Paramagnetism-Assisted Nuclear Magnetic Resonance Analysis of Dynamic Conformations and Interactions of Oligosaccharides

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Abstract

Nuclear magnetic resonance (NMR) spectroscopy has considerable potential for describing dynamic oligosaccharides at the atomic level in solution and in complexes with other biomolecules. However, conformational restraints primarily provided through measurements of nuclear Overhauser effect and spin–spin coupling, which act as information sources about local conformation, are insufficient, limiting the adequate carbohydrate characterization by conventional NMR approaches. Recently developed paramagnetism-assisted NMR

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techniques have addressed this issue by exploiting the effects of magnetic dipole–dipole interactions between nuclei and an unpaired electron. This method is utilized to describe the overall conformation of oligosaccharides. Specifically, paramagnetic probes, such as a lanthanide-chelating tag and a nitroxide radical, are employed to determine the geometrical arrangements of individual nuclei in the target oligosaccharides relative to the paramagnetic center position. Inspection of paramagnetic effects such as pseudocontact shift and relaxation enhancement, in conjunction with molecular dynamics simulations, gives atomic long-distance information – extremely valuable data for characterization of conformational dynamics and interactions of oligosaccharides.

Keywords
Conformational dynamics • Ganglioside • High-mannose-type oligosaccharide • Lanthanide ion • Molecular dynamics simulation • NMR • Paramagnetic effect • Paramagnetic relaxation enhancement • Pseudocontact shift • Spin label

Introduction

Nuclear magnetic resonance (NMR) spectroscopy is a powerful tool for structural analyses of oligosaccharides in solution. Conventional NMR approaches based on the observation of nuclear Overhauser effects (NOE) and spin–spin coupling (*J* coupling) have been widely used to characterize oligosaccharide conformations and dynamics. NOE and *J* coupling measurements offer information on inter-proton distances and dihedral angles, respectively, to determine glycosidic linkage conformations. However, oligosaccharides display low-proton density and small *J* values, often hampering the accurate and precise description of their overall conformation. Recently developed paramagnetism-assisted NMR approaches complement the NOE- and *J* coupling-based methods by providing longer-distance information, thus facilitating the elucidation of specific nuclear arrangements in oligosaccharides.

Principle of Paramagnetic Effects

The introduction of a paramagnetic center into a target biomolecule can dramatically affect the NMR spectra because it generates magnetic dipole–dipole interactions between nuclei and an unpaired electron. These paramagnetic effects can modulate the chemical shift and relaxation of each signal depending on the geometrical relationship between individual nuclei and the unpaired electron (Luchinat and Parigi 2010; Fig. 1a). The pseudocontact shift (PCS) is a well-known paramagnetic effect observed in the presence of ions exhibiting anisotropic magnetic susceptibility. Interactions between a paramagnetic center and nuclei also increase NMR relaxation rates in an effect known as paramagnetic relaxation enhancement (PRE).

a

$$\Delta\delta^{PCS} = \frac{1}{12\pi r^3}\left[\Delta\chi_{ax}(3\cos^2\theta - 1) + \frac{2}{3}\Delta\chi_{rh}\sin^2\theta\cos 2\phi\right] \quad (1)$$

$$R_2^{PRE} = \frac{1}{15}\left(\frac{\mu_0}{4\pi}\right)\frac{\gamma_I^2 g^2 \mu_B^2 S(S+1)}{r^6}\left\{4\tau_c + \frac{3\tau_c}{1+(\omega_I\tau_c)^2}\right\} \quad (2)$$

