

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

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Key Words

- Finnigan™ LTQ™
- Finnigan vMALDI™ source
- Enhanced protein and sequence coverage

Introduction

Electrospray and matrix-assisted laser desorption ionization (MALDI) 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.^{1,2}

Goal

This study utilizes Thermo's Finnigan LTQ (linear ion trap) mass spectrometer with the combined workflow (Figure 1) of nano LC-MS/MS (electrospray mode) and LC-MALDI/MS/MS with the vMALDI source to evaluate the individual and complementary benefits of both ionization techniques for the characterization of simple standard and complex protein mixtures such as human cerebrospinal fluid (CSF).

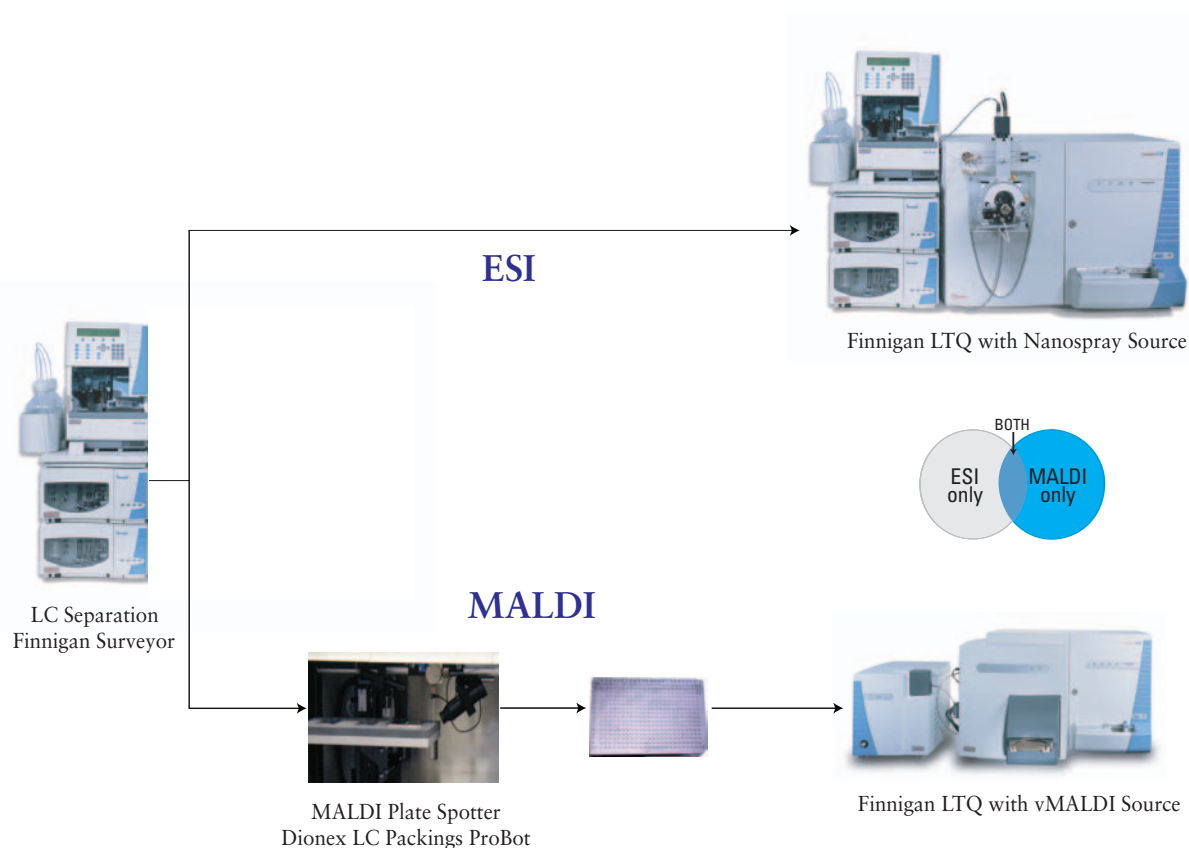


Figure 1: Combined electrospray and MALDI workflows using the Finnigan LTQ mass spectrometer

Experimental Conditions

Protein Samples

Human CSF proteins (Vital Products) were denatured with 8M urea and separated into six fractions using a strong anion exchange spin column with a pH step gradient (ProteinChip® Serum Fractionation kit) followed by an enzymatic digest.

Human serum (SIGMA®) was also reduced and digested according to the above protocol with no fractionation.

A standard six protein mix digest (Dionex®, LC Packings) was also evaluated.

