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ANALYSIS OF CAPILLARY REVERSED-PHASE HPLC SEPARATIONS OF 1-D GEL DIGESTS BY MALDI MS/MS

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Overview

Purpose:

Evaluate efficacy of 1-D SDS PAGE for protein fractionation prior to tandem mass spectrometry of proteolytic digests.
Compare MALDI Q-Tof™ MS/MS analysis of gel band digests without and with HPLC separation.
Compare MALDI Q-Tof and ESI Q-Tof MS/MS analysis of gel band digests.
Evaluate effects of peptide sequence on MALDI-MS/MS spectra.

Methods:

An *E. coli* cytosolic protein fraction was separated by 1-D SDS PAGE and the gel band digests then subjected to MALDI-MS/MS and ESI-MS/MS analysis. A heated capillary nebulizer interface, the LC-MALDIprep™, was used to collect separations to the MALDI targets.

Introduction

Various techniques to interface liquid phase separations to MALDI mass spectrometry have been attempted including vacuum deposition [1] and electrospray [2]. In this work the separations are collected to a MALDI target using a heated capillary nebulizer [3-7] with co-deposition of 4-HCCA MALDI matrix. The nature of MALDI-MS/MS fragmentation [8] is explored and compared to ESI-MS/MS.

Methods

Sample Preparation: The cytosolic protein fraction from Escherichia coli was prepared by French Press using 20 mM Tris at pH 8.0 and centrifugation at 100,000 RCFG. The protein concentration was 50 mg/mL
1-D SDS PAGE: Proteins were separated on Criterion™ gels [15%: 10—100 kDa] (BIO-RAD) and visualized with Bio-Safe™ Coomassie Blue (BIO-RAD). Bands were excised and then the proteins digested using an automated protocol on a Waters MassPREP™ Station.

LC Conditions:

LC-MALDI

Trap:	Opti-Pak™, 0.35x5 mm, 5 µm, Symmetry300™ C_{18}
Column:	0.32x150 mm, 5 µm, Symmetry® C_{18}
Flow Rate:	3 µL/min
Injection Vol.:	6 µL
Gradient:	3 to 53% acetonitrile over 25 minutes A: 98% H_2O, 0.1% TFA, B: 80% Acetonitrile, 0.1% TFA

LC-ESI

Trap:	Opti-Pak™, 0.35x5 mm, 5 µm, Symmetry300™ C_{18}
Column:	0.075x100 mm, 3.5 µm, Symmetry® C_{18}
Flow Rate:	400 nL/min
Injection Vol.:	6 µL
Gradient:	5 to 60 % acetonitrile over 60 minutes A: 98% H_2O, 0.1% FA B: 98% Acetonitrile, 0.1% FA

MS Conditions:

MALDI Q-Tof

Source Pressure: 3.5E-1 torr
Inter-scan delay: 0.1 s
Survey scan, from 300 to 2500 m/z
MS/MS scan from 50 to m+50 m/z
Lock Mass: Glu-fibrinopeptide B, 1570.6774

ESI Q-Tof

Cone Voltage: 35 V
Inter-scan delay: 0.1 s
Survey scan from 300 to 1800 m/z
MS/MS scan from 50 to 1800 m/z
ESI Mass Spectrometry: MS performed on a Waters® Micromass® Q-Tof micro™ with 6000 resolution (FWHM) and argon collision gas. Survey data was acquired with a 1 s scan and MS/MS data with a 2 s scan. The emitter was a PicoTip™ with P200P coating (New Objective).

MALDI Mass Spectrometry: MS performed on a Waters Micromass MALDI Q-Tof Ultima™ with 10,000 resolution (FWHM) and argon collision gas. Survey data was acquired with a 1 s scan and MS/MS data with a 2 s scan. Nitrogen gas at 11 PSI produced a source pressure of 3.5E-1 torr. A nitrogen laser (Laser Science Inc. VSL-337I, 337 nm) operated at a repetition rate of 10 Hz with 300 µL per pulse was utilized.

LC-MALDI Sample Deposition: The eluent was sprayed onto the MALDI plate using the Waters LC-MALDIprep™ heated capillary nebulizer with 36 x 0.5 minute time fractions per separation. 4-HCCA matrix (Sigma) was tee'd in at 1 mg/mL and 2 µL/min.

