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Ab initio and NMR studies on the effect of hydration on the chemical shift of hydroxy protons in carbohydrates using disaccharides and water/methanol/ethers as model systems

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Received 26th September 2003, Accepted 13th November 2003
First published as an Advance Article on the web 10th December 2003

Density functional theory (DFT) and Hartree–Fock (HF) quantum mechanical calculations have been performed on the disaccharides, β-L-Fuc*p*-(1⟶4)-α-D-Gal*p*-OMe, β-L-Fuc*p*-(1⟶4)-α-D-Glc*p*-OMe, and β-L-Fuc*p*-(1⟶3)-α-D-Glc*p*-OMe. The Δδ-values (difference between the chemical shift in the disaccharide and the corresponding monosaccharide methyl glycoside) for the exchangeable hydroxy protons have been calculated and compared to experimental values previously measured by NMR spectroscopy for samples in aqueous solutions. The calculations performed on molecules in vacuum showed that hydroxy protons hydrogen bonded to the neighboring ring oxygens have large positive Δδ-values, indicating that they are deshielded relative to those in the corresponding methyl glycoside. The NMR experiments showed instead that these hydroxy protons close to the neighboring ring oxygens were shielded. This discrepancy between calculated and experimental data was attributed to solvent effects, and this hypothesis has been confirmed in this work by monitoring the chemical shift of the hydroxy proton of methanol in water, ethers and water/ether solutions. Shielding of the hydroxy proton of methanol is observed for increased ether concentrations, whereas deshielding is observed for increased concentration of water. The shielding observed for hydroxy protons in disaccharides is a consequence of reduced hydration due to intermolecular hydrogen bonding or steric effects. In strongly hydrated systems such as carbohydrates, the hydration state of a hydroxy proton is the key factor determining the value of the chemical shift of its NMR signal, and the Δδ will be a direct measure of the change in hydration state.

1. Introduction

Chemical shifts, unlike other NMR parameters such as spin–spin couplings and NOEs, have so far played little role in conformational studies by NMR spectroscopy. Chemical shifts are however very sensitive to steric and electronic effects, and, as has been shown for proteins, to secondary and tertiary structure effects. Therefore, it is of considerable interest to understand the origin of the chemical shift behavior, and it is also of practical importance for obtaining information about the structures of biomolecules. In carbohydrates the chemical shifts of non-exchangeable protons are mainly dependent on the type of sugar and glycosidic linkage and not on conformational effects. The chemical shifts of signals from exchangeable hydroxy protons are more difficult to interpret since they are sensitive to many different effects.

We have previously reported the use of hydroxy protons in NMR studies of di-,[1–3] tri-[4,5] and tetra-saccharides[6] in aqueous solutions. Hydroxy protons close to acetal oxygen atoms *i.e.* ring or glycosidic oxygens, or situated in sterically crowded regions were found to be shielded relative to the corresponding protons in the monosaccharide methyl glycosides. Hydroxy protons hydrogen bonded to ring oxygens were also shielded, although it is known that hydroxy protons which are hydrogen bonded are normally deshielded[7], with the largest deshielding for protons involved in the strongest hydrogen bonds. On the other hand, hydroxy protons close to and/or hydrogen bonded to other hydroxy groups were found to be deshielded.[2,5,8] Since the shielded hydroxy protons also showed a slower exchange with water protons, the shielding was attributed to an alteration of the hydrogen bond network between the hydroxy groups and water.

To verify this hypothesis, we have calculated, in the first part of this paper, the NMR chemical shifts of hydroxy proton signals from three disaccharides, β-L-Fuc*p*-(1⟶4)-α-D-Gal*p*-OMe (**1**), β-L-Fuc*p*-(1⟶4)-α-D-Glc*p*-OMe (**2**), and β-L-Fuc*p*-(1⟶3)-α-D-Glc*p*-OMe (**3**) (Fig. 1) using the gauge independent atomic orbital (GIAO) method available in the Gaussian 98 program.[9] These three compounds were chosen as model compounds for calculations due to their moderate size and because

