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Note

NMR study on the hydroxy protons of the Lewis X and Lewis Y oligosaccharides

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Abstract—The ^{1}H NMR chemical shifts and NOEs of hydroxy protons in Lewis X trisaccharide, β-D-Gal*p*-(1 → 4)[α-L-Fuc*p*-(1 → 3)]-β-D-Glc*p*NAc, and Lewis Y tetrasaccharide, α-L-Fuc*p*-(1 → 2)-β-D-Gal*p*-(1 → 4)[α-L-Fuc*p*-(1 → 3)]-β-D-Glc*p*NAc, were obtained for 85% H_2O/15% $(CD_3)_2CO$ solutions. The OH-4 signal of Gal*p* in Lewis X and OH-3, OH-4 signals of Gal*p*, and OH-2 signal of Fuc*p* linked to Gal*p* in Lewis Y had chemical shifts which indicate reduced hydration due to their proximity to the hydrophobic face of the Fuc*p* unit linked to Glc*p*NAc. The inter-residue NOEs involving the exchangeable NH and OH protons confirmed the stacking interaction between the Fuc*p* linked to the Glc*p*NAc and the Gal*p* residues in Lewis X and Lewis Y.

Keywords: Conformation; Hydration; Hydroxy protons; Lewis X; Lewis Y; NMR

Previous NMR studies on the structures of disaccharides,[1–3] trisaccharides[4,5] and cyclodextrins[6] have shown that the chemical shifts of hydroxy protons could be used as conformational probes. Large negative Δδ-values ($\Delta\delta = \delta_{\text{Oligosaccharide}} - \delta_{\text{Monosaccharide}}$) were obtained for hydroxy protons close to acetal oxygens whereas positive Δδ-values were obtained for hydroxy protons close to other hydroxy groups. An NMR study on the more rigid Lewis b tetrasaccharide[7] showed that hydroxy protons situated in amphiphilic regions also had large negative Δδ-values. The results suggested that in strongly hydrated systems, the interaction between bulk water and hydroxy protons is the key factor contributing to the values of chemical shifts.[8] Thus, when the hydration of a hydroxy group is reduced due to interaction with acetal oxygens or to steric factors, the hydroxy proton is shielded.

In this work, the ^{1}H NMR chemical shifts and NOEs for the hydroxy protons of the Lewis X trisaccharide (Le^x), [β-D-Gal*p*-(1 → 4)[α-L-Fuc*p*-(1 → 3)]-β-D-Glc*p*NAc and the Lewis Y tetrasaccharide (Le^y), [α-L-Fuc*p*-(1 → 2)-β-D-Gal*p*-(1 → 4)[α-L-Fuc*p*-(1 → 3)]-β-D-Glc*p*NAc] (Scheme 1) have been measured, as they have similar rigid core structures[9] as Lewis b (Le^b). In Le^b, OH-3 and OH-4 of Gal*p*, with large negative Δδ-values,[7] are important in key polar interactions with the lectin IV glycoprotein of GS4.[10] The same hydroxy groups are also found in the epitope of Le^y binding to the monoclonal antibody BR55-2,[11] and in this work, we wanted to determine if the signals for corresponding hydroxy protons also have large Δδ-values.

The hydroxy proton resonances in Le^x and Le^y were assigned from the combination of two-dimensional DQF-COSY, TOCSY, ROESY, HSQC-TOCSY and HSQC-DEPT spectra. The ^{1}H NMR chemical shifts (δ, ppm) and Δδ-values are given in Table 1. The chemical shifts for the hydroxy proton signals of the methyl monosaccharides have been reported previously.[5] The OH-4 signal of Gal*p*(3) has a large negative Δδ-value of −0.520 ppm in Le^x and −0.649 ppm in Le^y. This negative Δδ indicates that the OH-4 is shielded in the oligosaccharides if compared to the same proton in the corresponding methyl monosaccharide. The 3D structures of Le^x and Le^y, obtained using reported glycosidic

