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Hydration of Glucose and Galactose Derivatives

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The spin-lattice relaxation times, T_1, of $H_2{}^{17}O$ have been measured for aqueous solutions of glucose and galactose derivatives, which are substituents of the 2-OH or 6-OH of these sugars, as a function of the concentration at 25 °C. The values of the coordination numbers, n_h, have been estimated on the basis of the water-accessible surface areas (ASA) of the solute molecules. The rotational correlation times, $\tau_c{}^h$, of water molecules around the solute were estimated and compared with that of pure water, $\tau_c{}^0$. All values of the $\tau_c{}^h/\tau_c{}^0$ for the derivatives were smaller than those of the parent sugars, and the value of $\tau_c{}^h/\tau_c{}^0 = 1.26$ for glucosamine was the smallest among these derivatives. The greater the hydration of the parent sugars is, the more the hydration layer will be disturbed by the substituted groups.

Monosaccharide derivatives are distributed widely in nature as the constituents of polysaccharides and muco-polysaccharides. These biopolymers can form gels under some conditions.[1] The physical properties of these gels depend on the hydration of their constituents.[2] The complex oligosaccharides at the cell surfaces, which contain *N*-acetylglucosamine and *N*-acetylgalactosamine, play important roles in biological processes such as cell recognition. In the binding of an inhibitor (sugar or sugar derivative) by an enzyme, dehydration/hydration turnover occurs.[3,4]

The tertiary structure of protein in water is stabilized by the saccharides,[5,6,7] and this stabilizing effect is closely related to their hydration. We found that the temperatures of thermal denaturation of lysozyme and ovalbumin increased linearly with increasing mean numbers of equatorial hydroxy groups *n*(e-OH) per molecule of mono- and oligo-saccharides.[6,8] Furthermore, it has been shown that the values of *n*(e-OH) and the physical properties of the hydration of the monosaccharides are expressed by the linear equation of the dynamic hydration number, n_{DHN}, which was obtained by measuring the ^{17}O relaxation rates of water in aqueous saccharide solutions.[9,10] These results mean that the solute molecules with larger n_{DHN} values more strongly inhibit the thermal motion of water molecules around them.

However, few physical properties of monosaccharides derivatives have been investigated except for those of glucosamine hydrochloride, sodium glucuronate,[11,12] and methyl pyranosides,[13,14] in spite of their biological importance.[15] The hydration of a solute is the important factor which determines the physical properties of the solution. The overall hydration of the solute depends on the numbers and the positions of the functional groups in the solute molecule.[9,10] Of the thermodynamic and the dynamic methods available for the experimental investigation on the hydrations, the nuclear magnetic relaxation method of $H_2{}^{17}O$ is extremely powerful to detect the slight difference between the hydration properties.[9,10]

Recently, we obtained the values of the dynamic hydration numbers of amines, diamines, polyamines, alcohols, and diols from the measurement of the ^{17}O relaxation rate of water in aqueous solutions of these compounds.[16,17] From the results, we revealed that the hydrophobic hydration layer around a solute molecule is disturbed by the adjacent hydrophilic group in the molecule. The dynamic hydration number is an important physical quantity which describes the hydration characteristics of a solute.[18]

In this paper, we report the relaxation rate measurements of natural-abundance ^{17}O nuclei of water in aqueous solutions of monosaccharide derivatives at 25 °C We obtain the values of the n_{DHN} of these sugars. We calculate the water-accessible surface areas (ASA) and the coordination number n_h of these sugars, and obtain the rotational correlation times $\tau_c{}^h$ of water molecules around the solute molecule from n_{DHN}. The hydration of these sugars is discussed in relation to the hydration of the parent sugars.

Method

The T_1 of $H_2{}^{17}O$ in aqueous solutions is adequately represented by an empirical equation of the form

$$T_1{}^0/T_1 = 1 + Bm + Cm^2 \tag{1}$$

where $T_1{}^0$ is the spin-lattice relaxation time of $H_2{}^{17}O$ in pure water and *m* is the molality of the solute.

