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Observation of Hydroxyl Protons of Sucrose in Aqueous Solution: No Evidence for Persistent Intramolecular Hydrogen Bonds

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Abstract: We have measured temperature coefficients of the chemical shifts, scalar coupling constants, and exchange rates for the hydroxyl protons in sucrose in mixtures of water and acetone. The values measured are virtually the same for all the hydroxyl protons in sucrose. These observations indicate that there are no persistent hydrogen bonds in sucrose in aqueous solution.

Introduction

The solution conformation of sucrose (Figure 1) remains the subject of continuing interest. An early interpretation of proton NMR data and hard sphere exoanomeric calculations was that the solution structure was "rather rigid", being essentially the same as the crystal structure, including the persistence in solution of the OH-1^f to O-2^g hydrogen bond seen in the crystalline solid.[1] Detailed ^{13}C relaxation and NOE studies have been interpreted similarly as supporting a rigid conformation in solution.[2,3]

More recently, several groups have begun to question the rigidity of sucrose in aqueous solution. Perez and co-workers[4] have argued that the observed proton–proton NOEs are not consistent with any single conformation. Using molecular mechanics and molecular dynamics, Petillo[5] and Tran and Brady[6] have found several conformations for sucrose lower in energy, by ~10 kJ/mol, than the crystal structure. Most recently, Poppe and van Halbeek have demonstrated further discrepancies between proton NOEs and ROEs actually observed and those predicted for a rigid molecule.[7]

If sucrose were to have a rigid structure in aqueous solution, and particularly one other than the most stable conformation, some means of maintaining this structure must be found. The most likely candidate would be one or more interresidue hydrogen bonds, as is seen in the crystal structure and in nonaqueous solution.[1,8] However, Williams and co-workers have recently calculated the energetics of a hydroxyl-to-hydroxyl hydrogen bond to be virtually the same as that for a hydroxyl-to-water bond.[9] This makes it questionable whether the interresidue hydrogen bonds could in fact supply the needed stabilization.

High-resolution NMR has been used in several different ways to infer the existence of hydrogen bonds. (i) In studies of model compounds such as alcohols, it has been observed that hydrogen bonding causes a downfield change in chemical shifts of the involved protons, and that increasing temperature causes an upfield change.[10] Some previous studies have reported smaller temperature coefficients for hydrogen-bonded hydroxyl protons in sugars.[11,12] (ii) Coupling constants and nuclear Overhauser enhancement (NOE) measurements may be used to establish whether or not a particular hydrogen bond is sterically possible. (iii) Further indirect evidence may come from the correlation times of ^{13}C nuclei, if intramolecular hydrogen bonds restrict their motions.[1-3]

Until recently these types of information were obtainable only from ring protons or carbons, or from hydroxyl protons in nonaqueous solution, because of the difficulty in observing hydroxyl protons. But inferring the existence of hydrogen bonds from indirect evidence is not infallible. For example, while relaxation times can be used to detect restricted motion, the restriction may arise from something other than an intramolecular hydrogen bond. Ideally, the most direct evidence for hydrogen bonds would come from NMR parameters for nuclei directly involved in the hydrogen bond.

It has been difficult to observe directly the hydroxyl protons in H_2O because of their rapid exchange with bulk water, as well as the dynamic range problem of observation in H_2O. Recently, several groups have reported success in observing these elusive protons, by lowering the temperature to slow exchange, and by using pulse sequences that suppress the water signal.[12-14] We have used ^1H NMR of hydroxyl protons to seek evidence for intramolecular hydrogen bonds in sucrose in mixtures of water and acetone-d_6.

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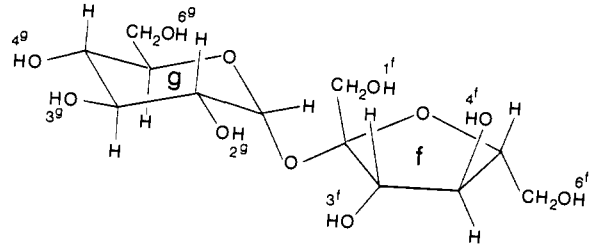

Figure 1. Sucrose, with hydroxyl protons labeled.

