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Abstract of Doctoral Dissertation

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Title of Doctoral Dissertation

Anionic Glycan Diversity in Waterfowl Egg Whites through
Glycoblotting-based Sulphoglycomics Approach
(グライコブロットイング連動型スルフォグライコムクス法による
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*-tolyltriazen (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.