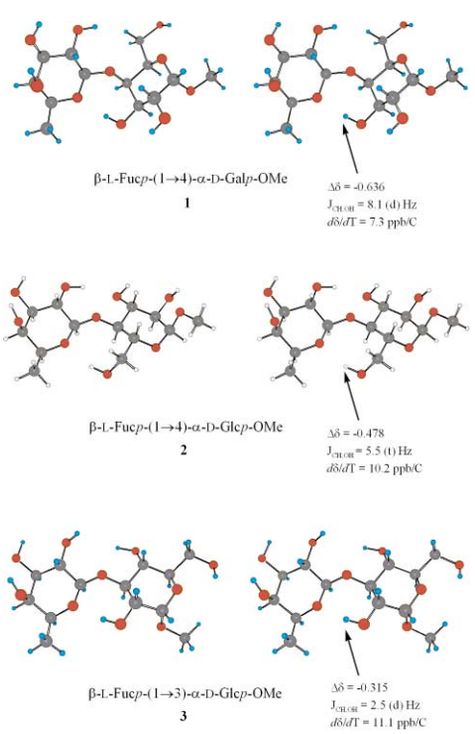


Fig. 1 Stereoviews and experimental data[2] (Δδ, $^3J_{CH,OH}$, and dδ/dT) of the structures of the disaccharides **1**, **2**, and **3**.

DOI: 10.1039/b311852e

Table 1 Calculated and experimental chemical shift differences ($\Delta\delta$), oxygen proton bond lengths (O–H) and distances from hydroxy protons to closest oxygen atoms (H ⋯ O), the valence angle to closest oxygens (O–H ⋯ O), and the oxygen atoms closest to hydroxy protons (O(X))[a]

	HF[b] $\Delta\delta$	B3LYP[b] $\Delta\delta$	$\Delta\delta_{(exp)}$[d]	B3LYP[c]			
				O–H	H ⋯ O	O–H ⋯ O	O(X)
β-L-Fuc-(1⟶4)-α-D-Gal-OMe **(1)**							
O(2′)H	−0.037	−0.058	0.040	0.971	2.571	97.2	O(4)
O(3′)H	0.160	0.217	0.079	0.972	2.316	110.8	O(2′)
O(4′)H	0.030	−0.002	0.132	0.974	2.152	114.8	O(3′)
O(2)H	0.915	1.162	0.099	0.974	2.249	114.9	O(3)
O(3)H	1.535	1.113	−0.636	0.978	1.899	153.8	O(5′)
O(6)H	0.316	0.249	0.031	0.973	2.216	110.8	O(5)
β-L-Fuc-(1⟶4)-α-D-Glc-OMe **(2)**							
O(2′)H	2.510	2.473	−0.084	0.979	2.047	156.5	O(3)
O(3′)H	−0.013	−0.007	0.052	0.973	2.356	109.9	O(2′)
O(4′)H	−0.084	−0.116	0.054	0.973	2.172	114.9	O(3′)
O(2)H	0.136	0.155	0.096	0.974	2.169	113.5	OMe
O(3)H	0.285	0.299	0.029	0.974	2.413	109.4	O(2)
O(6)H	2.348	2.201	−0.478	0.976	1.941	162.8	O(5′)
β-L-Fuc-(1⟶3)-α-D-Glc-OMe **(3)**							
O(2′)H	−0.229	−0.149	−0.034	0.969	2.663	90.2	O(3)
O(3′)H	0.109	0.163	0.084	0.972	2.341	110.5	O(2′)
O(4′)H	−0.033	−0.046	0.135	0.974	2.157	114.4	O(3′)
O(2)H	2.299	2.314	−0.315	0.976	1.947	156.2	O(5′)
O(4)H	−0.257	−0.123	0.119	0.972	2.398	108.4	O(3)
O(6)H	0.146	−0.141	0.068	0.972	2.266	109.2	O(5)

[a] For each disaccharide, the hydroxy protons with primed numbers designate the hydroxy groups on fucopyranosyl substituents, whereas those without primes show the hydroxy groups on methyl galacto- and glucopyranosides. [b] Chemical shift differences are calculated by subtracting the chemical shifts of the corresponding monosaccharide methyl glycosides from the chemical shifts of the hydroxy protons of **1**, **2**, and **3**. [c] The geometrical parameters were taken from the structures fully optimized at the level of B3LYP/6-31G(d). The distances and angles are given in Å and degree respectively. [d] Data from reference 2.