In the extreme motional narrowing condition i.e. $\omega^2\tau_c^2 \ll 1$, we obtain the ^{17}O relaxation rate,[9,10,19]

$$\frac{1}{T_1} = \frac{3}{125}\left(1+\frac{\eta^2}{3}\right)\left(\frac{e^2qQ}{h}\right)^2 \tau_c \qquad (2)$$

where η is an asymmetric parameter of the electric field gradient, e^2qQ / h is the quadrupole coupling constant (^{17}OQCC), and τ_c is the correlation time of molecular reorientation. The contribution of η will be omitted in the following discussion, since the value is usually small.[9,10]

In aqueous solution, the ^{17}O nuclei of water molecules are distributed between two motional states with different intrinsic relaxation rates. Since the exchange of water molecules between the hydration water in the co-sphere (mole fraction x_h) and the bulk water is fast, the following relation is obtained :[7,9,10,16]

$$\frac{1}{T_1} = \frac{(1-x_h)}{T_1^0} + \frac{x_h}{T_1^h} \qquad (3)$$

where T_1^h is the relaxation time of the hydration water, $x_h = n_h m / 55.5$, and n_h is the coordination number of the solute molecule. Combining Eqs. 2 and 3, we obtain[9,10]

$$\frac{T_1^0}{T_1} = 1 + n_h\left(\frac{\tau_c^h}{\tau_c^0}-1\right)\frac{m}{55.5} \qquad (4)$$

where the superscripts h and 0 refer to the water of the hydration layer and to the bulk water, respectively. n_h is determined by the geometrical size of a solute molecule and τ_c^h/τ_c^0 depends on the solute -water interaction. We define n_{DHN} as follows :[9,16]

$$n_{DHN} = 55.5B = n_h\left(\frac{\tau_c^h}{\tau_c^0}-1\right) \qquad (5)$$

In aqueous solutions, it can be assumed that the value of ^{17}O QCC for hydration water is equal to that for bulk water.[9,10,16,17,20]

The physical meaning of n_{DHN} is as follows.[9,18] Consider a hypothetical molecule of the same size and shape as the solute molecule, which interacts with water in the same manner as would a water–water interaction. In effect, n_{DHN} expresses the difference in the dynamic states of the water molecules in the hydration layers of the solute and of the hypothetical molecule. The value of n_{DHN} corresponds thermodynamically to the entropy difference of water molecules in the two hydration layers. Thus, n_{DHN} is the quantity which represents the hydration characteristics of a solute.

Since it is very difficult to obtain the n_h values experimentally, these have been determined from the accessible surface area (ASA) of the solute molecules.[16,21,22] We calculated the ASAs by drawing spheres with radius ($r_v + r_w$) around each atom, where r_v is the van der Waals radius of each atom in the solute molecule and r_w is the radius of a water molecule (r_w = 0.15 nm). The computer program QCPE No. QCMP 132 was used for the ASA calculation. Considering the packing density of water molecules in the co-sphere, we calculated the n_h from the ASA. The calculated values of the ASA and n_h for monosaccharide derivatives are shown in the 3rd and 4th columns of Table 1, respectively.

Experimental

Glucosamine (GlcN), *N*-acetylglucosamine (GlcNAc), *N*-trifluoroacetylglucosamine (TFGlcNAc), glucuronic acid (GlcA), *β*-D-glucose-6-monophosphate (Glc-6-P) sodium salt, *N*-acetylgalactosamine (GalNAc), and D-galacturonic acid (GalA) were purchased from Tokyo Kasei. All of the sugars were of G.R. grade and were used without further purification. The solutions of glucosamine, glucuronic acid, and galacturonic acid were maintained at pH 3.5.[9]

All natural abundance O-17 NMR experiments were performed using a JEOL GX-500 spectrometer operating at 67.8 MHz. Details of the experiments are described elsewhere.[9] The temperature was maintained at 25 ± 0.3 °C by means of a gas thermostat.

Results and Discussion

In Figs. 1 and 2, the values of T_1^0/T_1 for $H_2{}^{17}O$ in aqueous solutions of glucose and galactose derivatives are plotted against their concentrations. The solid lines in these figures are those calculated by the least-squares method. The numbers on the lines in all the figures denote the same monosaccharide derivatives as in Table 1.

