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Structure-activity relationships in carbohydrates revealed by their hydration☆

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ABSTRACT

One of the more intriguing aspects of carbohydrate chemistry is that despite having very similar molecular structures, sugars have very different properties. For instance, there is a sensible difference in sweet taste between glucose and trehalose, even though trehalose is a disaccharide that comprised two glucose units, suggesting a different ability of these two carbohydrates to bind to sweet receptors. Here we have looked at the hydration of specific sites and at the three-dimensional configuration of water molecules around three carbohydrates (glucose, cellobiose, and trehalose), combining neutron diffraction data with computer modelling. Results indicate that identical chemical groups can have radically different hydration patterns depending on their location on a given molecule. These differences can be linked with the specific activity of glucose, cellobiose, and trehalose as a sweet substance, as building block of cellulose fiber, and as a bioprotective agent, respectively. This article is part of a Special Issue entitled "Recent Advances in Bionanomaterials" Guest Editors: Dr. Marie-Louise Saboungi and Dr. Samuel D. Bader.

1. Introduction

Carbohydrates are among the most important naturally occurring biomolecules, with the premier role in metabolism of all living species. These molecules also have a myriad of other functions, working as bioprotective agents against dehydration and other environmental pressures and are important to the food industry as well as being a potential source of fuels.

One of the more intriguing aspects of carbohydrate chemistry is that despite having very similar molecular structures – which often only vary with respect to stereochemistry – sugars have very different properties which affect their ability to bind to protein receptors. For instance, there is a sensible difference in sweet taste between glucose and trehalose [1], even though trehalose is a disaccharide that comprised two glucose units (linked by an $\alpha-1,1$-glycosidic bond). Further, the intermolecular hydrogen bonding between glycol OH groups, obviously present both in glucose and in trehalose, and the taste bud receptor site is considered the primary mechanism for sweet taste response by humans [2] and as such should be sufficient to elicit sweet response [2], even though in practice it does not.

In the present work, we have considered the possibility that the different properties of carbohydrates, namely their ability to interact with receptors and/or other proteins, might be related to their different hydration shells. To tackle this issue, the hydration shell of glucose in solution has been determined using neutron diffraction experiments, enhanced by isotopic substitution (NDIS). Results are compared with the hydration shell of two disaccharides, namely trehalose [3] and cellobiose [4], which are both made up by two glucose units yet linked by a different glycosidic bond. The rationale behind this approach is to compare the hydration pattern of the same building block, namely a single glucose unit, in three different situations: for glucose as a monomer, and when it is in two glucose-containing disaccharides with a different glycosidic bond between the glucose monomers, namely an $\alpha-1,1$-glycosidic bond for trehalose and a $\beta-1,4$-glycosidic bond for cellobiose. Interestingly and relevant in the present context, this latter carbohydrate is reported as tasteless. NDIS can measure the hydrogen bonding of molecules in aqueous solutions on the atomic length scale and as such is one of the

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premier techniques for structural determinations of hydrogen bonding in hydrogen-containing solutions [5–8], including a number of biological molecules [9–17] in aqueous solutions. This is due, in part, to the fact that hydrogen and deuterium give a strong yet different signal in a neutron diffraction pattern. H/D substitution techniques allow the average structure and number of hydrogen bonding interactions to be assessed, with no need for extrapolation of structure at the atomic scale from dynamical properties of the sample.

The NDIS experiments have been augmented by two different tools: Empirical Potential Structure Refinement (EPSR) [18] and ANGULA [19,20]. EPSR is a computational technique which has been explicitly designed to provide atomic scale detail for disordered systems, using a set of NDIS data to constrain the 3-dimensional model of the solution in question [21–23]. While EPSR does not necessarily provide the only possible interpretation of the structural data, it does provide a model that is consistent with the measured diffraction data. The program ANGULA was used to characterize the position of neighboring water molecules around the solute molecules – glucose, trehalose or cellobiose. The rationale behind such a combined experimental and computer simulation approach is to reveal similarities and differences between the local hydration of molecular sites and the hydration shells of glucose, cellobiose, and trehalose, linking the hydrated structure of each solute, on the atomic length scale, with its specific activity as a sweet substance, as building block of cellulose fiber, and as a bioprotective agent, respectively.

Linking the hydration of a sugar to its subsequent function could not only unveil the link between structure and function of carbohydrates in solution, but it could also help to unravel the biochemistry of sweet taste, a process that is yet to be fully understood [24]. It is relevant here to underline that to provide a full picture of the structure-function relationship presented by carbohydrates in solution, dynamical aspects of carbohydrate-water interactions and their implications in the sweetness reception should also be taken into consideration. While a very large body of information is available in the literature on the dynamical aspects of carbohydrate hydration (see for instance [25,26] and references therein), nothing is available on the sugar-sweet receptor dynamics of docking, possibly due to the lack of crystallographic structure of the receptor-ligand complex.

