage.** Many more peptides identified. The combination of both techniques in average increased the sequence coverage by at least 20%.
- Better protein coverage.** Many more unique proteins. This was particularly true for low abundance proteins in complex samples. For example: independent identification of 205 and 240 proteins in CSF fraction 5 by ESI or MALDI respectively, increases to 360 when results are combined. This number increases to 577 when combined with ESI and MALDI results from fraction 6.
- Complementarity of results.** Only about 35% of proteins identified in this analysis were found by both ESI and MALDI – the results from both methods are highly complementary.

References

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