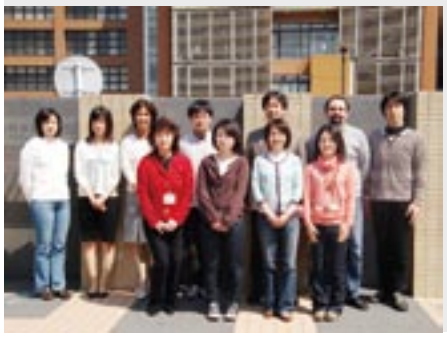
Elucidation of Dynamical Structures of Biomolecules toward Understanding the Mechanisms Underlying Their Functions

Department of Life and Coordination-Complex Molecular Science
Division of Biomolecular Functions



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Our biomolecular studies are based on detailed analyses of structures and dynamics of various biological macromolecules and their complexes at atomic level, primarily using nuclear magnetic resonance (NMR) spectroscopy. In particular, we conducted studies aimed at elucidating dynamic structures of glycoconjugates and proteins for integrative understanding of the mechanisms underlying their biological functions. For this purpose, we use multidisciplinary approaches integrating the methodologies of molecular and cellular biology and nanoscience along with molecular spectroscopy.

1. Molecular Basis for Glycoprotein-Fate Determination in Cells[1–3)]

Accumulating evidence indicates that a variety of lectins are involved in folding, transport and degradation of glycoproteins in cells. These intracellular lectins are supposed to recognize the *N*-linked oligosaccharides when they act as molecular chaperones, cargo receptors, or ER-associated degradation factors in the quality control system of glycoproteins. To understand the details of the structural and molecular basis of the mechanisms underlying quality control of glycoproteins, we characterize the sugar-binding specificities of the intracellular lectins with a series of intermediates of high-mannose-type oligosaccharides, which are generated through the actions of specific glycosidases in the endoplasmic reticulum. Our frontal affinity chromatographic analyses revealed that molecular chaperone CRT, cargo receptor VIPL, and ER-associated degradation factor OS-9 exhibit distinct sugar-binding specificities (Figure 1). On the basis of these data, we conclude that intracellular lectins recognize distinct 'glycotopes' located on the different site of the high-mannose-type oligosaccharides.

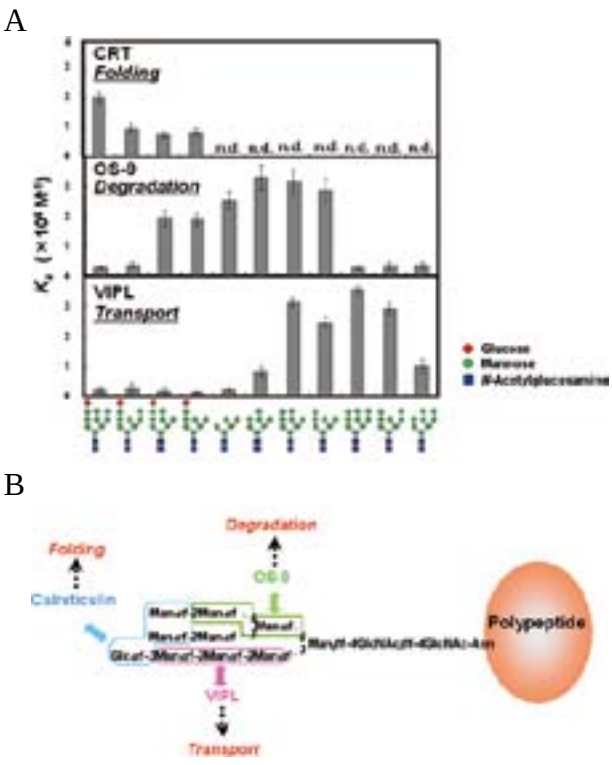

Figure 1. (A) K_a values for CRT, VIPL, and OS-9 determined by the frontal affinity chromatographic analyses. While CRT specifically interacts with monoglucosylated oligosaccharide, VIPL has higher affinity for deglucosylated oligosaccarides. On the other hand, Mannose-trimmed glycans are specifically recognized by OS-9. (B) Schematic representation of a glycoprotein indicating the glycotopes recognized by the intracellular lectins.

