Synthesis of Singly Spin-labeled Mono- and Disaccharides and Their EPR Spectra in Different Solvents

Injae Shin,* Hyuk-jun Jung, and Sanghyuk Lee†

Department of Chemistry, Yonsei University, Seoul 120-749, Korea
†Department of Chemistry, Ewha Womans University, Seoul 120-750, Korea
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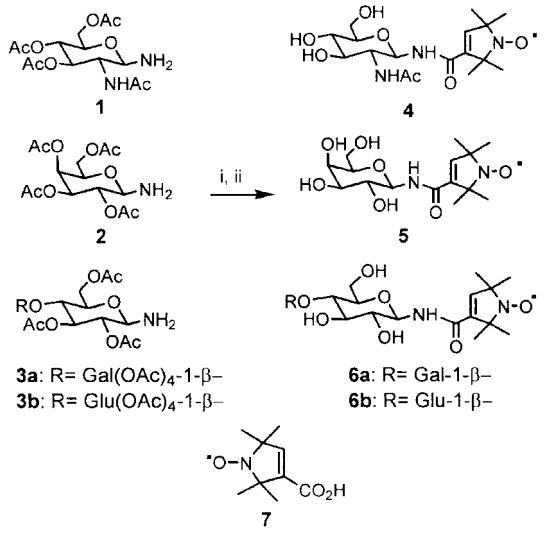
Over a decade, the biological functions of oligosaccharides in glycoconjugates have been extensively investigated by chemists, biochemists and biologists. The cell-surface oligosaccharides are known to contribute to many important roles including cell-cell communication, cell adhesion, cell growth, bacterial and viral infection.[1] Carbohydrates of glycoproteins also influence the intrinsic properties of proteins and thus result in proper folding of proteins, improved thermal stability and resistance to proteases. Many of their biological roles come from initial recognition of oligosaccharides through carbohydrate-protein interactions.[2] Approaches to understand these interactions have been primarily performed with NMR,[3] circular dichroism (CD)[4] and fluorescence resonance energy transfer (FRET)[5] as well as affinity labeling experiments as a biochemical tool.[6]

Spin-labeled protein/peptides and nucleic acids have been employed to investigate their biological processes by EPR spectroscopy. Singly or doubly spin-labeled peptides and proteins have provided valuable informations on their local and global mobility, conformational change by interactions with small ligands or receptors, and stability of secondary and tertiary structures.[7] They have been also used to elucidate conformational change of membrane-bound proteins and to determine intrahelix residue-residue distances.[7] The conformational change and local dynamics of DNA double helices have been studied by using nitroxide-based spin-labeled nucleic acids.[8] Therefore, studies to use spin-labeled carbohydrates may be an alternative method to understand protein-carbohydrate interactions at the molecular level. To our knowledge, biological studies using spin-labeled oligosaccharides on protein-carbohydrate interactions by EPR have never been performed.[9] Herein we report the facile synthesis of singly nitroxide-labeled mono- and disaccharides and their EPR spectra in three solvents as a model study for the more complex systems.

A nitroxide-spin label was incorporated into an anomeric position via an amide linkage to minimize structural alterations of carbohydrates and perturbation of binding of carbohydrates to lectins or antibodies by a spin label. Sugars containing spin labels were prepared by the reaction delineated in Scheme 1. *N*-Acetylglucosamine, galactose, lactose and cellobiose were converted to the corresponding acetobromosugars followed by nucleophilic substitution of bromide by azide and reduction of azide to amine to provide corresponding acetylated glucosaminylamine (**1**), galactosylamine (**2**), lactosylamine (**3a**) and cellobiosylamine (**3b**), respectively.[10] Spin-labeled acid **7** obtained from 2,2,5,5-tetramethyl-3-pyrroline-3-carboxamide in two steps[11] was coupled to acetylated glycosylamines in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide · HCl (EDC) and *N*-hydroxybenzotriazole (HOBt) as coupling reagents. Finally, the removal of acetyl protecting groups was performed by reacting with anion exchange resin to afford spin-labeled sugars **4**-**6**.[12]



Scheme 1. *Reagents and conditions*: i) **7**, EDC, HOBT, TEA, DMF, 25 °C. ii) Amberlite IRA 900 (OH^-), MeOH, 25 °C.

The EPR spectra of nitroxide-labeled sugars **4**-**6** and their simulated spectra in three solvents (MeOH, DMF, H_2O) are shown in Figure 1, and the EPR parameters for each spin in these simulation are summarized in Table 1. Line shape is analyzed by nonlinear least square procedure using NLSL program developed by Freed and coworkers.[13] A triplet structure in EPR spectra by $S = 1$ of a nitrogen atom of N-O$^{\cdot}$ $\leftrightarrow$ $^{\cdot}$N-O is observed for all spin-labeled sugars. The *g*-tensor is assumed to be isotropic, and the experimentally measured values are used in the simulation of spectra. The hyperfine and the rotational diffusion tensors are assumed to be axially symmetric. It is further assumed that the axes of *g*-, hyperfine, and rotational diffusion tensors coincide to simplify the spectral analysis. Additional sources of inhomogeneous line broadening are included as gib (gaussian inhomogeneous broadening) parameter in these simulations.

