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Doctoral Dissertation Evaluation Review

Degree requested: Doctor of Life Science　　Applicant's name: Jurgen Teodosio Sanes

Examiner:

Chief examiner	Professor Shin-Ichiro Nishimura
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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）

Results of Evaluation of the Doctoral Dissertation (Report)

With the advent of studies related to glycobiology, a correctly defined glycan and protein component is necessary to understand the role of glycosylation in many biological processes. An alternative to glycan synthetic methodology is to use glycans from natural sources to eliminate tedious stereochemical constraints related to chemically synthesized carbohydrates. The current PhD work provides details from screening natural glycans for providing an application of chemo-enzymatically synthesized glycopeptides.

Screening glycans present in quail egg whites using the laboratory-developed glycoblotting methodology establishes glycan profiles of the 4 Galliformes quail (Blue-scaled, Bobwhite, Japanese, and Mountain Quail) egg white species studied that may be due to different evolutionary and selection pressures. The results reveal abundant and distinct glycans as described in Chapter 2 of the dissertation. Distinct high-mannose structures, the 1686 *m/z* $Hex_7HexNAc_2$ and 1848 *m/z* $Hex_8HexNAc_2$ glycan were only present in Japanese quail, while, the 1850 *m/z* $Hex_3HexNAc_6$ tetra-antennary glycan is in the other 3 species. Confirmation of the structures was conducted through MS fragmentation analysis.

Screening glycans in egg whites not only gave insights into glycan diversity, but, another goal was to screen it for functional carbohydrate structures essential for glycopeptide synthesis. Particularly, the truncated version of $Man_3GlcNAc_2$ glycan, $Man_3GlcNAc$, was isolated as a template for *N*-glycopeptide production as it can be easily extended using various types of glycosyltransferases to prepare different glycoforms. The truncated version can be easily purified, converted to oxazoline,

and attached to different glycosyl acceptors as presented in Chapter 3 of the work. Two glycoconjugates, Fmoc-Asn-OH and IgG1 tryptic glycopeptide with $Man_3GlcNAc_2$ attached were synthesized with less purification steps and was almost a one-pot strategy owing to the effective choice of Endo-D and the use of a filter molecular-weight-cut-off eliminating multiple fraction collection and identification. Coupled with improvements in glycan conjugation as in oxazoline conversion and use of endoglycosidase mutants for transglycosylation reaction, a direct glycoconjugate strategy as stated in the dissertation was accomplished in 5-steps including HPLC purification. The direct glycoconjugate preparation strategy developed with the conserved *N*-glycan attached is simple, doable, and a brilliant procedure.

Synthesized glycoconjugates can be used in a variety of purposes for structural-activity assays and to serve as standards for quantitation purposes as shown in Chapter 4 of the dissertation. IgG glycopeptides was optimized in LC-MS/MS under Multiple Reaction Monitoring (MRM) and a calibration curve was prepared to quantitate the presence of such biomolecules in human serum. The results are important as they provided an absolute-glycoform-specific quantitation in femtomolar quantities that may be useful for diagnosis and monitoring of biomarkers.

Since the procedures require numerous amounts of enzymes to release and attach glycans to form different glycoconjugates, the last part of the PhD work in Chapter 5 involved a strategy to reuse the enzyme multiple times by conjugating onto an immobilization support. Endoglycosidase enzymes need no recombinant engineering to be immobilized to chitosan nanoparticles. The research project on enzyme immobilization addresses the difficulty in acquiring enough glycan structures for assays by being able to reuse expensive endoglycosidases that would be supplement protein/enzyme expression systems.

Glycosylation is an important post-translational modification involved in many biological processes. The PhD dissertation by Jurgen T. Sanes is geared towards deciphering the biological significance of dynamic glycosylation on glycan variants using mass spectrometry as an instrumentation technique. As a summary, the research projects dealt with glycan diversity, screening natural glycans, glycoconjugate preparations, provided an immediate application for glycopeptide standards, and endoglycosidase enzyme immobilization for glycopeptide synthesis. In conclusion, the author provided a wide array of methodologies and findings that will contribute vastly to the glycocommunity. Therefore, we acknowledge that the author is qualified to be granted a Doctorate of Life Science from Hokkaido University.