MultiDimensional Protein Identification Technologies Mass Spectrometry of Mouse Brain Proteins

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Objective

To present a MultiDimensional Protein Identification Technology for identification of mouse brain proteome; and a possible alternative methodology as a comparison.

Introduction

MultiDimensional Protein Identification Technology has been developed by Yates and coworkers for various applications of proteomics [1, 2]. Typically, protein digests are separate by strong cation exchange (SCX) in the first dimension and reverse phase (RP) in the second dimension, then analyzed by mass spectrometry. Here two different versions of MuDPIT are compared on proteins extracted from the cortex region of mouse brain. We explore the possibility of strong cation exchange separation in the 1st dimension and FT-ICR [3] MS as the 2nd dimension in peptide separation.

Instrumentation

Our 9.4 Tesla Bruker Apex Qe FTMS "Combi" system (Fig. 1A) is equipped with a combination of Matrix Assisted Laser Desorption Ionization [MALDI] and conventional and as well as nano Electrospray ionization [nESI] sources. Multiple fragmentation techniques (Collision Induced Dissociation [CID, MS^n], Infrared MultiPhoton Dissociation [IRMPD], and Electron Capture Dissociation [ECD]) can be selected to enhance determination of post translational modified sites and sequencing of peptides and intact proteins. Like the QSTAR XL MS instrument (Fig. 1B), the use of various forms of hyphenated techniques (LC-MS, 2DLC-MS, LC-MS/MS, etc.) are possible with this platform for a variety of proteomics applications.

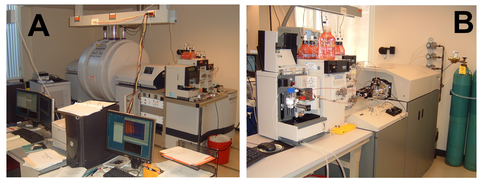

Figure 1. A) 9.4Tesla Bruker Apex Qe Fourier Transform Ion Cyclotron Resonance Mass spectrometer and B) Applied Biosystems/MDS SCIEX QSTAR XL Hybrid LC/MS/MS at WM Keck Foundation Biotechnology Resource Center.

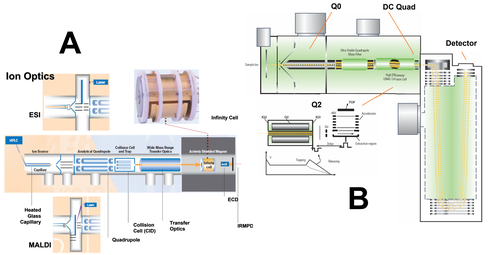

Figure 2. Ion optics of A) 9.4T FT-ICR MS and B) QSTAR LC/MS/MS. A Combi-source (MALDI and ESI) and Q-mass filter interface is configured for the FTMS system. The QSTAR instrument is equipped with several quadrupole for collisional focusing, trapping, and mass filtering prior to ions being introduced into the flight tube where they are detected with a four-anode detector. The FTMS system can fragments ions by CID (either in the collision cell or the Infinity Cell), IRMPD, or ECD.

Method

The concentration of proteins extracted from the cortex region of the mouse brain was determined by Amino Acid Analysis. Initial 2D-PAGE indicates ~400 unique protein spots. The extracted proteins were subjected to in-solution tryptic digest at 37 ºC overnight, then aliquot into several fractions for the various studies. The first experiment involves separation of digested proteins mixture by a 5µm PolyLC 2.1 mm id SCX column on a Vision-BioCAD Perfusion Chromatography system. A 90-min gradient from 2% ACN to 78% ACN in 0.1%HOAc and 0.01% TFA separated the peptides into 75 180µL-fractions. Each fraction was subjected to LC MS/MS analysis with ABI QSTAR XL LC/MS/MS system containing an LC Packings 5mm PepMap C18 trap and an Atlantis nano100 µm 15cm RP column. The LC MS/MS results were search against in-house IP mouse database with Sequest and Mascot search engine. Search results were summarized with Protein Prophet pl v2.0 based on p-value scores. SCX fractions which contain large number of peptides with p-values scores above 0.75 were further analyzed by nESI FT-ICR MS after C18 ZipTip cleanup in a second set of experiments. FTMS spectral data were analyzed by Bruker Xmass and Data Analysis software.

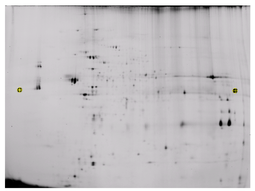

Figure 3. 2D-PAGE image (Gel214) of proteins extracted from mouse brain. ~400 protein spots are visualized on the gel.

