Panel Discussion

Strategy for functional analysis of glycans based on synthesis: preparation of homogeneous glycans and reconstruction of glycan environment mimics

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Koichi Fukase was graduated from Department of Chemistry, School of Science, Osaka University in 1982 and received his Ph. D. in 1987 from Osaka University (Prof. Tetsuo Shiba). After spending 1 year as a JSPS Postdoctoral Fellow (Prof. Tetsuo Shiba), he obtained the position as an Assistant Professor (1988–1996) at Department of Chemistry, School of Science, Osaka University (Prof. Shoichi Kusumoto). In 1994, he joined the group of Prof. W. Clark Still at Columbia University as a Ministry of Education Research Fellow. After going back to Osaka in 1995, he was promoted to Lecturer (1996), Associate Professor (1998), and became a full Professor at Department of Chemistry, Graduate School of Science, Osaka University in 2004.
He has been a board member of the Japanese Society of Carbohydrate Research from 2007 and became a President of the Japan Society of Carbohydrate in 2017.
His research interest is in the field of carbohydrate chemistry and glycobiology, synthetic organic chemistry, bio-imaging, and innate immunity.

Diversity is the essence of glycans

Glycans are the most abundant biomass on the earth. Innovation in glycoscience will encourage social system transformation using natural glycans as renewable material feedstocks for production of biofuels, bioplastics, new fabrics, medical materials, and etc. Glycoscience is also indispensable for the comprehensive understanding of life and the medical technology advances, since glycans are one of the three fundamental elements (nucleic acids, proteins, glycans) of life and participate in many key biological processes including molecular trafficking and clearance, receptor activation, signal transduction, endocytosis, and various recognition processes such as cell adhesion, cell differentiation and proliferation, infection, inflammation, immunity, cancer progression and metastasis, fertilization, and etc.

Structural complexity and heterogeneity are major features of glycans. Most classes of glycans exist as complex glycoconjugates such as glycoproteins, glycolipids, glycosaminoglycans, and etc. Glycans even at specific glycosylation site on glycoproteins are heterogeneous. These diversities are called glycoforms, which perturb and regulate the function of glycoconjugates via various glycan recognition proteins. Abnormal cell surface glycoforms and/or glycan profiles are associated with diseases such as cancer, atherosclerosis, neurodegenerative diseases, and etc. Therefore, reliable biomarkers have been developed for the disease diagnosis[1]. Bioinformatics will help the development of reliable biomarkers, although the complexity of glycan-related information restricted the utilization of bioinformatics in glycan biomarker research. The author expects that the biomarker researches will be dramatically accelerated by the use of “artificial intelligence” in conjunction

with glycomics research using mass spectrometry, lectin microarrays, glycan microarrays, surface plasmon resonance (SPR) analysis, and etc [2),3)].

On the other hand, the biological functions of glycans including bio-functional role of glycoforms have not yet been sufficiently elucidated due to their inherent complexity. Synthetic studies of glycans have greatly contributed to the functional studies of glycans by supplying homogeneous glycans. It is often difficult to obtain homogeneous glycans from nature, and the possibility of contamination of other active substances cannot be excluded even after extensive purification, when glycans from natural source are used. Therefore, the use of chemically synthesized compounds without contamination of other active substances is essential in order to determine the active principle or identify "glycocode" as shown later. In recent years, the synthesis of glycan has made great progress. It has been becoming possible to investigate the physiological significance of glycoforms by using synthetic glycoproteins and glycolipids as a homogeneous form.

Multivalent interaction between glycans and glycans recognition molecules such as lectins is also important feature in glycan function. Recognition via multiple sites can allow for the high affinity and high selectivity. In addition, interaction between multiple glycans and multiple lectins leads to pattern recognition of cells; in other words, glycans add a certain personality to cells, extracellular microparticles, glycoproteins, and etc. Synthetic studies can also contribute to the analysis of such complex systems by providing the reconstruction models such as synthetic glyco-clusters, glyconanoparticles, and model glycoconjugates. Furthermore, reconstruction of glycan environment on living cells by using chemical probes can give opportunities for *in vivo* functional analysis based on live cell imaging [4),5)].

