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Note

High-field, proton-nuclear magnetic resonance study of hydration in concentrated solutions of monosaccharides

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The solvation of sugars in water is of considerable biochemical importance. An impressive array of physicochemical measurements[1–10] has shown that sugar molecules, in dilute aqueous solution, are surrounded by an ordered, long-lived solvational sheath of water molecules. However, the question of sugar–sugar interactions at high concentrations, and whether sugar hydroxyl groups give up specific hydration in favor of sugar–sugar hydrogen-bonds, is unanswered[11,12]. An answer to this question is important for understanding biochemically important molecular interactions involving polysaccharides or immunologically significant glycoproteins. We report here that high-field (360 MHz) proton-nuclear magnetic resonance (n.m.r.) spectroscopy permits the direct observation of both "fully hydrated" and "less hydrated" forms of several D-aldopyranoses at high concentration, or in the presence of pyridine.

EXPERIMENTAL

Sugars were of high commercial grade and were used as supplied. Maximally concentrated solutions were prepared by dissolving weighed portions of sugars in a known volume of ^2H_2O (99%); lesser concentrations were prepared by further dilution with ^2H_2O. Proton-n.m.r. spectra were obtained with a Nicolet NT-360 360-MHz, pulsed f.t. spectrometer using up to 16 K points over a 5-KHz bandwidth. The free-induction decays were apodized by exponential multiplication prior to Fourier transformation. In some cases, resolution enhancement by double exponential multiplication (Nicolet routine, d.m. = 3)[13] was used.

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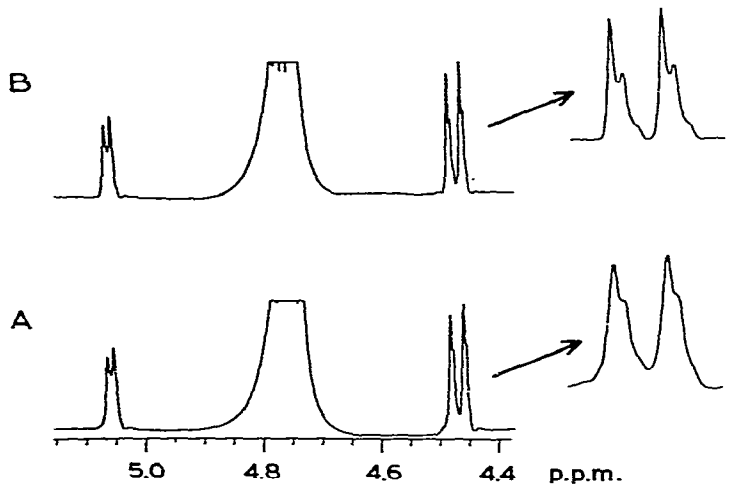

Fig. 1. Proton-n.m.r. spectra (360 MHz) of D-glucopyranose, 2.7M in ^2H_2O. A, regular spectrum; B, as in A, but with double exponential multiplication (d.m. = 3)[13].

RESULTS

The anomeric-proton n.m.r. signals of several D-aldopyranoses clearly revealed the presence of both "hydrated" and "less hydrated" forms at high concentration, or in the presence of added pyridine. As illustrated in Fig. 1, the H-1 resonances of both the α and β anomers of 2.7M D-glucopyranose appear as doublets, as expected from coupling with H-2, but each doublet is accompanied by at least one other doublet, weaker than the first, and with ~0.005 p.p.m. (2 Hz) upfield shift in each case. The relative amplitude of these upfield shifts is concentration-dependent (see later). Both sets of upfield resonances appear in the fully coupled spectrum (A), and are apparent in the broadness of the peaks in the decoupled spectra (not shown) where the H-2 signals of the α and β anomers have been irradiated separately. The doubling of peaks does not arise from long-range coupling, because it occurs at both the α- and β-proton signals, and would require an unreasonably large coupling-constant. Resolution enhancement by double exponential multiplication[13] (d.m. = 3) resolved the upfield member(s) of each pair into two separate peaks (Fig. 1B); we take the more-intense, downfield peak to correspond to the anomeric proton of the fully hydrated form of glucose, and the other two to the anomeric protons of glucose having mono- and non-hydrated anomeric hydroxyl groups. Loss of hydrogen bonding to this hydroxyl group should lead to increased electron density on oxygen, greater shielding, and therefore an upfield shift for the anomeric proton.