a previous NMR study[2] has shown that in each of the disaccharides **1**–**3**, one hydroxy proton signal has a large negative $\Delta\delta$ ($\delta_{\text{disaccharide}} - \delta_{\text{monosaccharide}}$) attributed to the proximity of the hydroxy proton to the ring oxygen of the neighboring sugar. In this work, the chemical shift values obtained from calculations have been compared to the experimental values.[2] In the second part of the study, the chemical shift of the hydroxy proton of methanol in different mixtures of methanol and water, diethyl ether, tetrahydrofuran (THF) and dioxane were measured. These systems were selected to depict the interactions of hydroxy protons with water (methanol/water) and ring oxygens (methanol/ethers). To model the effect of a ring oxygen on the hydration of a hydroxy group in a disaccharide, methanol in water/dioxane mixtures were studied. The goal of the work was (i) to correlate the results of the calculations to the NMR experimental data and (ii) to gain understanding on the origin of the upfield shift measured for hydroxy protons in carbohydrates.

2 Results

(1) Calculations on disaccharides 1–3

The lowest energy geometries of MM3 calculations[10] were taken as starting conformers for a full optimisation using a density functional theory (DFT) method with the standard 6-31G(d) basis set and B3LYP hybrid functional. The theoretical chemical shifts of hydroxy protons in disaccharides **1**–**3** were calculated by the Hartree–Fock (HF) and density functional theory (DFT) methods and the results are reported as $\Delta\delta$s in Table 1. The $\Delta\delta$ values obtained from HF and DFT calculations were comparable, although the proton chemical shift (δ) calculations using the HF method systematically gave more upfield values than those of the DFT method. In each disaccharide, one or two hydroxy protons showed a large positive $\Delta\delta$ (a positive $\Delta\delta$ indicates a downfield shift in the disaccharide relative to the monosaccharide). These hydroxy protons were O(2)H and O(3)H in **1**, O(2′)H and O(6)H in **2**, and O(2)H in **3**. They also showed geometrical parameters *i.e.* bond lengths, hydrogen bond distances and O–H ⋯ O angles, indicating involvement in hydrogen bonding (Table 1). The hydrogen bond acceptor for O(3)H in **1**, O(6)H in **2**, and O(2)H in **3** was the ring oxygen (O5′) of the neighboring sugar unit. The hydrogen bond acceptor of O(2)H in **1** and of O(2′)H in **2** was O(3) (Table 1). The dependence of chemical shift values on hydrogen bonding is expected, since many studies have shown that protons involved in strong hydrogen bonds are deshielded due to more delocalization of the electrons.

These hydrogen bond interactions are in good agreement with the interatomic distances obtained for the same compounds using HSEA/GESA methods.[11–13] The O(6)H–O(5′) hydrogen bond in **2** and the O(2)H–O(5′) hydrogen bond in **3** have been also observed by X-Ray in the solid state.[14,15] We have previously shown[2] that in aqueous solution, O(3)H in **1**, O(6)H in **2** and O(2)H in **3** have large negative $\Delta\delta$s (indicating a shielding of the hydroxy proton in the disaccharide relative to that in the monosaccharide methyl glycoside) although they are found to be in close proximity to the neighboring O(5′) (Fig. 1). Experimental evidence for the involvement of O(3)H in a transient hydrogen bond interaction with O(5′) in disaccharide **1** was obtained[2] from its large $^3J_{CH,OH}$-value and its relatively small temperature coefficient ($d\delta/dT$). The existence of a weak and transient hydrogen bond between O(2)H and O(5′) in disaccharide **3** was also proposed[2] from the small $^3J_{CH,OH}$-value of O(2)H (Fig. 1). The hydrogen bond interaction involving the two hydroxy groups O(2′)H and O(3)H in **2** were not observed experimentally by NMR spectroscopy, probably because the interaction did not persist long enough or was too weak due to the strong solvation of the hydroxy groups. In the calculation, only one minimum energy conformation was considered, while the disaccharides are not rigid molecules, but instead have conformational flexibility.