Elucidation of the glycocode is the main object of glycan synthesis

The glycan chain encodes the glycocode (glycan code) that includes crucial information about the recognition, localization in cell, distribution and circulation in the body and tissues, and age of glycoproteins [6)]. The glycocode is encoded by a series of glycosidases and carbohydrate transferases. The glycocode is deciphered by glycan-binding proteins such as lectins. The lengths of the glycocodes generally are ca. 1 to 5 residues per one carbohydrate recognition domain. These are actually appropriate sizes for the synthesis, considering the synthetic easiness and industrial applications. Therefore, chemical synthesis has contributed the identification of the minimal structure required for recognition (glycocode) by lectins and other glycan recognition proteins. There have been a number of reports regarding the identification of glycocode by using synthetic or naturally derived glycans. Unraveling the glycocode has been getting more and more important but still difficult task considering the diversity and complexity of glycans. Therefore, the efficient methods for the synthesis of various-type of glycans will be continuously developed including stereoselective glycosylation, protection-deprotection strategy for regioselective control in glycosylation, solid-phase synthesis, automated synthesis, microflow synthesis, enzymatic and chemoenzymatic synthesis, and so on [7)].

One of the remarkable successes is the development of Fondaparinux, which is an anticoagulant related to low molecular weight heparins and is marketed by GlaxoSmithKline (**Figure 1**). Many synthetic studies of have been carried out by Seeberger, Hung, Boons, Bonnaffé, Tamura, Suda, and so on to investigate protein interactions with synthetic glycosaminoglycan fragments [8)]. For example, Hung identified GlcNS–IdoA2S as the minimum requirement for fibroblast growth factor-1 (FGF-1) interaction with 48 disaccharide heparan sulfate fragment library.

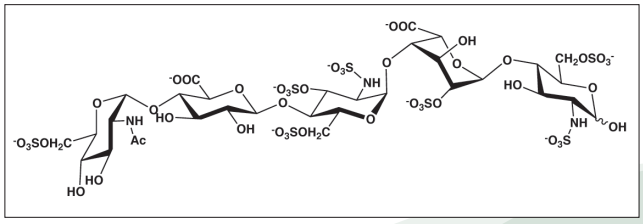

Figure 1
The structure of Fondaparinux

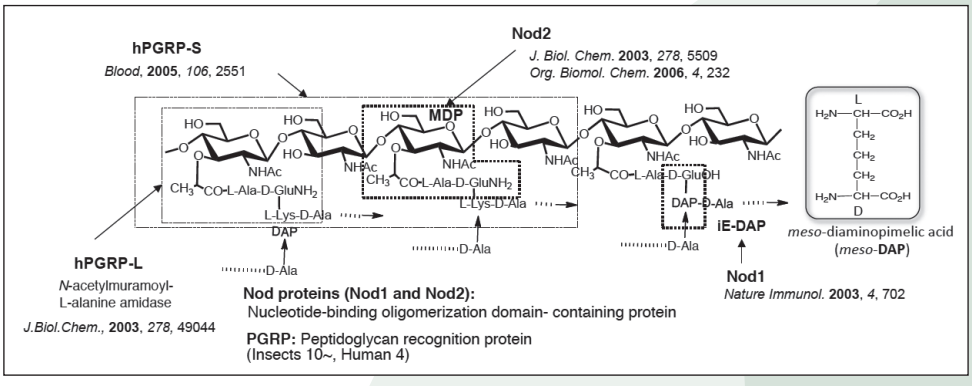

Figure 2
Peptidoglycan partial structures required for binding with the recognition proteins

Our group, Boons, and Mobashery have synthesized various partial structures and derivatives of peptidoglycan (PGN), which is a major component of cell wall and has been well-known stimulant of innate immunity. Interaction of PGN with various proteins was revealed by using the synthetic fragments (**Figure 2**) [9),10)].

