IX-Q Structural Analyses of Biological Macromolecules by Ultra-High Field NMR Spectroscopy

Our research seeks the underlying molecular basis for the function of biological macromolecules. In particular, we are interested in the function of molecular machines that work in the cellular processes involving protein folding, transport and degradation, and of glycoproteins playing important roles in the humoral and cellular immune systems. By use of ultra-high field NMR spectroscopy, we aim to elucidate the three-dimensional structure, dynamics, and interactions of proteins and glycoconjugates at the atomic level. A knowledge gained in this project will provide the structural basis for the rational design of drugs and biomolecular engineering that contribute towards a detailed understanding of biological systems.

IX-Q-1 Ultra-High Field NMR Study of Carbohydrate-Protein Interactions

KAMIYA, Yukiko[1]; YAMAGUCHI, Yoshiki[2]; SASAKAWA, Hiroaki[1]; KATO, Koichi[1]
([1]IMS and Nagoya City Univ.; [2]Nagoya City Univ.)

NMR analyses of glycans have been hampered by spectral overlap and low sensitivity. It is obviously advantageous to measure NMR spectra of these molecules at an ultra-high magnetic field because of high resolution and high sensitivity. We demonstrate that the 920 MHz NMR spectra provide us with atomic information on complicated glycoconjugates.

The vesicular integral protein of 36 kDa (VIP36) is an intracellular animal lectin, which act as a putative cargo receptor recycling between the Golgi and the endoplasmic reticulum. Although VIP36 has been reported to recognize glycoproteins carrying high mannose-type oligosaccharides, very little has been known about structural aspects of the sugar-binding modes of this cargo receptor. We have therefore analyzed the interactions between a recombinant carbohydrate recognition domain of VIP36 (VIP36-CRD) and chemically synthesized oligosaccharides by use of nuclear magnetic resonance spectroscopy.

Figure 1a shows the anomeric regions of the 500 MHz ^{1}H NMR spectra of $Man_9GlcNAc_2$. The signals derived from Man-D1, Man-D2 and Man-D3 are severely degenerated. In contrast, these peaks are perfectly separated in the 920 MHz NMR spectrum (Figure 1b).

The one-dimensional ^{1}H NMR spectra of $Man_9GlcNAc_2$ titrated with VIP36-CRD were observed for identification of sugar residues involved in binding to this lectin domain. Upon titration with VIP36-CRD, selective line broadening was observed for the signals originating from the mannose residues at positions 4, C, and D1, which correspond to the D1 arm of the $Man_9GlcNAc_2$ (Figure 1c,d). On the basis of these data, we conclude that VIP36-CRD interacts predominantly with the D1 arm of high-mannose oligosaccharides.

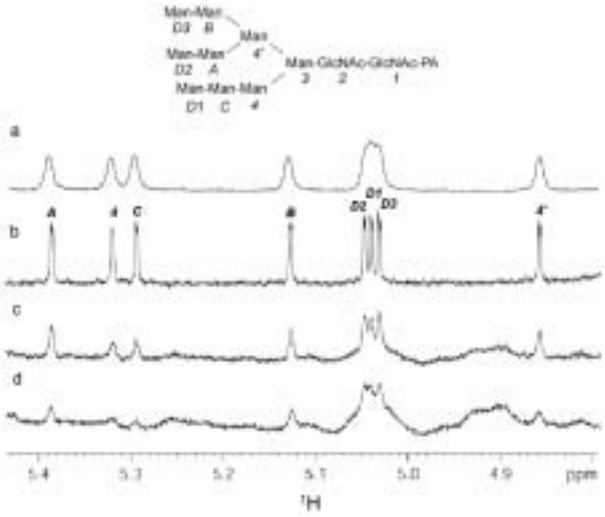

Figure 1. ^{1}H NMR spectra of oligosaccharide, $Man_9GlcNAc_2$ (anomeric region). These spectra were recorded at the ^{1}H frequency of 500 MHz(a) and 920 MHz(b–d) in the absence (a, b) and presence of 0.2 equiv.(c) and 0.5 equiv.(d) of VIP36-CRD.