The intensity of the smaller, upfield resonances depends on the concentration of glucose, on temperature, and on the concentration of added pyridine, (which is a hydrogen-bond-accepting co-solvent). Upon dilution, the intensity of the smaller, upfield peaks decreases as a function of glucose concentration. There is a general

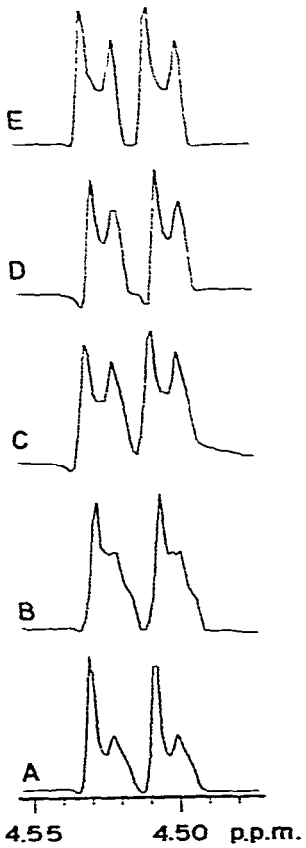

Fig. 2. Proton n.m.r. spectra (360 MHz) of the β-anomeric proton region of D-glucopyranose in ^2H_2O, with addition of increasing aliquots of pyridine; [pyridine]/[sugar] ratios are, A, 0.661; B, 1.327; C, 1.989; D, 3.316; and E, 6.625. Owing to a gradual, downfield shift of the resonances upon addition of pyridine, the chemical shifts given apply only to spectrum A. The initial concentration of glucose was 1.87M.

downfield shift of the anomeric-proton peaks with dilution[6]. Harvey and Symons have suggested that most hydroxyl groups of sugars are doubly hydrogen-bonded in water[6], and this implies a primary hydration shell of ~11 water molecules per glucose, with a possible second hydration shell of 10–20 additional water molecules, hydrogen-bonded to the primary layer. Thermodynamic measurements show that saccharide solutions become more "structured" with increasing concentration; however, a partial retrogradation of hydration occurs as water is partitioned between the bulk and sugar-solvation spheres[12]. In this model, water:glucose ratios of $< \sim 20$ should involve considerable disruption of the hydration scheme that undoubtedly operates in more-dilute solutions. Our results are consistent with this expectation; the anomeric hydroxyl group is not fully hydrated at water:glucose ratios below ~20. Pyridine, which has specific hydration requirements of its own, should compete for water and disrupt hydration of glucose. In Fig. 2, the proportion of the smaller to

