Abstract of Doctoral Dissertation

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Anionic Glycan Diversity in Waterfowl Egg Whites through Glycoblotting-based Sulphoglycomics Approach (グライコブロッティング連動型スルフォグライコミクス法による水鳥卵白中のアニオン性糖鎖多様性に関する研究)

Sulfated *N*- and *O*-glycans exist in trace levels which are challenging to detect, particularly in the presence of abundant neutral and sialylated glycans. Current MALDI-TOF MS-based sulfoglycomics approaches employ permethylation to discriminate sulfated glycans from sialylated glycans and charge-based separation to isolate the sulfated glycans from the rest of the permethylated neutral and sialyl-glycans. However, these methods suffer from concomitant sample losses during cleanup steps. In this study, we describe Glycoblotting as a straightforward complementary method that offers a seamless platform for glycan purification, enrichment, methylation, and labeling to address sulfated glycan enrichment, sialic acid methylation, and sample loss. Glycoblotting's on-bead chemoselective ligation of reducing sugars with hydrazide demonstrates excellent recovery of sulfated glycans and allows the detection of more sulfated glycan species. The on-bead methyl esterification of sialic acid using 3-methyl-1-*p*-tolyltriazene (MTT) effectively discriminates sulfated glycans from sialylated glycans. Furthermore, MTT facilitates simultaneous detection and differentiation of sulfate and phosphate groups in isobaric *N*-glycan species.

Additionally, we investigate the expression of acidic *N*-glycans, specifically sulfated and phosphorylated *N*-glycans, in the egg whites of 72 avian species belonging to the Order Anseriformes (waterfowls). Employing the Glycoblotting-based sulphoglycomics approach, we elucidated the diversity of acidic *N*-glycans and their implication in protecting embryos from infections. Our findings revealed family-specific variations in waterfowl egg whites sulfated and phosphorylated *N*-glycan profiles. Different waterfowl species exhibit distinct expressions of sulfated trans-Gal(+) and trans-Gal(-) *N*-glycan structures. Moreover, species-specific expression of phosphorylated *N*-glycans was also observed. Notably, waterfowl species with a high virus prevalence expressed a higher abundance of phosphorylated hybrid and high-mannose *N*-glycans on their egg whites.

The Glycoblotting-based sulphoglycomics approach presents a significant breakthrough in sulfated glycan analysis, simplifying the existing MALDI-TOF MS-based sulphoglycomics workflow and enabling comprehensive exploration of the complex glycome of biological samples. Furthermore, the findings of this study shed light on the importance of phosphorylated and sulfated *N*-glycans in understanding the role of acidic glycans in the Influenza A virus (IAV) propagation in waterfowl. These results hold immense potential for advancing our understanding of IAV propagation in avian species and guiding the development of targeted interventions to combat influenza.