A Density Based Sample Fractionation Technology: Folate Deficiency and Oxidative Stress Response in Brain and Liver

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Introduction

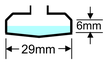
Folate deficiency (FD) alters hepatic methionine metabolism and is associated with increased hepatocellular apoptosis. Additionally, mice deprived of folate showed increased oxidative damage in brain tissue leading to cognitive impairment. Most previous studies have focused independently on either liver, the main tissue of folate storage and metabolism, or brain, where folate regulates neurogenesis and programs cell death. The aim of this study was to apply a powerful and rapid proteomics approach to understand potential subcellular correlations of folate deficiency in both brain and liver of the same rat. This approach combined a new density based sample fractionation technology (Edge™ Technology) with other conventional proteomics techniques, such as western blot analysis, 2DE and mass spectrometry.

Method

- Healthy adult rats (7-8 weeks old, 4 per group) were fed with a diet deficient in folate or a control diet. Rats were killed after 6 weeks, and then rat livers and brains were collected and snap frozen in liquid nitrogen.
- Rat tissues were homogenized, fractionated using Edge 200 separation system, and samples from each fraction were subjected to western analyses. Selected fractions were analyzed by 2DE. Interesting protein spots were excised, digested and identified by MALDI-TOF/TOF.
- Three animals from each group were used to evaluate inter-animal variation versus inter-group correlation.

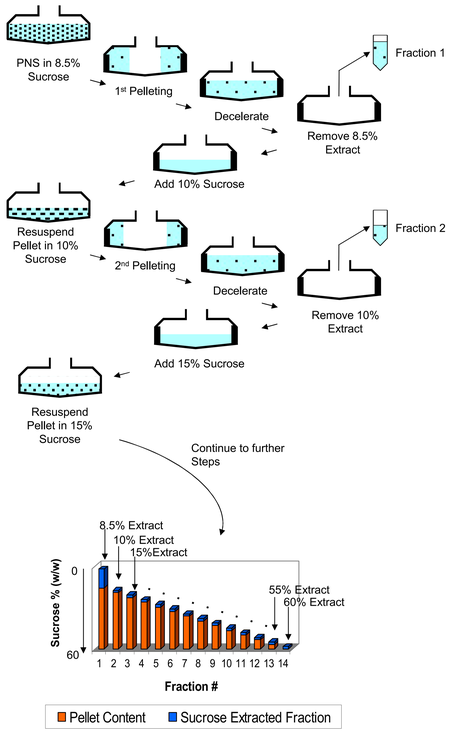
Edge 200 Separation System

Sample Rotor



Sample Container

Edge Technology



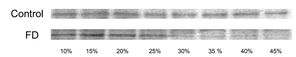
Edge Technology Benefits

- **Edge Technology** provides a powerful, selective sample fractionation method for proteomics analysis, including low abundance protein isolation and enrichment, biomarker discovery, as well as information on subcellular location.

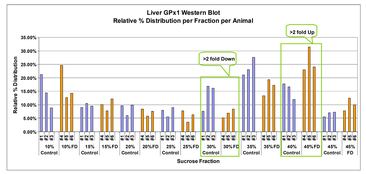
- Sample complexity is reduced significantly.
- Extraction may start at any density step of interest without the need for going through the whole gradient.
- The method DOES NOT need any density gradient medium and does not need any gradient mixer.
- The density of the extraction medium at each step is pre-defined and no other instrument is needed for density determination.
- Fractionation conditions are non-denaturing.
- Starting sample volume is less than 4 ml.
- Extraction volume is flexible, user-defined, and can be as low as 100 µl.
- Each extraction step takes about 5 minutes.
- The recovery yield is > 90%.

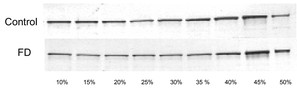
Results – FD Rat Liver Study

Western Blot Analysis of Fractionated FD Rat Liver

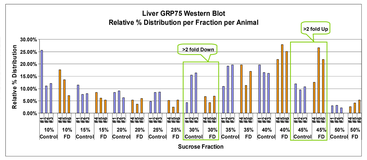


Liver GPx1 (rat #3 Ctrl, rat #6 FD)



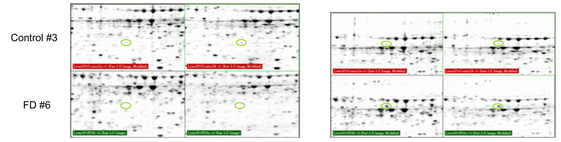


Liver GRP75 (rat #3 Ctrl, rat #6 FD)

