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Note

Heteronuclear coupling constants of hydroxyl protons in a water solution of oligosaccharides: trehalose and sucrose

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Abstract

Relatively few details are known about the conformational preferences of hydroxyl groups in carbohydrates in water solution, though these would be informative about solvation and H-bonding. We show that highly concentrated solutions of sucrose and trehalose exhibit surprisingly well-resolved ^{1}H NMR spectra in a deuterium oxide–water solvent mixture at subzero temperatures. Measurement conditions are suitable to extract nearly all homonuclear and, for the first time, heteronuclear coupling constants of OH groups of carbohydrates in their natural abundance. For $^{2,3}J_{HO,C}$ coupling constants new, powerful variants of HETLOC and HECADE techniques were applied. The present data do not support the presence of persistent H-bonds in these two cryogenic disaccharides.

Keywords: Hydration; Sucrose; Trehalose; NMR coupling constants

One of the most striking (and perhaps least understood) problems in the conformational analysis of carbohydrates is the orientation of their functional groups (OH) in water [1]. Intra- or intermolecular hydrogen bonding may prefer particular OH conformations, and, for example, the hydroxymethyl conformation is of particular importance in the theoretical studies of hexopyranoses. Recent molecular dynamics studies demonstrate that water molecules in the first hydration shell have a longer residence time due to the multitude of hydrogen bonds between the water molecules and the hydroxyl groups [2,3]. To the best of our knowledge, no methods or data have been reported on the heteronuclear couplings of OH protons in pure water solution, though they could be reporters of conformational preferences. In this work, we determine the heteronuclear coupling constants [4], relevant to OH conformations [5], for two cryogenic disaccharides.

Vicinal H–O–C–H proton–proton couplings were measured a long time ago using organic solvents [6] and Karplus parameters [6a], while the dihedral angle dependence was suggested for this particular case. Such cou-

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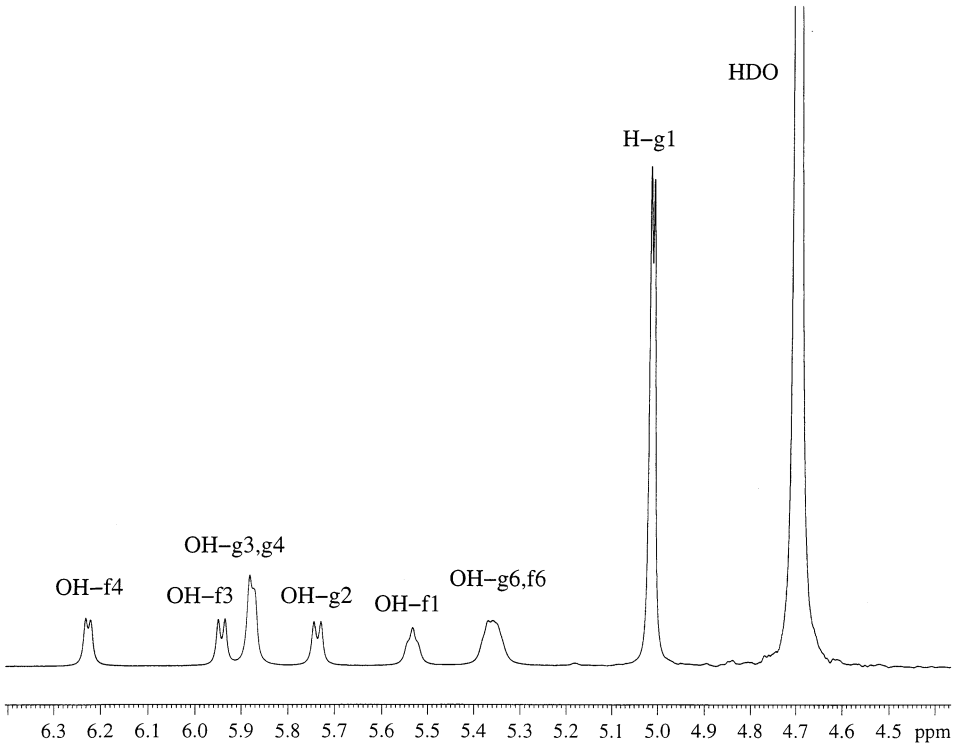

