Glycomics & Lipidomics

Extended Abstract

Glycomics profile analysis by MALDI TOF/MS in human CSF

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Abstract

Protein glycosylation is important for human brain development and function. The majority of the >100 known subtypes presents with intellectual disability. To explore possible defects in protein glycosylation in the brain, we developed comprehensive glycomics analysis of N-glycome, O-glycome and free glycome by MALDI TOF/MS in cerebrospinal fluid (CSF). 0.4 ml CSF was filtered to separate free oligosaccharides from glycoprotein. N-glycans were released via PNGase F digestion and O-glycans were released separately by reductive beta-elimination reaction. After purified, glycans were permethylated and analyzed by MALDI TOF/MS. Thirty eight different N-glycan species, 85 free oligosaccharide and 25 O-glycan species were identified in 10 control CSF samples. Compared with plasma glycomics data, CSF has much more O-glycan species including O-mannosylated glycans and polysialylated O-glycans. Comparing 166 CSF samples from patients with undiagnosed neurological disease from the NIH Undiagnosed Diseases Program (UDP) with 10 control CSF samples, about 20 UDP patients have CSF glycomics profiles significantly deviated from the profiles of control CSF samples. Four of the UDP patients have known genetic disorders that alter protein glycosylation in human brain, including one patient with putative CAD deficiency, a defect in uridine biosynthesis. Interestingly, one of the known patients has a completely normal plasma glycomics profile and urine oligosaccharide profile, while both his CSF N and O-glycomics profiles are abnormal. Our results suggest that CSF glycomics analysis could be a useful tool to discover new diseases or disease mechanism.

We present the methodology currently applied in our laboratory for the structural elucidation of the cerebrospinal fluid (CSF) N-glycome. N-glycans are released from denatured carboxymethylated glycoproteins by digestion with peptide-N-glycosidase F (PNGase F) and purified using both C18 Sep-Pak® and porous graphitized carbon (PGC) HyperSep™ Hypercarb™ solid-phase extraction (SPE) cartridges. The glycan pool is subsequently permethylated to increase mass spectrometry sensitivity. Molecular assignments are performed through matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF MS) analysis considering either the protein N-linked glycosylation pathway or MALDI TOF MS/MS data. Each stage has been optimized to obtain high-quality mass spectra in reflector mode with an optimal signal-to-noise ratio up to m/z 4800. This method has been successfully adopted to associate specific N-glycome profiles to the early and the advanced phases of Alzheimer's disease.

CSF diagnostics has proved to be a formidable testing ground for N-glycoproteomic analysis of neurological diseases. To characterize specific N-glycan profiles of CSF in early and advanced phases of Alzheimer's disease, as well as in lysosomal storage disorders such as Tay-Sachs disease, we set up in our lab a robust and feasible protocol by coupling bioanalytical methods and mass spectrometry analysis.Starting from a few microliters of CSF, after protein denaturation, reduction, and alkylation, N-glycans are released from glycoproteins using the peptide-N-glycosidase F (PNGase F) and purified. The analysis of permethylated N-glycans by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and MALDI-TOF MS/MS allowed us to identify specific glyco-structures and also to distinguish between isobaric N-glycans.

Glycosylation represents the most common of all known protein post-translational modifications. Carbohydrates can modulate the biological functions of a glycoprotein, protect a protein against hydrolysis via protease activity, and reduce or prevent aggregation of a protein. The determination of the carbohydrate structure and function in glycoproteins remains one of the most challenging tasks given to biochemists, as these molecules can exhibit complex branched structures that can differ in linkage and in the level of branching. In this review, we will present the approach followed in our laboratory for the elucidation of N- and O-glycan chains of glycoproteins. First, reduced/carboxamidomethylated glycoproteins are digested with a protease or a chemical reagent. N-Glycans are then released from the resulting peptides/glycopeptides via digestion with peptide N-glycosidase F (PNGase F). Oligosaccharides released by PNGase F are separated from peptides and glycopeptides using a C18 Sep-Pak, and their methylated derivatives are characterized by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF-MS). O-Glycans are released by reductive elimination, which are permethylated, purified on a Sep-Pak C18 cartridge, and analyzed with MALDI-TOF-MS. Finally, to confirm the structures N-glycans released by PNGase F are characterized using MALDI-TOF-MS following on-plate sequential exoglycosidase digestions. The clean-up procedures of native and permethylated oligosaccharides for an efficient MALDI-TOF-MS analysis will also be described. This strategy was applied to calf fetuin and glycoproteins present in human serum.

We present a detailed protocol for the structural analysis of protein-linked glycans. In this approach, appropriate for glycomics studies, N-linked glycans are released using peptide N-glycosidase F and O-linked glycans are released by reductive alkaline beta-elimination. Using strategies based on mass spectrometry (matrix-assisted laser desorption/ionization-time of flight mass spectrometry and nano-electrospray ionization mass spectrometry/mass spectrometry (nano-ESI-MS-MS), Chemical derivatization, sequential exoglycosidase digestions and linkage analysis, the structures of the N- and/or O-glycans are defined. This approach can be used to study the glycosylation of isolated complex glycoproteins or of numerous glycoproteins encountered in a complex biological medium (cells, tissues and physiological fluids).

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