Materials and Methods

All chemicals used were reagent grade. Sucrose was purchased from Amend Drug and Chemical Company and used without further purification. Deuterated solvents were purchased from Aldrich Chemical Company. H_2O used in sample preparation was distilled, deionized, ultrafiltered to a resistance greater than 18 MΩ using a Millipore Milli-Q apparatus, and then degassed at aspirator pressure in an ultrasonic bath, followed by saturation with N_2(g). The degas/saturate cycle was repeated three times.

In order to minimize the presence of contaminants that could catalyze exchange, NMR sample tubes were prepared in the following manner. After being thoroughly rinsed, tubes were soaked for a minimum of I h in a 50 mM solution of phosphate buffer, pH 7. Then the tubes were rinsed with the degassed water described above and dried under a stream of nitrogen.

The samples themselves were prepared so as to avoid possible contamination from atmospheric CO_2, as well as from glass surface impurities. All solutions were stored in closed plastic containers in a glovebag maintained under a nitrogen atmosphere, and all sample preparation was done in this bag. All transfers were done using plastic labware. The NMR tubes, after the preparation described above, were filled and sealed in the glovebag, using an Omni-Fit sealing NMR tube cap (Wilmad Glass, Buena, NJ).

Exchange rates were measured using the saturation transfer method described in ref 15, on a Varian UNITY 500 spectrometer. Intensity vs spin-lock time data were fit using software supplied by Varian. The reported error of 15% in exchange rates was calculated by assuming a 10% uncertainty in determination of the peak amplitude in a saturation transfer experiment, and then propagating this error through the calculation of the exchange rate.

Results and Discussion

We have examined the temperature coefficients, coupling constants, and exchange rates of the hydroxyl protons in sucrose, looking for evidence of hydrogen bonding. Our underlying assumption is that a hydrogen bond will decrease the temperature sensitivity of the chemical shift, possibly restrict the rotation about the C–O bond, and reduce the exchange rate with water for such nuclei. As discussed below, interpretation of these NMR parameters solely in terms of hydrogen bonding is not straightforward. Other factors may contribute to, or counteract changes in, chemical shifts, coupling constants, and exchange rates. However, at the least, if any of these NMR parameters is different for any of the hydroxyl protons in sucrose, it would be suggestive of involvement in a hydrogen bond.

Chemical Shift Temperature Coefficients. The changes with temperature of the chemical shifts of the hydroxyl protons in sucrose are shown in Figure 2, for a range of acetone-d_6:H_2O ratios. As can be seen in the figure, the coefficients for OH-1^f, -4^f, -6^f, -3^g, -4^g, and -6^g are virtually the same. The coefficient is slightly lower for OH-2^g and higher for OH-3^f. This near equivalence of temperature coefficients constrasts sharply with a recent report by Poppe and van Halbeek,[12] in which a proton clearly involved in an intramolecular hydrogen bond had a temperature coefficient which was an order of magnitude smaller than those for other hydroxyls.

It is noteworthy that, for all the hydroxyl protons we observe in sucrose, there is no change on going from a solvent composition which is largely nonaqueous (4:1 v/v acetone-d_6/water) to one which has water as the major component. Data (now shown) for 1:9 v/v acetone-d_6/water shows that this trend continues, there being at this solvent composition no change in either relative or absolute temperature coefficients of the chemical shifts.