Fig. 1. Partial ^{1}H NMR spectrum of a 1.7 M sucrose 22:3 solution of deuterium oxide–water at 270 K. No water suppression or resolution enhancement was applied. The chemical shift scale is arbitrarily referenced to 4.7 ppm for the water signal. OH protons are labeled with 'g' or 'f' for the glucopyranose or fructofuranose residues, respectively. H–g1 is the anomeric proton in the glucopryranosyl ring. OH-signals were assigned in a DQ-filtered COSY experiment, based on known skeleton proton assignment.

pling constants in branched trisaccharides have been reported recently in mixed solvents (water–$(CD_3)_2CO$) at -8 °C [7] and large (~ 9 Hz) homonuclear couplings were attributed to H bonding. Heteronuclear couplings of OH groups in α-D-mannofuranoside rings were measured in dimethyl sulfoxide-$^{2}H_6$ (Me_2SO-d_6) [6b] solution, and more recent NMR methods were applied to measure $^{n}J_{C,2'-OH}$ couplings of ribonucleosides [6c] in the same solvent. Long-range intra-ring or inter-residue $^{1}H-^{13}C$ couplings have been reported using a variety of NMR techniques for mono- and oligosaccharides [8] and nucleic acids [9], sometimes using ^{13}C labeling [10,11]. Me_2SO-d_6 is a useful solvent for unprotected carbohy-

Table 1
Hetero- and homonuclear couplings of OH protons of sucrose in water solution [a]

OH ($^{3}J_{HH}$)	C–f1	C–f2	C–f3	C–f4	C–f5	C–f6
f1 (6.0)	−3.4(0.6)	*				
f3 (7.5)		*	−1.9(0.4)	+2.0(0.4)		
f4 (5.6)			+1.9(0.8)	−2.9(1.0)	+3.9(0.8)	
f6 (5.2)					+1.5(0.8)	−2.9(1.2)
	C–g1	C–g2	C–g3	C–g4	C–g5	C–g6
g2 (7.4)	+2.4(0.4)	−2.6(0.8)	+2.1(0.9)			
g3 (6.3)		+1.9(0.4)	*	*		
g4 (6.3)			*	*	+1.6(0.8)	
g6 (5.1)					N/A	−3.1(0.9)

[a] Homonuclear couplings were measured from 1D experiments. Heteronuclear coupling constants and error estimates are obtained from the average of three SEHETLOC and three HECADE experiments. Couplings to quaternary carbon–f2 or to overlapping OH–g3 and OH–g4 were only detected (* not evaluated) in an HMBC experiment.