Data Processing and Databank Searching: Data was processed using Waters Micromass ProteinLynx™ Global SERVER 2.0 and the *Escherichia coli K-12* protein databank.

Results

A 1-D gel separation was used to create twenty-six fractions from the *E. coli* cytosolic proteins (Figure 1). Digests were initially screened using the MALDI Q-Tof with no chromatographic separation. With this approach approximately fifty proteins were identified (I.D.). The molecular weights (MW) from the databank protein I.D.'s were compared to those predicted by the position in the gel relative to the protein standards (Figures 1 and 2) and showed a strong linear correlation (R^2 = 0.96). Most predicted values are within 10% of the calculated values. This information can be quite useful as a filter to eliminate incorrect protein identifications produced from the fragmentation data, with the caveat that some proteins can appear as fragments or have post translational modifications leading to

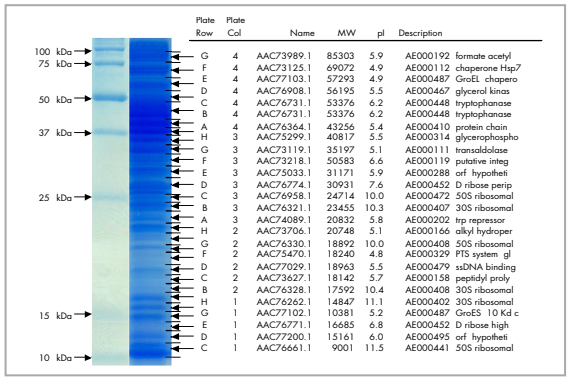

Plate Row	Plate Col	Name	MW	pI	Description
G	4	AAC73989.1	85303	5.9	AE000192 formate acetyl
F	4	AAC73125.1	69072	4.9	AE000112 chaperone Hsp7
E	4	AAC77103.1	57293	4.9	AE000487 GroEL chapero
D	4	AAC76908.1	56195	5.5	AE000467 glycerol kinas
C	4	AAC76731.1	53376	6.2	AE000448 tryptophanase
B	4	AAC76731.1	53376	6.2	AE000448 tryptophanase
A	4	AAC76364.1	43256	5.4	AE000410 protein chain
H	3	AAC75299.1	40817	5.5	AE000314 glycerophospho
G	3	AAC73119.1	35197	5.1	AE000111 transaldolase
F	3	AAC73218.1	50583	6.6	AE000119 putative integ
E	3	AAC75033.1	31171	5.9	AE000288 orf hypotheti
D	3	AAC76774.1	30931	7.6	AE000452 D ribose perip
C	3	AAC76958.1	24714	10.0	AE000472 50S ribosomal
B	3	AAC76321.1	23455	10.3	AE000407 30S ribosomal
A	3	AAC74089.1	20832	5.8	AE000202 trp repressor
H	2	AAC73706.1	20748	5.1	AE000166 alkyl hydroper
G	2	AAC76330.1	18892	10.0	AE000408 50S ribosomal
F	2	AAC75470.1	18240	4.8	AE000329 PTS system gl
D	2	AAC77029.1	18963	5.5	AE000479 ssDNA binding
C	2	AAC73627.1	18142	5.7	AE000158 peptidyl proly
B	2	AAC76328.1	17592	10.4	AE000408 30S ribosomal
H	1	AAC76262.1	14847	11.1	AE000402 30S ribosomal
G	1	AAC77102.1	10381	5.2	AE000487 GroES 10 Kd c
E	1	AAC76771.1	16685	6.8	AE000452 D ribose high
D	1	AAC77200.1	15161	6.0	AE000495 orf hypotheti
C	1	AAC76661.1	9001	11.5	AE000441 50S ribosomal

Figure 1. 1-D SDS PAGE of Escherichia coli cytosolic proteins and top identifications by MALDI Q-Tof with no LC separation (100 µg load per lane).

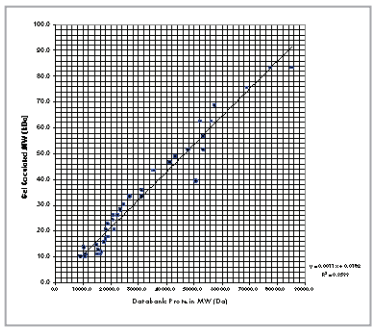

Figure 2. How good is the gel at predicting protein MW? Correlation between 1-D SDS PAGE and databank protein molecular weights.

erroneous gel predicted MW's.

