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Carbohydrate Research 337 (2002) 441–450

CARBOHYDRATE RESEARCH

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Effect of molecular structure on thermodynamic properties of carbohydrates. A calorimetric study of aqueous di- and oligosaccharides at subzero temperatures

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Received 17 July 2001; accepted 13 December 2001

Abstract

For aqueous solutions of di- and oligosaccharides thermodynamic properties have been investigated at subzero temperatures using differential scanning calorimetry. The amount of unfrozen water observed is found to increase linearly with the glass transition temperatures of anhydrous carbohydrates. Furthermore, the amount of unfrozen water shows a linear relationship with known solution properties of aqueous carbohydrates, such as partial molar compressibility and heat of solution. The different effectiveness among various di- and oligosaccharides to avoid ice formation is associated with the combination of constitutive monosaccharides and attendant molecular structure features including the position and type of the glycosidic linkage between the constituent units. More unfrozen water is induced in the presence of a carbohydrate having a poorer compatibility with the three-dimensional hydrogen-bond network of water. A series of these results obtained imply that there is a common key of carbohydrate stereochemistry governing several different thermodynamic amounts of a given system involving carbohydrates. In this context, a modified stereospecific-hydration model can be used to interpret the present results in terms of stereochemical effects of carbohydrates.

Keywords: Disaccharides; Oligosaccharides; Unfrozen water; Glass transition temperature; Heat of solution; Partial molar compressibility; A modified stereospecific hydration model

1. Introduction

Di- and oligosaccharides are of academic and industrial importance since they have the ability to protect biological substances and organs against freezing stress. In nature, trehalose is distributed in various freeze-tolerance organisms such as polar biota[1] and overwintering insects.[2] Sucrose plus oligosaccharides, raffinose, and/or stachyose, are accumulated in the cells of some kinds of plants, like populus[3] and others,[4–7] during cold seasons to acquire freezing tolerance. Sucrose, trehalose, and raffinose can be also artificially added protectants for cryopreservation of plant cells and organs.[8–12] In a practical view, these carbohydrates have potential utility as protective agents in both freeze–thawing[13–17] and freeze-drying[14,15,17–19] treatment of proteins[13–15,18] and membranes.[14,16,17,19] For freeze-drying processes, carbohydrate-induced stabilization of given materials during drying and subsequent storage has been often focused and emphasized.[14,15,17–19] It must be still kept in mind, however, that added carbohydrates also serve as protectants in the freezing step.[13–17] Apart from this, in addition, for the purpose of devising a strategy of successful freeze-drying, it is indispensable to know the freezing behavior of samples to be freeze-dried.[20]

Cryoprotective effects of carbohydrates on proteins have been explained by the so-called preferential exclusion hypothesis,[13a,13b,13c,14,15] which was originally developed to describe protein stabilization in non-frozen aqueous medium.[21,22] According to the hypothesis, the mechanism by which added carbohydrates preserve proteins against freezing damage is not the direct interaction between a protein and added carbohydrates, but is due to water-mediated effects in the presence of the additives. On the other hand, membrane protection

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0008-6215/02/$ - see front matter
PII: S0008-6215(01)00332-9

during freeze-thawing has been interpreted to involve a direct interaction, probably through hydrogen-bond formation, between a membrane and a carbohydrate.[16,17] In this situation, the membrane-bound carbohydrate is able to make a steric barrier between membranes, preventing aggregation and fusion of membranes. At the same time, however, there is a possibility that a given carbohydrate, regardless of its binding to a membrane or not, alters the physicochemical properties of the aqueous medium.[16a,16b] Hitmi et al. demonstrated that in the cryopreservation of plant cells, using a carbohydrate like sucrose as a protectant, the concomitant increased unfrozen water content in cells was a key factor behind their enhanced survival during frozen storage.[12] Recently Magazu et al. suggested that carbohydrate–water interactions were a significant factor in understanding biological protective functions of carbohydrates, through a neutron scattering study of aqueous disaccharides, trehalose, sucrose, and maltose above room temperature.[23]