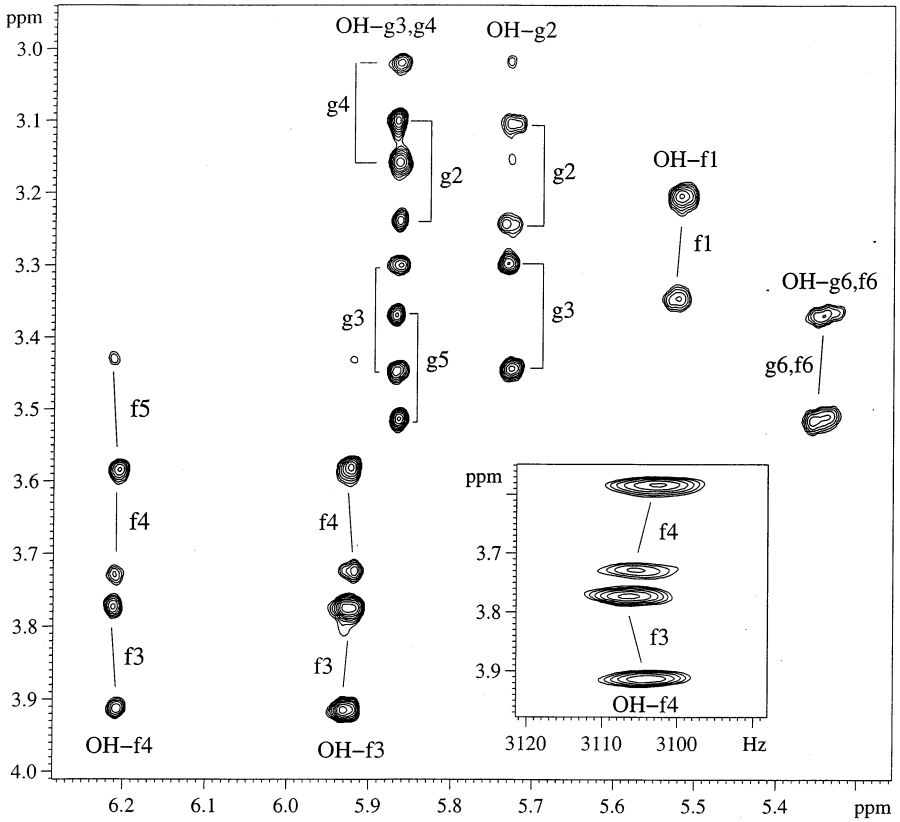

Fig. 2. Partial $^{13}C(\omega 1)$-filtered, gradient enhanced (echo–antiecho) TOCSY[11] spectrum of a 1.7 M sucrose 22:3 solution of deuterium oxide–water at 270 K. One-bond coupling constants are scaled by a factor of 0.5 in the f1 dimension in order to reduce spectral overlap. The Watergate solvent suppression scheme was applied at the end of the pulse sequence. Thirty-two scans were accumulated in each of the 256 experiments. A relaxation delay of 0.8 s was allowed, acquisition time was 0.25 s and resulted in 4 h total measuring time. The DIPSI-2 sequence lasted 48 ms for magnetization transfer. Squared cosine and cosine weighting functions were applied in the t2 and t1 domains (2K × 1K data table). No baseline correction or other post-processing tools were applied.

drates. However, it should also be kept in mind that H-bonds that persist in Me_2SO may disappear in water [12]. In order to reduce fast OH exchange, mixtures of acetone-d_6–water can be used between 250 and 273 K [13,14]. However, sometimes the hemiacetal form of acetone can produce a disturbing signal in the OH region of the ^1H NMR spectrum. 'Supercooling' of carbohydrates [15] in a 9:1 solution of water–deuterium oxide is a rare method, since sudden formation of crystallization centers is probable. Homonuclear OH couplings [16] were shown to have little dependence on solvent composition in cryogenic media in the

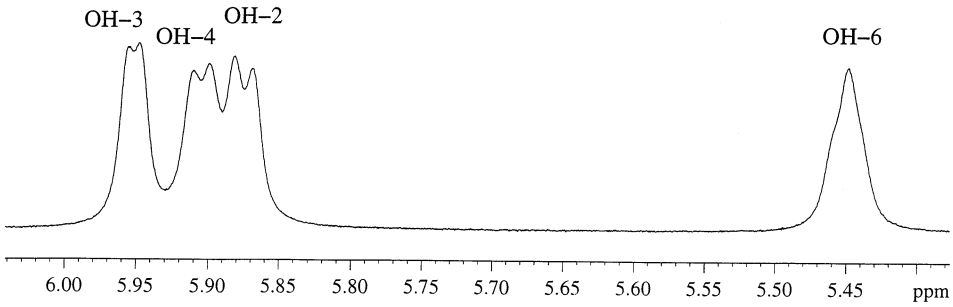

Fig. 3. Partial ^1H NMR spectrum of a 1.7 M trehalose 97:3 solution of deuterium oxide–water at 270 K. No water suppression or resolution enhancement was applied. The chemical shift scale is arbitrarily referenced to 4.7 ppm for the water signal. OH-signals were assigned in a DQ-filtered COSY experiment, based on known skeleton proton assignments.

