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Lanthanide-assisted NMR evaluation of a dynamic ensemble of oligosaccharide conformations†

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A novel methodology is presented for evaluating a dynamic ensemble of oligosaccharide conformations by lanthanide-assisted NMR spectroscopy combined with molecular dynamics (MD) simulations. The results obtained using the GM3 trisaccharide demonstrated that pseudocontact shift measurements offer a valuable experimental tool for the validation of MD simulations of highly flexible biomolecules.

Conformational flexibility is an important property of biological molecules functioning in living systems, as best exemplified by oligosaccharides, which possess significant degrees of motional freedom, thereby exhibiting conformational adaptability upon interacting with various target molecules in molecular recognition events.[1,2] Therefore, atomic descriptions of dynamic oligosaccharide conformations are important not only for understanding the quantitative energetics of carbohydrate–protein and carbohydrate–carbohydrate interactions but also for designing drugs targeting these interacting systems. However, although recent advancements in computational approaches have enabled large-scale molecular dynamics (MD) simulations of oligosaccharides in solution with improved force fields,[3–6] the experimental methodology to evaluate their conformational dynamics has not yet been fully developed.

NMR spectroscopy has immense potential to deal with such dynamics issues in various ranges of spatial and time scales.[7–10] In conformational analyses of flexible biomolecules, NMR data should be interpreted as a weighted average of two or more conformational states. Such analyses have been attempted in interpretations of NOE and residual dipolar coupling (RDC) data of oligosaccharides,[11–14] although low-proton density, a low number of independent bond vectors, and smaller magnitudes of these parameter values in oligosaccharides often preclude the achievement of precision and accuracy in measuring these NMR parameters.

Another potentially useful tool involves the introduction of paramagnetic lanthanide ions with an anisotropic magnetic susceptibility tensor ($\Delta\chi$ tensor), which induces pseudocontact shifts (PCSs) as chemical shift changes related to the spatial position of the observed nuclei with respect to the paramagnetic center.[15–17] Despite the relative ease of their quantitative observation, the application of PCSs to carbohydrate conformational analyses has been quite limited probably because of the lack of a conventional method to attach lanthanide ions to oligosaccharides. Recently, we and other groups have reported protocols for the lanthanide tagging of disaccharides to enable their NMR conformational analyses.[18–20] In particular, Erdélyi *et al.* demonstrated that the PCS data for lactose could be effectively reproduced by a population-optimized combination of selected low-energy conformers.[19] In view of this situation, we herein attempt to combine the lanthanide-assisted NMR method with MD simulations for the evaluation of dynamic conformational ensembles of highly flexible oligosaccharides that exhibit shallow and broad energy minima in their conformational space.

We used the trisaccharide of the GM3 ganglioside αNeu5Ac-(2–3)-βGal-(1–4)-βGlc as a model system because this trisaccharide is the common core structure shared among gangliosides. They are glycosphingolipids having a sialyl oligosaccharide, which act as targets for various pathologically relevant proteins.[21–23] There have been several reports describing NMR and computational analyses of this trisaccharide conformation.[24–28] The key to lanthanide tagging is to introduce a metal-chelating unit into the reducing end of the oligosaccharide, which can form a stable complex with a paramagnetic lanthanide ion. A phenylenediamine-based lanthanide-chelating tag was newly designed to improve the rigidity of the tag, which is crucially important for the accurate interpretation of PCS data in terms of carbohydrate dynamics.

This novel tag was successfully attached to the trisaccharide, as shown in Scheme 1. The reducing terminus of the sugar moiety was selectively aminated in good yield; subsequently, it was connected to the tag through an amide linkage. By ^{1}H NMR titration experiments, it was confirmed that the

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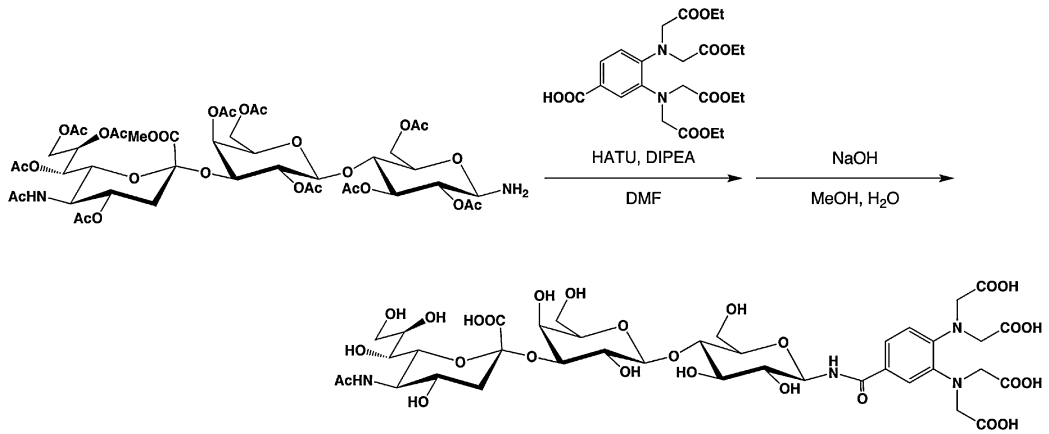

Scheme 1 Introduction of the lanthanide-chelating tag into the GM3 trisaccharide.

lanthanide ions were selectively bound to the tag moiety of the trisaccharide, giving rise to a stable 1 : 1 complex.