Since the *ab initio* calculations were performed in vacuum, the effects of the solvent on the chemical shifts were not taken

into account. The discrepancy between the sign of the $\Delta\delta$ obtained from calculations and experiments is therefore likely to be associated with interactions with the solvent. In the calculations, the $\Delta\delta$-values would only represent the structural changes occurring while forming a disaccharide from the corresponding monomers.

(2) Dependence of the ^{1}H NMR chemical shift of the hydroxy proton of methanol on the concentration of water, diethyl ether, tetrahydrofuran, and dioxane

To determine whether the shielding measured for some hydroxy protons in disaccharides in aqueous solution is caused by disruption of the hydrogen bond network between hydroxy groups and water, a series of binary model systems was analyzed by ^{1}H NMR spectroscopy. The systems were methanol/water, methanol/diethyl ether, methanol/THF and methanol/dioxane. The solvents were selected to represent the interactions of a hydroxy proton with water (methanol/water) and acetal oxygens (methanol/diethyl ether, methanol/THF and methanol/dioxane). NMR studies on binary mixtures involving water as the main component and alcohols, amines, and ethers have previously been reported.[16–20] It was desirable, however, to use the same experimental conditions as those used for disaccharides **1**–**3** in ref. 2, thus the NMR spectra were recorded at −10 °C with 15% acetone-d_6 added to the samples. The experimental data is summarized in Table 2 and in Fig. 2. The chemical shift of the signal from the hydroxy proton of methanol changed from that of the pure methanol, δ 5.245 ppm, downfield with increasing amount of water and upfield upon addition of the ethers (Fig. 2). The effect of the water content on the chemical shift of the hydroxy proton of methanol was less pronounced as compared to that of the ethers, ~0.3 ppm downfield shift with water compared to ~2 ppm upfield shift with all ethers. The chemical shift of the water proton signal changed as the concentration was varied with the same magnitude and direction as did the OH of methanol. Since the magnitude of the shifts is related to the strength of hydrogen bonding[7], these data show that hydrogen bonding between water and methanol is more efficient than hydrogen bonding between ether and methanol. It also shows that hydrogen bonding between water and methanol

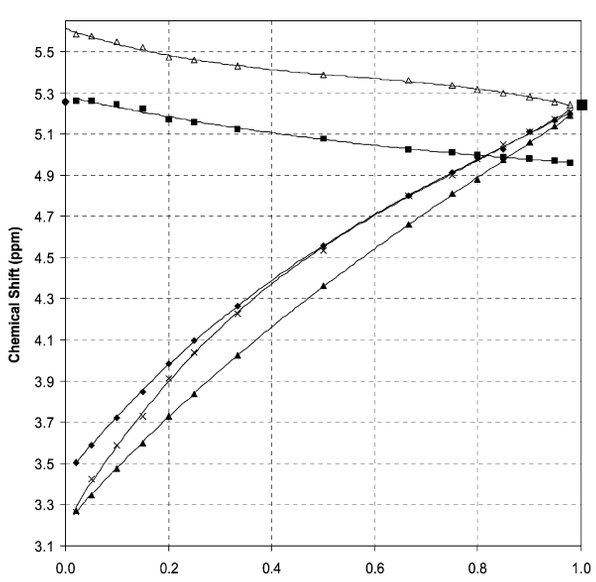


Fig. 2 Chemical shift of the hydroxy proton of methanol as a function of the mole fraction of methanol in water (△), diethyl ether (×), tetrahydrofuran (◆) and dioxane (▲). The chemical shift of water proton as a function of the mole fraction of methanol is also shown (■). The chemical shift of the hydroxy proton of methanol alone, and the chemical shift of water alone are designated by larger filled square (■) and larger tilted filled square (◆) respectively. The fitted lines are to show the trends of change.