2. ^{13}C-Detection NMR Approach for Large Glycoproteins[4)]

NMR spectroscopy has great potential to provide us with information on structure and dynamics at atomic resolution of glycoproteins in solution. In larger glycoproteins, however, the detrimental fast ^{1}H transverse relaxation renders the conventional ^{1}H-detected NMR experiments difficult. ^{13}C direct detection potentially offers a valuable alternative to ^{1}H detection to overcome the fast T_2 relaxation. Furthermore, ^{13}C-detected experiments are expected to have several advantages for the NMR spectral assignments of the carbohydrate peaks of glycoproteins. In general, the ^{1}H signals of glycans except for those originating from the anomeric position are severely overlapped in a narrow spectral region (3–4 ppm) while most ^{13}C signals are dispersed in a wide range (50–110 ppm). Secondly, the information of ^{13}C–^{13}C shift correlation obtained from the ^{13}C-detected experiments can be helpful in classifying the carbohydrate signals simply by comparing with the reported ^{13}C chemical shift values (Figure 2A). This is due to the fact that ^{13}C chemical shift values of a sugar residue primarily depend on the covalent structures but are not largely affected by the glycosidic linkages conformations and any other environmental factors in contrast to the ^{1}H chemical shifts, which are influenced, for example, by the interactions with the polypeptide chains. We applied ^{13}C-detected NMR methods to observe the NMR signals of ^{13}C-labeled glycans attached to Fc fragment of immunoglobulin G with a molecular mass of 56 kDa. We successfully demonstrated that ^{13}C-detected ^{13}C–^{13}C NOESY experiment is highly useful for spectral assignments of the glycans of large glycoproteins because ^{13}C–^{13}C magnetization transfer is efficiently achieved through dipolar-dipolar interaction in a large glycoprotein due to slower molecular tumbling (Figure 2B). This approach would be in part complementary to ^{13}C–^{13}C TOCSY and ^{1}H-detection experiments.

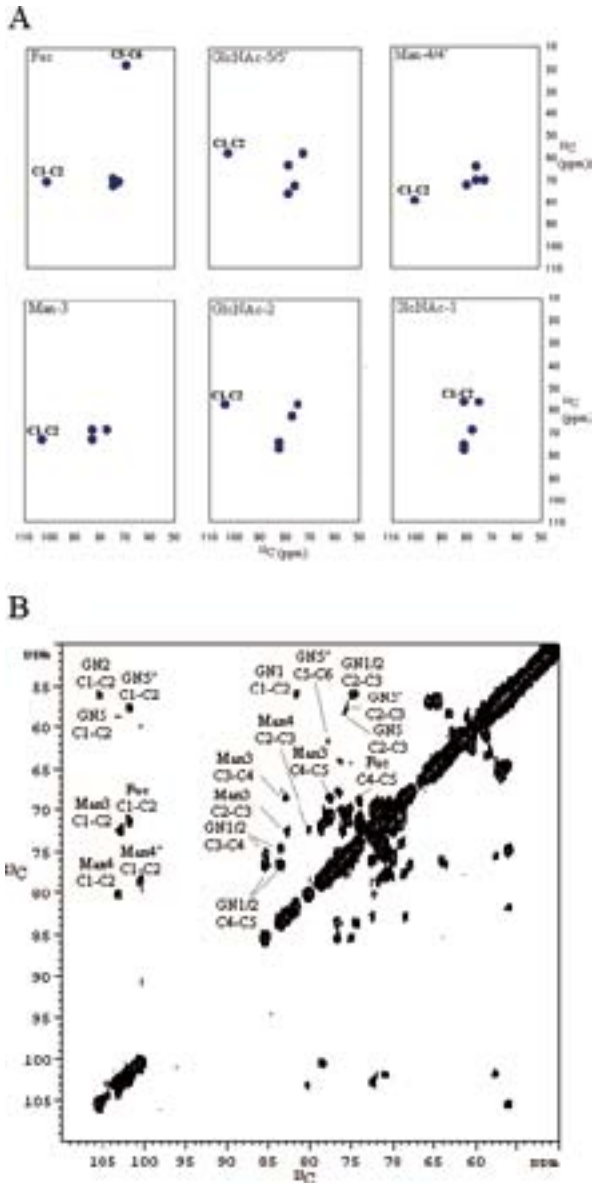

Figure 2. Use of two-dimensional ^{13}C–^{13}C NOESY spectrum for the assignment of IgG-Fc glycans. (A) Two-dimensional ^{13}C–^{13}C NOESY patterns expected for the sugar residues, Fuc, GlcNAc-5/5', Man-4/4', Man-3, GlcNAc-2 and GlcNAc-1, in a biannntenary complex type oligosaccharide based on the ^{13}C chemical shift values of the sugar residues in glycopeptides derived from ^{13}C-labeled IgG-Fc. (B) Two-dimensional ^{13}C–^{13}C NOESY spectrum of ^{13}C-labeled IgG-Fc.

References

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Award

KAMIYA, Yukiko; Poster Presentation Award, Annual meeting in Protein Community-organization and maintenance of protein functions (2008).

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