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Abstract of Doctoral Dissertation

博士の専攻分野の名称　博士（生命科学）　氏 名

Degree requested　Doctor of Life Science　Applicant name　RAVI KUMAR H V

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）

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. Two general strategies are used for oligosaccharide production- enzymatic (including chemoenzymatic) synthesis and chemical synthesis. In enzymatic and chemoenzymatic routes, saccharide intermediates are elaborated with enzymes, typically glycosyltransferases or glycosidases, to generate oligosaccharides. Chemoenzymatic synthesis by its reliance on both chemical synthesis and enzymatic transformations. Enzymes can be used to effect glycosylation with absolute regio- and stereo-control. If the necessary enzyme is available, the desired bond can be formed, often with high efficiency. Availability of specific enzymes and price of the enzymes turns difficulty in the synthesis. The chemical synthesis offers exceptional flexibility. Natural and non-natural saccharide building blocks can be assembled with natural and non-natural linkages. The chemical synthesis of oligosaccharides is formidable, it requires stereochemical and regiochemical control in glycosidic linkage formation. 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 β-mannosylation and α-sialylation which had used to be recognized as difficult reactions. 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-β-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. Both glycosyltransferases and glycosidases have been vigorously studied for synthetic purposes. In comparison with glycosyltransferase-catalyzed reaction that uses complex sugar nucleotide as the donor and usually has very stringent substrate specificity, glycosidase-catalyzed transglycosylation has several advantages, including the use of readily available donor substrates, the relaxed substrate specificity for acceptors, the easy access to the enzymes, and the potential for a single-step block oligosaccharide transfer (as in the case of endoglycosidases). 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. Moreover, protein engineering, including directed evolution coupled with elegant screening methodology has led to the discovery of an expanding number of novel glycosynthases with enhanced transglycosylation activity and/or altered substrate specificity.

In conclusion, ENGase catalyzed transglycosylation produced varieties of glycoconjugates. But the main disadvantage is the chemical synthesis of the *N*-glycan core Manß1,4GlcN disaccharide needs stereocontrolled ß-glycosylation, followed by selective inversion of the *Glc* C-2 configuration and protecting group manipulations, makes the glycans synthesis most difficult and practical synthesis is impossible. Insertion of gal as well as Neu5Ac group at non reducing end also makes the glycans synthesis much difficult. In this thesis, I challenged to overcome this problem and tried to synthesize hyper branched *N*-glycopeptides by using novel synthetic route and the application of ENGase catalyzed transglycosylation. In chapter-2, I described the highly efficient synthesis of *N*-glycan core Manß1,4GlcN disaccharide from natural abundant polysaccharide locust bean gum. Then di, tri and tetra antennary n-glycans synthesis were demonstrated using core Manß1,4GlcN disaccharide in chapter-3. The synthesis of glycopeptides using di, and tetra antennary *N*-glycans by the application of ENGase catalyzed transglycosylation in chapter-4.

EPO-related tetra antennary *N*-glycopeptide synthesis is the first demonstration towards the synthesis in homogeneous form and 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 endoglycosidases-catalyzed transglycosylation protocols.