Table 2 ^{1}H NMR chemical shifts (δ, ppm) of the proton signals observed for the methanol/water, methanol/diethyl ether, methanol/tetrahydrofuran, and methanol/dioxane mixtures with varied mole fractions[a]

	Methanol/water			Methanol/diethyl ether				Methanol/tetrahydrofuran				Methanol/dioxane		
	Methanol		Water	Methanol		Diethyl ether		Methanol		THF		Methanol		Dioxane
Mole frac.	OH	CH_3	H_2O	OH	CH_3	CH_2	CH_3	OH	CH_3	CH_2[b]	CH_2[c]	OH	CH_3	CH_2
0.02	5.585	3.363	5.258	3.266	3.454	3.559	1.300	3.502	3.435	3.792	1.956	3.268	3.441	3.750
0.05	5.573	3.363	5.258	3.424	3.451	3.558	1.296	3.589	3.436	3.791	1.955	3.346	3.442	3.750
0.10	5.545	3.363	5.243	3.590	3.451	3.555	1.293	3.722	3.437	3.792	1.955	3.475	3.441	3.748
0.15	5.517	3.364	5.221	3.728	3.451	3.554	1.290	3.847	3.438	3.791	1.954	3.597	3.440	3.748
0.20	5.470	3.365	5.173	3.914	3.451	3.555	1.287	3.981	3.439	3.790	1.952	3.730	3.442	3.744
0.25	5.457	3.367	5.156	4.041	3.452	3.553	1.285	4.096	3.440	3.790	1.951	3.835	3.442	3.743
0.33	5.430	3.372	5.124	4.229	3.451	3.553	1.282	4.264	3.440	3.790	1.950	4.027	3.442	3.742
0.50	5.388	3.384	5.076	4.530	3.447	3.551	1.273	4.555	3.440	3.791	1.948	4.361	3.441	3.739
0.67	5.357	3.390	5.025	4.800	3.444	3.550	1.267	4.800	3.439	3.793	1.948	4.657	3.440	3.736
0.75	5.337	3.398	5.012	4.899	3.442	3.551	1.263	4.913	3.438	3.793	1.947	4.811	3.439	3.734
0.80	5.315	3.408	4.995	4.980	3.440	3.552	1.262	4.983	3.437	3.795	1.947	4.879	3.438	3.733
0.85	5.297	3.414	4.987	5.047	3.438	3.552	1.260	5.027	3.436	3.796	1.948	4.973	3.436	3.733
0.90	5.278	3.419	4.977	5.112	3.435	3.554	1.258	5.108	3.435	3.798	1.949	5.058	3.434	3.732
0.95	5.256	3.426	4.967	5.171	3.433	3.556	1.256	5.166	3.434	3.800	1.949	5.139	3.434	3.731
0.98	5.240	3.430	4.959	5.206	3.433	3.558	1.256	5.201	3.433	3.801	1.950	5.189	3.433	3.731

[a] All measurements were done at −10 °C. 15% Acetone-d_6 was included in all samples. For the only methanol sample, the proton chemical shifts were found at δ 5.245 and 3.432 ppm for OH and CH_3, respectively. [b] α and [c] β methylene protons of THF.

Table 3 ^{1}H NMR chemical shifts (δ, ppm) of the proton signals observed for the methanol/water/dioxane ternary mixtures with varied mole fractions[a]

Mole fraction (methanol 0.04)		Methanol			
Water	Dioxane	OH	CH_3	Water	Dioxane
0.96	0.00	5.576	3.363	5.257	—
0.94	0.02	5.522	3.360	5.199	3.756
0.92	0.04	5.473	3.356	5.141	3.750
0.88	0.08	5.391	3.356	5.038	3.747
0.78	0.18	5.230	3.361	4.821	3.741
0.68	0.28	5.081	3.372	4.644	3.738
0.58	0.38	4.944	3.383	4.503	3.737
0.48	0.48	4.769	3.393	4.345	3.737
0.38	0.58	4.573	3.401	4.178	3.738
0.28	0.68	4.349	3.410	3.988	3.740
0.18	0.78	4.077	3.418	3.758	3.742
0.08	0.88	3.699	3.424	3.746	3.746
0.04	0.92	3.516	3.433	3.268	3.749
0.02	0.94	3.434	3.434	3.188	3.750
0.00	0.96	3.304	3.440	3.078[b]	3.750
Mole fraction (methanol 0.4)		Methanol			
Water	Dioxane	OH	CH_3	Water	Dioxane
0.60	0.00	5.416	3.376	5.106	—
0.58	0.02	5.381	3.377	5.052	3.744
0.56	0.04	5.349	3.379	5.003	3.743
0.54	0.06	5.311	3.379	4.947	3.740
0.48	0.12	5.223	3.385	4.827	3.737
0.42	0.18	5.120	3.394	4.708	3.736
0.36	0.24	5.019	3.401	4.593	3.735
0.30	0.30	4.913	3.407	4.520	3.735
0.24	0.36	4.798	3.416	4.440	3.736
0.18	0.42	4.671	3.421	4.347	3.736
0.12	0.48	4.519	3.428	4.243	3.737
0.06	0.54	4.371	3.434	4.150	3.739
0.04	0.56	4.317	3.436	4.112	3.739
0.02	0.58	4.249	3.439	4.067	3.741
0.00	0.60	4.210	3.441	4.050[b]	3.741

