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Figure 1.0 - Overview of the ProteomeLab™ Initiative

Abstract

The genomic revolution has been invaluable to scientific research and discovery generating a wealth of information that allows us to relate genotype with disease phenotypes. However, it is ultimately our understanding of the linkage of genomics with proteomics in a functioning cellular environment that will be critical to unraveling the complexities associated with disease state. To generate such a comprehensive data set requires the use of a multi-technology strategy. This poster presents practical aspects of using chromatographic and capillary electrophoresis methodology to fractionate and characterize complex proteomes, providing generic robust methodology for protein solubilization, fractionation and characterization.

Introduction

Although the term "Proteome" was first introduced in the mid 1990's its definition has since expanded to include the identification, characterization and quantification of all proteins in a biological pathway that can be studied in concert to provide comprehensive data on that pathway. A proteome can be studied at many levels - organism, organ, tissue, cell or sub-cellular organelle. Analysis involves not only the characterization and quantification of proteins, but also the study of interactions between proteins and between proteins and nucleic acids and small molecules. The ultimate goal of proteomics research is to identify specialized molecules that will be good diagnostic markers of disease or targets for therapeutic drug development. While the field of protein characterization is not new, the demand for more integrated, highly automated approaches is.

While the genome provides the blue-print for what will be - the proteome essentially becomes the functional expression of what really is. The large abundance of data being generated from the human genome has further fueled the demand for more advanced strategies and analytical tools to streamline and automate this field of protein research. At Beckman Coulter we have created a new Systems Biology center to focus on simplifying, automating and standardizing processes from the first identification of cellular difference - to the ultimate diagnosis of disease. Figure 1.0 illustrates this vision of our proteomics initiative, beginning the process at cellular level and identifying common segments that make up the experimental workflow. In this poster we focus on both the fractionation and characterization steps of the ProteomeLab™ initiative.

Fractionate

Once unique differences are identified, compartmentalized and the protein lysate created, our goal is to fractionate the thousands of proteins that potentially may be present in a given tissue, cell or organelle. This is a tremendously challenging process because it involves separating and detecting proteins at very low and very high concentrations, proteins that are very basic from those very acidic as well as separating very polar from very hydrophobic (membrane bound) proteins. The ProteomeLab™ PF 2D protein fractionation system is an automated, two-dimensional fractionation platform that resolves and collects proteins by isoelectric point in the first dimension and by hydrophobicity in the second dimension. The system allows you to visualize the complex protein pattern with a 2 dimensional protein map and the system's differential display allows the user to highlight differences in protein pattern expression between normal and abnormal states. All components are then either isolated into a 96 well plate as a liquid phase - ideal for introduction to mass spectrometry or to electrophoresis based characterization processes.

1st Dimension Separation, Chromatofocusing:

Column and buffers: ProteomeLab HPCF chromatofocusing column, Start Buffer (pH 8.5), Eluent Buffer (pH 4.0), 1M NaCl and water.

Method: The first dimension separation is done at ambient temperature with a flow rate of 0.2 ml/min and absorbance of the column effluent was measured at 280 nm. The column is automatically equilibrated with 30 column volumes of start buffer and then up to 5.0 mg of protein sample is typically injected - at which time the chromatofocusing gradient is generated by switching to the eluent buffer. Fractions are automatically collected every 0.3 pH units (user selectable). Once the column effluent reaches pH 4.00, the system automatically switches to the wash with 10-column volumes of 1M NaCl followed by 10-column volumes water with continued fraction collection until the absorbance at 280 nm reached baseline.

2nd Dimension Separation, Reversed Phase:

Column and buffers: ProteomeLab HPRP reversed phase column, 0.1% TFA in water (solvent A) and 0.08% TFA in acetonitrile (solvent B).

Method: The second dimension separation is performed at 50° C with a flow rate of 0.75 ml/min and absorbance of the column effluent was measured at 214 nm. The column is automatically equilibrated with 10 column volumes of solvent A prior to each fraction injection. From each first dimension fraction, 200 µL is automatically injected and the proteins eluted with a gradient of 0-90% solvent B over 30 minutes. Figure 2.0 illustrates the graphical differential display of a selection of pI fractions from two different colon cancer cell lines samples - before and after treatment.

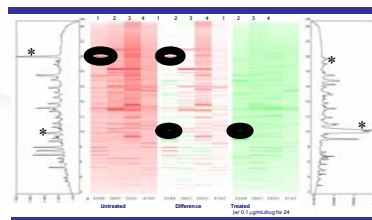


Fig 2.0 Differential display of partial UV/pI maps of colon cancer cell line, before and after treatment.



