IX-O 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. Here we report stable-isotope-assisted NMR studies of Ufm1, a ubiquitin-like modifier, and IgG-Fc glycoprotein.

IX-O-1 Solution Structure and Dynamics of Ufm1, a Ubiquitin-Fold Modifier 1

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The ubiquitin-fold modifier 1 (Ufm1) is one of various ubiquitin-like modifiers and conjugates to target proteins in cells through Uba5 (E1) and Ufc1 (E2). The Ufm1-system is conserved in metazoa and plants, suggesting its potential roles in various multicellular organisms. Herein, we analyzed the solution structure and dynamics of human Ufm1 (hsUfm1) by nuclear magnetic resonance spectroscopy. Although the global fold of hsUfm1 is similar to those of ubiquitin (Ub) and NEDD8, the cluster of acidic residues conserved in Ub and NEDD8 does not exist on the Ufm1 surface. ^{15}N spin relaxation data revealed that the amino acid residues of hsUfm1 exhibiting conformational fluctuations form a cluster at the C-terminal segment and its spatial proximity, which correspond to the versatile ligand-binding sites of Ub and other ubiquitin-like proteins (Ubls). We suggest that Ub and other Ubl-modifiers share a common feature of potential conformational multiplicity, which might be associated with the broad ligand specificities of these proteins.

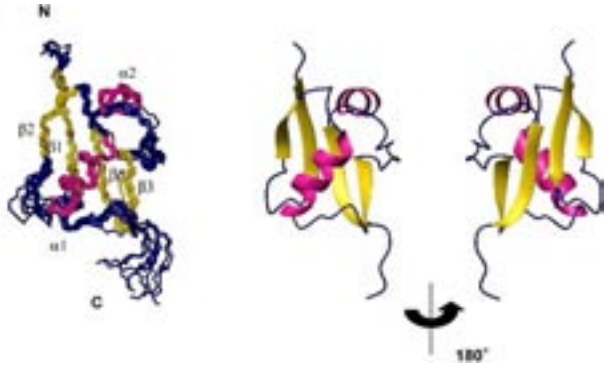

Figure 1. Backbone atom superposition of the final 10 structures (left) and ribbon representation of the lowest energy structure (right) of human Ufm1.

IX-O-2 Glycoform-Dependent Conformational Alteration of the Fc Region of Human Immunoglobulin G1 as Revealed by NMR Spectroscopy

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The Fc portion of immunoglobulin G (IgG) expresses the biantennary complex type oligosaccharides at Asn297 of the C_H2 domain of each heavy chain with microheterogeneities depending on physiological and pathological states. These N-glycans are known to be essential for promotion of proper effector functions of IgG such as complement activation and Fcγ receptor (FcγR)-mediated activities. To gain a better understanding of the role of Fc glycosylation, we prepared a series of truncated glycoforms of human IgG1-Fc and analyzed their interactions with human soluble FcγRIIIa (sFcγRIIIa) and with staphylococcal protein A by surface plasmon resonance and nuclear magnetic resonance (NMR) methods. Progressive but less pronounced reductions in the affinity for sFcγRIIIa were observed as a result of the galactosidase and subsequent N-acetylhexosaminidase treatments of IgG1-Fc. The following endoglycosidase D treatment, giving rise to a disaccharide structure composed of a fucosylated GlcNAc, abrogated the affinity of IgG1-Fc for sFcγRIIIa. On the other hand, those glycosidase treatments did not significantly affect the affinity of IgG1-Fc for protein A. Inspection of stable-isotope-assisted NMR data of a series of Fc glycoforms indicates that the stepwise trimming out of the carbohydrate residues results in concomitant increase in the number of amino acid residues perturbed thereby in the C_H2 domains. Furthermore, the cleavage at the GlcNAcβ1-4GlcNAc glycosidic linkage induced the conformational alterations of part of the lower hinge region, which makes no direct contact with the carbohydrate moieties and forms the major FcγR-binding site, while the conformation of the C_H2/C_H3 interface was barely perturbed that is the protein A-binding site. These results indicate that the carbohydrate moieties are required for maintaining the structural integrity of the FcγR-binding site.

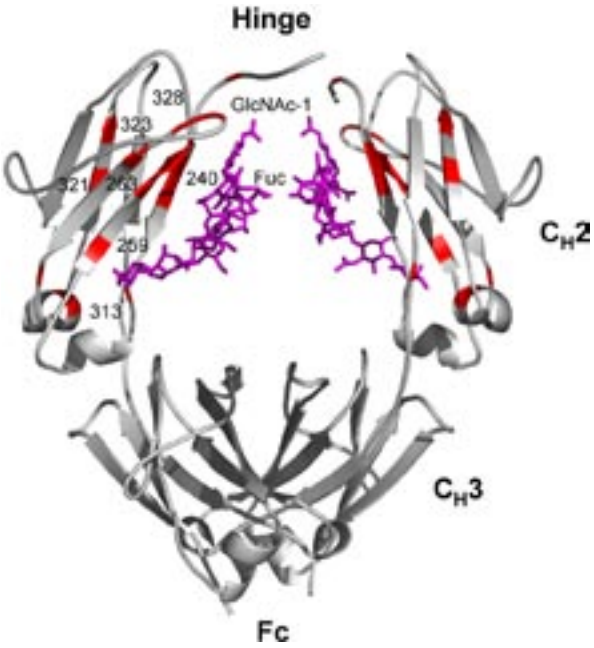

Figure 1. Mapping on the crystal structure of IgG1-Fc of the amino acid residues perturbed upon deglycosylation of carbohydrate chains attached onto Fc.