The hyperfine tensor of singly spin-labeled mono- and disaccharides is found to be 1-6 gauss for the perpendicular component ($a_\perp$) and 35-39 gauss for the parallel component

*To whom correspondence should be addressed. Tel: +82-2-2123-2631; Fax: +82-2-364-7050; E-mail: injae@alchemy.yonsei.ac.kr

Table 1. EPR parameters for each spin probe used in these simulation

compounds	N-acetylglucosamine (**4**)			Galactose (**5**)			Lactose (**6a**)			Cellobiose (**6b**)		
solvents	MeOH	DMF	H_2O	MeOH	DMF	H_2O	MeOH	DMF	H_2O	MeOH	DMF	H_2O
g_0	2.0060	2.0061	2.0059	2.0060	2.0059	2.0060	2.0061	2.0061	2.0063	2.0061	2.0061	2.0062
$a_\perp$ (gauss)	3.4	3.8	6.1	1.2	3.0	4.7	3.2	3.0	4.5	3.3	3.0	4.5
$a_\parallel$ (gauss)	37.1	35.4	35.6	37.0	37.0	38.0	37.0	37.1	38.7	37.1	37.1	38.7
log $R_\perp$ (sec^{-1})	8.55	8.50	8.70	8.40	8.57	8.73	8.52	8.73	8.69	8.61	8.57	8.69
gib (gauss)	4.00	2.49	0.98	7.72	2.39	1.60	4.58	2.61	1.16	4.48	2.46	1.30

g_0: isotropic g-value, $a_\perp$: perpendicular hyperfine tensor, $a_\parallel$: parallel hyperfine tensor, $R_\perp$: perpendicular component of rotational rate, gib: gaussian inhomogeneous broadening

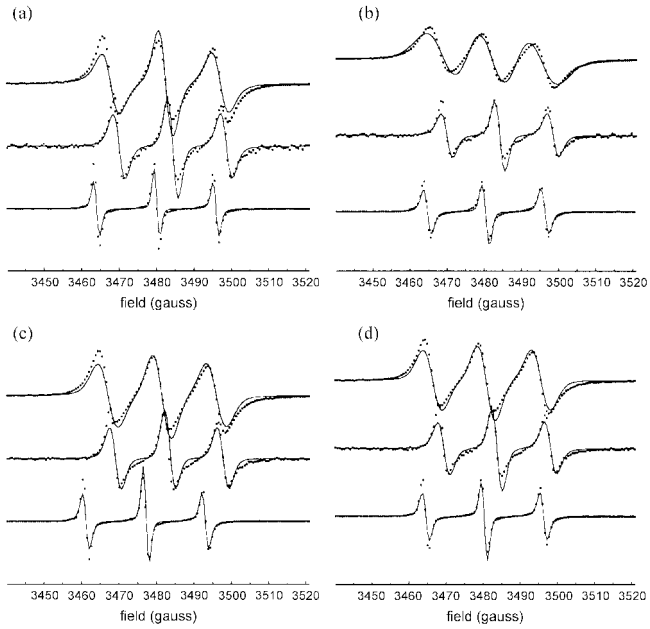


Figure 1. EPR spectra of mono- and disaccharides containing spin labels in different solvents. The dot lines represent the experimental data and the solid lines show the simulated spectra. The used solvents to obtain spectra are MeOH, DMF and H_2O from top to bottom in each figure. (a) *N*-acetylglucosamine (**4**) (b) galactose (**5**) (c) lactose (**6a**) (d) cellobiose (**6b**).

($a_\parallel$). The perpendicular component of rotational (tumbling) rate ($R_\perp$) ranged from 2.5×10^8 sec^{-1} to 5.4×10^8 sec^{-1}. However, the spectral shape was relatively insensitive to the parallel rotational rate, which is arbitrarily set to 1.0×10^{10} sec^{-1}. It was observed that EPR spectra are dependent on solvents. In general, the perpendicular rotational rate is slightly faster in water solvent than MeOH and DMF and inhomogeneous line broadening is smallest in H_2O. This is quite unexpected because the viscosity of water is larger than that of MeOH or DMF.[14] It seems that the hydrogen bonding between the carbohydrate unit and the solvent molecules, which may potentially have significantly influence on the conformations of carbohydrates or the structure of the solvents, affects the rotational rate. It was suggested that some of the solvation effects on the conformation of sugars appear to be the direct consequence of specific hydrogen bonds rather than dielectric effects.[15] The dependence of hyperfine constants on solvent supports this assumption.

For the spin probes used in this prototypical work, the rotational motion belongs to fast-motional regime. Therefore, information that can be obtained from line shape analysis is rather limited. However, when protein-carbohydrate interaction is present, line shape is expected to reflect the local environment sensitively.

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