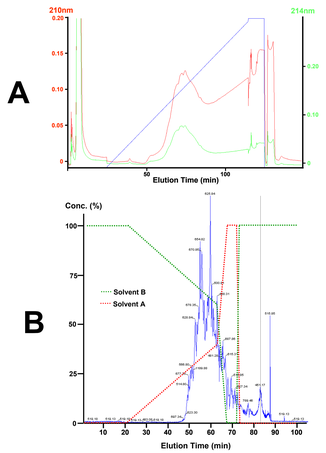

Figure 4. Elution profiles from A) strong cation exchange column and B) reverse phase nanoLC column. The blue line in A represents the KCl salt gradient (from 0 to 1M KCl with KH_2PO_4 buffer) implemented for peptide separation based on charge. The red dotted line in B represents the increased in organic concentration for elution of peptides into the MS for online LC MS/MS analysis. The RP elution profile shown corresponds to fraction 36 of the SCX fractions collected.

Results

Protein Prophet pvalue	Protein Name	# of Peptides Used in ID
1	- Bassoon	47
1	- Similar to RaS GTPaSe-activating protein SynGAP (Synaptic RaS-GTPaSe-activating protein 1) (Synaptic RaS-GAP 1) (Neuronal RaSGAP)	39
1	- ATP synthase beta chain, mitochondrial precursor	38
1	- Splice Isoform HK1-SA of Hexokinase, type I	35
1	- Tubulin, beta 2	35
1	- Tubulin beta-5 chain	33
0.99	- 118 kDa protein	32
1	- Splice Isoform 1 of Mitochondrial inner membrane protein	29
1	- Tubulin beta-3 chain	28
1	- Spectrin beta chain, brain 1	27
1	- Spectrin alpha 2	27
1	- Tubulin, beta 4	27
1	- Splice Isoform 3 of Mitochondrial inner membrane protein	27
1	- Na+/K+-ATPase alpha 3 subunit	26
1	- Sodium/potassium-transporting ATPase alpha-1 chain precursor	25
1	- ATP synthase alpha chain, mitochondrial precursor	25
1	- Splice Isoform CNPI of 2',3'-cyclic-nucleotide 3'-phosphodiesterase	24
1	- Piccolo	24
1	- Tubulin alpha-1 chain	24
1	- 133 kDa protein	23
1	- Tubulin alpha-4 chain	23
1	- ATPase, Na+/K+ transporting, alpha 2 polypeptide	22
1	- Contactin 1 precursor	22
1	- Ubiquinol-cytochrome-c reductase complex core protein 2, mitochondrial precursor	20
1	- Solute carrier family 25 (mitochondrial carrier, adenine nucleotide translocator),	20
1	- Calcium/calmodulin-dependent protein kinase II, delta	20
1	- Discs, large homolog 4	19
1	- Heat shock cognate 71 kDa protein	19
1	- Actin, cytoplasmic 1	19
1	- Creatine kinase, ubiquitous mitochondrial precursor	18
1	- NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial precursor	18
1	- Splice Isoform Alpha CaMKII of Calcium/calmodulin-dependent protein kinase type II	18
1	- Splice Isoform Synapsin IB of Synapsin-1	18
1	- Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial precursor	17
1	- Splice Isoform 5 of Myelin basic protein	16
1	- Splice Isoform 1 of Homer protein homolog 1	16
1	- Calcium-binding mitochondrial carrier protein Aralar1	16
1	- Ubiquinol-cytochrome-c reductase complex core protein I, mitochondrial precursor	16
1	- Splice Isoform 3 of Calcium/calmodulin-dependent protein kinase type II gamma chain	15
1	- Splice Isoform Mt-VDAC1 of Voltage-dependent anion-selective channel protein 1	15
1	- STOP protein	15
1	- Myosin Va	14
1	- Brain-specific angiogenesis inhibitor 1-associated protein 2	14
1	- Splice Isoform A1-III of Vacuolar proton translocating ATPase 116 kDa subunit a	14
1	- NADH-ubiquinone oxidoreductase 39 kDa subunit, mitochondrial precursor	14
1	- Similar to Spectrin alpha chain, brain (Spectrin, non-erythroid alpha chain) (Alpha-II Spectrin) (Fodrin alpha chain)	14
1	- Pyruvate dehydrogenase E1 component beta subunit, mitochondrial precursor	13
1	- Guanine nucleotide-binding protein G(o), alpha subunit 2	13
1	- Syn2 protein	12
1	- Mitochondrial 2-oxoglutarate/malate carrier protein	12
1	- Synaptotagmin-1	11
1	- Splice Isoform 1 of Syntaxin binding protein 1	11
1	- BAP	11
1	- Splice Isoform 5 of Dynamin-1	11
1	- Glutamate [NMDA] receptor subunit epsilon 2 precursor	11
1	- NADH-ubiquinone oxidoreductase 51 kDa subunit, mitochondrial precursor	11
1	- ADP,ATP carrier protein 2	11
1	- Splice Isoform 1 of Channel associated protein of synapse-110	10
1	- Cytochrome c oxidase subunit IV isoform 1, mitochondrial precursor	10
1	- Splice Isoform A of Adapter-related protein complex 2 alpha 1 subunit	10
1	- Camk2d protein	10
1	- Voltage-dependent anion-selective channel protein 2	10
1	- Gprin1 protein	10
1	- 51 kDa protein	9
1	- Glutamine synthetase	9
1	- Thy-1 membrane glycoprotein precursor	9
1	- N-ethylmaleimide sensitive fusion protein	9
1	- Visinin-like 1	9
1	- NADH-ubiquinone oxidoreductase 49 kDa subunit, mitochondrial precursor	9
1	- ATP synthase oligomycin sensitivity conferral protein, mitochondrial precursor	9
1	- ATP synthase gamma chain, mitochondrial precursor	9
1	- Similar to denSin-180	9
1	- Ras-related C3 botulinum toxin substrate 1	9
1	- 268 kDa protein	8
1	- Uncharacterized hematopoietic stem/progenitor cells protein MDS029 homolog	8