Asparagine-linked oligosaccharides (*N*-glycans) on glycoproteins have high diversity and complexity and are involved in a variety of important physiological events. Various syntheses of *N*-glycans have been studied to investigate their biological functions [11)]. α-Sialylation and β-mannosylation have been the key issues for the synthesis of *N*-glycans. Kinetic solvent effect of nitrile found by Kanie, Kiso, and Hasegawa has been mainly used for α-sialylation, whereas intramolecular aglycon delivery developed by Ito, Ishiwata, Stork, and Hindsgaul or Crich's S_N2-like glycosylation has been used for β-mannosylation. Danishefsky succeeded in synthesizing various types of *N*-glycans, including a core-fucosylated glycan and a triantennary glycan. Ito synthesized high-mannose/complex-type glycans and has clearly showed the biological role of *N*-glycan in protein folding and protein quality control in ER. Unverzagt et al. reported the syntheses of *N*-glycans with various structures, including core fucose. Chemoenzymatic approaches using a variety of glycosidases or glycosyltransferases have been employed for the synthesis of *N*-glycans by Ito, Boons, Wang, and Wong. Kajihara reported the semi-synthetic approach for the synthesis of triantennary glycan from natural biantennary glycan. Schmidt et al. carried out the synthesis of complex-type *N*-glycans not only in liquid phase but also on solid phase. Our research group has reported the solid-phase synthesis of a sialic acid containing glycan as well as the solution synthesis of core-fucosylated *N*-glycan. In the latter, glycosyl-asparagine was first synthesized by *N*-glycosylation, and the oligosaccharide chains were then elongated. We reinvestigated α-sialylation, β-mannosylation, and *N*-glycosylation by using microflow system to reveal that precise temperature control was essential for these glycosylations. Intermolecular hydrogen bonds involving acetamide groups were found to reduce the reactivity in glycosylations: the protection of NHAc as NAc_2 dramatically improved the reactivity.

Reconstruction of glycan environments

It has been difficult to elucidate the bio-functions of glycans because of the structural diversity and heterogeneity of glycans as well as the multivalent recognition of glycans with various recognition proteins as mentioned above. Reconstructing glycan environments using homogenous synthesized glycans can facilitate the investigation and control of the glycan functions. A number of glycoconjugates or clusters have reported on dendrimers, liposomes, nanoparticles, and protein templates for biodistribution studies. Glycoclusters containing *N*-glycan structures have been used for bio-distribution studies. André, Gabius, and Unverzagt have prepared albumin connecting various di-, tri-, and tetra-antennary *N*-glycans, but introducing ratio of *N*-glycan to albumin were not high enough to observe the cluster effects. Our group reported the synthesis of polylysine-based glycodendrimers with 16 molecules of biantennary *N*-glycans via the Cu-mediated Huisgen cycloaddition reaction, and we have successfully mimicked the cluster effects of *N*-glycans and visualized the sialic acid-dependent circulatory residence by Positron Emission Tomography (PET) [12)].