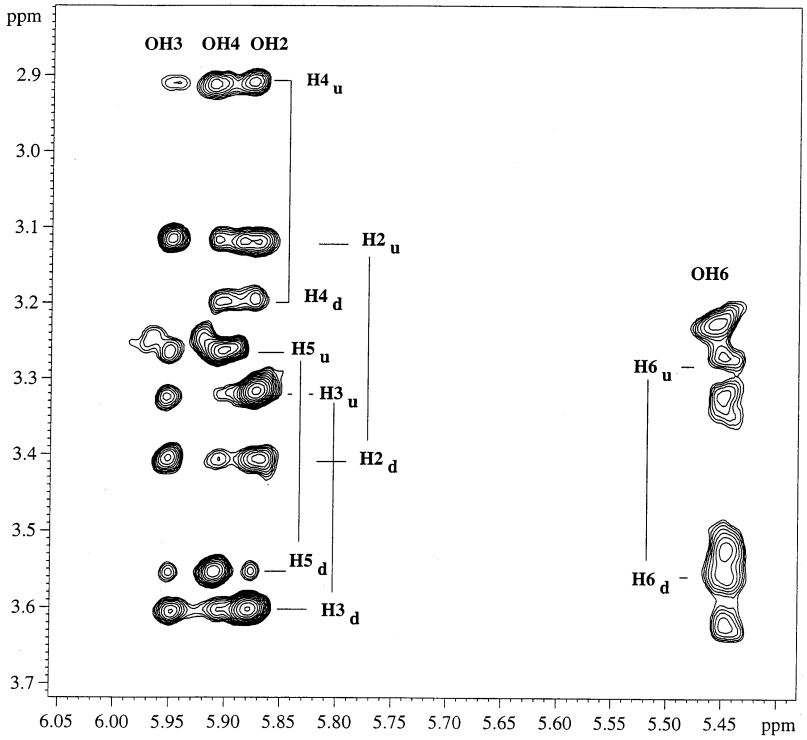

Fig. 4. Partial $^{13}C(\omega 1)$-filtered, gradient enhanced (echo–antiecho) TOCSY[11] spectrum of a 1.7 M α,α-trehalose 97:3 solution of deuterium oxide–water at 270 K. No *J*-scaling or water suppression was applied. Eighty scans were accumulated in each of the 256 experiments. A relaxation delay of 1 s was allowed; acquisition time was 0.25 s. The DIPSI-2 sequence lasted 52 ms for magnetization transfer. Squared cosine or cosine weighting functions were applied in the $t2$ and $t1$ domains, respectively (2K × 1K data table). Linear baseline correction was applied in both directions in the plotted region.

case of simple monosaccharides and sucrose. However, this may not be the case for more complex oligomers or when carbohydrates are involved in molecular recognition processes.

In this work, we suggest suitable experiments for measuring homo- and heteronuclear long-range OH couplings of carbohydrates in water–deuterium oxide solutions at low temperature. We can utilize two distinct features of carbohydrates, namely their enormous solubility in water and their potential to decrease the freezing point of water. As examples, we chose sucrose and α,α-trehalose due to the fact that at high concentration their water solutions can be cooled down to ca. 265–270 K. The reduced exchange rate sharpens the proton lineshapes, which more than overpowers inhomogeneous broadening due to increased rotational correlation times (1.5–2 ns for the particular solutions). In addition, apparent exchange rates can be further reduced by decreasing the concentration of the 'reactant' water. Our 1.7 M sucrose 22:3 solution of deuterium oxide–water still gives more than a 0.2 M concentration for exchangeable OH protons. As a consequence, radiation damping becomes less serious and water suppression is not a must. We found that excellent ^1H resolu-

Table 2
Hetero- and homonuclear couplings of OH protons of α,α-trehalose in water solution [a]

OH ($^3J_{HH}$)	C-1	C-2	C-3	C-4	C-5	C-6
2-OH (6.8)	N/A	−2.8(0.6)	+2.6(0.8)			
3-OH (5.1)		+1.9(0.5)	−3.2(1.2)	+1.5(0.8)		
4-OH (6.6)			+1.8(1.1)	−2.5(1.2)	+1.6(1.0)	
6-OH (5.3)					N/A	−4.2(1.0)

[a] Homonuclear couplings were obtained in 1D experiments (Fig. 3). The heteronuclear couplings are the average of two unscaled SEHETLOC (Fig. 4) and a HECADE experiment.

tion and lineshape can be obtained at 270 K, as shown in Fig. 1.

Basically, two recent methods proved to be applicable to resolve long-range heteronuclear couplings of slowly exchanging OH groups. Both are based on the resolving power of heteronuclear E-COSY [17] related experiments. One is a new X(ω1)-filtered, sensitivity enhanced, F1 decoupled, (optionally *J*-scaled) TOCSY experiment [18] (also abbreviated as 'SEHETLOC' for brevity), the other one is the so-called HECADE [19] method, which uses accordion time evolution and ^{13}C chemical shifts in ω1 dimension. The former experiment has higher sensitivity, the latter exhibits better resolving power. We also measured the homonuclear coupling constants in sucrose and found them to be similar to those reported by Adams and Lerner in a mixed solvent [20] (Table 1). This observation warrants that OH proton exchange is sufficiently slow to measure small heteronuclear couplings. Solute–solute interactions are supposed to be negligible since full hydration (10–14 water molecules) is still possible. Furthermore, a low-temperature (270 K) off-resonance ROESY [21] experiment (spin lock time ~ 0.1 s/axis tilt angle $\theta = 55°$) with Watergate suppression scheme shows that the direct exchange is the dominant mechanism between the OH and water protons [7][1].