The n_{DHN} values for glucose and galactose derivatives were obtained from Eqs. 1 and 5. Since sodium *β*-D-glucose-6-monophosphate includes Na$^+$ ion, the n_{DHN} value for Glc-6-P was derived as described elsewhere,[16] taking n_{DHN} (Na$^+$) = 4.1 into account.[23] The values of n_{DHN} and τ_c^h/τ_c^0 for all the sugars are presented in the 5th and 6th columns of Table 1, respectively. The corresponding values for glucose and galactose[9] are presented in Table 1 for reference, since monosaccharide de-

Table 1. Hydration Characteristics of Monosaccharide Derivatives at 25 °C

No.	Compounds	ASA/Å^2	n_h	n_{DHN}	τ_c^h/τ_c^0
1	Glucose	339	24	18.6	1.76
2	Glucosamine	339	24	6.37	1.26
3	*N*-Acetylglucosamine	408	29	10.4	1.35
4	TF-*N*-Acetylglucosamine[a)]	472	34	22.4	1.66
5	Glucuronic acid	336	24	13.0	1.54
6	*β*-D-Glucose-6-monophosphate	453	32	20.7	1.65
7	Galactose	339	24	16.6	1.68
8	*N*-Acetylgalactosamine	408	29	15.1	1.52
9	Galacturonic acid	336	24	15.6	1.65

a) TF: trifluoro.

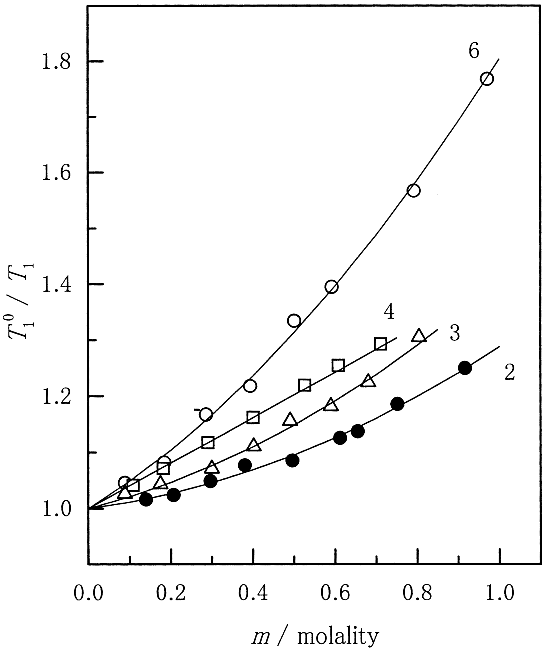

Fig. 1. T_1^0/T_1 of $H_2{}^{17}O$ in aqueous solutions of monosaccharide derivatives as a function of the molality at 25 °C.
2. −●− GlcN; 3. −△− GlcNAc; 4. −□− TFGlcNAc; 6. −○− Glc-6-P.

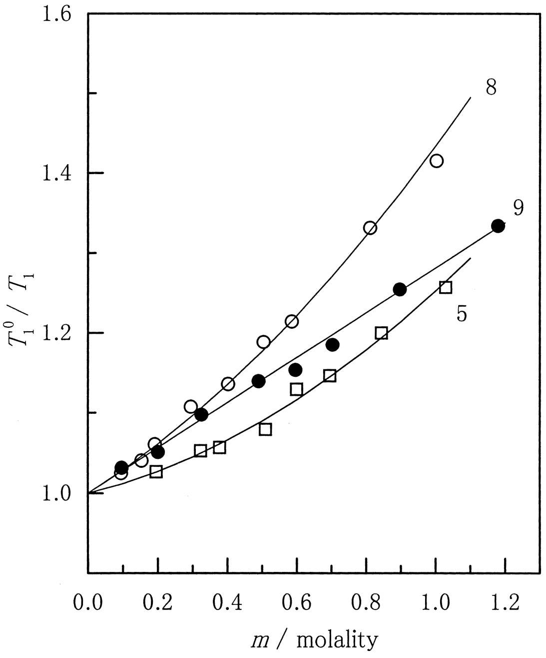

Fig. 2. T_1^0/T_1 of $H_2{}^{17}O$ in aqueous solutions of monosaccharide derivatives as a function of the molality at 25 °C.
5. −□− GlcA; 8. −○− GalNAc; 9. −●− GalA.

rivatives used in this work are the derivatives of these two sugars. In aqueous solutions, carbohydrates are present in several conformers which are at equibrium,[24] and the experimental values are the mean of the existing conformer mixtures.