SDS-PAGE

CSF fractions (~20 μ L) 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.

Reversed Phase Nano LC Conditions with a Post Column Flow Split

HPLC System: Finnigan Surveyor™ MS pump with a flow splitter
Column: C18 column (150 μ m ID \times 15 cm)
Injection Volume: 2-10 μ L
Flow Rate: 2 μ L /min, post split to 1:1 (1 part for ESI, 1 part for LC-MALDI spotting)
Mobile Phase: A: Water, 0.1% formic acid
B: Acetonitrile, 0.1% formic acid
Gradient: 0–85% B in 20 min for six 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

ESI/MS/MS Conditions

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)

MALDI MS/MS Conditions

Mass Spectrometer: Finnigan LTQ with vMALDI source
Matrices: 1 μ L 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.

Database Search

BioWorks™ 3.2 incorporating SEQUEST® database search algorithm was used to identify proteins with following filters:

Xcorr vs Charge State 1.5-1; 2-2; 2.5-3
Minimum of two different peptides per protein
SwissProt and Human protein sequence databases were used.

Results

In order to optimize the LC separation and MS/MS conditions for subsequent analysis of the CSF sample, a simple six protein mixture digest was analyzed by LC/ESI and LC-MALDI/MS/MS according to the above workflow. As shown in Table 1, maximum sequence coverage was achieved by combining both the ESI-MS/MS results with MALDI data.

The total number of identified peptides was higher from nano ESI-MS/MS analysis probably due to difference in scan rates of the LTQ in ESI mode (16,700 m/z per second) across 460-1600 m/z compared with 900-4000 m/z for MALDI, therefore there were more MS/MS experiments using the ESI technique.

This was confirmed with the analysis of whole human serum digest with different acquisition times per spot.

When collecting data for 10 min per spot, 61 peptides were identified by MALDI as compared with 54 peptides from ESI from human serum albumin (Figure 2). If the time was limited to 4 min per spot, only 35 peptides were identified.

However, if the acquisition time per spot was reduced to 4-5 min, the total number of identified proteins was decreased just by 5% (data not shown), but the total experimental time was reduced by at least two times. Thus, from the practical standpoint, all other experiments were run using an acquisition time of 5 min per spot.

This optimized protocol for LC-ESI combined with LC-MALDI-MS/MS was subsequently utilized to identify proteins in CSF separated according to the workflow shown in Figure 3a and 3b.

Protein	Peptides Identified			# Different Peptides
	Combined ESI and MALDI	ESI Only	MALDI Only	
Bovine Serotransferrin	37	33	28	4
Bovine Serum Albumin	40	35	30	5
Bovine Cytochrome C	9	7	7	2
<i>E. coli</i> Beta Galactosidase	31	23	15	8
Chicken Lysozyme	11	6	7	4
Yeast Alcohol Dehydrogenase 1	11	10	5	6

Table 1: Number of peptides identified in the digest of a six protein mixture from a 20 minute gradient HPLC run

From the gel, fractions 5 (eluted at pH 3) and 6 (eluted with organic solvents, 33.3% isopropanol/16.7% acetonitrile/0.1% trifluoroacetic acid) were selected for subsequent MS/MS analysis after digestion (reduced, not alkylated).

For MALDI analysis, sample collection was based on the ESI-LTQ base peak chromatograms (Figure 4A and 4B), set between 30 and 95 min. A total of 273 and 360

proteins (fractions 5 and 6 respectively) were identified from the complementary LC-ESI and LC-MALDI-MS/MS analysis using the Swiss Prot database and search filters as specified in the “Methods” section. Some overlap in the numbers of proteins identified by both ESI and MALDI techniques was observed as shown by the Venn diagrams (Figure 4C and 4D) which is in accordance to data obtained on CSF and serum digests on ESI and vMALDI MS/MS analysis on the LTQ.³