The PCSs were measured as the differences between the ^{1}H and ^{13}C chemical shifts of the compound chelated to the paramagnetic ion and those observed with the diamagnetic ion in their ^{1}H–^{13}C HSQC spectra (Fig. 1 and Table S1, ESI†). In this comparison, most of the CH groups exhibited PCS values sufficiently large for a quantitative conformational analysis, as summarized in Table 1. Significantly larger PCS values were observed for the H8 of Neu5Ac and its proximal atoms, suggesting a contribution of some bent conformations of this trisaccharide, which is consistent with a previously reported NMR study.[26] These experimentally determined PCS values were compared with those calculated from a conformational ensemble of the GM3 trisaccharide.

To generate atomic coordinates of the conformationally fluctuating trisaccharide, 10 MD simulations were performed in explicit water for 12 ns with the GLYCAM_06 force field.[4] Consistent with the previously reported calculation, torsion angle pairs of this trisaccharide show high flexibility especially around the glycosidic linkage between the Neu5Ac and Gal residues (Fig. 2).

All MD runs were combined after excluding the first 2 ns of trajectories. Subsequently, 2000 trisaccharide conformers were extracted at equal intervals to create an ensemble model, which involved harmonic motions in a local minimum as well as transitions from one low-energy region to another in the energy landscape (Fig. 2). The coordinate of the average position of the paramagnetic center with respect to the innermost Glc residue was defined from additional MD calculations of the tag moiety (see ESI†).

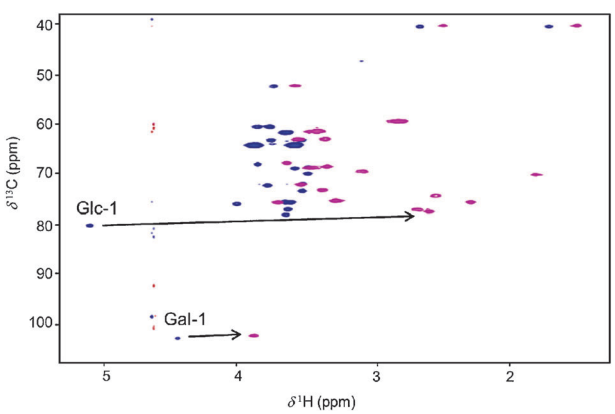

Fig. 1 ^{1}H–^{13}C HSQC spectra of the sugar tagged with Tm^{3+} (magenta) and La^{3+} (blue). Chemical shift differences of the anomeric CH groups are indicated by arrows.

Table 1 Values of PCSs (ppm) derived from the Tm^{3+} ion

	Neu5Ac		Gal		Glc	
	$\Delta\delta_{^{13}C}$	$\Delta\delta_{^{1}H}$	$\Delta\delta_{^{13}C}$	$\Delta\delta_{^{1}H}$	$\Delta\delta_{^{13}C}$	$\Delta\delta_{^{1}H}$
1	—	—	−0.62	−0.56	−2.83	−2.51
2	—	—	−0.45	−0.42	−1.87	−1.73
3	−0.16	−0.17/−0.20	−0.34	−0.30	−1.22	−1.12
4	−0.19	−0.14	−0.31	−0.23	−1.12	−0.98
5	−0.14	−0.17	−0.36	−0.34	−1.45	−1.35
6	−0.19	−0.15	−0.27	−0.23/−0.23	−1.12	−1.04/−0.98
7	−0.20	−0.15	—	—	—	—
8	−0.23	−0.25	—	—	—	—
9	−0.20	−0.21/−0.17	—	—	—	—

A single $\Delta\chi$ tensor was determined for the conformational ensemble by inspection of the experimentally obtained PCSs with the assumption that every conformer contributes equally to the PCSs. The anisotropy values of the $\Delta\chi$ tensors $\Delta\chi_{ax}$ and $\Delta\chi_{rh}$, derived from the Tm^{3+} ion for the ensemble, were estimated to be 8.1×10^{-23} m^3 and 3.5×10^{-23} m^3, respectively. The results for the back-calculated PCSs were in excellent agreement with the experimental data: the Q value§ was 0.05 (Fig. 3(a)). Similarly, a low Q (0.06) was obtained using Tb^{3+} as a lanthanide probe (Fig. 3(b)). Such low Q values could be obtained neither by employing most single conformers nor by using a combination of selected low-energy conformers (Table S2, ESI†). Furthermore, a conformational ensemble derived from only one trajectory (12 ns), which does not include a low-populated conformational cluster in Gal–Glc linkage ($\Psi \approx 180^{\circ}$), gave a compromised Q value (Fig. S1, ESI†).