[a] All measurements were done at −10 °C. 15% Acetone-d_6 was included in all samples. [b] Small amount of residual water was detected.

is stronger than between methanol molecules. The chemical shifts of signals of the protons bonded to carbon atoms (CH_3 and CH_2 groups) did not change significantly upon changes in concentration of the ethers or water. These observations indicate that the influence on the chemical shifts occurs mainly through hydrogen bonding interactions directly affecting the hydrogen bond donor and acceptor sites of the molecules. Therefore, the alteration of hydrogen bond interactions can be monitored by the chemical shift changes observed for the hydroxy proton signals.

In the above systems, the individual effect of water and of ethers on the chemical shift of the hydroxy proton signal of methanol was investigated. To study simultaneously the influence of both water and ring oxygen on the chemical shift of a hydroxy proton signal in a disaccharide, water/dioxane/methanol ternary mixtures were also investigated by NMR spectroscopy. Two series of ternary mixtures of methanol/water/dioxane were prepared, one with constant 0.04 mole fraction of methanol and the other 0.40 (Table 3, Fig. 3). As observed in the binary mixtures, an increased concentration of dioxane causes a shielding of the hydroxy proton of methanol. The shielding of the hydroxy proton of methanol is dependent on the methanol concentration, and is larger at low methanol concentration than at high methanol concentration. For a mole fraction of dioxane ($n_{dioxane}/(n_{dioxane} + n_{H_2O})$) < 0.2, the hydroxy proton signal in the low methanol concentration sample occurs

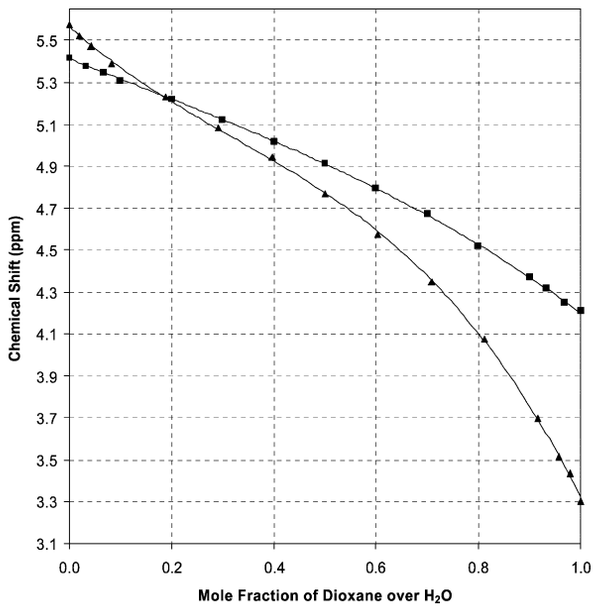


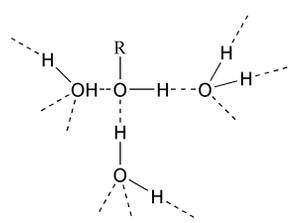
Fig. 3 Chemical shift of the hydroxy proton of methanol as a function of the mole fraction of dioxane over H_2O ($n_{dioxane}/(n_{dioxane} + n_{H_2O})$). The triangles show the data points for the series of mixtures having 0.04 mole fraction of methanol whereas the squares represent the data points for 0.4 mole fraction of methanol. The fitted third degree polynomial curves are to show the trend of change.

slightly downfield relative to that in the high methanol concentration. At a mole fraction of dioxane ($n_{dioxane}/(n_{dioxane} + n_{H_2O})$) > 0.2, the hydroxy proton signal in the low methanol concentration sample becomes more shielded than the one in the high methanol concentration. At high concentration, methanol can be both donor and acceptor of hydrogen bonds and can compete with dioxane and solvate itself, while at low concentration, methanol is mainly solvated by dioxane which can only act as a hydrogen bond acceptor.