The carbohydrate features of cryoprotectants function to modify water behavior at subzero temperatures. Important thermodynamic parameters include the unfrozen water—in other words, the solute concentration in the unfrozen fraction, and the glass transition temperature, both of which are measurable by differential scanning calorimetry (DSC). Numerical data of the corresponding physical parameters have been accumulated for aqueous solutions of sucrose from the early stages in the field of DSC studies.[24–35] In the past decade trehalose has also received increasing attention.[19e,35–40] A larger amount of unfrozen water has been observed in the aqueous solution of trehalose as compared with aqueous sucrose.[35,37–39] The glass transition temperature has been found higher for aqueous trehalose than for aqueous solutions of sucrose.[19e,32,35,36,38,39] Such a characteristic aspect has been interpreted to link to the more bioprotective efficiency of trehalose relative to sucrose.[19e,35–39] Roos provided thermodynamic information on aqueous solutions of various small carbohydrates including several disaccharides.[32] A recent DSC study of aqueous media in the presence of trehalose, sucrose, glucose, and fructose, respectively, has suggested that there is a certain relationship between modifying effects of carbohydrates on freezing behavior of water and their intrinsic properties and/or molecular structure.[35] Through these investigations, details are known of thermodynamic nature for aqueous carbohydrates, particularly sucrose and trehalose. However, there is little or no study including a systematic view of the effects of molecular structure of di- and oligosaccharides in regard to their different capacities to prevent ice formation. Our previous paper reported that different degrees of anti-freeze effectiveness among monosaccharides can be related to their stereochemistry with respect to hydroxyl configurations.[41] The purpose of the current research is to get insight into molecular structure dependence di- and oligosaccharides exhibit regarding their altering effects on aqueous behavior at subzero temperatures. Relevant thermodynamic events were investigated with DSC measurements for aqueous solutions of 17 di- and eight oligosaccharides. Those were characterized by the amount of unfrozen water, U_w, and glass transition temperatures, T'_g. The U_w data are provided in terms of moles of unfrozen water per mole of a given carbohydrate, and T'_g observed here will be taken as onset values. We will examine, for carbohydrates including monosaccharides studied in our previous work,[41] the relationships not only between U_w and T'_g but also between U_w and T_{gc}, where T_{gc} represents the glass transition temperatures of anhydrous carbohydrates. In addition, U_w will be correlated with other known physicochemical properties of aqueous carbohydrates, such as partial molar compressibility and heat of solution. Through these comparative works it will be shown there is a possibility that the unit configuration in the molecular structure of each carbohydrate plays a key role in determining several different thermodynamic parameters in a given system, including carbohydrates.

2. Experimental

Kojibiose and nigerose were purchased from Wako Co. Neotrehalose, sophorose, laminaribiose, isomaltose, maltortiose, and erlose were from Sigma Chemical Co. All of the other carbohydrates studied were from Fluka. All reagents were received in the best quality available and used without further purification. Their moisture contents were checked using a Karl Fischer coulometric titrimeter (Mitsubishi). Aqueous solutions of carbohydrates at desired concentrations were prepared by weighing with deionized distilled water. If necessary, the solution to be prepared was heated carefully up to 65 °C for complete dissolution. Carbohydrate concentration was changed from 10 wt.% up to a maximum of 45 wt.% in 5 wt.% increments. A series of aqueous solutions prepared were sealed in independent vials and kept at 25 °C for 24 h in an incubator for allowing them to reach a steady state.

A Mac-Science DSC3100 apparatus was used to observe thermodynamic events which occur in aqueous carbohydrates at subzero temperatures. The apparatus was calibrated using indium, gallium, and zinc. Aqueous samples prepared were placed in 20-μL aluminum DSC pans (TA instruments), which were then hermetically sealed. An empty aluminum pan was used as a reference. Temperatures were cooled down to −70 °C from rt at 8 °C/min, and then heating run was immediately performed at 5 °C/min to 30 °C. Dry nitrogen gas was streamed at 130 mL/min to prevent con-

densation of moisture within the DSC furnace. For all carbohydrates studied, DSC measurements were carried out for at least triplicate samples at each concentration.

Heat of fusion of ice, H_f, was obtained by integration of a melting endotherm observed at a heating scan. The amount of unfrozen water, U_w, was determined by extrapolating H_f, which was linearly dependent on carbohydrate concentration (wt.%), to zero. Glass transition temperatures were taken as onset values.

3. Results and discussion

Amount of unfrozen water.—Heat of fusion of ice, H_f, was measured for aqueous solutions of a given carbohydrate as a function of its weight fraction (wt.%). H_f correlated well to the concentration, with a correlation coefficient r^2 of as high as 0.99, for each carbohydrate studied. This is exemplified by the result of sucrose as shown in Fig. 1. The linear dependence of H_f on the concentration means the equal amount of unfrozen water formation within the covered range of sucrose concentration. In other words, the carbohydrate concentration of the freeze-concentrated liquid fraction, C'_g, is independent of its initial concentration. The corresponding freeze-concentrated concentration is provided as an intersection point of the extrapolated linear regression line with abscissa, from which the amount of unfrozen water, U_w, is obtainable in terms of moles of water per mole of carbohydrate. The extrapolation shown in Fig. 1 yields $C'_g = 69.1\%$. This value is reasonable as the sucrose concentration in the unfrozen liquid phase, was consistent with the following previous findings: (i) that in DSC thermograms an endothermic event corresponding to ice melting was observed for aqueous sucrose of which the upper-limit concentration was 68 wt.%,[28] to which similar findings were reported in other literature reports;[29,34] and (ii) that no conventional freezing and melting peaks were observed in DSC thermograms for aqueous solution of sucrose with its 10.9% mole fraction, i.e.,70 wt.% concentration.[35]