As shown in Table 1, it is noteworthy that the τ_c^h/τ_c^0 value of each monosaccharide derivatives is smaller than that of the corresponding parent sugar. There is a low correlation between τ_c^h/τ_c^0 and n_h, which is proportional to the molecular size. For example, the n_h values for glucose and TFGlcNAc are 24 and 34, respectively, but the τ_c^h/τ_c^0 value for glucose is larger than that for TFGlcNAc. Although n_h values for GlcNAc and GalNAc are equal to each other, the τ_c^h/τ_c^0 value for the latter is larger than the former. A similar tendency is seen for glucuronic and galacturonic acids too. These results show that the hydration of derivatives depend on the kind of substituted group and on the hydration of the parent sugars. In the following section we discuss the relation of the hydration properties between the substituents and the parent sugars.

Hydration of Glucose Derivatives. As shown in Table 1, the τ_c^h/τ_c^0 values for GlcN, GlcNAc, and TFGlcNAc, which are substituents on 2-OH of glucose, are smaller than that for glucose. In particular, the τ_c^h/τ_c^0 value of GlcN is the smallest of the monosaccharide derivatives in Table 1.

The sugar hydration is affected by the mean value of the number of equatorial OH groups[n(e-OH)] of the various conformers, which coexist at equilibrium in solution.[9,10] The e-OH groups are able to interact with water in a manner which forms a long-lived hydration structure, since e-OH groups on pyranose sugars match the unperturbed water structure.[9,13,24–26] For monosaccharides, a sugar with more e-OH groups shows a larger value of τ_c^h/τ_c^0.[9] D-glucose has the largest value of n(e-OH), 4.6, and exerts the strongest stabilizing effect on the water structure of any of the monosaccharides (Table 1).[9] The 2-OH of glucose is an equatorial and may cooperatively stabilize the water structure with the 1-, 3-, and 4-OH groups. The effect of the substitution of the OH group of glucose by another group on the water structure can be divided into following two effects: (1) The hydration behavior of the sugar derivative will be influenced by the interaction between water and the individual substituted group. The τ_c^h/τ_c^0 value of the derivative may reduce or increase corresponding to the hydration characteristics of the substituted group. (2) As the three-dimensional hydrogen-bonded water structure is in good compatibility with the conformation of the glucose molecule,[1,9,10] the water structure around the derivative will be always disturbed by the mismatch between water structure and the conformation of the derivative with substituted group.

To clarify the distinction of the hydration behavior of GlcN and glucose, we must pay special attention to the interaction between -NH_2 group and water. We obtained the values of n_{DHN} and τ_c^h/τ_c^0 for amino acids, glycine peptides[27] and amines, diamines, amides,[14] and showed that the amino group breaks the water structure. Thus, because of both the above-mentioned effect (2) and the structure-breaking effect of the -NH_2 group, the thermal motion of water molecules around the glucosamine molecule becomes more vigorous than that around glucose: that is, both effects result in a considerable decrease of the τ_c^h/τ_c^0 value for glucosamine.

Since GlcNAc and TFGlcNAc have hydrophobic groups, the effect of the substituted groups on the water structure is different from that of the -NH_2 group. We can think that the hydration of the -$NHCOCH_3$ group is approximately equal to that of acetamide. The τ_c^h/τ_c^0 values for methanol, ethanol, and acetamide are 1.48, 1.71, and 1.34, respectively.[17,16] These results show that the thermal motion of water molecules around the methyl group is affected by the adjacent hydrophilic one.[16,17] Since the degree of hydration of the -$NHCOCH_3$ group is not so large, its stabilizing action on the water structure does not exceed the above mentioned effect (2). The τ_c^h/τ_c^0 value of GlcNAc, therefore, is smaller than that of glucose.

The hydrophobicity of perfluorocarbons is greater than that of the corresponding alkanes,[28] and accordingly the thermal motion of water molecules around the -CF_3 group is more inhibited than that around the -CH_3 group. Moreover, the water structure around the bulky hydrophobic group is hardly affected by the adjacent hydrophilic group.[16,17] The τ_c^h/τ_c^0 value of TFGlcNAc is consequently larger than that of GlcNAc as shown in Table 1.