Heteronuclear coupling constants of sucrose (Table 1) were extracted from *J*/2- and *J*/3-scaled, and unscaled, sensitivity enhanced ^{13}C(ω1)-filtered TOCSY experiments [18] (Fig. 2). No fitting of the shifted pattern was used and the couplings are obtained from shifted cross sections of cross peaks. OH couplings to the quaternary carbon–f2 could only be detected in a separate HMBC experiment. Because of the overlap of g3–g4, and g6–f6 hydroxyl proton pairs, HECADE and its *J*/2-scaled variant were invaluable for measurement of $^2J_{OH,C}$ within hydroxymethyl groups.

Several theoretical and experimental studies [22] have been published recently on the structure of α,α-trehalose in water. The present homo- and heteronuclear couplings (Table 2)[2] suggest, similar to that of sucrose and monosaccharides [23], no persistent intramolecular H-bond exists in the water solution of trehalose. Three-bond proton–proton couplings are in the 5–7 Hz region, which is expected for freely rotating OH groups [15]. It is known for carbohydrates that vicinal $^1H-^1H$ couplings rarely exceed 10 Hz, and this limit is ca. 5–6 Hz for $^1H-^{13}C$ long-range couplings through an interglycosidic oxygen atom [24]. Our measured heteronuclear three-bond couplings scatter between ca. $+1$ and $+3$ Hz, which also suggests that OH–s cannot be antiperiplanar with respect to vicinal carbons [25a][3]. Consequently, secondary hydroxyl groups are not likely to form a ring of persistent cooperative hydrogen bonds clockwise or counter-clockwise about the pyranose rings in trehalose [25b]. Two-bond $OH-^{13}C$ couplings were found to be negative in both disaccharides and measured between -3 and -4 Hz. In summary, we have demonstrated that the methods presented here are capable of measuring long-range couplings between OH and skeleton carbons in simple disaccharides. Presumably, such couplings can be measured in higher oligomers in water solution, which may be particularly important when proton couplings are difficult to resolve.

1. Experimental

A Bruker DRX 500 NMR spectrometer operated at 500.13/125.89 MHz ($^1H-^{13}C$) was used in all experiments. $^1H-^{13}C$ 90° pulses were 13 and 11.8 μs, respectively. The temperature was controlled by the manufacturer's B-VT 2000 unit. ^1H chemical shifts on the figures are arbitrarily referenced to HDO (4.7

[1] Interestingly, some weak, direct OH–OH exchange persists between OH-g6 and OH-g3 or OH-g4, and produces a cross peak of ca. 5–10% of the diagonal OH signals. Poppe and van Halbeek [15] also reported such an effect between OH-g2 and OH-1f under different conditions. However, interpretation of the weak direct exchange between OHs is difficult, and is not necessarily related to H-bonding.

[2] When a 4:1 water–deuterium oxide solvent was used, the homonuclear couplings remained unresolved in trehalose. However, heteronuclear couplings were still measurable from the tilted multiplet pattern.

[3] In the same experiment, we measured a $^3J_{H1,C3}$ 5.1 Hz value according to the known conformation of the glucopyranose ring (approximate dihedral angle is 175°).

ppm). For accurate chemical shifts at 270 K, an extra +0.34 ppm correction should be added according to the $\delta = 7.83 - T/96.9$ formula. OH assignments are based on DQF-COSY experiments. Generally 0.8–1 s delay times were allowed between scans. In the SE-HETLOC sequence, sine bell-shaped z-gradient pulses of 1 ms duration and 5 G/cm were applied with alternating sign for echo–antiecho coherence selection. The G-BIRD sequence element accomplished broadband homonuclear decoupling in the F1 dimension. Optional J-scaling in F1 was applied as described [18]. DIPSI-2 mixing times of 50–90 ms were applied for efficient TOCSY transfer. In the HECADE experiments, a 10 G/cm gradient was inserted for final coherence selection and the single-bond J-splittings in the F1 dimension were scaled by a factor of 0.5–0.6 in order to reduce spectral overlap. DIPSI-2 mixing times of 35–50 ms were applied. Coupling constants can be read in both experiments from the shifted CH doublet pattern in the F2 (acquisition) dimension.

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