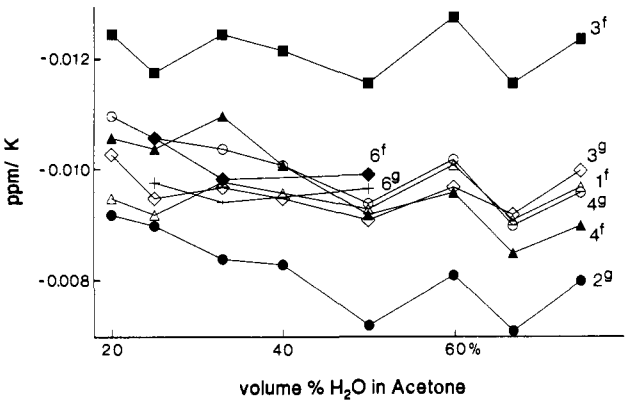

Figure 2. Temperature coefficient of the chemical shift (ppm/K) for hydroxyl resonances in sucrose in mixtures of H_2O/acetone-d_6, as a function of volume percent H_2O. ^1H spectra were obtained at 500 MHz, using the 1–1 echo sequence as described in Figure 3. Temperature was controlled by the varian variable-temperature unit supplied with the spectrometer.

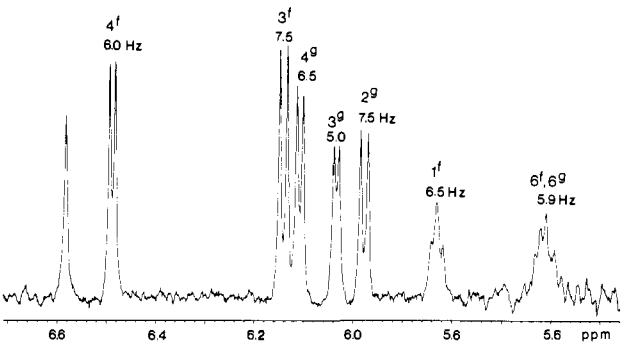

Figure 3. Region of the ^1H spectrum of sucrose, at 500 MHz, temperature 253 K, showing hydroxyl proton resonances. Resonances are labeled with their assignments and coupling constants (between hydroxyl and ring protons). The sample was 50 mM sucrose, in 1:2 acetone-d_6/H_2O. The spectrum was obtained using the 1–1 echo sequence developed by Sklenar and Bax,[18] with a spectral width (SW) of ±2600 Hz, 5212 data points zero-filled to 16384 points. Gaussian apodization was applied. The single peak at ~6.58 ppm appears in all samples prepared with acetone-d_6, but not in samples prepared with acetonitrile-d_3 or DMSO-d_6. Its size increases with increasing volume percent acetone-d_6 in the sample. This signal exchanges with water but shows no other correlations in a TOCSY spectrum.[15] Therefore, we suggest that it is a signal from the hemiacetal form of acetone. The coupling constant of 5.9 Hz reported for OH-6^f and OH-6^g was obtained by inspection of numerous other spectra (not shown) with better signal-to-noise than the spectrum shown here.

Ring–Hydroxyl Coupling Constants. Because the scalar coupling of hydroxyl protons to vicinal ring protons has a Karplus-type dependence on dihedral angle, a hydrogen bond which enforced some particular angle could be reflected in a deviation of the coupling constant for that hydroxyl from the rotationally averaged value. Scalar coupling constants (J_{HCOH}) between hydroxyl and ring protons were measured, by simple first-order analysis, for several different solvent compositions. Values are shown above their appropriate resonances in Figure 3, a one-dimensional spectrum of 50 mM sucrose in 3:1 acetone-d_6/water. The range of values, 6.5 ± ~1 Hz, is typical for free rotation about the C–O axis.[16] As with the temperature coefficients, the hydroxyl–ring coupling constants are all independent of solvent composition. They are also independent of temperature and exchange with solvent.