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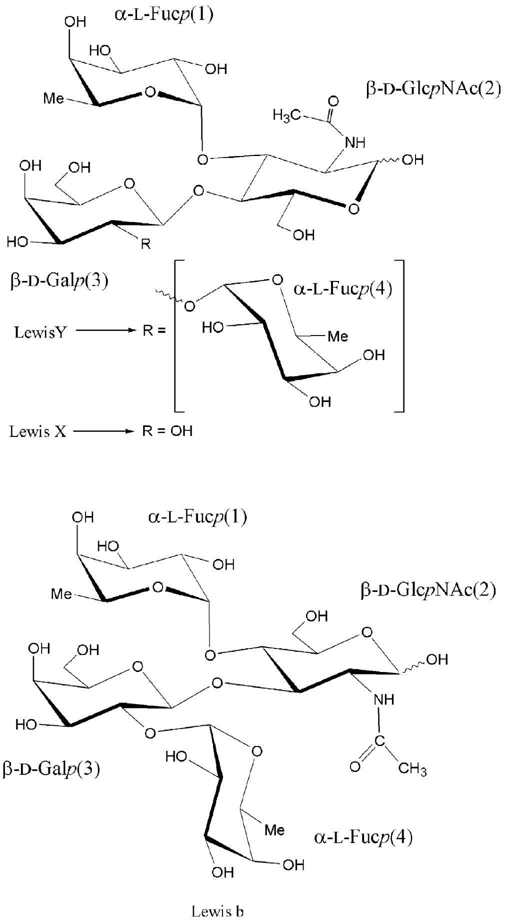

Scheme 1. Structures of Le^x, Le^y and Le^b.

angles[9] (Fig. 1), shows that in both Le^x and Le^y, the OH-4 of Gal*p*(3) has a close interaction with the hydrophobic face of the Fuc*p* (1) residue. The water accessibility at the hydroxy group is reduced leading to an upfield shift of the hydroxy proton signal. In Le^y, although the signals for OH-3 of Gal*p*(3) and OH-2 of Fuc*p*(4) have relatively large negative Δδ-values (−0.184 and −0.111 ppm, respectively), these Δδ-values are smaller than those for Le^b (−0.401 and −0.323 ppm, respectively). These differences in Δδ may reflect small structural differences between Le^y and Le^b such as different stacking interactions. In Le^x, there is no steric hindrance for hydration of OH-3 of Gal*p* by Fuc*p*(4) as in Le^y, and the OH-3 proton has a very small Δδ of −0.004 ppm. The OH-2 and OH-3 of Fuc*p*(1) in Le^x have Δδ-values of −0.118 and 0.177 ppm, respectively. The upfield shift of the OH-2 signal is probably due to steric hindrance of hydration of the OH-2 group by the 2-acetamido group. The positive Δδ-value of OH-3 of Fuc*p*(1) is more difficult to interpret but could be due to an interaction between the two hydroxy groups, OH-2 and OH-3 of Fuc*p*(1).[6]

The observation of hydroxy protons in NMR spectra provides the possibility to obtain additional inter-residue NOEs. The NOEs involving the nonexchangeable ring protons in Le^x were the same as previously published[12] with only one inter-residual NOE between H-5 Fuc*p*(1) and H-2 Gal*p*(3). However, six inter-residual NOEs (Table 2) were observed for the exchangeable OH and NH protons of Le^x trisaccharide. To discriminate between the cross-peaks due to dipolar relaxation and those due to chemical exchange, ROESY experiments were also performed. Five of the six inter-residual NOEs are long-range NOEs between residues, which are not directly connected. These long-range NOEs are more sensitive to structural changes since the distances between residues alter drastically by small conformational changes in the glycosidic bonds connecting them. These data together with the values of the chemical shifts of the hydroxyl proton signals are also in good agreement with the conformations in the solid state found by X-ray crystallography[13] and in solutions by NMR[14–16] and characterized by stacking between the fucose and galactose rings. For Le^y, nine inter-residue NOEs could be detected (Table 2). Four of them, OH-4 Gal*p*(3) to H-3 Fuc*p*(1), H-4 Fuc*p*(1), H-5 Fuc*p*(1), and to Me Fuc*p*(1) are long-range NOEs, supporting the stacking interaction between Fuc*p*(1) and Gal*p*(3).

The OH-3 and OH-4 of Gal*p*(3) had large negative Δδ-values and were involved in key polar interaction when Le^b binds to lectin IV glycoprotein of GS4.[10] These two hydroxy groups were also found in the epitope of Le^y binding to the monoclonal antibody BR55-2.[11] These results suggest that the Δδ-values could give information about the potential role of a hydroxy group in binding processes. For rigid molecules, hydration can affect the affinity of the ligand for the protein.[17] When an interaction between protein and ligand takes place, water molecules have to be displaced, and it is easier to displace water molecules from the less polar regions.[17] Thus, chemical shifts could become a conformational probe used to identify the hydroxy protons of a rigid carbohydrate that could be recognized by proteins.