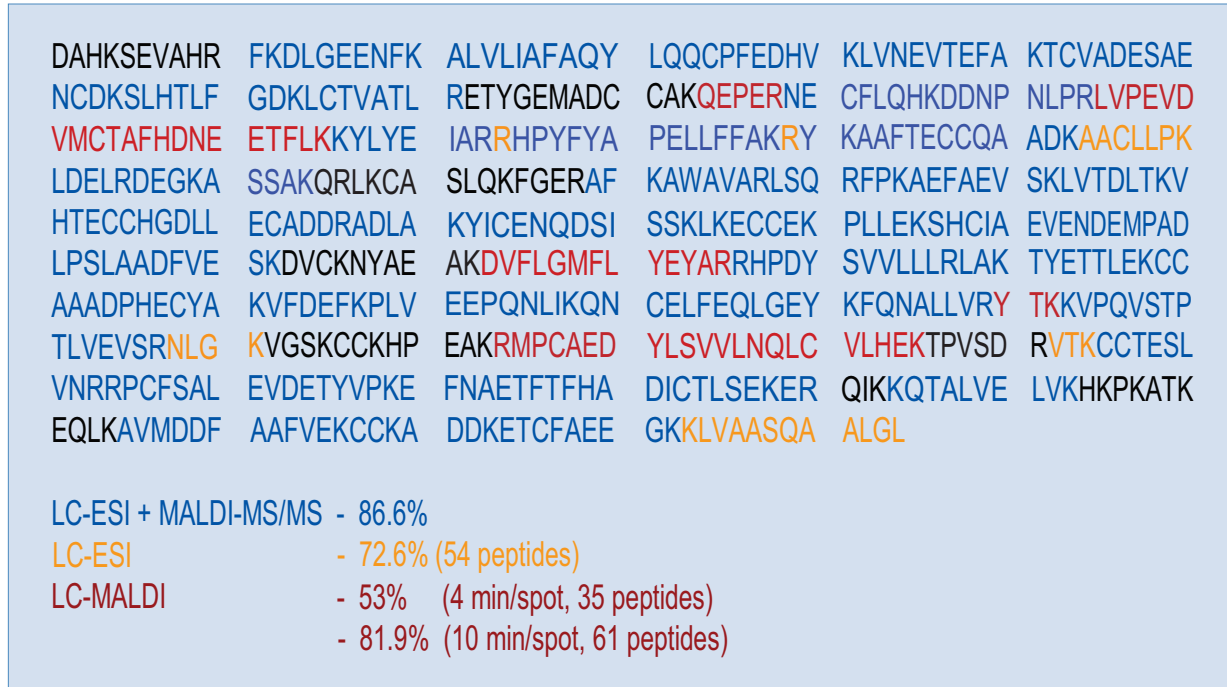


Figure 2: Combined human serum albumin sequence coverage for LC ESI+MALDI-MS/MS from serum digest

