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Comprehensive O-Glycan Analysis by Porous Graphitized Carbon Nano-Liquid Chromatography-Mass Spectrometry

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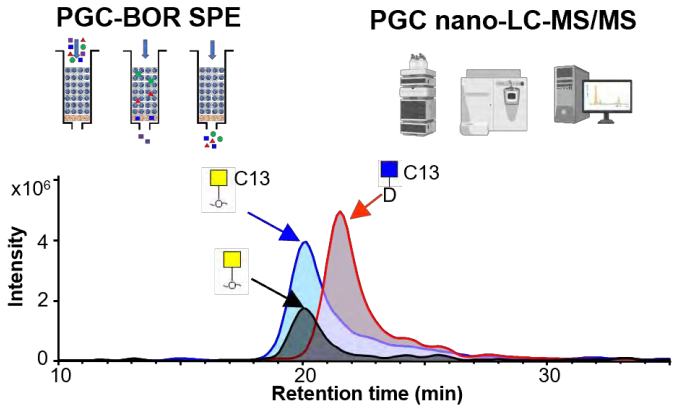
Summary

The diverse and unpredictable structures of *O*-GalNAc-type protein glycosylation present a challenge for its structural and functional characterization in a biological system. Porous graphitized carbon (PGC) liquid chromatography (LC) coupled to mass spectrometry (MS) has become one of the most powerful methods for the global analysis of glycans in complex biological samples, mainly due to the extensive chromatographic separation of (isomeric) glycan structures and the information delivered by collision induced fragmentation in negative mode MS for structural elucidation [1,2]. However, current PGC-based methodologies fail to detect the smaller glycan species consisting of one or two monosaccharides, such as the Tn (single GalNAc) antigen, which are broadly implicated in cancer biology. This limitation is caused by the loss of small saccharides during sample preparation and LC. Here, we upgraded the conventional PGC nano-LC-MS/MS-based strategy for *O*-glycan analysis, enabling the detection of truncated *O*-glycan species and improving isomer separation. This was achieved by the implementation of 2.7 µm PGC particles in both the trap- and analytical LC columns, which provided an enhanced binding capacity and isomer separation for *O*-glycans. Furthermore, a novel mixed-mode PGC-boronic acid-solid phase extraction during sample preparation was established to purify a broad range of glycans in an unbiased manner, including the previously missed mono- and disaccharides. Taken together, the optimized PGC nano-LC-MS/MS platform presents as a powerful component of the toolbox for comprehensive *O*-glycan characterization and revealing biological function of *O*-glycosylation in immune responses and cancer research.



References

[1] P.H. Jensen, N.G. Karlsson, D. Kolarich, N.H. Packer, Structural analysis of N- and O-glycans released from glycoproteins, Nat. Protoc. 7 (2012) 1299–1310.

[2] T. Zhang, K. Madunić, S. Holst, J. Zhang, C. Jin, P. ten Dijke, N.G. Karlsson, K. Stavenhagen, M. Wuhrer, Development of a 96-well plate sample preparation method for integrated N- and O-glycomics using porous graphitized carbon liquid chromatography-mass spectrometry, Mol. Omics 16 (2020) 355–363.