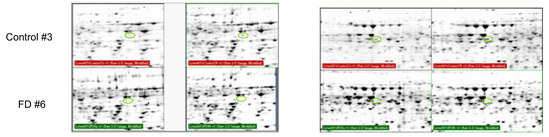


2D Gel Images of Selected Liver Fractions

(Animals #3 and #6)



Liver 30% fraction, spot 3305 Liver 30% fraction, spot 3607



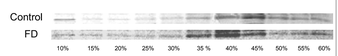
Liver 40% fraction, spot 8507 Liver 40% fraction, spot 4505

Protein ID of Selected Spots Using MALDI-TOF/TOF

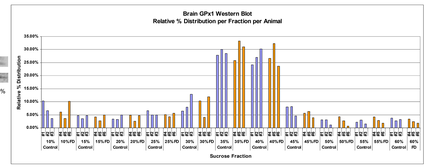
	Spot	Protein	Accession #	Protein Score	Total Ion Score	Relative Expression
Fr 30%	0012	similar to NADH dehydrogenase:Ubiquinone Fe-S protein 8	gi|27661165	339	286	Down
	4305	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex 10	gi|46391108	498	410	Down
	3305	frutose-1,6-biphosphatase 1	gi|51036635	568	435	Unique to Control
	5001	Calreticulin	gi|11693172	772	718	Unique to Control
	0201	Annexin A5	gi|51858950	851	726	Unique to FD
	3607	similar to alpha-1 major acute phase protein prepeptide	gi|57526868	835	690	Up
Fr 40%	1202	regucalcin	gi|13928740	178	136	Down
	2214	estrogen sulfotransferase isomer 3	gi|2144059	202	123	Down
	4008	chain D, rat transthyretin	gi|3212535	289	222	Down
	4505	4-trimethylaminobutyraldehyde dehydrogenase	gi|7248632	464	353	Unique to Control
	8507	similar to 6-phosphogluconate dehydrogenase,decarboxylating	gi|34872479	295	241	Unique to Control
	9611	fibrinogen, beta polypeptide	gi|56971493	684	534	Unique to FD
	3702	Hpx protein	gi|60688311	472	382	Up
	2411	unknown	gi|44890246	112	37	Up
	8208	haptoglobin	gi|59808182	483	395	Up

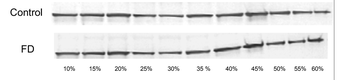
Results – FD Rat Brain Study

Western Blot Analysis of Fractionated FD Rat Brain

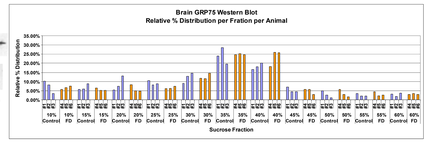


Brain GPx1 (rat #2 Ctrl, rat #5 FD)



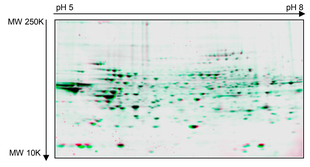


Brain GRP75 (rat #2 Ctrl, rat #5 FD)



- Western blot analyses of brain fractions show <25% change between control and FD rats.
- Western blot analyses of brain PNS before fractionation show 10 to 30% up regulation for both markers (data not shown). This is consistent with literature report (Shea TB, et al, *Free Radic Biol Med.* 2002, **33**(8):1115-20).

2D Gel Overlay of Control and FD Brain Fraction 40%



2D gel analysis of brain fraction 40% shows very little change between control and FD rats.

Conclusions

- Edge technology provides a rapid fractionation and screening method for various biomarkers.
- Changes in relative percentage distribution of markers per fraction indicate their potential translocation in the cell.
- Rat liver shows much higher response to folate deficiency than does rat brain with little or no correlation between organs.
- Fractionation reveals more than 2 fold up regulation for GPx1 in FD rat liver fraction 40%, and more than 2 fold down regulation for GPx1 in FD rat liver fraction 30%.
- Fractionation reveals more than 2 fold up regulation for GRP75 in FD rat liver fraction 45%,and more than 2 fold down regulation for GRP75 in FD rat liver fraction 30%.
- Several unique and theoretical proteins found by MALDI demonstrate the power of Edge technology for low abundance protein isolation and biomarker discovery.
- Further investigation is needed to explore the biological roles of those unique proteins identified by MALDI.
- Edge technology provides a powerful tool to see the significant differences between the treated and non-treated groups, as well as some inter-animal variation within 3 FD and 3 control animals.
- This is the first report of folate deficiency using multiple organ, same animal analysis.

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