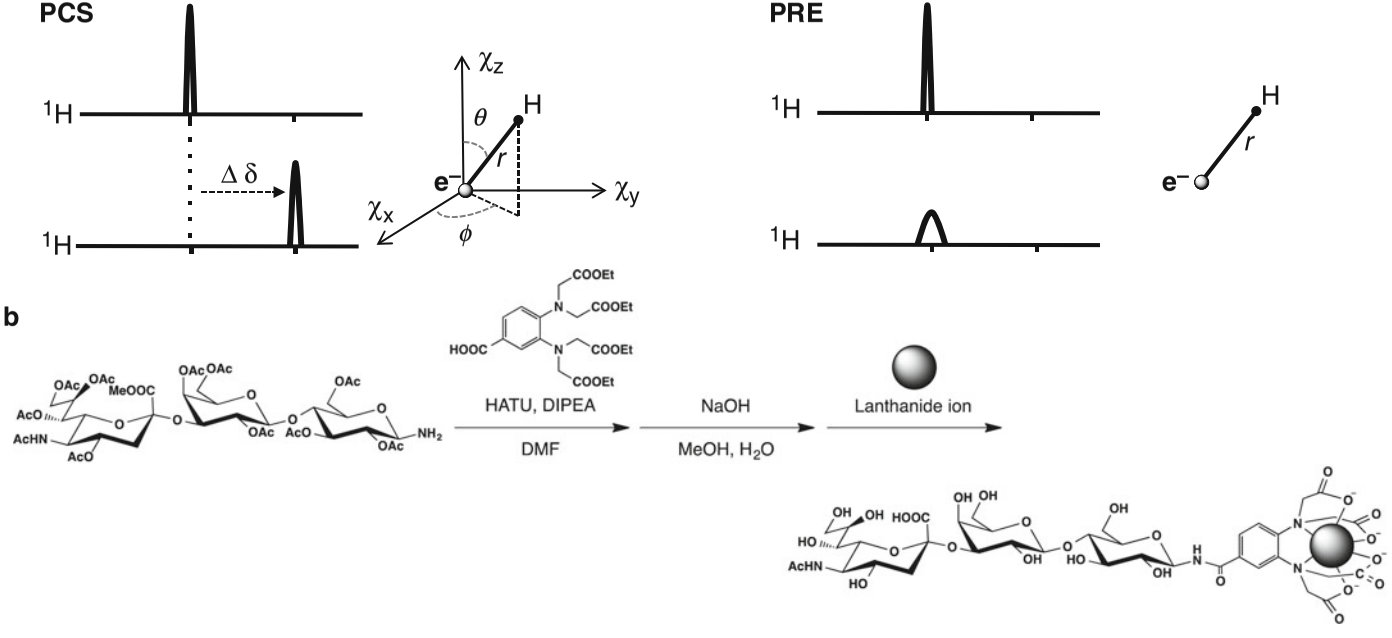

Fig. 1 (continued)

