Elucidation of Dynamical Structures of Biomolecules toward Understanding the Mechanisms Underlying Their 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, using NMR spectroscopy and X-ray crystallography in conjunction with other biophysical, biochemical and molecular biology techniques. Here we report our recent studies of conformations, dynamics, and interactions of oligosaccharides and glycoconjugates along with proteins involved in the ubiquitin (Ub)-proteasome system.

1. Lanthanide-Assisted NMR Analyses of the Conformational Ensemble of Oligosaccharides in Conjunction with Molecular Dynamics Simulations

Conformational flexibility is an important property of biological molecules functioning in living systems, as best exemplified by oligosaccharides. We attempted to combine the lanthanide-assisted NMR method with molecular dynamics (MD) simulations for the evaluation of dynamic conformational ensembles of highly flexible oligosaccharides. In this approach, a metal-chelating tag was covalently attached to the reducing end of the oligosaccharide moieties of gangliosides, which form integral parts of cellular membranes, for observing pseudocontact shifts (PCSs). Upon complexation with paramagnetic lanthanide ions, the tagged GM3 trisaccharide, which is the common core structure shared among the gangliosides, exhibited NMR spectral changes due to PCSs according to the relative positions of the individual atoms with respect to the lanthanide ion coordinated at the tag. The observed PCS values were in excellent agreement with those back-calculated from the vast conformational ensemble of the trisaccharide derived from MD simulations (Figure 1). Thus, the PCS measurements offer a valuable experimental tool for the validation of MD simulation of highly flexible biomolecules.[1)] Furthermore, this approach was successfully applied to the characterization of the conformational dynamics of the branched tetrasaccharide of ganglioside GM2.[2)] The interbranch interactions responsible for the conformational differences between the GM2 tetrasaccahride and the GM3 trisaccharide were identified by the paramagnetic NMR method in conjunction with MD simulations. These results demonstrated the utility of our approach in the evaluation of dynamic conformational ensembles of oligosaccharides, considering their minor conformers in a systematic manner.

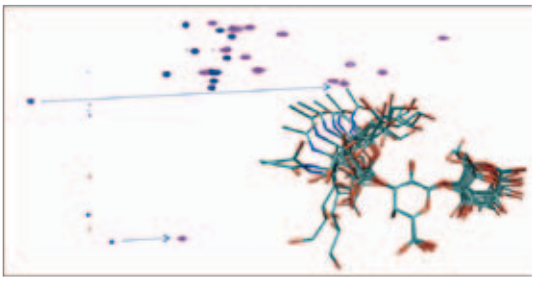

Figure 1. ^{1}H–^{13}C HSQC spectral change of the GM3 trisaccharide tagged with a paramagnetic ion and snapshots of the sugar from an MD-simulated trajectory.

2. Structural Basis for Improved Effector Functions of Antibodies by Engineering of Their Glycans

More than half of proteins in nature are estimated to be modified by sugar chains, which affect the physical and biological properties of proteins. The effector functions of immunoglobulin G (IgG) critically depend on *N*-glycosylation of its Fc region. Removal of the fucose residue from the *N*-glycans of IgG-Fc results in a dramatic enhancement of antibody-dependent cellular cytotoxicity (ADCC) through improved affinity for Fcγ receptor IIIa (FcγRIIIa). We successfully determined the crystal structure of the complex formed between non-fucosylated IgG1-Fc and a soluble form of FcγRIIIa (sFcγRIIIa) with two *N*-glycosylation sites (Figure 2a).[3] The crystal structure demonstrates that one of the two *N*-glycans of sFcγRIIIa mediates the interaction with the *N*-glycan of non-fucosylated Fc, thereby stabilizing the complex. However, fucosylation of the Fc *N*-glycans impairs this interaction because of steric hindrance. On the other hand, our NMR data demonstrated that Tyr296 of the non-fucosylated Fc glycoform exhibits conformational multiplicity in its uncomplexed state, suggesting that conformational selection is governed by the presence or absence of the fucose residue of the Fc *N*-glycan. These findings offer a structural basis for improvement in ADCC of therapeutic antibodies by defucosylation.

3. Conformational Dynamics of Proteins Involved in the Ubiquitin-Proteasome System

While recent progresses have been made in understanding intra-domain conformational fluctuations of proteins, the evaluation of the relative motions of individual domains of multi-domain proteins is still a challenge. We successfully characterized conformational dynamics of Lys-48-linked Ub dimer (diUb) in solution using NMR spectroscopy.[4] Comparison of a chemical shift of wild-type diUb with that of monomeric Ub and cyclic diUb, which mimic the open and closed states (Figure 2b), respectively, with regard to the exposure of hydrophobic surfaces to the solvent indicates that wild-type Lys-48-linked diUb in solution predominantly exhibits the open conformation (75% at pH 7.0), which becomes more populated upon lowering pH. The intrinsic properties of Lys-48-linked Ub chains to adopt the open conformation may be advantageous for interacting with Ub-binding proteins. We also characterized interaction modes of the Ub-like domains of HOIL-1L and HR23 with their specific binding-partners by NMR spectroscopy.[5,6]

Furthermore, we developed a novel technique for real-time monitoring of subunit exchange in homooligomeric proteins, using deuteration-assisted small-angle neutron scattering, and applied it to the tetradecamer of the proteasome α7 subunit.[7]

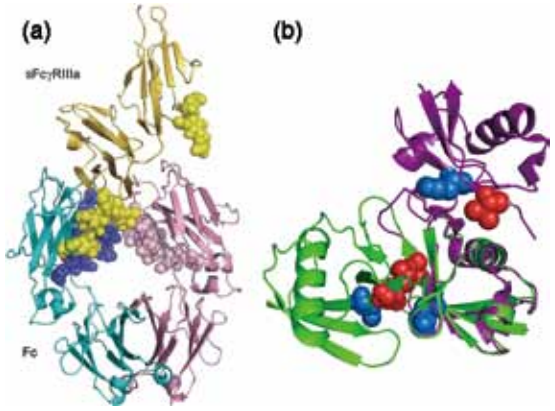

Figure 2. 3D structures of (a) sFcγRIIIa bound to non-fucosylated Fc and (b) wild-type Lys-48-linked diUb. The open form of diUb (purple) was superposed on the closed state (green).

References

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Awards

YAMAMOTO, Sayoko; Young Scientists Poster Awards, The International Symposium on Nuclear Magnetic Resonance 2011 (2011).
KATO, Koichi; The Erwin von Bälz Prize 2011 (First Prize) (2011).
YAMAGUCHI, Takumi; CSJ Presentation Award 2012, The 92nd Annual Meeting of The Chemical Society of Japan (2012).
ZHANG, Ying; FY2012 Sokendai President's Award (2012).
KUMOI, Kentaro; Young Poster Award, The 12th Annual Meeting of The Protein Science Society of Japan (2012).

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