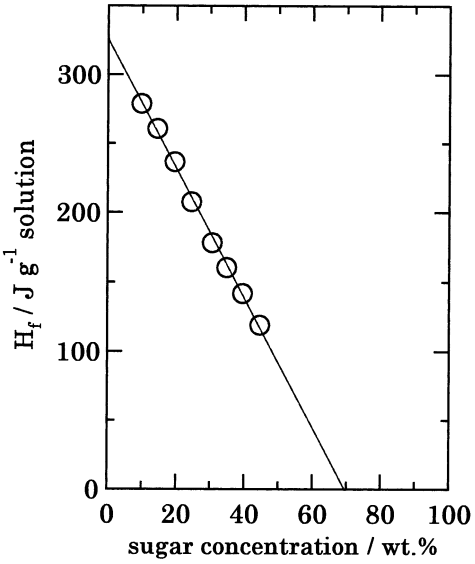

Fig. 1. Plot of H_f vs. concentration of sucrose.

Present U_w data evaluated in such a way as exemplified by Fig. 1 are summarized in the sixth column in Table 1, showing a good agreement with the corresponding literature data available listed in the seventh column, except for the case of comparison with the data from Ref. 16b. As an overall tendency, the U_w values estimated are comparable to the number of hydroxyl groups within a molecule of interest. It was reported in Ref. 37 that for aqueous solutions of trehalose, maltose, and sucrose, respectively, these U_w values, 7.95, 6.50, and 6.33, respectively, were in parallel with the number of equatorial hydroxyl groups, Ne_{OH}, of which numerical data are given in the fifth column in Table 1. A similar relationship is found for the current U_w values with Ne_{OH} for the three disaccharides. However, a comprehensive comparison of numerical values between U_w and Ne_{OH} listed in Table 1 shows that in a few cases the relationship holds. Further discussion of stereochemical aspects regarding U_w will be provided in another section which follows.

Glass transition temperatures.—Glass transition temperature, T'_g, measured and the corresponding published data available are listed in the eighth and last columns of Table 1, respectively. T'_g values determined here are lower than those cited from Ref. 32. It should be pointed out that in Ref. 32 T'_g values were observed for samples subject to annealing treatment, an isothermal holding at a temperature $T'_g < T < T'_m$ (ice melting point) for the purpose of promoting more ice formation to reach their maximally freeze-concentrated states. Under such treatment the residual unfrozen liquid is concentrated to a greater extent, which leads to an elevated T'_g.[32] The corresponding treatment was not performed in the present experiment. It is possible that lower T'_g values obtained here are due to non-maximum ice formation. Apart from the absolute values of T'_g, however, it is worth noting that our T'_g values mostly follow the literature ones in the relative magnitude among different carbohydrates.

Amount of unfrozen water versus glass transition temperature.—The relationship between U_w (or C'_g) and T'_g will be discussed with the aid of the glass transition curve which is usually drawn in the state diagram about a given carbohydrate–water system, where the glass transition temperature, T_g, and the weight fraction of the carbohydrate, w_c, are given as ordinate and abscissa, respectively. The intersection point of the T_g curve and a freezing-point curve yields the values of T'_g and C'_g for the maximally freeze-concentrated aqueous carbohydrate. The T_g curve has been formulated as

Table 1
Results of amount of unfrozen water and glass transition temperatures for aqueous solutions of di- and oligosaccharides

