7. NMR Structural Glycomics

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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 glycoproteins 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 as a model system. On the basis of NMR data, structure and dynamics of the carbohydrate moieties as well as the polypeptide chains of Fc in solution will be discussed. Accumulating evidence shows that carbohydrate moieties contribute to folding, transport, and degradation of glycoproteins in cells *via* interactions with a variety of intracellular lectins. To gain insights into the mechanisms of the recognition of glycoproteins in those systems, we applied the stable-isotope-assisted NMR techniques to analyses of sugar binding of the intracellular lectins, which include the cargo receptor VIP36, and the ubiquitin ligase SCF^{Fbs1}. On inspection of NMR spectral data, the amino acid and the sugar residues involved in the carbohydrate-protein interactions were identified. To perform NMR analyses, glycoforms of target proteins have to be described in advance. The multi-dimensional HPLC mapping technique combined with mass spectrometric methods is powerful for determination of covalent structures of target glycoproteins. We have made a web application “GALAXY” (http://www.glycoanalysis.info/) based on the HPLC map to facilitate identification *N*-linked oligosaccharides expressed on proteins.