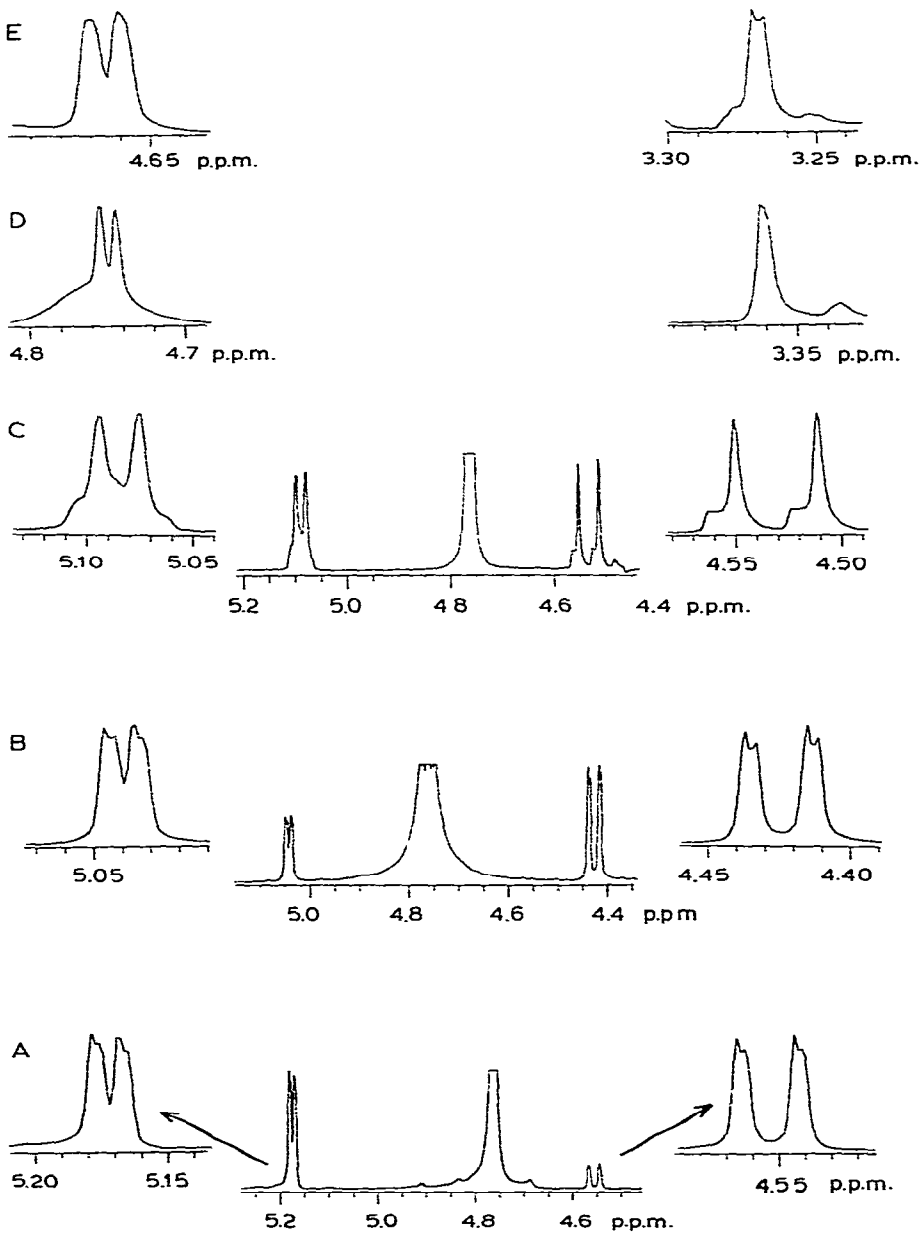

Fig. 3. Proton n.m.r. spectra (360 MHz) of monosaccharides in ^2H_2O. A, 2.755M xylose, added as the α anomer; B, same sample as in A, but with pyridine added to give a [pyridine]/[xylose] ratio of 0.3 (Note that mutarotation has caused increased formation of the β anomer); C, 1.54M 6-deoxy-D-glucose with a [pyridine]/[sugar] ratio of 0.41; D, 2.13M methyl α-D-glucoside; and E, 2.13M methyl α-D-glucoside with a [pyridine]/[sugar] ratio of 0.5. Expansions to the left and right of the full spectra are the α and β anomeric protons, respectively, except for D and E, where the right-hand expansion is the methoxyl peak. Spectrum D also shows a broad, water peak centered at 4.76 p.p.m.

larger peaks for 1.87M glucose is shown to increase as a function of pyridine concentration. There is an upfield shift of the H-1 resonances relative to the water line, and addition of pyridine to ^2H_2O (containing a trace of dimethyl sulfoxide as internal reference) failed to show any shift difference for the water peak. The intensity changes shown in Fig. 3 are not a pH effect, because addition of a small amount of triethylamine, which renders the solution more basic than the large amounts of pyridine, did not cause any change in the anomeric-proton resonances of glucose.

Decreasing the temperature to 4° enhanced the intensity of the anomeric-proton resonances by decreasing their line-widths (not shown). The resolution was improved and the separation between the peaks was more apparent. However, the relative intensities of the peaks were not markedly altered, and neither were they altered when the temperature was raised to 50°.

The presence of separate resonances for the fully hydrated and less hydrated forms of glucose requires that the rate of solvent transfer between them be low on the n.m.r. time-scale, and that the primary hydration-shell of a carbohydrate be long-lived. As proton transfers occur between hydrogen-bonded pairs, the rate of proton exchange within the pair must be equal to or greater than the rate of hydrogen-bond cleavage. The rate of proton exchange at lower temperatures is low enough to permit the direct observation[6-9] of hydroxyl-protons resonances at temperatures near 0° and, at these temperatures, the primary hydration shell must be long-lived in an n.m.r. sense. The surprising result that non-hydrated forms of glucose are long-lived at 24° may be ascribed to cooperative interaction between pairs in some sort of sugar–sugar aggregation. However, we have ascertained that the whole phenomenon is not attributable purely to sugar–sugar aggregation, because no doubling of peaks was observed in a 1.6M solution of D-glucose in dimethyl sulfoxide.

Other D-aldopyranoses also reveal hydrated and less hydrated anomeric hydroxyl groups, as illustrated in Fig. 3. The effect is most pronounced in xylose, but may also be seen in 6-deoxy-α-D-glucopyranose, and methyl α-D-glucopyranoside (with added pyridine). The last compound exhibits only one "less-hydrated" form, as expected from the replacement of the anomeric hydroxyl proton by a methyl group.

A preferential desolvation of the anomeric hydroxyl group is consistent with the earlier, low-field n.m.r. studies of hydration of glucose, which demonstrated that the anomeric hydroxyl group is especially sensitive to increased sugar–sugar interactions. At low temperatures, the individual hydroxyl resonances may be resolved. All hydroxyl-proton resonances are shifted downfield, with the anomeric hydroxyl signals being shifted the most because the inductive effect of the ring-oxygen atom renders them more acidic than the remaining hydroxyl protons[6]. The chemical shift of the anomeric hydroxyl proton is more dependent on sugar concentration than are other sugar hydroxyl resonances. The proton spin–lattice relaxation times (T_1-values) for both α- and β-D-glucopyranose anomeric protons depend upon concentration and temperature, and it has been suggested that the anomeric proton of α-D-glucopyranose is more susceptible than the other sugar protons to intermolecular interactions.

We have demonstrated that the hydration of D-glucopyranose, and other aldopyranoses, may be studied by high-field n.m.r. spectroscopy, and that the concentration dependence of sugar hydration fits a model where dehydration occurs preferentially at the anomeric hydroxyl group in water:sugar ratios of $<20:1$. The unusual kinetic stability of "less hydrated" forms may involve cooperative interactions between partially hydrated sugars.

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