The addition of a separation to the MALDI analysis produced a large improvement in the number and quality of protein I.D.'s. The score for a sample peptide MS/MS databank hit increased from 84 to 120 when an LC separation was used (Figure 3). Also the number of proteins I.D. from gel band D4 increased from two to thirteen. The total number of proteins I.D. using the separation was 300 whereas only 50 were I.D. from the unseparated digests. The potential for successful *de novo* sequencing is

increased due to the higher quality of MALDI-MS/MS spectra resulting from the separation. A sample peptide from the D4 gel band digest underwent *de novo* sequencing (Figure 4) with the top scoring peptide being T289-T300 [Tryptophanase Esc.]. The spectrum shows a complete y-ion series. Interestingly the MALDI-MS/MS spectra shown (Figures 3 and 4) illustrate the typical augmentation of y-ions with cleavage of the amide bond C-terminal to aspartic (D-X) and glutamic acid (E-X) where an arginine is present [8]. The sequence dependence of MALDI-MS/MS spectra is further highlighted when typical LC-MALDI and LC-ESI MS/MS spectra are compared (Figure 5). The MALDI spectra are again characterized by a strong bias towards E/D-X, y-ions whereas ESI spectra are characterized by y-ions of similar intensity. The MS/MS spectra identifying Glycerol Kinase E (Figure 6) show a different pattern. Each spectrum shows a fairly complete series of y-ions of roughly equal intensity but no bias is seen for the E residue in the LC-MALDI: T221-233 spectrum. This may be due to the presence of a less basic lysine (K) at the C-terminus, rather than R [8,9].

From gel band D4 thirteen proteins were identified by LC-ESI-MS/MS and thirteen identified by LC-MALDI-MS/MS (Table 1). Nine of these proteins were common to both methods while four were unique to each method. The peptides that identified these proteins were different depending on whether ESI or MALDI was employed. In the case of Glycerol Kinase E, the ESI method identified five peptides with an average MW of 1402, pI of 5.4 and HPLC index of 45.6. For the same protein the MALDI method identified six peptides with an average MW of 1127, pI of 5.8 and HPLC index of 13.6. Only one peptide was common to both ionization methods. These data indicate that LC-MALDI-MS/MS favors detection of more basic and soluble peptides relative to LC-ESI-MS/MS.

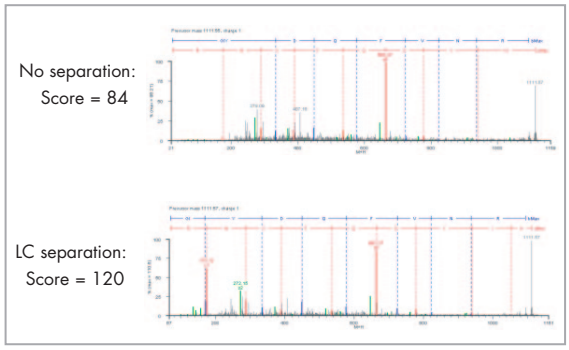

Figure 3. Comparison between MALDI Q-Tof results for gel band D4 with and without a chromatographic separation.

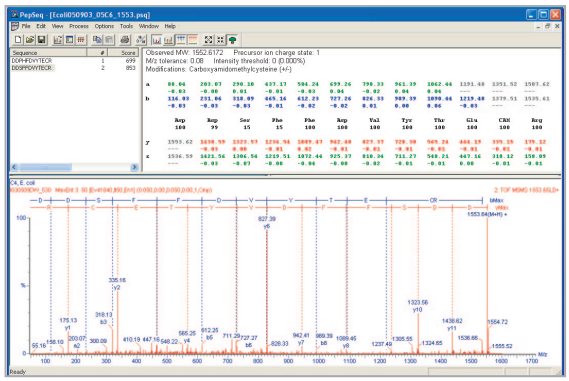

Figure 4. Improved MALDI-MS/MS fragmentation spectra from LC separations enable more effective de novo sequencing of peptides

AAC76731.1, Tryptophanase Esc, T289-300, Carbamidomethyl C (11).