The effect of adding acetone to the solvent on the NMR data has been previously studied. In β-cyclodextrin,[8] where hydrogen bond interactions are found between the O(2)H and O(3)H groups, the chemical shifts of the hydroxy proton signals were comparable in 95% H_2O/5% D_2O and 85% H_2O/15% $(CD_3)_2CO$ solutions. Another study[21] on hydrogen bonding in dicarboxylic acids has shown that even in 90% $(CD_3)_2CO$/10% H_2O, the water is sufficient to allow full solvation of the intramolecular hydrogen bonded species.

3 Discussion

Water/alcohol systems are complicated because of the different types of hydrogen bonds that can be formed, and there are still many uncertainties about the nature of the hydration structure of alcohols in water solutions. Water has the highest solvation number with two acceptor sites and two donor sites for hydrogen bonding, and it has been suggested[20] that methanol molecules dissolved in water form three hydrogen bonds as shown in Scheme 1. When the concentration of methanol increases, loss of the hydrogen bond from the third water molecule[20] will occur, and the NMR signal of the methanol hydroxy proton



Scheme 1 Schematic representation of the solvation of methanol in water (taken from ref. 15).

shifts upfield toward the value observed for methanol alone. Thus, the chemical shift of the hydroxy proton is dependent on the hydrogen bond network surrounding it.

In aqueous solutions of carbohydrates, the chemical shift of hydroxy proton signals will represent the hydration state, or in other words, the total number and the lifetime of the hydrogen bonds available to the hydroxy proton under consideration. Thus, the shielding of certain hydroxy protons in an oligosaccharide, as observed for several di-,[1–3] tri-saccharides[4,5] and a Lewis b tetrasaccharide,[6] can be attributed to reduced accessibility of water (Fig. 4). Due to steric factors or intramolecular hydrogen bonds in the sugar residues, the surrounding water molecules around a particular hydroxy proton are excluded, leading to a reduced hydration and a consequent upfield shift of the hydroxy proton signal relative to that in the monosaccharide.

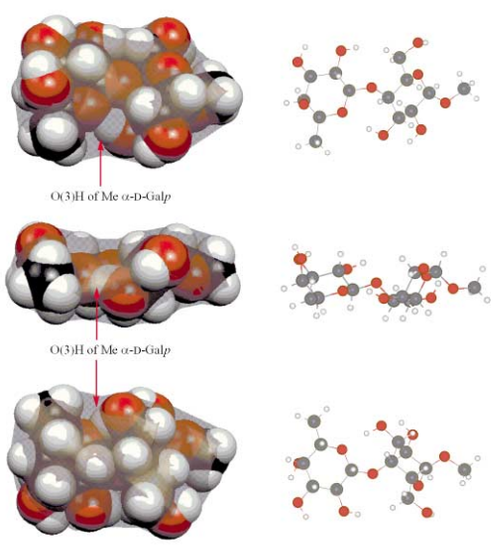


Fig. 4 Ball-and-stick and space filling models of disaccharide **1** in three orientations showing the solvent inaccessible surface[28,29] slightly shaded. The O(3)H hydroxy proton is located within the space surrounded by this surface.

