学 位 論 文 審 査 の 要 旨
Doctoral Dissertation Evaluation Review

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学 位 論 文 題 名
Title of Doctoral Dissertation

Synthetic Studies on Highly Complex *N*-glycans and Glycopeptides
（高度に複雑なアスパラギン結合型糖鎖と糖ペプチドの合成研究）

博士学位論文審査等の結果について（報告）
Results of Evaluation of the Doctoral Dissertation (Report)

The glycosylation is one of the most common post-translational modifications. Encountered in around 50% of proteins are glycosylated and is understood to play a pivotal role in numerous vital processes, including protein folding, cell-cell recognition, immune surveillance, hormone activities, and inflammatory reactions. The biological properties of glycoproteins are influenced by the structure of their oligosaccharide. However, the relationship between structure and function of *N*-glycans remains mostly unclear due to the structural complexity and heterogeneity in the dynamic posttranslational modification at the potential *N*-glycosylation sites. The diversity of natural *N*-glycans is thus imparing the isolation of these compounds from glycoproteins. Therefore, the synthesis of *N*-glycans has been used to overcome the shortage of material for biological studies. The chemical synthesis offers exceptional flexibility. The chemical synthesis of oligosaccharides is formidable, it requires stereochemical and regiochemical control in glycosidic linkage formation. Stereo chemical control can be achieved by employing stereospecific activation methods, using protecting groups that direct the orientation of the glycosidic bond through intermolecular (neighbouring group participation) or intramolecular (tethered aglycone delivery) participation, altering the steric environment around the anomeric position to bias the desired outcome, or exploiting the intrinsic stereoelectronic preferences for reaction at the anomeric position. On the other hand the endo-ß-N-acetylglucosaminidase (ENGase) catalyzed oligosaccharide transfer for glycopeptide synthesis is attracted, because it allows the attachment of a large oligosaccharide to a pre-assembled, unprotected GlcNAc-peptide /protein in a single step in a regio- and stereospecific manner, thus providing a highly convergent approach. Nevertheless, the use of glycosidases in synthesis is subject to two major limitations: the low transglycosylation yield and the product hydrolysis. Significant progresses have been made in recent years to overcome these problems. A major breakthrough in the field is the invention of glycosynthases, a class of novel glycosidase mutants that can promote glycosidic bond formation when a suitable activated glycosyl donor is provided, but do not hydrolyze the newly formed glycosidic linkage.

As chemical synthesis of glycans depends on the subtle balance of various factors; protecting groups, anomeric leaving groups, activator systems, solvents and so on, even careful tunings of systems are needed, such as ß-mannosylation and α-sialylation which had used to be recognized as difficult reactions for the construction of glycans and glycoconjugates with practical yields. There is a need to develop a novel protocol to obtain enough quantity of glycans and glycoconjugates in homogeneous form for the detailed structural and functional studies

In this study, based on the status of previous research and importance, the author performed research on "synthetic studies on highly complex *N*-glycans and glycopeptides" and he succeeded to develop a novel protocol for the construction of various *N*-glycans. In the present study, at first he investigated the degradation products of two abundant polysaccharides, guar gum and locust bean gum, under various hydrolytic conditions. Consequently, he discovered that the degradation of locust bean gum by treating with pectinase from *Aspergillus aculeatus* at 50ºC for 48 h in 50 mM acetate buffer (pH5.0) gives dominantly a disaccharide Manß(1→4)Man and subsequent per-*O*-acetylation of the crude product facilitates the isolation of ß1,4-mannobiose octaacetate by silica gel chromatography in 32% overall yield (51 g) from crude locust bean gum (100 g). As anticipated, ß1,4-mannobiose acetate allowed for highly efficient synthesis of the key intermediate by a general reaction procedures. Then he have developed a modular set of building blocks, that can be used in a generally applicable double regio- and stereoselective glycosylation approach for multiantennary *N*-glycans from two to four antennaery. The yields of the convergent glycosylation reactions were high and in all cases only the formation of desired stereo- and regioisomers was found. In the case of the tri- and tetra-antennary products the glycosylation reactions were free of steric hindrance. This demonstrates a high compatibility of the donors with the corresponding acceptors. After, he performed enzymatic galactosylation of partially protected sugars by Gal-T with UDP-Gal; the galctosylation went smoothely even with protection at reducing end. After one-pot deprotection afforded the free *N*-glycans. Finally he demonstrated that when a sufficient amount of biantennary complex-type sugar oxazoline was used, the corresponding glycopeptides could be synthesized in high yield by the Endo-M-N175Q-catalyzed transglycosylation, without hydrolysis of the product. After, it was demonstrated, for the first time, that the reaction between tri, tetra antennary sugar oxazoline as donors and EPO-related GlcNAc peptide as acceptor was conducted by recombinant endo-M-N175Q preceded significantly and afforded the desired EPO-related glycopeptides in moderate yield.

As mentioned above, this new approach for the synthesis of hyper branched *N*-glycans /glycopeptides from abundant polysaccharide locust bean gum is a useful methodology for practical synthesis of varieties of *N*-glycans. It will open new door for the scientists, who wish to synthesize varieties of *N*-glycans and glycopeptides for the drug discovery. It should be emphasized that the present strategy should allow for the synthesis of a variety of glycopeptides and glycoproteins having homogeneous and highly complicated *N*-glycans when combined with endoglycosidase-catalyzed transglycosylation protocols.

Therefore, we acknowledge that the author is qualified to be granted the Doctorate of (Life Science from Hokkaido University.