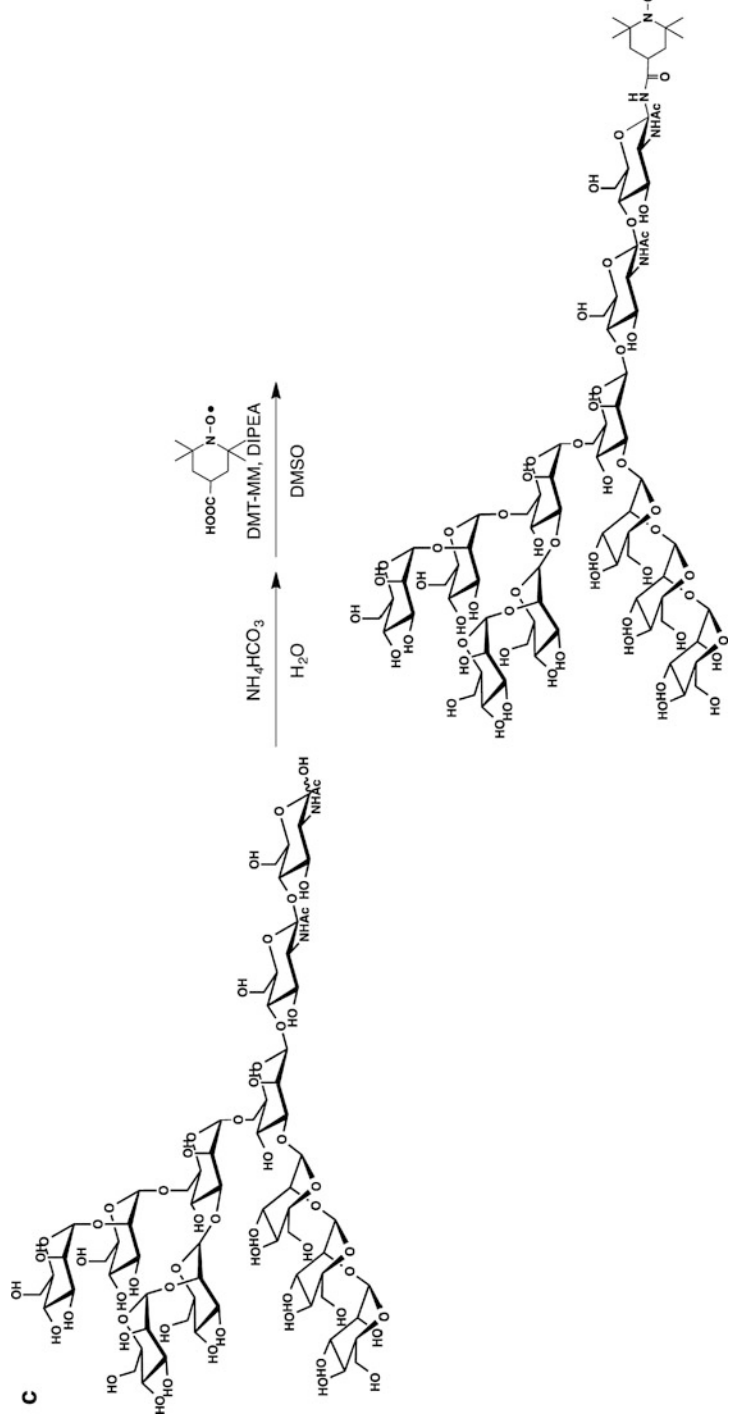

Fig. 1 (**a**) NMR spectral perturbations caused by paramagnetic effects. In Eq. 1 for PCS, $\Delta\chi_{ax}$ and $\Delta\chi_{rh}$ are the axial and rhombic components, respectively, of the anisotropic magnetic susceptibility ($\Delta\chi$) tensor. The polar coordinates of the nucleus, r, θ, and φ, are defined with respect to the paramagnetic center and the principal axis of the $\Delta\chi$ tensor. The R_2 enhancement through dipole–dipole interactions is shown in Eq. 2, where μ_0 is the permeability of vacuum, γ_I is the gyromagnetic ratio of the nucleus, $\omega_I/2\pi$ is the Larmor frequency of the nucleus, g is the electronic g-factor, μ_B is the Bohr magneton, S is the spin, r is the distance between the paramagnetic center and the nucleus, and τ_c is the correlation time. The correlation time is defined as $1/\tau_c = 1/\tau_r + 1/\tau_e$, where τ_r and τ_e

Because an unpaired electron displays a large magnetic moment, through-space dipole interactions perturb not only the signals from the nuclei proximal to the paramagnetic center but also those distributed in wide area. These perturbations show an r^{-3} (PCS) and r^{-6} dependence (PRE) with respect to the paramagnetic spin–nuclei distance (r). PCS values also depend on angular coordinates regarding the paramagnetic center and the principal axis of the magnetic susceptibility tensor. Hence, the PCS and PRE measurements provide valuable atomic long-distance information for the characterization of biomolecular conformations and interactions.

Pseudocontact Shift

To exploit PCS effects, a paramagnetic tag harboring a metal ion is covalently attached to the target sugar chain (Zhang et al. 2013). The choice of ion species and the design of the ion coordinating units are important for appropriate PCS measurements and quantitative data interpretation. Lanthanide ions, such as Tm^{3+} and Dy^{3+}, are useful paramagnetic probes for PCS observation in oligosaccharides. These ions are introduced in the reducing terminus of target oligosaccharides using ion-chelating tags derived from ethylenediaminetetraacetic acid and phenylenediamine (Yamamoto et al. 2011; Zhang et al. 2013).

