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, primarily using NMR spectroscopy. Here we report NMR methods we recently developed for detailed conformational characterization of oligosaccharides and an NMR study of intermolecular interaction of amyloid β (Aβ) promoted on GM1 micelles.

1. Paramagnetic Lanthanide Tagging for NMR Conformational Analyses of *N*-Linked Oligosaccharides[1)]

Although NMR spectroscopy has great potential to provide information on structure and dynamics of oligosaccharides, the applicability of the NOE-based approach, widely used for protein-structure determination, is limited by the insufficiency of distance-restraint information as a consequence of the low proton density in oligosaccharides and the exceedingly low number of proton-proton NOEs. Hence, to develop NOE-independent approaches for determining the oligosaccharide conformations and dynamics, we employed paramagnetic effects using novel lanthanide tags attached to the reducing end of an *N*-linked oligosaccharide.

Paramagnetic effects, such as pseudocontact shifts (PCSs) induced by lanthanide ions with an anisotropic magnetic susceptibility tensor, offer long-distance information on conformations and dynamics of biological macromolecules. For the development of a general method, we focused on the common core structure shared among all *N*-linked oligosaccharides, *i.e. N,N'*-diacetylchitobiose. An EDTA derivative designed to serve as the paramagnetic tag by chelating a lanthanide ion was attached to *N,N'*-diacetylchitobiose through amide linkage mimicking the '*N*-linked' oligosaccharides.

By ^{1}H-^{13}C HSQC experiments using a series of paramagnetic lanthanide ions, the PCS values were measured as the differences of ^{1}H and ^{13}C chemical shifts from those of the complex with the diamagnetic La^{3+} ion as a reference. For quantitative validation of our approach, the experimentally obtained PCS values were compared with those calculated from the 3D model of the complex based on a reported conformation of *N,N'*-diacetylchitobiose. The back-calculated PCS values are in excellent agreement with the experimental data demonstrating the utility of our approach. These results indicate that the common innermost part of the *N*-linked oligosaccharides exhibits a rigid conformation, which is little affected by the attachment of the tag (Figure 1). The conformational rigidity of the glycosidic linkage of this disaccharide agrees with results from molecular dynamics simulation. This success opens the door to conformational studies of a variety of sugar chains of biological interest.

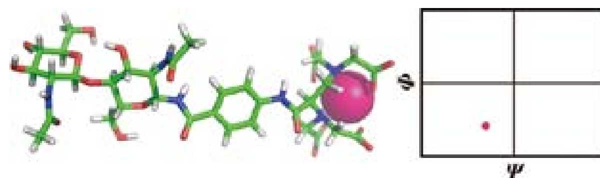

Figure 1. 3D structural model of the lanthanide-tagged *N,N'*-diacetylchitobiose and its glycosidic torsional angles.

2. Development of Metabolic ^{13}C-Labeling Techniques for Carbohydrate NMR Analyses Using Genetically Engineered Yeast Strain[2)]

Applicability of NMR approach to carbohydrate conformational analyses will be strengthened by combining it with stable isotope labeling of the oligosaccharides. However, methodology of stable isotope labeling of sugar chains has been largely unexplored, especially for larger, branched oligosaccharides. In view of the situation, we developed a novel method for overexpression of homogeneous oligosaccharides with ^{13}C labeling using genetically engineered yeast strain.

We employed engineered *Saccharomyces cerevisiae* cells, in which a homogeneous high-mannose-type oligosaccharide accumulates because of deletions of genes encoding three enzymes involved in the processing pathway of *N*-linked oligosaccharides in the Golgi complex. Using uniformly ^{13}C-labeled glucose as the sole carbon source in the culture medium of these engineered yeast cells, high yields of the isotopically labeled $Man_8GlcNAc_2$ (M8B) oligosaccharide could be successfully harvested from glycoprotein extracts of the cells. This enabled to observe ^{1}H-^{13}C HSQC spectrum at a proton observation frequency of 920.7 MHz. Furthermore, ^{13}C labeling at selected positions of the sugar residues in the oligosaccharide could be achieved using a site-specific ^{13}C- enriched glucose as the metabolic precursor, facilitating NMR spectral assignments (Figure 2). The ^{13}C-labeling method thus established provides the technical basis for NMR analyses of structures, dynamics, and interactions of larger, branched oligosaccharides.

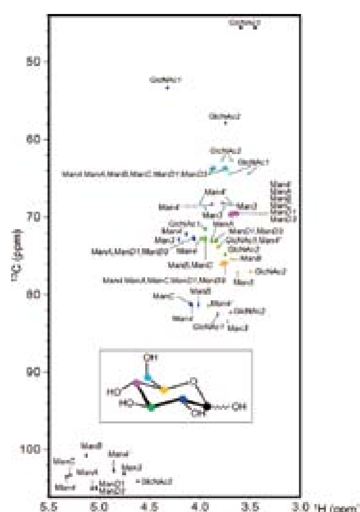

Figure 2. ^{1}H-^{13}C HSQC spectra of the pyridylamino derivative of M8B, metabolically ^{13}C-labeled using site-specific ^{13}C-enriched glucose isotopomers.

3. Spectroscopic Characterization of Intermolecular Interaction of Amyloid β Promoted on GM1 Micelles[3)]

Clusters of GM1 gangliosides act as platforms for conformational transition of monomeric, unstructured Aβ to its toxic β-structured aggregates. We previously reported that Aβ(1-40) is accommodated on the hydrophobic/hydrophilic interface of the ganglioside cluster exhibiting an α-helical conformation under ganglioside-excess conditions. To gain further insights into the underlying mechanisms of the amyloid formation of Aβ, it is necessary to characterize the conformational transition from α-helices to β-structures of Aβ on the ganglioside clusters.

We characterized conformational states of Aβ(1-40) in the presence of varying amounts of GM1 aqueous micelles using stable-isotope-assisted NMR spectroscopy in conjunction with synchrotron-radiation vacuum-ultraviolet CD spectroscopy. We found that GM1 micelles induce distinct secondary structures of Aβ(1-40) depending on the Aβ/GM1 ratios. Furthermore, it was revealed that the thioflavin T (ThT)-reactive β-structure is more populated in Aβ(1-40) under conditions where the Aβ(1-40) density on GM1 micelles is high. Under this circumstance, the C-terminal hydrophobic anchor Val39-Val40 shows two distinct conformational states that are reactive with ThT, while such Aβ species were not generated by smaller lyso-GM1 micelles (Figure 3). These findings suggest that GM1 clusters promote specific Aβ–Aβinteractions through their C-termini coupled with formation of the ThT-reactive β-structure depending on sizes and curvatures of the clusters.

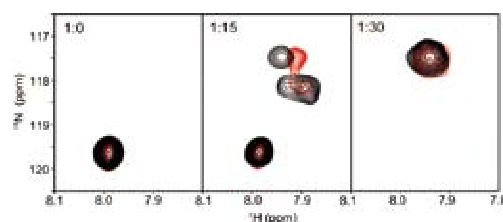

Figure 3. ^{1}H-^{15}N HSQC peak originating from Val39 of Aβ(1-40) titrated with GM1 micelles in the presence or absence of ThT. The spectra measured in the absence (black) and presence (red) of ThT are overlaid.

References

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Award

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