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ISCHEMIA-INDUCED CHANGES IN PROTEIN EXPRESSION IN RAT AND MOUSE BRAINS

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OVERVIEW

- Quantitative proteomic analyses were performed using peak areas of peptides in control vs. ischemic samples.
- Peptides were identified by accurate mass and fragmentation induced by alternating collision energy (CE) between a low and elevated energy state[1] (MS^E).
- Four different experiments were performed to determine changes in protein expression due to stroke.
  1. Ischemic and internal control rat brains digested in-gel
  2. Ischemic and sham control rat brains digested in-gel
  3. Ischemic and control rat brains digested in-solution
  4. Ischemic and control mouse brains digested in-solution
- Consistent changes in protein expression can be seen across all four studies.

METHODS

Samples were analyzed using a Waters nanoACQUITY™ UPLC system and a Micromass™ Q-Tof API US.

LC method:
- 180 µm by 20 mm trapping column packed with 5 µm Symmetry C_{18} (Waters)
- 75 µm by 100 mm analytical column packed with 1.7 µm BEH C_{18} (Waters)
- 60 minute gradient from 5-30% ACN at 300 nl/min

MS Method:
- 0.6 sec MS scan with CE at 10 eV
- 0.6 sec MS^E scan with CE stepped from 23-33 eV
- Lockmass channel sampled every 30 sec

Data Analysis:
- Protein Lynx Global Server (PLGS) version 2.2 with Expression Informatics
- Peptides tracked across triplicate injections
- Ions detected in 2 of 3 replicates chosen for databank search

Identification of Peptides

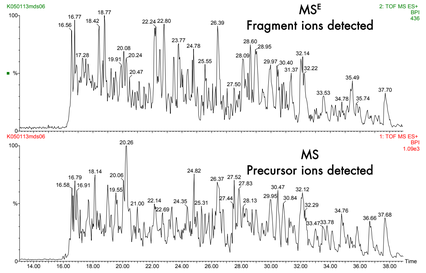

Figure 1: Chromatograms with alternating the collision energy between a low and elevated energy state to collect precursor and fragment ions. This sample was from a control mouse brain homogenate.

SAMPLE PREPARATION

Internal Control (Within same brain)
- Focal ischemia induced by occluding the middle cerebral artery in one side
- The other side of the brain used as control

Sham Control (Different brain)
- Animals underwent surgery with the occlusion of the middle cerebral artery
- Control animals had surgery with no occlusion

Digestions
- For in-solution digestions, proteins were solubilized with Rapigest™
- Gel bands were destained prior to digestion
- All proteins were reduced and alkylated prior to tryptic digestion

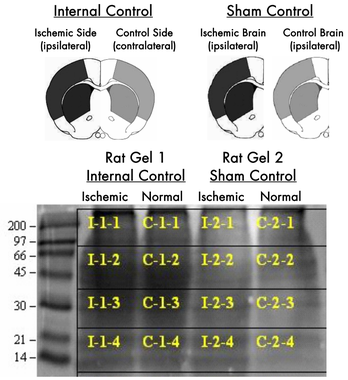

Figure 2: Coomassie-stained SDS-PAGE of control and ischemic rat brain homogenates. Experiments using internal control and sham brain control were both run on this gel.

Quantitation of Peptides

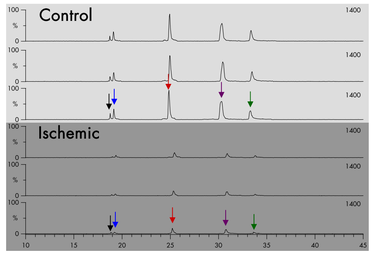

Figure 3: Extracted ion chromatograms of five peptides identified and quantified from calcium calmodulin dependent kinase II alpha from the digestion of control and ischemic mouse brains. The peptides were tracked across these 6 injections by PLGS software.

RESULTS

- Rat In-gel Digestions
  - 402 Proteins Identified and Quantified with average >6 peptides/protein
  - Expression ratios ranged from 2.8 to 0.3 with internal controls
  - Expression ratios ranged from 6.7 to 0.08 with sham controls
- Rat In-solution Digestion
  - 235 Proteins Identified and Quantified with average >4 peptides/protein
  - Expression ratios ranged from 2.2 to 0.29 with internal controls
- Mouse In-solution Digestion
  - 195 Proteins Identified and Quantified with average >4 peptides/protein
  - Expression ratios ranged from 10.8 to 0.19 with internal controls

Key:
- Significantly Overexpressed in Ischemic Sample
- Only Observed in Ischemic Sample
- Significantly Underexpressed in Ischemic Sample
- Only Observed in Control Sample

Serum Albumin

Rat Gel1
Average Ratio 2.8, 16% CV

Rat Gel 2
Average Ratio 6.7, 20% CV

Rat In-solution
Average Ratio 1.9, 28% CV

Mouse In-solution
Average Ratio 10.8, 30% CV

Calcium Calmodulin Dependent Kinase II alpha (CAM II α)

Rat Gel 1
Average Ratio 0.33, 20% CV

Rat Gel 2
Average Ratio 0.18, 23% CV

Rat In-solution
Average Ratio 0.32, 19% CV

Mouse In-solution
Average Ratio 0.16, 33% CV

Figure 4: Sequence coverage for two proteins found in all experiments. Both of these proteins change in the direction previously described during ischemia[2,3]. Identified peptides are highlighted and colored according to expression ratio.

Protein	Expression	Rat Gel 1 Ratio	Peptides	Rat Gel 2 Ratio	Peptides	Rat Ratio	Peptides	Mouse Ratio	Peptides
Serum Albumin	↑	2.8	15	6.7	20	1.9	11	11	14
Malate Dehydrogenase	↔	1.0	10	1.2	8	1.0	4	1.2	7
Heat Shock Protein 90	↔	0.91	13	1.0	11	1.1	5	1.1	8
Tubulin α-4	↓	0.77	12	0.66	5	0.73	3	0.61	10
CAM II α	↓	0.33	12	0.18	5	0.32	7	0.16	8

Table 1: Expression ratios for selected proteins identified and quantified in all experiments. The number of detected peptides were typically greater for gel experiments.

CONCLUSIONS

- Reliable protein expression results can be obtained from biological samples using a label-free method that employs continuous acquisition of accurate mass precursor ion and fragment ion intensities for both normal and treated samples.
- Many of the proteins that changed in expression by more than 30% are connected to ischemia in literature. Several novel changes were also observed.
- Separating the proteins into four fractions with PAGE resulted in more protein and peptide identifications. 402 (in-gel) vs. 235 (in-solution) protein IDs
- As expected, experiments using the sham brain controls led to greater expression differences than those using internal controls.
- In-gel digestions led to very similar quantitative results as in-solution digestions of the same samples.
- Individual peptides within the same proteins were detected reproducibly, showing that the best ionizing peptides are repeatedly identified in multiple experiments.

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