The incorporation of the phenylenediamine-based lanthanide-chelating tag into the GM3 trisaccharide is shown in Fig. 1b. Selective amination and subsequent acylation link the tag to the trisaccharide through a rigid amide linkage. Deprotection under basic conditions gives rise to an active chelating unit. The rigidity of the tag and its affinity for the lanthanide ion are crucial for the accurate quantitation of observed PCSs because they prevent potential ambiguities in data interpretation related to the possible motional freedom of the tag moiety. An appropriate spacer is also required between the ion and the oligosaccharides to suppress unfavorable Fermi contact shift and relaxation perturbation in the sugar moiety upon ion chelation.

The pH of the aqueous tagged oligosaccharide solution should be 7.0 or higher for strong ion binding. Phosphate buffer should not be used because it forms insoluble lanthanide salts. Upon titration of a paramagnetic lanthanide ion, the ^1H NMR spectra of the oligosaccharide in D_2O show the appearance of a new set of

Fig. 1 (continued) are the rotational correlation times of the molecule and the effective electron relaxation time, respectively. (**b** and **c**) Attachment of the paramagnetic probes to the target oligosaccharides. (**b**) Lanthanide-tagging of the GM3 trisaccharide and (**c**) spin labeling of the M9 undecasaccharide (HATU = 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b] pyridinium 3-oxid hexafluorophosphate, DIPEA = *N*,*N*-diisopropylethylamine, DMT-MM = 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride) (Reprinted from Zhang et al. (2013) and Yamamoto et al. (2012) with permissions from The Chemical Society of Japan and The Royal Society of Chemistry, respectively)

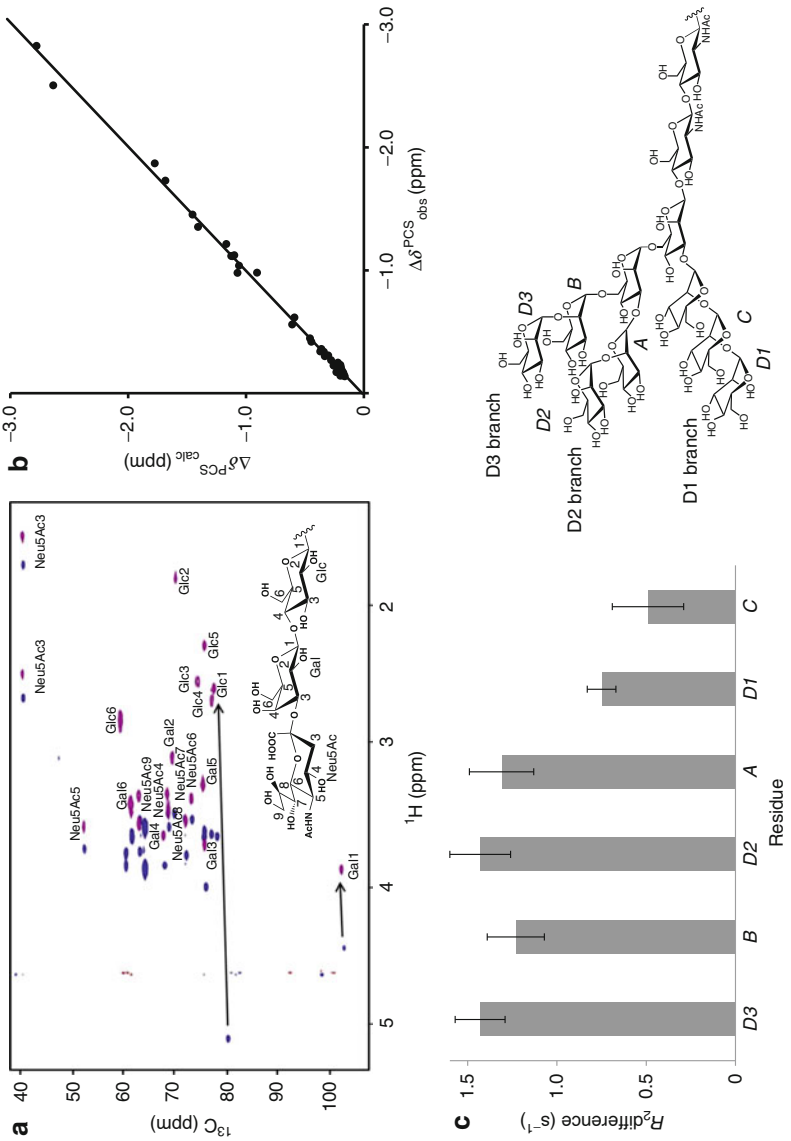

Fig. 2 (continued)

peaks concomitant with the disappearance of the original peaks, confirming the generation of a stable 1:1 complex. The PCS values are estimated as the differences in ^{1}H and ^{13}C chemical shifts between the oligosaccharide–paramagnetic ion complex and the diamagnetic reference La^{3+} ion by ^{1}H–^{13}C heteronuclear chemical shift correlation experiments (Fig. 2a).

