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A Novel Experimental Scheme to Fully Separate Permethylated Glycans and Allow Online RP-LC-MS with Simplified ESI-MS Analysis

Jesse Hines
Complex Carbohydrate Research Center

Shujuan Tao
University of Georgia

Ron Orlando
University of Georgia

Stacy D. Brown
East Tennessee State University, browsd03@etsu.edu

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BIOMARKERS

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High Performance Protein Enrichment Using Streptavidin Magnetic Beads

Lena Hornsten, Maria Björner, Ann Bergh, Gunnar Glad, Therese Granér, Helena Hedlund, Ulf Hellberg, Kristina Uhlén

GE Healthcare Bio-Sciences AB, Uppsala, Sweden

The binding of streptavidin to biotin is one of the strongest known non-covalent biological interactions and hence a powerful tool used in affinity chromatography. Biomolecules can easily be fused with biotins, to which immobilized streptavidin ligands on chromatography matrices can bind. The usage of magnetic beads for affinity based purifications simplifies small-scale purifications and provides high flexibility with scales ranging from µl to ml.

In this study, we show that the magnetic bead medium Streptavidin Mag Sepharose™ offers leading performance concerning binding capacity and achieved purity. Binding capacity of Streptavidin Mag Sepharose for biotinylated rabbit monoclonal IgG was 1.7 mg/ml bead slurry. Immunoprecipitation of 7.5 µg/ml transferrin in a background of 5 mg/ml E.coli protein showed a 420-fold enrichment. Scaling up immuno-precipitation experiments 10 times or changing sample concentration 100 times, resulted in equal purity and recovery (%).

The characteristics of the Mag Sepharose beads are; simplify handling by reducing the risk of beads sticking on tubes and pipette tips. Moreover, the beads respond quickly to magnetic fields resulting in rapid separation of the beads from the sample, completed within seconds.

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Proteomics Profiling of Human Melanoma, Breast Cancer, Glioblastoma, Lung carcinoma, Osteosarcoma and Cervical Cancer Cell Lines

Vadiraja B. Bhat[1], Christine Miller[2]

[1]*Agilent Technologies Inc.,* [2]*Agilent Technologies Inc.*

Most often, global proteomic analysis is performed using one type of cell line. However, protein expression may differ from cell line to cell line within a single organism. In this study, we investigated over 22 different types of human cell lines by 1D-LC-MS/MS analysis using nanoLC coupled to a q-TOF mass spectrometer. 100µg each of protein samples from 22 different types (7 melanoma, 8 breast cancer, 4 glioblastoma, 1 non-small cell lung carcinoma, 1 osteosarcoma, 1 cervical cancer) of human cancer cell lysates were reduced, alkylated and trypsin digested. After drying, digests were reconstituted in 0.1% formic acid for LC-MS analysis. 1-2µg of tryptic peptides from different types of cancer cell lysates were analyzed in triplicates on HPLC-Chip/ 6520 or 6550 QTOF Mass Spectrometer using 90 min gradient. The data was analyzed and semi-quantified using Spectrum Mill bioinformatics tool. In this study, we demonstrated a label free 1D-LC-MS/MS approach for proteomics phenotyping of several different types of human cancer cell lines. About 5000 unique human proteins and 50000 unique peptides were identified in the preliminary analysis of about 22 different human cancer cell lines. Proteomic comparison of melanoma and breast cancer cells showed about 30% of the total proteins unique to each cell type and about 68% of proteins were differentially expressed. Overall about 75% of the total proteins were differentially expressed between different types of cancer cell lines. In this large scale proteomics profiling study we found that protein expression between different types of cells varies significantly.

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IFNbeta-1b Treatment Alters the Composition of Circulating B Cell Subsets and Leads to Changes in the B Cell Cytokine Secretion Profile in an Ex-vivo Stimulation Assay for Patients with Relapsing-Remitting Multiple Sclerosis

Alan Bergeron, Daniel Mielcarz, Ph.D., John DeLong, Kathleen Smith, Alexandra Heyn, Karen Mack, Brant Oliver, Lloyd Kasper, M.D., Jacqueline Channon, Ph.D.

Dartmouth College

Recent studies provide strong evidence for a role for B cells in the pathology of relapsing-remitting multiple sclerosis (RRMS). To explore the effect of IFNbeta-1b treatment on B cell phenotype and function in these patients, blood was drawn from 10 RRMS patients before treatment and again 2 and 6 months after daily injections of IFNbeta-1b. Cryopreserved PBMCs were thawed and stained with panels of antibodies against B cell surface antigens. PBMCs were also stimulated with CpG-B. Stimulated cell surface phenotype as well as intracellular IL10 and IL6 were determined by 10-parameter flow cytometry. PBMCs from age and gender matched normal healthy donors were examined in parallel. At baseline, RRMS patients have significantly increased frequencies of naïve B cells and decreased frequencies of memory B cells when compared with healthy controls. CpG-B-stimulated PBMCs from patients treated with IFNbeta-1b show an increase in IL-10 production and a decrease in IL-6 production by naïve, memory and B1 cells (a recently-described subset of autoantibody producing cells that are CD20+CD27+CD43+). These changes in cytokine production are indicative of a change from a pro- to an anti-inflammatory phenotype. In addition, this treatment alters the composition of circulating B cell subsets, leading to an increase after six months in circulating naïve B cells and a decrease in both memory and B1 cells, both cell types of which are potentially pathogenic in RRMS. Although the number of subjects in this study was limited, these findings suggest that alterations in B cell phenotype and function may be a mechanism by which IFNbeta-1b reduces disease activity.