Particularly noteworthy is the appearance of OH-1^f as a triplet (seen more clearly in spectra taken under conditions of even slower exchange than shown), implying relatively free rotation about the

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Table I. Exchange Rates[a] for Hydroxyl Protons in Sucrose at 273 K in Mixtures of Acetone-d_6/Water

acetone-d_6/ water	hydroxyl proton					
	1^f	3^f	4^f	2^g	3^g	4^g
3:1	9.33	3.98	3.90	2.93	5.32	3.48
1:3	11.9	nm[b]	12.6	11.2	11.4	nm

[a] In s^{-1}. [b] Not measured.

$C1^f$–O bond, and thus implying that this hydroxyl cannot be held rigidly in any one conformation.

Hydroxyl Proton Exchange Rates. Protons engaged in strong intramolecular hydrogen bonds exchange more slowly with solvent.[17] Solvent accessibility could be reduced by participation in a hydrogen bond, although other causes are possible.

The exchange rates for sucrose hydroxyl protons in mixtures of 3:1 and 1:3 acetone-d_6/water are listed in Table I. The uncertainty in each value is no more than 15%. (Exchange rates for OH-6^f and OH-6^g could not be quantified: first, because their signals were overlapping; second, being closest to the water resonance, they were somewhat affected by the selective pulses.) The measured exchange rates, while strongly dependent on solvent type and composition,[14] are all of the same order of magnitude, further lack of evidence for the presence of intramolecular hydrogen bonds. In 1:3 v/v acetone-d_6/water, there are clearly no differences among those rates which can be measured. (Resonances for OH-3^f and OH-4^g overlap at most temperatures in this solvent composition, preventing independent determination of their exchange rates.) In 3:1 v/v acetone-d_6/water, OH-1^f is exchanging notably faster than others, just the reverse of the expected effect, since decreasing the water content would favor intramolecular H-bonding, which would slow exchange. The reason for this is that OH-1^f is a primary hydroxyl, more accessible to solvent. The 6^f and 6^g hydroxyls also show an elevated exchange rate under these conditions. For example, in Figure 3, the broadening of the OH-1^f and OH-$6^f/6^g$ resonances is caused by exchange with water. A more detailed analysis of exchange data (to be presented elsewhere) shows that the enthalpies and entropies of activation for the exchange with water are very similar for all hydroxyls, and the free energies of activation are virtually identical.

Poppe and van Halbeek[7] have presented evidence for the direct exchange of OH-1^f and OH-2^g in 1:6 v/v acetone-d_6/water, from which it may be inferred that the hydrogen bond observed between these two positions exists at least transiently in solution. The exchange rate they observe is much less than that we observe for the exchange of either of these hydroxyl protons with water. In all of our experiments, exchange with water completely dominates. Most significantly, in a 2-D TOCSY experiment,[15] we observed exchange cross peaks only between hydroxyl protons and water. The absence of significant interresidue cross peaks between hydroxyls in sucrose indicates an exchange rate on a time scale much slower than the reciprocal of the mixing time (100 ms).

Conclusions

On the basis of the criteria of temperature coefficients, coupling constants, and exchange rates, our conclusion is that none of the hydroxyl protons in sucrose is involved in a hydrogen bond long-lived enough to affect these NMR parameters. Rather, all the hydroxyl protons are essentially equivalent in terms of the parameters we have measured. The best explanation for this is that all the sucrose hydroxyl protons are interacting with the solvent, presumably hydrogen bonding to water molecules.

At this point, virtually every possible means for detecting the presence of hydrogen bonds has been applied to the sucrose molecule, and virtually every atom in the molecule has been examined for clues. The last source of information, the hydroxyl protons themselves, has been explored in this work. The NMR parameters for these protons do not support the existence of intramolecular hydrogen bonds for sucrose that persist in aqueous solutions. Consequently, such hydrogen bonding cannot be invoked as a possible means of conferring rigidity to sucrose in aqueous solution. Intramolecular hydrogen bonds could occur in a flexible molecule, breaking and reforming as the molecule undergoes conformational changes. We cannot exclude the possibility that intramolecular hydrogen bonds exist transiently, but our measurements indicate that hydrogen bonds to water predominate.

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