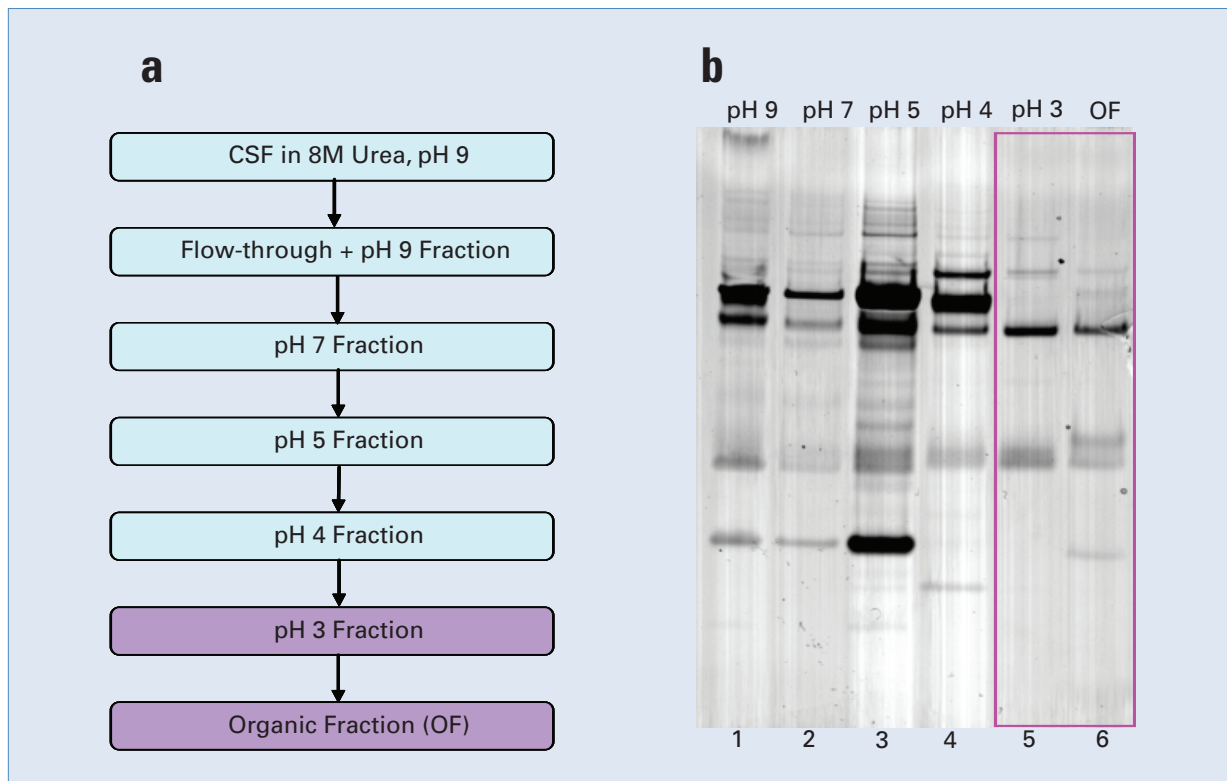


Figure 3: CSF fractionation on Q-Hyper D F strong anion exchange spin column (a) and SDS-PAGE of CSF fractions (b)

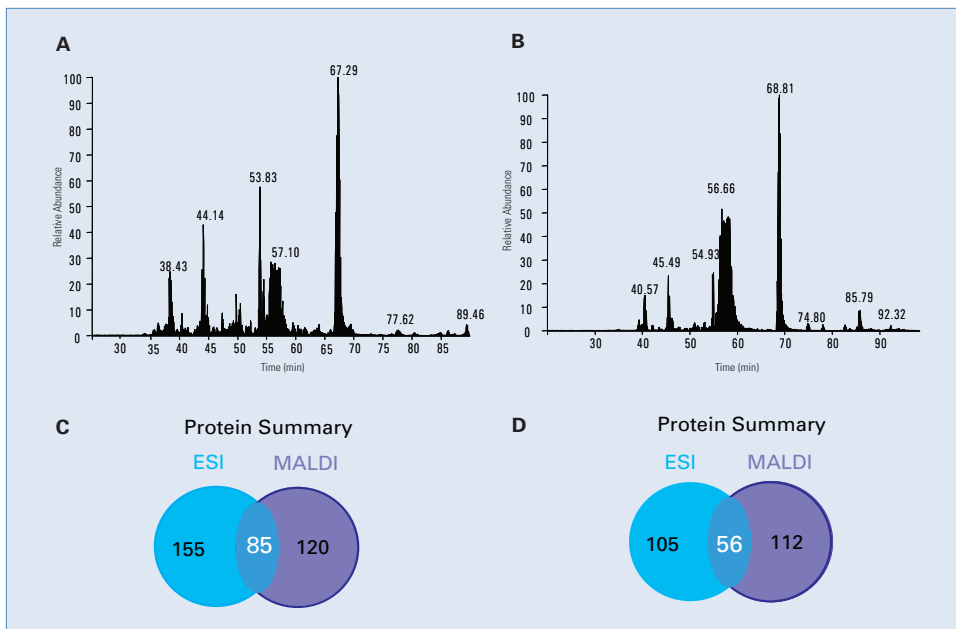


Figure 4: Base peak chromatograms
A organic fraction
B pH 3 fraction and Venn diagrams showing complementary identifications using ESI and MALDI
C organic fraction and
D pH 3 fraction

Protein	% Sequence Coverage (Number of 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 (Q92608)	1.3 (2)	1 (2)	2 (4)
Total Number of Proteins	240	205	360

Table 2: Benefits of the combined ESI and MALDI workflow in the analysis of the CSF proteome

Detailed analysis of data from the 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 comparable to the simple protein mixture (Table 1).

However, in case of low abundant proteins such as Q14573 and Q92608, for example, combining the two ionization techniques increased the protein sequence coverage at least by 20% even when same mass analyzer (LTQ) and same chromatographic conditions were applied.

Since the LTQ mass spectrometer was utilized with different peptides identified by either MALDI or ESI, these differences are a direct result of the ionization technique used. These results illustrate the clear benefit of using both ESI and MALDI techniques in order to achieve the highest confidence in protein identification and the highest number, i.e. depth of analysis in a complex sample.

Conclusion

Using the Finnigan LTQ enabled both with ESI and vMALDI sources, a greater depth of protein identification was achieved by combining the data:

- Enhanced sequence coverage: All types of samples from simple proteins to complex mixtures such as CSF when analyzed both with LC/ESI and LC/MALDI MS/MS increased sequence coverage by 20%
- Enhanced protein coverage: More unique proteins were identified with this combination, in particular for low abundance proteins
- Complementarity of both ESI and MALDI: Approximately 35% of proteins were identified by both ESI and MALDI.

References

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