These results indicate that minor conformers significantly contribute to the observed PCS values. The minor "anti" conformations about the Gal–Glc linkage were barely detected in the MD simulation restrained by rotating frame Overhauser effect data that were obtained using ^{13}C-enriched GM3 trisaccharide.[27] These results demonstrated the utility of our

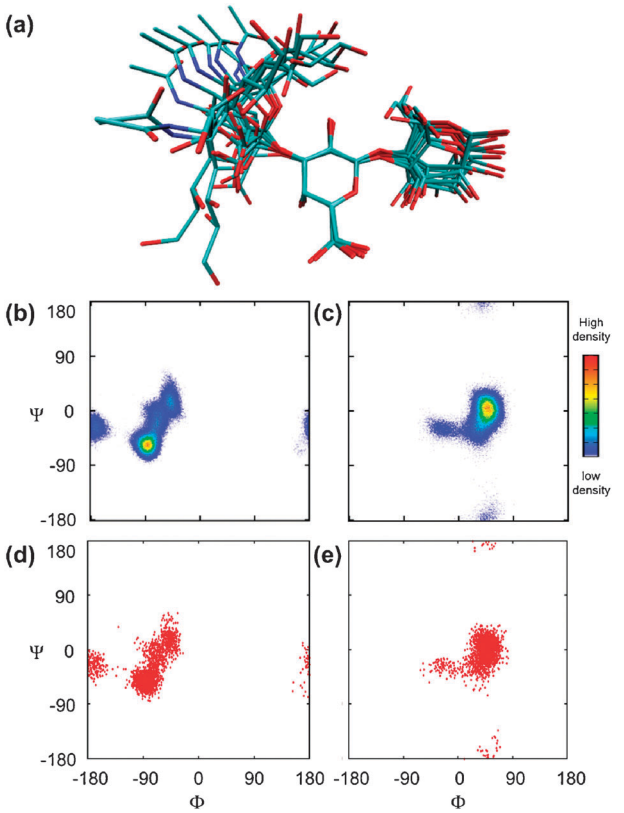

Fig. 2 Conformational ensemble of the GM3 trisaccharide. (a) Snapshots of the GM3 trisaccharide from a simulated trajectory superimposed on the ring atoms of the Gal residue. All hydrogen atoms are omitted. Torsion angle density maps of MD trajectories of (b) the Neu5Ac–Gal linkage and (c) the Gal–Glc linkage. Scattered plots of torsion angles of (d) the Neu5Ac–Gal linkage and (e) the Gal–Glc linkage of the ensemble for PCS analysis. The NMR definitions of Φ and Ψ were used, namely, for the Neu5Ac–Gal linkage, Φ = C1–C2–O′3–C′3 and Ψ = C2–O′3–C′3–H′3 and for the Gal–Glc linkage, Φ = H1–C1–O′4–C′4 and Ψ = C1–O′4–C′4–H′4.

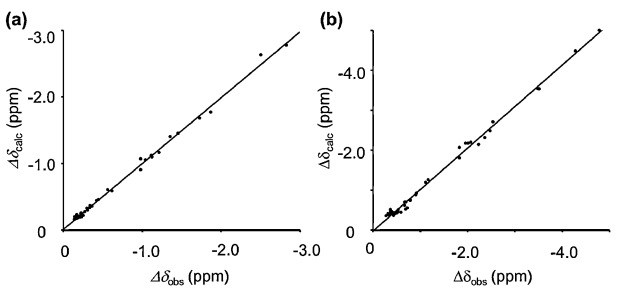

Fig. 3 Correlations between the experimentally observed PCS values with (a) Tm^{3+} and (b) Tb^{3+} and back-calculated PCS values.

lanthanide-assisted NMR method in conjunction with MD simulations in the evaluation of dynamic conformational ensembles of highly flexible oligosaccharides, considering their minor conformers in a systematic manner.

In summary, we successfully acquired PCSs derived from the GM3 trisaccharide by the lanthanide-tagging method and interpreted the PCS data by inspecting a vast conformational ensemble of this flexible trisaccharide generated from MD simulations. The lanthanide-assisted NMR approach serves as an experimental tool for validating MD simulations of not only oligosaccharides but also other flexible biomacromolecules such as intrinsically disordered proteins.

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Notes and references

§ $Q = \mathrm{rms}(\Delta\delta_{calc} - \Delta\delta_{obs})/\mathrm{rms}(\Delta\delta_{obs})$. $\Delta\delta_{calc}$ is given by following equation; $\Delta\delta_{calc} = \sum_{i=1}^{N} (p_i/12\pi r_i^3 [\Delta\chi_{ax}(3\cos^2\vartheta_i - 1) + 3/2\Delta\chi_{rh}(\sin^2\vartheta_i \cos 2\varphi_i)])$, where p_i is the population of each structure (set to 0.0005), N is the number of each conformers, and $(r_i, \theta_i, \varphi_i)$ defines the position vector for conformer i of the nucleus in polar coordinates with respect to the metal center and principal axis of a $\Delta\chi$ tensor.

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