1. Experimental

1.1. Sample preparation

The NMR tubes were soaked for 3 h in a 50 mM solution of phosphate buffer, pH 7, to minimize adsorption of impurities from glassware.[18] The compounds Lewis X and Y were purchased from Dextra Laboratories Ltd. The 1 mg samples were dissolved in 80 μL of 85% H_2O/15% $(CD_3)_2CO$ to give a sample concentration of 15 and 20 mM for Lewis Y and X, respectively. The addition of acetone to the samples allowed lowering the sample temperature to −10 °C without freezing. The pH was adjusted by addition of minute amounts of HCl and NaOH in order to obtain sharp hydroxy protons signals.

Table 1. ^{1}H NMR chemical shifts (δ) and chemical shift differences ($\Delta\delta$) for the hydroxy protons of Le^x and Le^y

		Lewis Y		Lewis X	
		δ (ppm)	$\Delta\delta$[a]	δ (ppm)	$\Delta\delta$[a]
α-L-Fuc*p*(1)	OH-2	6.145	0.009	6.018	−0.118
	OH-3	6.051	0.121	6.107	0.177
	OH-4	5.905	−0.073	6.020	0.042
D-Glc*p*NAc(2)	OH-6(α)	6.016	−0.027	6.068	0.025
	OH-6(β)	6.140	−0.056	5.999	−0.085
	OH-1(α)	7.208	−0.107	7.325	0.010
	OH-1(β)	7.932	−0.043	7.977	0.002
β-D-Gal*p*(3)	OH-2			6.551	−0.020
	OH-3	5.938	−0.184	6.118	−0.004
	OH-4	5.253	−0.649	5.382	−0.520
	OH-6	6.143	0.021	6.196	0.074
α-L-Fucp(4)	OH-2	6.025	−0.111		
	OH-3	6.022	0.092		
	OH-4	5.901	−0.077		

The measurements were performed at −10 °C in 85% H_2O/15% $(CD_3)_2CO$.

[a] $\Delta\delta$: Chemical shift in the oligosaccharide minus chemical shift in the corresponding monosaccharide methyl glycoside. A positive difference indicates a downfield shift.

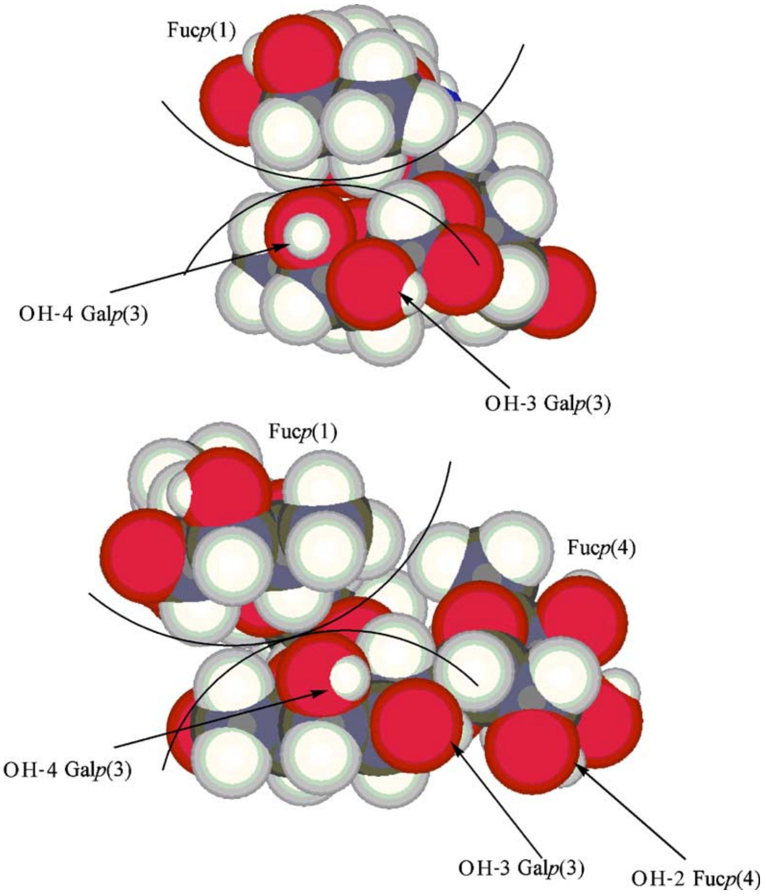

Figure 1. Space filling models of Le^x and Le^y, showing the stacking interactions between Fuc*p*(1) and Gal*p*(3). The OH-3, OH-4 of Gal*p*(3) and OH-2 of Fuc*p*(4) are indicated by arrows.