Another question that has to be addressed is the origin of the downfield shifts measured for hydroxy protons close to other hydroxy groups. We propose that a deshielding is observed when the total number of hydrogen bonds to the hydroxy group is the same as in the monosaccharide, but an intermolecular water–hydroxy proton hydrogen bond that was present in the monosaccharide is replaced by an intramolecular OH–OH hydrogen bond. The total number of hydrogen bonds is the same but the intramolecular hydrogen bond has a longer lifetime than that of the intermolecular hydrogen bond to water. Therefore, the electron density at the hydroxy proton is slightly lower, leading to a (small) downfield shift. This has been observed in α-, β-, and γ-cyclodextrins, where the deshielded O(3)H proton was found to be the donor in a hydrogen bond interaction with O(2).[8]

The chemical shift of a hydroxy proton signal in carbohydrates will be a balance between two opposite contributions: (i) a downfield shift due to hydrogen bond interactions and (ii) an upfield shift due to reduced hydration. The chemical shift of hydroxy protons can be used as a probe to monitor the hydration and/or the hydrogen bonding interactions of hydroxy groups in carbohydrates. An upfield shift will indicate reduced hydration due to steric hindrance or intramolecular hydrogen bonding to a ring oxygen, while a downfield shift will show proximity to another hydroxy group. The presence of intramolecular hydrogen bond interactions cannot be deduced from the chemical shifts of hydroxy proton signals, but can be determined from the measurements of temperature coefficients, exchange rates and coupling constants.

For conformationally restrained molecules, hydration can affect the affinity of the ligand for the protein.[22] Before association, the polar groups of both the ligand and receptor are all hydrogen bonded to water, and the water molecules have to be displaced for the complex to be formed.[22] If it is easier to displace water molecules from the less polar regions, the chemical shifts could become a conformational probe used to identify the hydroxy protons of a carbohydrate that could be recognized by proteins.

4 Experimental

Sample preparation for NMR experiments

The compounds for the NMR experiments were commercially available (purities better than 99% for all compounds) and used as supplied. The NMR sample tubes were soaked for more than 1 day in a 50 mM solution of sodium phosphate buffer, pH 7, to minimize adsorption of impurities from glassware[23]. Acetone-d_6 was added to all samples as 15% by volume. It was observed that the shape of the water signal in methanol/water mixtures changed with time. Therefore, prior to the experiments, each sample was shaken and left to equilibrate for at least 5 min. The same procedure was also applied to the ternary mixtures except that they were first shaken vigorously, left to equilibrate for more than 1 h and shaken again prior to NMR measurements.

NMR spectroscopy

NMR experiments were performed on BRUKER DRX-400 and 600 MHz spectrometers. All spectra were recorded at −10 °C with 16 FID's of 32 K data points. The recycle delay was 1.5 s. The ^{1}H NMR spectra were referenced by setting the residual acetone-d_5 signal to $\delta_H = 2.204$ ppm.

Calculations

All calculations were performed on an SGI R10000 workstation, using the Gaussian 98 program package[9]. Using the molecular mechanics program MM3[24], fully relaxed Φ–Ψ energy maps of the disaccharides, **1**, **2**, and **3**, have been prepared[10] by checking the three classical rotamers (*gauche* +, *gauche* −, and *trans*) of all possible dihedrals of the exocyclic substituents and clockwise and counter-clockwise sets of hydroxy protons of secondary alcohols. The lowest energy geometries of MM3 calculations were then taken as starting conformers for a full optimisation using a density functional theory (DFT) method with the standard 6-31G(d) basis set and B3LYP hybrid functional.[25] The functional and basis set were used as supplied in the Gaussian 98 program.[9] The level of theory and basis set configuration (B3LYP/6-31G(d)) has previously been shown to be appropriate for NMR calculations.[26] For all geometry optimisations, the TIGHT keyword calling more restricted convergence criteria was used to achieve better accuracy. The resulting geometries were verified to be the structures of minimum energies *via* frequency calculations. Subsequently, NMR chemical shift calculations using the gauge independent atomic orbital (GIAO)[27] method were performed on the optimised structures using two different model chemistries, HF/6-311++G(2d,2p) and B3LYP/6-311++G(2d,2p). The Hartree–Fock (HF) method was also used to check the computational robustness, since DFT NMR calculations make use of functionals where no intrinsic parameters for magnetic field susceptibilities are involved. All the input and output geometries together with the detailed computational results are available on request.

Acknowledgements

Dr. Roland Stenutz is gratefully acknowledged for valuable discussions. This work was supported by grants from the Swedish Research Council.

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