2. Experimental and data analysis

Neutron diffraction experiments have been performed using the SANDALS neutron diffractometer, installed at the ISIS Facility (U.K.) [35], on an isotopomeric series of glucose-water solutions each at two concentrations corresponding to 1 solute molecule per 12.5 water molecules (∼ 4.4 M), and to 1 solute molecule per 50 water molecules (∼ 1.1 M), at ambient temperature and pressure (298 K, 1 bar). NDIS data for trehalose [3] and cellobiose [4] have been already published. Glucose ($C_6H_{12}O_6$) samples were purchased from Sigma-Aldrich and used without further purification. The sample list included fully hydrogenated and fully deuterated glucose (labelled as H and D, respectively), along with partially deuterated glucose samples, labelled H7 (all hydroxyl groups deuterated), and D7 with all hydrogen atoms, but those in the hydroxyl groups, deuterated. To fully exploit the advantages of isotopic substitution, a set of isotopically labelled glucose solutions were prepared (see Table 1). Neutron diffraction data have been collected for the empty instrument, empty container and vanadium standard, in order to normalize the data for all investigated samples to an absolute scale. These data sets have been processed using the "Gudrun" suite of programs [27,28], which performs corrections for multiple scattering, absorption, inelasticity effects, and scattering from the samples. "Gudrun" also verifies that the measured scattered intensity is consistent with sample density and composition.

Table 1
Isotopic composition of the seven glucose solutions samples measured by neutron diffraction with isotopic substitution. The samples are labelled according to the format "xx/solvent" or "xx/yy/solvent"; for instance, the label of the first sample, xx/solvent = H7/D_2O, indicates a glucose solution made with $C_6H_7D_5O_6$ in D_2O, whose H/D composition is given by 42% D, 58% H, and 100% D_2O. Similarly, sample label xx/yy/solvent = D/H7/D_2O indicates a glucose solution obtained by mixing $C_6D_{12}O_6$ and $C_6H_7D_5O_6$ in D_2O, whose H/D composition is given by 71% D, 29% H, and 100% D_2O, and so on. All samples have been obtained by mixing H_2O, D_2O, $C_6H_{12}O_6$, $C_6D_{12}O_6$, $C_6H_7D_5O_6$, and $C_6H_5D_7O_6$ to obtain the desired solution concentration.

Sample label	%D	%H	% D_2O	% H_2O
H7/D_2O	42	58	100	0
D/H7/D_2O	71	29	100	0
D/D_2O	100	0	100	0
D7/D/HDO	79	21	50	50
HD/HDO	50	50	50	50
H/H_2O	0	100	0	100
D7/H_2O	58	42	0	100

The outputs of "Gudrun" are the total neutron-weighted interference differential cross sections (IDCS) defined as

$$F(Q) = \sum_{\alpha} \sum_{\beta \geq \alpha} w_{\alpha\beta}[S_{\alpha\beta}(Q) - 1] \quad (1)$$

where α and β label the atomic sites, Q is the magnitude of the change in the momentum vector by the scattered neutrons, defined as $Q = 4\pi sin\theta/\lambda$ where 2θ represents the scattering angle and λ the wavelength of scattered radiation. The functions

$$S_{\alpha\beta}(Q) = 4\pi\rho \int_0^{\infty} r^2 (g_{\alpha\beta}(r) - 1) \frac{\sin Qr}{Qr} dr, \quad (2)$$

called partial structure factor (PSF), are the Fourier transforms of individual site-site radial distribution function (RDF) $g_{\alpha\beta}(r)$, and ρ is the atomic number density of the solution. The individual PSFs are weighted in Eq. (1) by $w_{\alpha\beta} = c_\alpha c_\beta b_\alpha b_\beta (2 - \delta_{\alpha\beta})$, where c_α and c_β are the concentrations of the α and β nuclei, and b_α and b_β are their scattering lengths [29], respectively.

Thus, each experimental IDCS is a linear combination of many PSF of the individual site-site radial distribution functions. In liquids with a small number of distinct atoms, like H_2O, by measuring an array of different isotopically labelled samples, it is possible to directly extract all of the pair correlation functions from the experiment, giving a direct assessment of the hydrogen bonding present in the measured liquid. However, in more complex samples, like those investigated in the present report, it is not possible to isotopically label every atomic site. For this reason, we employ a simulation-assisted procedure that has been developed to convert IDCS data to real space, and extract a whole set of radial distribution functions. This is called empirical potential structure refinement (EPSR) [18,30] and is similar in principle to the methods routinely used in crystallography, which attempt to systematically refine a structural model to give best overall agreement with the diffraction data. Moreover, it should be noted that the larger is the number of isotopic contrast samples measured, the larger the number of constraints for the EPSR procedure. EPSR is required to fit all of the data sets, ensuring a physically reasonable model which is consistent with a set of measured diffraction data at the appropriate density and composition of each sample. Glucose in solution (at ambient pressure and temperature) undergoes mutarotation to give a 36:64 mixture of the anomers $\alpha - D$-glucose and $\beta - D$-glucose, respectively. To reproduce the experimental sample, our EPSR simulation box contains both glucose anomers at a ratio of $1\alpha/1.8\beta$ that closely resembles a glucose solution at the anomeric equilibrium [9]. The number of water molecules in the box and the box size is determined by the experimental sample