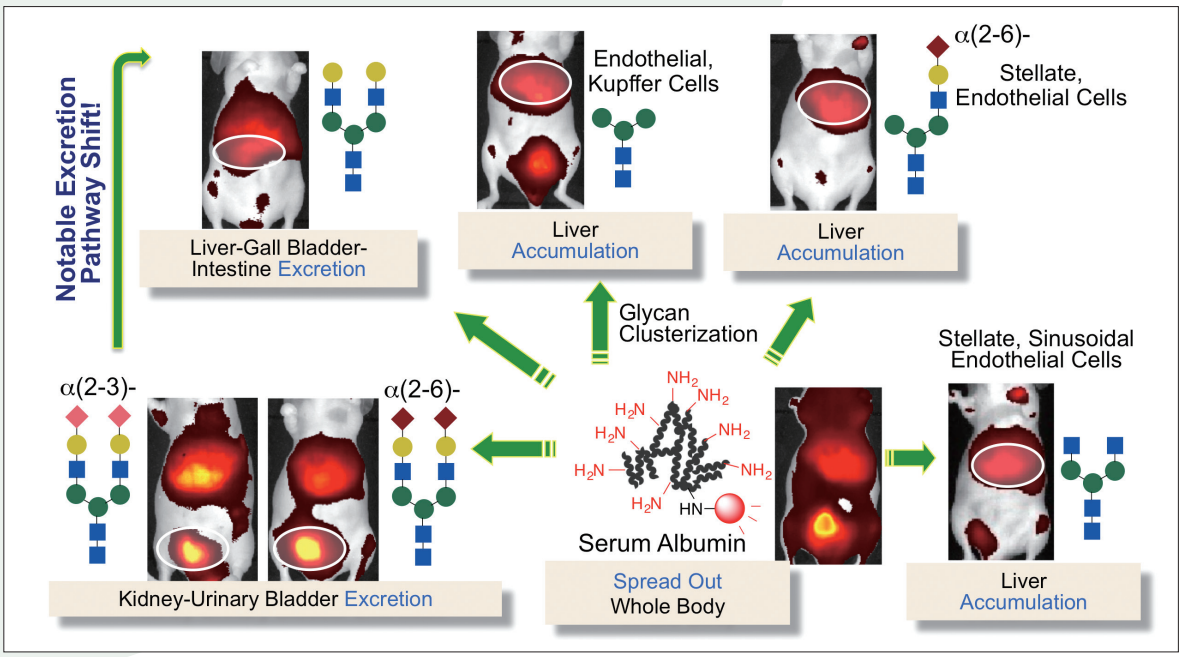

Figure 3
Fluorescent imaging of albumin conjugated with a series of *N*-glycans

However, the instability of *N*-glycan dendrimers in the serum as well as the difficulty of the synthesis hampered the further studies. Tanaka performed the systematic studies using human serum albumin (HSA), which is conjugated with a series of *N*-glycans, each sequentially trimmed from biantennary sialoglycans by using a method that combined strain-promoted alkyne-azide cyclization and 6π-azaelectrocyclization. Fluorescent imaging and dissection analysis revealed a glycan-dependent shift from urinary to gall bladder excretion mediated by sequential trimming of non-reducing end sialic acids. Albumins conjugated with GlcNAc- and hybrid biantennary-terminated congeners, were selectively taken up by sinusoidal endothelial and stellate cells in the liver. Tanaka also succeeded the preparation of glycoclusters having different *N*-glycans, which showed very interesting bio-distribution (**Figure 3**, See page 77). They also demonstrated the pattern recognition of cells by using the conjugates of *N*-glycans with other ligands on the cell surface.

Monodisperse glycooligomers carrying different sugar ligands (mannose, galactose, and glucose) at well-defined positions were synthesized [14]. Interestingly, all heteromultivalent structures showed high affinities toward Concanavalin A in comparison to their homomultivalent analogues.

The glycoclusters have the potential in various areas, e.g. as glycoprobes and novel biosensors for diagnosis and therapeutics such as tumor-targeting agents, anti-infectious drugs, vaccines, and etc.

Conclusions

As described above, chemical synthesis has contributed to uncover the glycan functions by supplying homogeneous bio-probes or reconstructing glycan recognition systems. Another important feature of chemical synthesis is that chemical synthesis can easily supply various partial structures, derivatives, and analogues. These chemically synthesized compounds have high potential as glycan-related drugs, since they can target specific proteins responsible for the pharmacological action.

Anti-infective glycan-related drugs such as anti-influenza drugs Tamiflu and Relenza have been realized, since glycans responsible for intercellular communication at the cell surface also serves as the window of infection of viruses and bacteria. It Is important to continue anti-infective drug discovery in order to overcome the drug-resistance, prevent and treat neglected tropical diseases, and deal with emerging and re-emerging infectious diseases. The α2,6-sialyl-gal glycan is necessary for human-type influenza virus infection, and ABO · Lewis blood group glycans for Norovirus, Lewis blood group type glycans for *Helicobacter pylori*, and etc.

Synthetic studies of glycans can also contribute to the development of preventive medicine such as vaccine. Carbohydrate antigens from pathogens such as bacteria, viruses, protozoans are promising vaccine candidates. Cancer vaccines using tumor-associated carbohydrate antigens (TACAs) have been extensively studied, but they have not been approved for the practical use. The low antigenicity of the carbohydrate antigens must be overcome for the effective immunization. Therefore, the use of carrier proteins or adjuvants is necessary to induce the effective immune responses. Development of new safe carbohydrate based vaccines as well as adjuvants will be realized in the near future.

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