

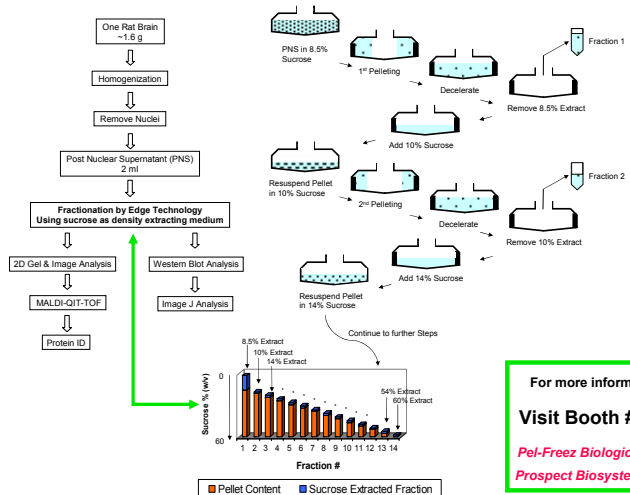
Differential Synaptic Protein Expression Profiling of Rats Fed with a High Fat Diet and Correlation with Brain Derived Neuronal Diseases Using a Proteomics Approach

Wenkui Lan & Marc J. Horn, Prospect Biosystems, LLC, Newark, NJ
Jun Q. Xia & Beverly Graham, Pel-Freez Biologicals Inc., Rogers, Arkansas

Abstract

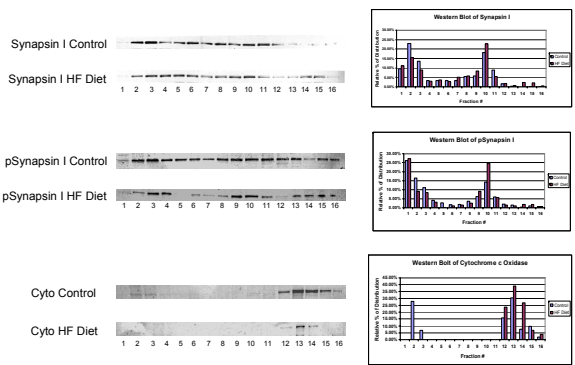
It is well known that a diet high in saturated fat (HF) not only leads to hyperlipidemia and hypercholesterolemia which increase the risk of cardiovascular diseases, but also increases oxidative stress in brains and leads to synaptic dysfunction. Despite tremendous progress, there are still some limitations for completely explaining the correlation of neuronal diseases and HF diet. Recently developed neuroproteomics has made it possible to understand the brain function in system-based approaches. However, the current proteomics technologies face a huge challenge due to the brain complexity on the protein level, such as large numbers of post-translational modifications and the wide dynamic range of protein abundance. We have developed a powerful and robust technology (Edge™) for proteomics sample fractionation to reduce sample complexity and increase possibility for low abundance protein isolation and biomarker discovery. In this study, we applied Edge technology and other proteomics techniques to perform a comparative analysis of synaptic protein expression profiles of rat brains with a HF diet and a control diet. Both rat brains from HF and control diet were homogenized and fractionated using Edge technology. Synapsin I and phospho-synapsin I (synaptic dysfunction markers) and cytochrome c oxidase (an oxidative stress marker) were analyzed by western blot. A lighter density fraction (3) and a heavier density fraction (15) were further separated by two-dimensional gel electrophoresis. Interesting protein spots were excised, digested and identified by MALDI-TOF/TOF. The results demonstrated a possible new avenue to understand the correlation between HF diet and many neuronal diseases. In addition, Edge technology combined with other proteomics techniques likely will result in the discovery of novel biomarkers for HF diet related neuronal diseases.

Method



Results

Western Blotting Analysis



In the above Western Blots:
1. A relative percentage (%) of each fraction (X) was based on the gel such that each lane contained the same amount of total protein.
2. After blotting, the absorbance (A₄₉₀) for the marker for each fraction (X) was measured.
3. Total Marker Absorbance for fraction (X) (TMA_X) was calculated: (TMA_X) = (A₄₉₀) * (W_X) * 100.
4. Relative % Distribution of Fraction (X) = (TMA_X) / (Σ TMA_X at fraction(s)) * 100.

Instrumentation

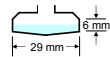
Edge 200 Separation System



Instrument



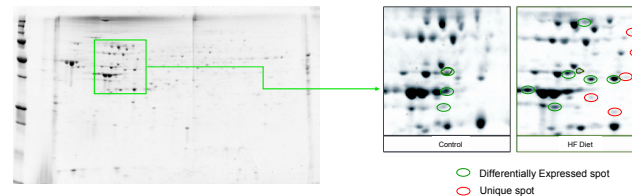
Sample Rotor



Sample Container
3-4 ml

Results

Differential Protein Regulation Analyzed by 2D Gel (Fraction 15)



Protein ID of Selected Spots Using MALDI-TOF/TOF (Fraction 3)

Spot	Protein	NCBI Accession #	Protein Score	Total Ion C. I. %	Relative Expression
1454	Brain lipid binding protein	gi 33549630	259	100	Up
793	Similar to gamma-actin-like protein	gi 34472636	235	100	Up
1027	Similar to CapP protease	gi 34822298	166	100	Up
323	TNF receptor-associated protein 1	gi 5747837	212	100	Up
274	Leukemia-inhibitory sensitive fusion protein	gi 13489397	51	99.291	Up
160	Unknown (protein for MGSC-94869)	gi 53734284	275	100	Up
127	IL-3 protein	gi 5716944	256	100	Up
343	Succinate dehydrogenase complex, subunit A, flavoprotein	gi 18426958	114	100	Down
904	Syntaxin 3-PEA	gi 251470	150	100	Down
1041	PDGF-A associated protein 1	gi 56971256	53	99.966	Down

Conclusions

- Biomarkers related to high fat diet may be rapidly screened and identified using Edge technology.
- Fractionation of rat brain PNS by density provides a rapid means of evaluating potential biomarkers for neuronal diseases.
- Changes in the relative percentage distribution by density of synapsin I and phospho-synapsin I (biomarkers for brain dysfunction) may indicate translocation of these proteins among subcellular compartments affected by high fat diet.
- Cytochrome c oxidase western results may provide validation of published reports proposing the biological relationship between high fat diet and mitochondrial dysfunction (Wu, et al. *Eur. J. Neurosci.* 19, 1699-1707, 2004).
- Further investigation is needed to explore the biological roles of those unique proteins identified in 2D gel analysis.

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

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