The 6-OH group of glucose is equatorial but it does not combine with the pyranose ring. Therefore, the hydration of the derivative, which is obtained by the chemical modification of 6-OH, mainly depends on the hydration characteristics of the modified group.

As shown in Table 1, the τ_c^h/τ_c^0 value of glucuronic acid is smaller than that of glucose. Recently, we measured T_1 of $H_2{}^{17}O$ in aqueous solutions of aliphatic carboxylic acids and obtained the τ_c^h/τ_c^0 values for these acids at 25 °C.[27] The τ_c^h/τ_c^0 value for acetic acid is 1.25 and is smaller than that for ethanol ($\tau_c^h/\tau_c^0 = 1.71$)[17]. From these results, it is thought that the degree of hydration of the -COOH group is less than that of -CH_2OH group. So, it is reasonable that the τ_c^h/τ_c^0 value of glucuronic acid is smaller than that of glucose.

The hydration of the glucuronate ion is of interest in this regard. Since the τ_c^h/τ_c^0 value of the acetate ion is 1.78,[29] the degree of hydration of the glucuronate ion is thought to be stronger than that of glucuronic acid. Glucuronic acid is the constituent of mucopolysaccharides such as hyaluronic acid. If the pH in the medium of a hyaluronic acid gel becomes higher for any reason, the glucuronic acid dissociates and forms glucuronate ions. The hydration state of the gel, therefore, may be greatly changed. This behavior of the gel with pH change is biologically important. The same situation may also occur in the case of mucopolysaccharides containing the galacturonic acid.

In the case of Glc-6-P, the thermal motion of water molecules around the -$OPO_3H_2^-$ group may be inhibited by electrostatic interaction. Moreover, these water molecules can make hydrogen bonds with the two OH groups of the -$OPO_3H_2^-$ group. Thus, it is presumed that the thermal motion of water molecules around Glc-6-P is strongly inhibited by these interactions. As shown in Table 1, in fact, the value of τ_c^h/τ_c^0 for Glc-6-P is larger than that for glucuronic acid, but smaller than that for glucose. This result shows that the interactions between the -$OPO_3H_2^-$ group and water molecules above mentioned are not so strong. One of the possible causes is as follows: the thermal motion of water molecules close to the -$OPO_3H_2^-$ group is not so strongly inhibited, since both interactions mentioned above are in competition with each other.

Hydration of Galactose Derivatives. In the case of the galactose derivatives, the degree of decrease of their τ_c^h/τ_c^0 values is not so small, compared with the glucose derivatives: still, the τ_c^h/τ_c^0 value of GalNAc is smaller than that of galacturonic acid. These results are explained as follows.

The value of the mean number of equatorial OH groups, n(e-OH), of galactose is 3.7,[9] and so its τ_c^h/τ_c^0 value is smaller than that for glucose (Table 1). This means that the stabilizing effect on water structure by galactose is weaker than that by glucose. Thus the effect of the mismatch with water structure above-mentioned is relatively weak and the hydration of galactose derivatives mainly depends on the hydration of the substituted groups. The hydration of GalNAc and galacturonic acid is larger than that of the corresponding derivatives of glucose.

A relation between the hydration behavior of the derivative and the mismatch with water structure is found thermodynamically. Galema and Høiland[13] measured the partial molar compressibility of glucose, galactose, methyl β-glucopyranoside, and methyl β-galactopyranoside at 25 °C and obtained -17.6×10^{-4}, -20.8×10^{-4}, -11.1×10^{-4}, and -17.1×10^{-4} $cm^3\ mol^{-1}\ bar^{-1}$, respectively. The differences of the compressibility of derivatives and the parent sugars are 6.5×10^{-4} and 3.7×10^{-4} $cm^3\ mol^{-1}\ bar^{-1}$, respectively. Hence, the hydration layer of glucose derivative is more strongly disturbed by the methoxy group than that of galactose.[13]

These results show that the effect of the mismatch always reduces the τ_c^h/τ_c^0 value and that the greater the hydration of the parent sugar is, the more the hydration layer will be disturbed by the substituted groups.

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