Table 2. Interresidual NOEs involving the exchangeable NH and OH protons of Le^x and Le^y

Le^x	NOEs	Le^y	NOEs
NHAc (2)	H-1 (1)	NHAc (2)	H-1 (1)
OH-4 (3)	H-3 (1)	OH-2 (1)	H-3 (2)
	H-4 (1)		H-4 (2)
	H-5 (1)	OH-3 (3)	H-1 (4)
	Me (1)	OH-4 (3)	H-3 (1)
OH-6 (3)	H-3 (1)		H-4 (1)
			H-5 (1)
			Me (1)

1.2. NMR spectroscopy

The NMR experiments were performed on a BRUKER DRX-600 spectrometer operating at 600.13 MHz for proton observation. All spectra were recorded at $-10\,^{\circ}$C. One and two-dimensional ^{1}H NMR spectra were acquired using the WATERGATE pulse sequence[19] for suppression of the water signal. The ^{1}H NMR spectra were referenced by setting the residual acetone-d_5 signal to 2.204 ppm. The 2D NMR proton spectra were recorded in the phase-sensitive mode using the TPPI method.[20] NOESY and ROESY spectra were recorded with mixing times (τ_m) of 150 and 300 ms with 512 spectra of $2K$ data points. For each FID, 80 scans were recorded using a repetition delay of 1.5 s. The data were zero filled to $2K \times 1K$ before applying a $\pi/2$ shifted sine-bell square window function in both dimensions. ^{1}H and ^{13}C chemical shifts are available on request.

Acknowledgements

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References

1. Sandström, C.; Magnusson, G.; Nilsson, U.; Kenne, L. *Carbohydr. Res.* **1999**, *322*, 46–56.
2. Ivarsson, I.; Sandström, C.; Sandström, A.; Kenne, L. *J. Chem. Soc., Perkin Trans. 2* **2000**, 2147–2152.
3. Kindahl, L.; Sandström, C.; Norberg, T.; Kenne, L. *J. Carbohydr. Chem.* **2000**, *19*, 1291–1303.
4. Sandström, C.; Baumann, H.; Kenne, L. *J. Chem. Soc., Perkin Trans. 2* **1998**, 2385–2393.
5. Sandström, C.; Baumann, H.; Kenne, L. *J. Chem. Soc., Perkin Trans. 2* **1998**, 809–815.
6. Bekiroglu, S.; Kenne, L.; Sandström, C. *J. Org. Chem.* **2003**, *68*, 1671–1678.
7. Bekiroglu, S.; Sandström, C.; Norberg, T.; Kenne, L. *Carbohydr. Res.* **2000**, *328*, 409–418.
8. Bekiroglu, S.; Sandström, A.; Kenne, L.; Sandström, C. *Org. Biomol. Chem.* **2003**, *2*, 200–205.
9. Imberty, A.; Mikros, E.; Koca, J.; Mollicone, R.; Oriol, R.; Pérez, S. *Glycoconjugate. J.* **1995**, *12*, 331–349.
10. Lemieux, R. U. *Chem. Soc. Rev.* **1989**, *18*, 347–374.
11. BlaszczykThurin, M.; Murali, R.; Westerink, M. A. J.; Steplewski, Z.; Co, M. S.; KieberEmmons, T. *Protein Eng.* **1996**, *9*, 447–459.
12. Geyer, A.; Hummel, G.; Eisele, T.; Reinhardt, S.; Schmidt, R. R. *Chem. Eur. J.* **1996**, *2*, 981–988.
13. Pérez, S.; Mouhous-Riou, N.; Nifant'ev, N. E.; Tsvetkov, Y. E.; Bachet, B.; Imberty, A. *Glycobiology* **1996**, *6*, 537–542.
14. Miller, K. E.; Mukhopadhyay, C.; Cagas, P.; Bush, A. *Biochemistry* **1992**, *31*, 6703–6709.
15. Haselhorst, T.; Weimar, T.; Peters, T. *J. Am. Chem. Soc.* **2001**, *123*, 10705–10714.
16. Azurmendi, H. F.; Martin-Pastor, M.; Bush, C. A. *Biopolymers* **2002**, *63*, 89–98.
17. Lemieux, R. U. *Acc. Chem. Res.* **1996**, *29*, 373–380.
18. Adams, B.; Lerner, L. *J. Am. Chem. Soc.* **1992**, *114*, 4827–4829.
19. Piotto, M.; Saudek, V.; Sklenar, V. *J. Biomol. NMR* **1992**, *2*, 661–665.
20. Marion, D.; Wüthrich, K. *Biochem. Biophys. Res. Commun.* **1983**, *113*, 967–974.