

Rapid and easy preparation of glycans from glycoproteins is a crucial step to glycomics. Preparation of N-glycans has been well-established, while O-glycan preparations have yet to satisfy the recent needs for glycan analysis in glycobiology research and biopharmaceutical industry. Here, we introduce a novel method of O-glycan liberation that can be executed in aqueous solution and completed within 20 min. O-Glycans could be released from glycoproteins by using 50% hydroxylamine and an organic superbase, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). The reaction condition was optimized by using bovine fetuin as a model glycoprotein. The optimized method afforded comparable recovery rates to those of gas-phase hydrazinolysis, but with less degraded products. Unlike hydrazinolysis, the glycans containing NeuGc could also be released without any degradation. Fluorescent tag such as anthranilic acid (2-AA) and 2-aminobenzamide (2-AB) was successfully introduced to label the released glycans by a reductive amination. To demonstrate the feasibility of the method, we analyzed O-glycans of mucins separated by supported molecular matrix electrophoresis (SMME) which is previously developed to characterize mucins. Mucins are hardly characterized by proteomics because of highly glycosylation and their inability to enter a polyacrylamide gel due to their large molecular size. SMME is a membrane electrophoresis using hydrophilic polymer soaking into porous membrane of polyvinylidene difluoride as the separation medium. Mucins can be separated as narrow bands by SMME. The bands are excised and subjected to the O-glycan liberation. We analyzed commercially available bovine submaxillary mucin and mucins extracted from porcine submaxillary gland using a combination of SMME and the developed O-glycan liberation as described above. 2-AA Labeled O-glycans obtained from each band generated upon SMME were successfully analyzed by LC-MS. Thus, the O-glycan liberation developed in this study demonstrated its rapidness and easiness, besides overcoming defects of gas-phase hydrazinolysis without sacrificing recovery rates. We believe that the method would be quite useful as a general technique of O-glycan liberation for glycomics, glycobiology research, and biopharmaceutical industry.

232. Increase in sialidase activity during cerebral infarction and its role on glutamate release in rat hippocampus

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Sialidase removes sialic acid residues in sialoglycoconjugates. We previously found that sialidase is essential for hippocampal memory and synaptic plasticity. Enzyme activity of sialidase is rapidly increased in response to neural excitation. Because sialic acid bound to gangliosides such as the tetra-sialoganglioside GQ1b is crucial for calcium signaling and neurotransmitter release, neural activity-dependent removal of sialic acid may affect hippocampal neurotransmission. In the present study, we investigated the desialylation by neural excitation during cerebral infarction and its role on glutamate release.

For in vivo monitoring of desialylation on the cell surface, the amount of free sialic acid collected from the hippocampal extracellular space was measured using in vivo microdialysis. The hippocampus was perfused with artificial cerebrospinal fluid (ACSF) and then injected with exogenous sialidase from *Arthrobacter ureafaciens* (AUSA). Neu5Ac, the main molecular species of sialic acid, in the perfusate was increased by AUSA injection. In contrast, Neu5Ac in the perfusate was decreased by novel sialidase inhibitor 2-deoxy-2,3-didehydro-D-N-propionylneuraminic acid. We next evaluated desialylation during cerebral infarction. The Neu5Ac level as well as glutamate level in the hippocampal extracellular space was significantly increased by was photochemically induced cortical infarction with the photosensitizing dye, rose bengal. In this study, we also investigated the role of endogenous sialidase in glutamate release in

the rat hippocampus. In the hippocampal CA3 region, the extracellular glutamate level measured by in vivo microdialysis was significantly increased by perfusion with sialidase inhibitors, 2-deoxy-2,3-didehydro-D-N-acetylneuraminic acid and 2-deoxy-2,3-didehydro-D-N-glycolylneuraminic acid. These results indicate that sialidase activity is increased by neural excitation during cerebral infarction, and that sialidase down-regulates glutamate release from hippocampal neurons. Neural activity dependent desialylation by sialidase may be a negative feedback factor against presynaptic activity.

233. Determination of serum proteins glycosylation in cancer before and after the treatment: lectin-based protein microarray and maldi-tof ms approaches

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Glycosylation status of glycoconjugates is one of the cancer-related markers which can significantly increase the informative value of protein biomarkers. We determined the differences in glycan composition in serum samples obtained from patients with various types of cancer (colorectal, breast, ovarian and others) before chemotherapy and 3 months after the end of the treatment. We used two glycoprofiling techniques: lectin-based protein microarray platform and MALDI-TOF MS. First of the platforms enables high-throughput glycoprofiling of biomarkers and recognition of glycan – lectin interactions. The serum samples were first depleted to remove abundant proteins (albumin and IgG) and then the samples were spotted into arrays on microarray slide and incubated with a panel of 16 chosen biotinylated lectins. The detection was performed after incubation with fluorescent conjugate of streptavidin using microarray scanner. MALDI-TOF MS was used for the direct glycoprofiling of serum apolipoprotein C-III without protein isolation or glycan release. The same approaches were used for the measurement of samples from control group of healthy individuals taken three months apart. Determined differences in signal intensities measured by lectin-based protein microarray showed statistically significant changes in glycosylation for the patients' samples. Although analytical assay based on lectin-glycan interactions does not allow identification of glycan structures, this platform is suitable for rapid screening and detecting glycosylation changes or abnormalities for a large number of samples and is complementary to MS methods enabling identification of glycan structures. It makes it very useful in biomarker research and diagnostics. We use this method also in another applications as e.g. glycoprofiling Congenital Disorders of Glycosylation (CDG) patients' samples, monitoring glycosylation changes related to the aging or determination of glycostructure of therapeutic proteins. Acknowledgement: this work was supported by the projects SK-KR-18-0004, SK-SRB-18-0028, APVV-17-0300, VEGA 2/0137/18, COST CA16113, COST CA18132, Centre for materials, layers and systems for applications and chemical processes under extreme conditions - Stage II (ITMS No.: 26240120021, R&D OP funded by ERDF).

234. Profiling of n-linked glycans from ultra-small samples by capillary electrophoresis with a large-volume dual preconcentration technique

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Structure of N-linked glycans is changed in response with pathogenesis like cancer. Glycomic profiling from limited number of cells in an early-stage tissue and/or progressed tumor micro-environment is essential for