Figure 3.0 illustrates the layout of the ProteomeLab PF 2D and PA 800 Systems

Characterize

The fractionation map yields the resolution and isolation of thousands of proteins which may be then rationally or methodically characterized with respect to their structure and function. As proteins fractionated with the ProteomeLab PF 2D are already in liquid phase, they are easily transferred to the characterization step. The new ProteomeLab PA 800, pictured in Figure 3.0, utilizes capillary electrophoresis technology to determine a protein's molecular weight, resolve differences in isoelectric point, generate high-resolution peptide maps and carbohydrate profiles, and provide front-end separation and introduction of these proteins to mass spectrometry. This same tool is used to assess and characterize protein-protein, protein-nucleic acid and protein-drug interactions. The following three figures highlight characterization steps run on the PA 800 system. Figure 4.0 illustrates the high resolution analysis of proteins by molecular size, using a new SDS-Gel sieving chemistry, that facilitates higher resolution analysis of proteins. This chemistry resolves proteins from 1,000 to 350,000 MW. Figure 5.0 illustrates identification of IgG isoforms using capillary isoelectric focusing while figure 6.0 highlights an N-Linked oligosaccharide mapping experiment utilizing the ProteomeLab CHO chemistry

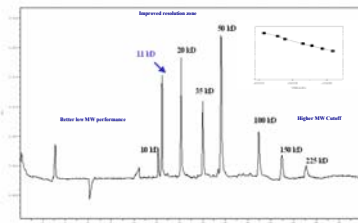


Figure 4.0. SDS-MW analysis of a Protein Standard ladder using the new SDS-Gel polymer. 75 µm x 20 cm (det) bare fused silica capillary, E.K. sample introduction

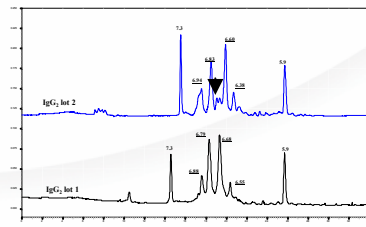


Figure 5.0 CIEF analysis of two different lots of monoclonal IgG_{2b}, being assessed as a standard control. The resolving power of CIEF clearly identifies differences in these lots

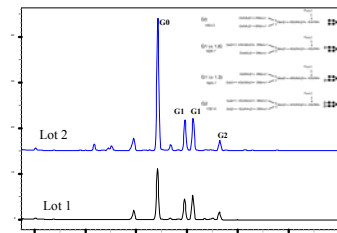


Figure 6.0 N-Linked Oligosaccharide profile comparing 2 lots of monoclonal IgG_{2b} being assessed as a standard control.

CE-MS/MS Characterization

Protein identification - using tryptic digests followed by CE-MS/MS provides rapid peptide sequence determination. Because trypsin cuts after Lysine and Arginine, the resultant peptides will be positively charged in acidic media, and as such are good candidates for MS in a positive ion mode. By incorporating the CE separation step we limit the number of ions being introduced to the detector at one time, which increases sensitivity; we also gain mobility information which can be helpful in identification of the peptides. The ProteomeLab PA 800 is configured to interface with an electro-spray ionization (ESI) device. In this poster we illustrate integration with an LCQ™ Advantage MS/MS system (ThermoFinnigan Inc) using integrated Xcalibur™ software to control both systems. In Figure 7.0 we highlight an example of this approach as we easily resolve differences between chicken and horse cytochrome c tryptic digests. In this case the N-terminal peptide is clearly different between the two species. The experiment used 50 µm X 80 cm-amine treated capillary, 100 mM NH₄OAc, pH 3.1, 30 KV/6.8 uA; 1.0 mg/ml digest/ triple spray; ESI: 4.5 KV/8-9 uA; gas flow: 8

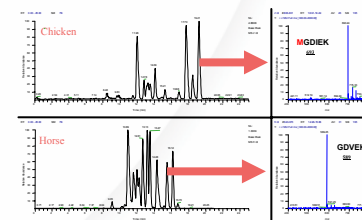


Figure 7.0. Horse and Chicken Cytochrome C Tryptic digests, resolved by the ProteomeLab PA 800 system interfaced with an LCQ Advantage MS/MS system.

Summary

The ProteomeLab initiative integrates Beckman Coulter systems, software and chemistries and leverages the company's technological strengths in the development of new products for protein research. In this poster we highlighted both the fractionation and characterization capabilities of the new ProteomeLab PF 2D and PA 800 systems. This initiative will continue to build on key technologies including automation, flow cytometry, electrophoresis, chromatography, centrifugation, spectrophotometry and microarrays. These solutions will address most aspects of the proteomics process, from first identifying cellular difference to the ultimate diagnosis of disease. Our focus here is one based on Systems Biology - to better enable the broader proteomics process, literally from Tissues to Targets