Experimental PCS values are compared with those calculated from the three-dimensional (3D) oligosaccharide molecular model to quantitatively validate the model conformation. Dynamic ensemble models of conformations derived from molecular dynamics (MD) simulations of the GM3 trisaccharide and the GM2 tetrasaccharide were evaluated through this approach (Yamamoto et al. 2012; Zhang et al. 2012). Anisotropic magnetic susceptibility tensor components were determined, and PCS values were back-calculated using a modified version of MSpin software (http://mestrelab.com/). As shown in Fig. 2b, the back-calculated PCS values of these oligosaccharides were in excellent agreement with the experimental data. This indicates that PCS-validated MD simulations are applicable to studies on the conformational dynamics of flexible and branched oligosaccharides.

Paramagnetic Relaxation Enhancement

Nitroxide radicals and Gd^{3+} ion are commonly utilized as paramagnetic probes for PRE observation. For example, spin labeling has been conducted by attaching a (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO) derivative to determine the 3D structure and interaction information of oligosaccharides by PRE analysis.

A triantennary undecasaccharide was subjected to such a PRE-based conformational analysis (Yamaguchi et al. 2013). The homogeneous high-mannose-type undecasaccharide M9 was overexpressed using *Saccharomyces cerevisiae* cells, which were genetically engineered by the deletion of the genes involved in the glycan processing pathway. The M9 oligosaccharides were isolated from the

Fig. 2 Observation of paramagnetic effects for NMR conformational analyses of oligosaccharides. (**a**) ^{1}H–^{13}C heteronuclear single quantum coherence spectra of the GM3 trisaccharide tagged with Tm^{3+} (*magenta*) and La^{3+} (*blue*). Chemical shift differences resulting from the PCS of the anomeric CH groups are indicated by *arrows*. (**b**) Correlations between experimental and back-calculated PCS values. Calculated values were determined for a vast conformational ensemble model of GM3 trisaccharide generated from MD simulations using Amber11 and GLYCAM06 force fields. Ten MD simulations were performed for the untagged trisaccharide in explicit water at 300 K for 12 ns with a 2 f. time step in NPT ensemble. Two thousand conformers extracted at equal intervals from the combined trajectory were employed to create the ensemble model. (**c**) R_2 differences in the anomeric proton resonances in spin-labeled M9 before and after radical quenching. The values represent the mean ± SD of three independent experiments (Reprinted and adapted with modifications from Yamamoto et al. (2012) and Yamaguchi et al. (2013) with permissions from The Royal Society of Chemistry and The Chemical Society of Japan, respectively)

glycoprotein mixture, which was harvested from the yeast cells, and treated with NH_4HCO_3 to achieve selective amination of the reducing end before spin labeling using TEMPO (Fig. 1c). This hybrid approach combining cell engineering and chemical techniques provides long-distance atomic information for the conformational characterization of high-mannose-type oligosaccharides in solution. The transverse relaxation rates (R_2) of the mannosyl anomeric protons were measured through Carr–Purcell–Meiboom–Gill experiments before and after radical quenching in the presence of L(+)-ascorbic acid (Fig. 2c). The R_2 differences between the paramagnetic and diamagnetic states reflect the spatial position of each proton with respect to that of the radical. The results showed that mannose residues located in D2 and D3 branches exhibited larger PRE than those in the D1 branch, indicating that the outer carbohydrate residues can access the reducing terminus. These foldback conformations are consistent with previous NOE-based data (Kamiya et al. 2013).