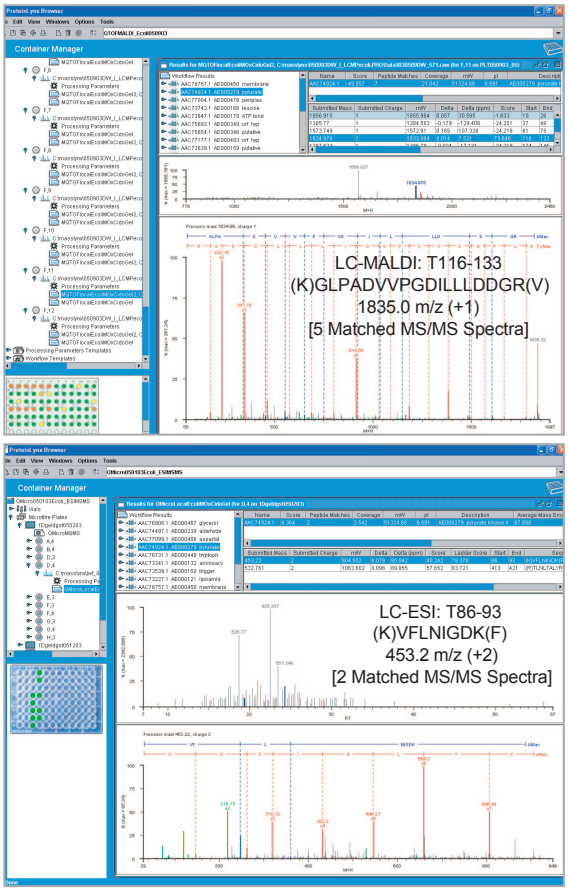

Figure 5. Best MALDI and ESI MS/MS spectra for peptide T116-133, (K)GLPADVVPGDILLLDDGR(V) and T86-93, (K)VFLNIGDK(F) respectively [Pyruvate Kinase II].

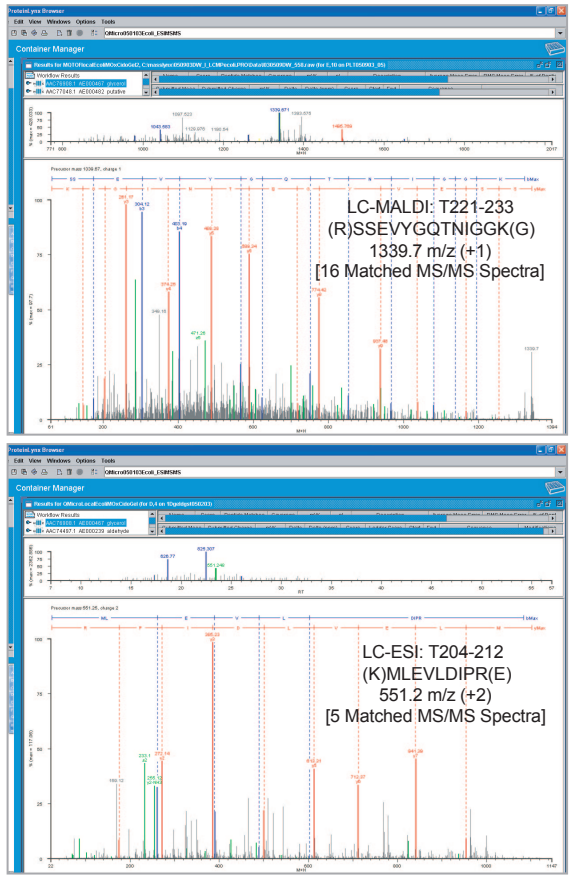

Figure 6. Best MALDI and ESI MS/MS spectra for peptide T221-233, (R)SSEVYGQTNIGGK(G) and T204-212, (K)MLEVLDIPR(E), {Oxidation M (1)} respectively [Glycerol Kinase E].

Discussion

The data indicate a significant advantage from using a separation in conjunction with MALDI-MS/MS, with the number of proteins identified increasing from fifty to three-hundred. Also, when an LC separation is used, the quality of the MALDI-MS/MS spectra is improved, enabling more effective *de novo* sequencing. Interesting differences are evident between MALDI and ESI spectra. MALDI-MS/MS generally favors y-ions with cleavage of the amide bonds C-terminal to aspartic (D) and glutamic acid (E), in arginine-containing peptides. ESI-based fragmentation is characterized by y-ions of similar intensity. The MALDI product ions are clearly sequence dependent as has been observed by others [8] and explained by the "mobile" proton model for peptide fragmentation [9].