IX-Q-2 Ultra-High Field NMR Study of Glycoproteins

NAGANO, Mayumi[1]; YAMAGUCHI, Yoshiki[2]; SASAKAWA, Hiroaki[1]; KATO, Koichi[1]
([1]IMS and Nagoya City Univ.; [2]Nagoya City Univ.)

Many of the proteins in the living systems express carbohydrate moieties. Although the biological importance of oligosaccharides covalently linked to proteins has been widely recognized, little is known about their specific roles from the structural aspect. This deficiency in our knowledge is largely due to the lack of an appropriate methodology to deal with glycoproteins as targets of structural biology. The carbohydrate moieties of glycoproteins generally exhibit microheterogeneities and possess a significant degree of freedom in internal motion, which hampers crystallization or interpretation of electron density. NMR spectroscopy can potentially provide us with information on structure and dynamics of glycoproteins in solution. However, there are few reports of structural determination of glycoproteins by NMR spectroscopy. In this situation, we have been developing NMR techniques for structural analyses at atomic resolution of glycoproteins in solution. In this methodology, the glycans and/or polypeptides of glyco-

proteins are uniformly or selectively labeled with stable isotopes (^{13}C, ^{15}N, and ^{2}H) by metabolic or enzymatic manners. We demonstrate our strategy using the Fc portion of immunoglobulin G (IgG) as a model system. A series of double and triple resonance experiments were performed for the uniformly $^{13}C/^{15}N$-labeled IgG-Fc. Figure 1 shows the 2D HSQC spectrum of IgG-Fc recorded on a 920 MHz NMR spectrometer. These spectra enabled us to assign the signals originating from the polypeptide backbone and the carbohydrate moieties. The NMR spectral data provide us with the basis for elucidation of structure and dynamics of the carbohydrate moieties as well as the polypeptide chains of the Fc glycoprotein in solution.

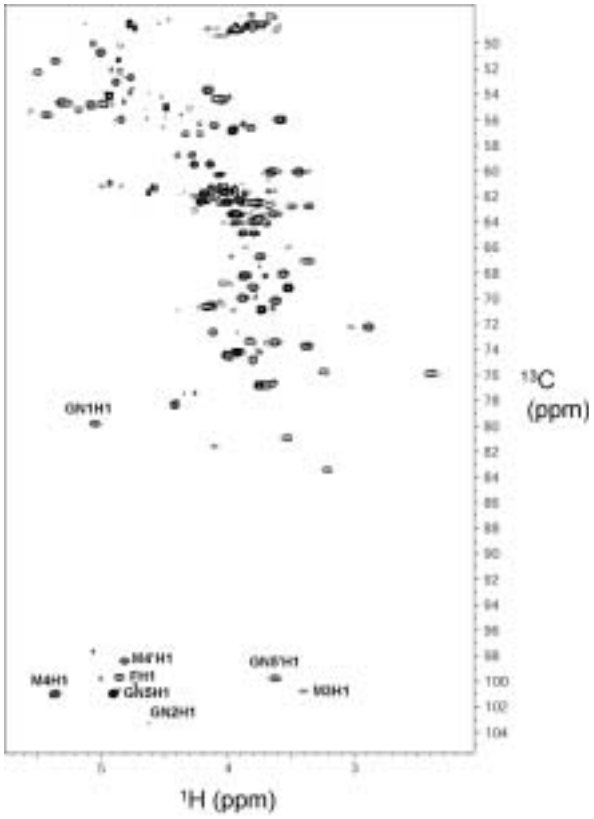

Figure 1. ^{1}H-^{13}C HSQC spectrum of the carbohydrate moieties of isotopically labeled IgG-Fc. The assignments of anomeric peaks are indicated in the spectrum.