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Abstract of Doctoral Dissertation

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Title of Doctoral Dissertation

Deciphering biological significance of dynamic glycosylation on glycan variants by mass spectrometry
質量分析法による動的で多様な糖鎖修飾の生物学的機能解明

With the advent of chemical glycobiology, the use of homogeneous glycoconjugate structures is crucial to correctly evaluate glycan roles and biological functions. My work involved the use of different methodologies to achieve this, particularly utilizing glycans from natural sources. Glycan profiling from Galliformes quail egg whites was first conducted to assess diversity of structures using glycoblotting methodology. With this procedure, distinct structures among species were identified. The actual interest was for the isolation of natural glycans for glycopeptide synthesis; of particular importance, the conserved $Man_3GlcNAc_2$ glycan structure found in quail egg whites. The truncation of the conserved *N*-glycan structure was conducted, dehydrated to an oxazoline intermediate, and directly conjugated to two synthesized glycosyl acceptors, Fmoc-Asn(GlcNAc)-OH and IgG-tryptic glycopeptide using endoglycosidase-M-N175Q. All the steps were achieved with less purification steps. The procedure provides for an isolation strategy of a glycan or a certain glycan class directly from a natural source depending on the endoglycosidase enzyme used. Starting with a conserved *N*-glycan structure is important for sugar elongation using different glycosyltransferases. The goal for chemoenzymatic synthesis of glycopeptides is to use it for different assays and as calibration standards. With this, different glycoforms of IgG1 and IgG2 tryptic glycopeptides were utilized to serve as standards and optimized for multiplexing quantitations in human serum. Since most of the reactions performed in this work is based on enzymatic transformations, reusing the endoglycosidase enzyme was necessary. As such, an endoglycosidase immobilization strategy on chitosan nanoparticles was conducted in the last part of the work wherein applications on glycan release and conjugation was shown. Overall, the methodologies mentioned in this work is of utmost importance in the preparation of homogeneous glycococonjugate structures that can be used for different applications.