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CARBOHYDRATE ANALYSIS

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A Novel Experimental Scheme to Fully Separate Permethylated Glycans and Allow Online RP-LC-MS with Simplified ESI-MS Analysis

Jesse Hines[1], Shujuan Tao[2], Ron Orlando[3], Stacy Brown[4]

[1]*Complex Carbohydrate Research Center, University of Georgia,* [2]*Department of Biochemistry and Molecular*

Biology, University of Georgia, [3]Department of Chemistry, University of Georgia, [4]Department of Pharmaceutical Sciences, East Tennessee State University

Permethylation of glycans is one of the steps used in glycoprotein analysis. Typical LC-MS configurations involve reverse-phase chromatography coupled online to ESI-MS systems. Historically, reverse phase chromatography has been unsuccessful in separating permethylated glycans. The interactions between the hydrophobic stationary phase and the glycans, which have similar monosaccharide compositions, are not distinct enough to enable their resolved separation. Therefore, ion chromatography is commonly used to separated these modified glycans.

In this work the authors have discovered a method that for the first time enables separation of permethylated glycans using reverse-phase media and mass spectrometry compatible mobile phases. The method, which involves heat activation in conjunction with use of small-particle size columns, has been successful in resolving both isomeric and structurally similar glycans. The results of this new separation technique are a significant improvement over ion chromatography and allow the LC to be directly coupled to the mass spectrometer for MS and MS/MS sequence analysis.

With electrospray ionization glycans and other large organic molecules greater than 2000 Da. are typically exhibited as charged ions within an ion series. In addition glycans can form multiple adducts resulting in complex spectra for just a single glycan. We observed that glycans separated with this new approach exhibited a very unusual behavior during electrospray ionization resulting in singly charged ions for all glycans even those with masses greater than 4000 Da. The presence of a single ion for each glycan greatly simplifies analysis of the mass spectra. In addition the lack of multiple-charged species results in improved mass accuracy by obviating the need to perform deisotoping calculations that can propagate mass errors.

These results and observations will be presented in the poster. In addition this initial discovery has spurred other research which builds on this technique using alternate reverse-phase media for quantitation of glycans.

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Development of a Fast HPAE-PAD Sialic Acid Analysis Method

Jeffrey Rohrer, Deanna Hurum

Thermo Fisher Scientific

Sialic acids are often determined in glycoprotein therapeutic products due to their critical role in product stability and/or efficacy. These carbohydrates play roles in glycoprotein bioavailability, function, stability, and metabolism. High-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) is a commonly used technique for accurate determinations of the two most common sialic acids found on glycoprotein oligosaccharides, N-acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid (Neu5Gc). After releasing the sialic acids either by acid hydrolysis or digestion with the enzyme neuraminidase, this assay typically requires 20 to 30 min per sample injection. With increasing demand to more quickly screen samples from expression cell line and growth condition optimization experiments, shorter analysis times are needed for all analyses, including sialic acid analysis. This presentation describes the development of a five minute HPAE-PAD assay for glycoprotein sialic acids after prior acetic acid hydrolysis. Acetic acid hydrolysis was chosen over other sialic acid release methods to eliminate the time required for sample lyophilization. In addition to measuring the common analytical figures of merit including method linearity, limits of detection, and intra- and inter-day reproducibility, we also evaluated method accuracy and ruggedness. Five common sialylated glycoproteins with published sialic acid content were assayed to evaluated accuracy and the same samples were used for spiked recovery experiments. Recoveries from the five glycoprotein hydrolyzate samples ranged from 81-96% for Neu5Ac and 82-106% for Neu5Gc, and the determined amounts agreed with published values, both suggesting method accuracy. The method proved to be rugged to column change and 1000 sample injections.

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Automated Glycan Structural Isomer Differentiation Using Bioinformatics Tool

Gurmil Gendeh[1], Julian Saba[1], Amy Zumwalt[1], Ningombam Sanjib Meitei[2], Arun Apte[2], Rosa Viner[1]

[1]*Thermo Fisher Scientific,* [2]*Premier Biosoft*

Mass spectrometry (MS) has emerged as a powerful tool for the structural elucidation of glycans. The use of permethylaytion in combination with multistage fragmentation (MSn) is a critical aspect for glycan structural characterization. Only MSn truly characterizes a glycan structure as it allows identification of branching patterns, linkages and resolution of isobaric structures which are otherwise indistinguishable in MS/MS spectra. However, MSn analysis is complicated by large number of spectra generated for a single structure. It is very common that one must acquire MS6 or MS7 level of fragmentation to differentiate potential glycan structural isomers. Here we present the use of a bioinformatics tool (SimGlycan) for glycan structural isomer differentiation from MSn data.

SimGlycan software was used in combination with MS^n and permethylation to differentiate structural isomers present in ovalbumin glycans. The ion trap MS profile was acquired for permethylated ovalbumin glycans and specific precursors were targeted for MS^n to differentiate structural isomers. Sequential MS^n data were imported into SimGlycan software and various structural isomers of ovalbumin glycans were differentiated. SimGlycan differentiated structural isomers were verified using manual assignment and previously published data.

CORE MANAGEMENT METHODS

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Methods for Tracking Core-contributed Publications and Increasing Acknowledgment and Authorship Compliance

Carol Lynn Curchoe

New York University Langone Medical Center