In addition, a water-soluble paramagnetic probe, Gd(III)-diethylenetriamine pentaacetic acid, and membrane-anchored radical 5-doxyl stearic acid have been used for the conformational analysis of ganglioside GM1 embedded in membrane-mimicking phospholipid bicelles. The observed PRE values were interpreted in conjunction with MD simulations to determine the conformation of GM1 in the membrane (DeMarco et al. 2010).

The examination of intermolecular PRE is also useful for the NMR characterization of interactions between oligosaccharides and proteins. The interactions of gangliosidic clusters with amyloid β (Aβ) protein, which are supposed to be crucial in its amyloid formation, were investigated using a spin-labeled Aβ interacting with lyso-GM1 micelles. Because of intermolecular PRE, the modified Aβ containing an extra nitroxide radical-linked C-terminal cysteine residue significantly attenuated the peaks originating from the sugar–lipid interface of the lyso-GM1 micelles (Yagi-Utsumi et al. 2010). This result was consistent with the up-and-down topology of Aβ molecules lying on amphiphilic GM1 clusters, which had previously been found by an NMR saturation transfer experiment (Utsumi et al. 2009).

Comments and Future Perspectives

Oligosaccharides exhibit a dynamic conformational behavior and primarily promote biological functions through weak interactions with other biomolecules. To better understand the energetics of these flexible biomolecular interactions, it is essential to quantitatively evaluate conformational dynamics of oligosaccharides at each step of interaction processes, such as the initial free state, early-encounter complex formation, and water-mediated complex stabilization. Combined with computational approaches, the paramagnetism-assisted NMR approach is expected to open the door for atomic descriptions of dynamic biomolecular systems involving a variety of interacting sugar chains at the atomic level.

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References

DeMarco ML, Woods RJ, Prestegard JH, Tian F (2010) Presentation of membrane-anchored glycosphingolipids determined from molecular dynamics simulations and NMR paramagnetic relaxation rate enhancement. J Am Chem Soc 132:1334–1338

Kamiya Y, Yanagi K, Kitajima T, Yamaguchi T, Chiba Y, Kato K (2013) Application of metabolic ^{13}C labeling in conjunction with high-field nuclear magnetic resonance spectroscopy for comparative conformational analysis of high mannose-type oligosaccharides. Biomolecules 3:108–123

Luchinat C, Parigi G (2010) Paramagnetic Systems in Biochemistry: Solution NMR Studies. Encyclopedia of NMR, Harris RK and Wasylishen RE ed., John Wiley, Chichester, U.K., vol.6, 3317–3323

Utsumi M, Yamaguchi Y, Sasakawa H, Yamamoto N, Yanagisawa K, Kato K (2009) Up-and-down topological mode of amyloid β-peptide lying on hydrophilic/hydrophobic interface of ganglioside clusters. Glycoconj J 26:999–1006

Yagi-Utsumi M, Kameda T, Yamaguchi Y, Kato K (2010) NMR characterization of the interactions between lyso-GM1 aqueous micelles and amyloid β. FEBS Lett 584:831–836

Yamaguchi T, Kamiya Y, Choo YM, Yamamoto S, Kato K (2013) Terminal spin labeling of a high-mannose-type oligosaccharide for quantitative NMR analysis of its dynamic conformation. Chem Lett 42:544–546

Yamamoto S, Yamaguchi T, Erdélyi M, Griesinger C, Kato K (2011) Paramagnetic lanthanide tagging for NMR conformational analyses of N-linked oligosaccharides. Chem Eur J 17:9280–9282

Yamamoto S, Zhang Y, Yamaguchi T, Kameda T, Kato K (2012) Lanthanide-assisted NMR evaluation of a dynamic ensemble of oligosaccharide conformations. Chem Commun 48:4752–4754

Zhang Y, Yamamoto S, Yamaguchi T, Kato K (2012) Application of paramagnetic NMR-validated molecular dynamics simulation to the analysis of a conformational ensemble of a branched oligosaccharide. Molecules 17:6658–6671

Zhang Y, Yamaguchi T, Kato K (2013) New NMR tools for characterizing the dynamic conformations and interactions of oligosaccharides. Chem Lett 42:1455–1462