Figure 5. Representative list of first 75 proteins ID (with most # of peptide matched) from MuDPIT on QSTAR LC/MS/MS analysis of proteins extracted from localized region of mouse brain. The above are search results from MASCOT search engine using IP mouse database with Protein Prophet software for analysis of search results. Sequest search engine was also used as a comparison (data not shown), and total number of proteins ID were similar (MASCOT~370 to SEQUEST~440 number of proteins).

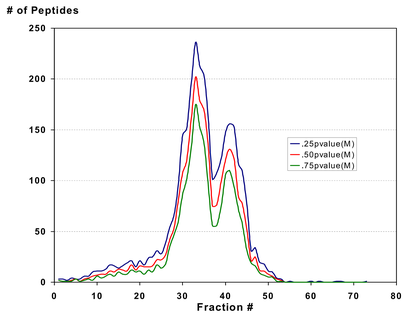

Figure 6. Plot of # of peptides ID in each SCX fraction. The blue (p-values<0.25), red (p-value <0.5), and green (p-value<0.75) lines suggests SCX fractions that will be further analyzed by FTMS for comparison.

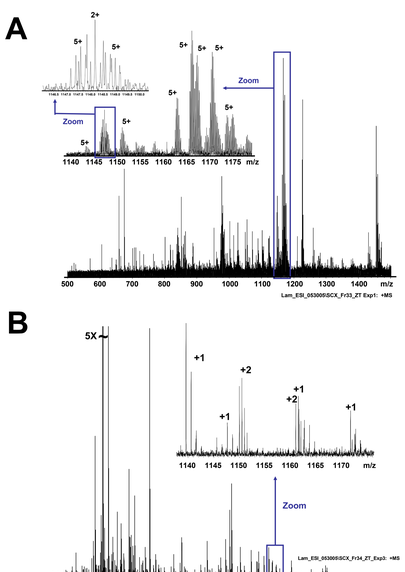

Figure 7. nESI FT-ICR MS spectra of SCX A) Fraction 33 and B) Fraction 34. Note the ability of FT-ICR MS to distinguish between peptides that overlap (zoom region in A) with mass accuracy less than 3 ppm with external calibration. This allows for high confidence in peptide fingerprint MS for protein identification. Also, note the comparison between the zoom regions of A and B; the difference in peptides show that SCX fractionation provides and added dimension of separation which will enhance FTMS separation capabilities.

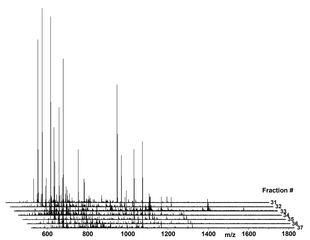

Figure 8. Staggered nESI FTMS mass spectra of 7 off line separated SCX fractions that have been desalted with C18 ZipTip.

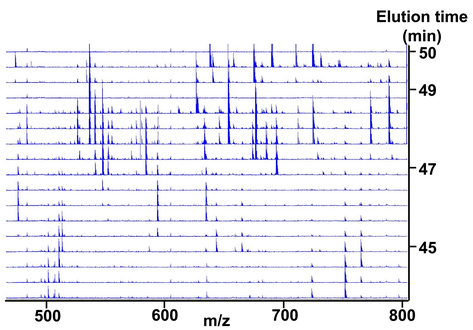

Figure 9. Sample stack plots of FT-ICR MS MuDPIT experiment. The plot is a zoomed in section of one of nine salt plug elution of a 2D LC MS run. Each row corresponds to a collected mass spectrum during peptide elution from a 75 um reverse phase C18 column. Note that average peptides elute within 2-4 spectra.

Conclusion

We are currently setting up for fractioning/spotting of 2D LC separated peptides onto 384-spots MALDI target plate for analysis by FT-ICR MS. Preliminary MALDI-FT-ICR MS experiments show low femtomole amount of protein tryptic digest were detected.

Acknowledgments

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