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This work demonstrates that the heated capillary nebulizer is an effective deposition interface between RP-HPLC separations and MALDI Q-Tof MS/MS. The results compare favorably to LC-ESI-MS/MS and due to the fact that unique proteins and peptides are identified by MALDI vs. ESI the two techniques are complementary.

ESI	Name	mW	pI	Description
	AAC76908.1	56195	5	AE000467 glycerol kinase E
	AAC74497.1	52240	5	AE000239 aldehyde dehydroge
	AAC77099.1	54030	6	AE000486 aspartate ammonia
	AAC74924.1	51325	7	AE000279 pyruvate kinase II
	AAC76731.1	53376	6	AE000448 tryptophanase Esc
	AAC73341.1	52882	5	AE000132 aminoacyl histidin
	AAC73539.1	48163	5	AE000150 trigger factor a
	AAC73227.1	50657	6	AE000121 lipoamide dehy
	AAC76757.1	55188	6	AE000450 membrane bound ATP
	AAC73707.1	57366	6	AE000166 alkyl hydroperoxid
	AAC76542.1	52651	5	AE000428 glutamate decarbox
	AAC73782.1	58324	6	AE000172 phosphoglucomutase
	AAC76612.1	59657	6	AE000436 aldehyde dehydroge
MALDI	Name	MW	pI	Description
	AAC76908.1	56195	5	AE000467 glycerol kinase E
	AAC74497.1	52240	5	AE000239 aldehyde dehydroge
	AAC74924.1	51325	7	AE000279 pyruvate kinas II
	AAC76731.1	53376	6	AE000448 tryptophanase Esc
	AAC76850.1	50144	6	AE000460 proline dipept
	AAC76757.1	55188	6	AE000450 membrane bound ATP
	AAC76867.1	51871	5	AE000462 glutamine synt
	AAC75457.1	53782	6	AE000328 glutamate tRNA
	AAC73227.1	50657	6	AE000121 lipoamide dehy
	AAC77099.1	54030	6	AE000486 aspartate ammonia
	AAC76612.1	59657	6	AE000436 aldehyde dehydroge
	AAC73972.1	62881	9	AE000190 ATP binding co
	AAC73707.1	57366	6	AE000166 alkyl hydroperoxid

Blue Protein Name = Common to both ESI and MALDI
Red Protein Name = Unique to ESI
Red Protein Name = Unique to MALDI

Table 1. Proteins identified from band D4 using LC-MALDI-MS/MS and LC-ESI-MS/MS.

Conclusions

The heated capillary nebulizer is an effective interface for LC-MALDI-MS/MS of 1-D SDS PAGE proteolytic digests.

The quality of MALDI-MS/MS spectra is improved when a separation is used enabling a greater number of proteins to be identified as well as increased confidence in protein identification and *de novo* sequencing.

LC-MALDI and LC-ESI MS/MS methods produced complementary information both in terms of proteins identified and peptides matched.

MALDI-MS/MS spectra exhibited sequence dependent fragmentation favoring E/D-X y-ions in arginine-containing peptides.

References

[1] Preisler, J., Foret, F., Karger, B. L., Anal. Chem. 1998, 70, 5278-5287.
[2] Hensel, R. R., King, R. C., Owens, K. G., Rapid Commun. in Mass Spectrom. 1997, 11, 1785-1793.
[3] Biemann, K., Gagel, J. J. U.S.A. Patent 4,843,243, June 27, 1989
[4] Prevost, T. H., Dwyer, J. L. U.S.A. Patent 5,772,964, June 30, 1998
[5] Biemann, K., Kochling, H. U.S.A. Patent 5770272, June 23, 1998
[6] Wall, D. B., Finch, J. W., Cohen, S. A. Gen. Eng. News, 22(9), May 1, 2002
[7] Wall, D. B., Berger, S. J., Finch J. W., Cohen, S. A., Richardson, K., Chapman, R., Drabble, D., Brown, J., Gostick, D. Electrophoresis, 23, 2002, 3193-3204
[8] Wattenberg, A., Organ, A. J., Schneider, K., Tyldesley, R., Bordoli, R., Bateman, R. H., J Am Soc Mass Spectrom 2002 Jul;13(7):772-83
[9] Wysocki, V. H., Tsaprailis, G., Smith, L. L., Breci, L. A., J. Mass Spectrom., 2000, 35, 1399-1406.

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