Compound	Carbohydrate	Molecular structure	N_{OH} [c]	Ne_{OH} [d]	U_w [a]		T'_g [b]	
					This work	Lit. [e]	This work	Lit. [f]
1	trehalose	α-D-Glc*p*-(1↔1)-α-D-Glc*p*	8	8	10.9 (0.7)	7.95 [g]	−43	−42
2	neotrehalose	α-D-Glc*p*-(1↔1)-β-D-Glc*p*	8	8	10.9 (0.7)		−45	
3	kojibiose	α-D-Glc*p*-(1→2)-D-Glc*p*	8	7.6 (7.5[h])	9.1 (0.6)		−45	
4	sophorose	β-D-Glc*p*-(1→2)-D-Glc*p*	8	7.6(7.4 [h])	9.7 (0.6)		−45	
5	nigerose	α-D-Glc*p*-(1→3)-D-Glc*p*	8	7.6 (7.6 [h])	9.8 (0.6)		−45	
6	laminaribiose	β-D-Glc*p*-(1→3)-D-Glc*p*	8	7.6 (7.6 [h])	11.6 (0.7)		−44	
7	maltose	α-D-Glc*p*-(1→4)-D-Glc*p*	8	7.6 (7.6 [h], 7.5 [i])	9.5 (0.6)	6.50 [g], 19.5 [j], 6.3 [k]	−45	−43
8	cellobiose	β-D-Glc*p*-(1→4)-D-Glc*p*	8	7.6 (7.6 [h])	13.1 (0.8)		−45	
9	isomaltose	α-D-Glc*p*-(1→6)-D-Glc*p*	8	7.6 (7.6 [h])	10.1 (0.6)		−47	
10	gentibiose	β-D-Glc*p*-(1→6)-D-Glc*p*	8	7.6 (7.7 [h])	11.3 (0.7)		−46	
11	sucrose	α-D-Glc*p*-(1↔2)-β-D-Fru*f*	8	6.3	8.5 (0.5)	6.33 [g], 8.1 [l], 7.0 [m]	−47	−46
12	turanose	α-D-Glc*p*-(1→3)-D-Fru*p*	8	6.5	8.6 (0.5)		−47	
13	leucrose	α-D-Glc*p*-(1→5)-D-Fru*p*	8	6.5	8.3 (0.5)		−46	
14	palatinose	α-D-Glc*p*-(1→6)-D-Fru*f*	8	6.5	8.8 (0.6)		−47	
15	melibiose	α-D-Gal*p*-(1→6)-D-Glc*p*	8	6.6	12.0 (0.7)		−46	−42
16	lactose	β-D-Gal*p*-(1→4)-D-Glc*p*	8	6.6 (6.5 [i])	14.4 (1.0)		−44	−41
17	lactulose	β-D-Gal*p*-(1→4)-D-Fru*f*	8	5.5	10.3 (0.6)		−47	
18	maltotriose	α-D-Glc*p*-(1→4)-D-α-Glc*p*-(1→4)-D-Glc*p*	11	10.6 (10.6 [h])	12.7 (0.9)	7.67 [g], 23 [j]	−40	
19	panose	α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→4)-D-Glc*p*	11	10.6	13.8 (0.9)		−40	
20	isomaltotriose	α-D-Glc*p*-(1→6)-D-α-Glc*p*-(1→6)-D-Glc*p*	11	10.6	12.2 (0.8)		−42	
21	melezitose	α-D-Glc*p*-(1→3)-β-D-Fruf(2↔1)-α-D-Glc*p*	11	8.3	15.8 (1.0)		−42	
22	erlose	α-D-Glc*p*-(1→4)-α-D-Glc*p*-(1↔2)-β-D-Fru*f*	11	9.3	11.3 (0.8)		−41	
23	raffinose	α-D-Galp(1→6)-α-D-Glc*p*-(1↔2)-β-D-Fru*f*	11	8.3	16.2 (1.0)		−40	
24	maltotetraose	α-D-Glc*p*-(1→4)-D-α-Glc*p*-(1→4)-α-D-Glc*p*-(1→4)-D-Glc*p*	14	13.6	13.3 (1.0)	12.01 [g]	−38	
25	stachyose	α-D-Gal*p*-(1→6)-α-D-Gal*p*-(1→6)-α-D-Glc*p*-(1↔2)-β-D-Fru*f*	14	10.3	19.8 (1.3)		−40	

[a] Unfrozen water in terms of moles of water per mole carbohydrate. Standard errors in numerical data are given in parentheses.
[b] Glass transition temperature given as onset values in °C.
[c] Total number of hydroxyl groups within each carbohydrate molecule.
[d] Averaged number of equatorial hydroxyl groups for each carbohydrate in water. The numerical value in each parenthesis was calculated using the anomeric composition data of the disaccharide. All of the other values for Ne_{OH} were calculated under the assumption that the anomeric composition of the reducing end was equal to that of the corresponding monosaccharide in its dilute aqueous solution at room temperature, of which numerical data in Ref. 42 were used.
[e] All literature values cited have been estimated in a similar way to the procedure used in the present study.
[f] Cited from Ref. 32.
[g] Cited from Ref. 37.
[h] Calculated from the anomeric composition data reported in Ref. 43, where the carbohydrate concentrations are as high as 20–35 wt.% in D_2O.
[i] Calculated from the anomeric composition data reported in Ref. 44 where the temperature is down to −9 °C and the carbohydrate concentrations are ca.1.2 M in buffer solution (sodium hydrogenmalate + maleic acid).
[j] Cited from Ref. 16b.
[k] Cited from Ref. 45. Annealing treatment was employed. The numerical value 6.3 for U_w was calculated from the literature data 0.33 g H_2O/g maltose.
[l] Cited from Ref. 25. The numerical value 8.1 for U_w was calculated from the literature data 70 wt.% obtained by extrapolation to $H_f = 0$.
[m] Cited from Ref. 28. Annealing treatment was employed. The numerical value 7.0 for U_w was calculated from the literature data 36.6 g H_2O/100 g sucrose.

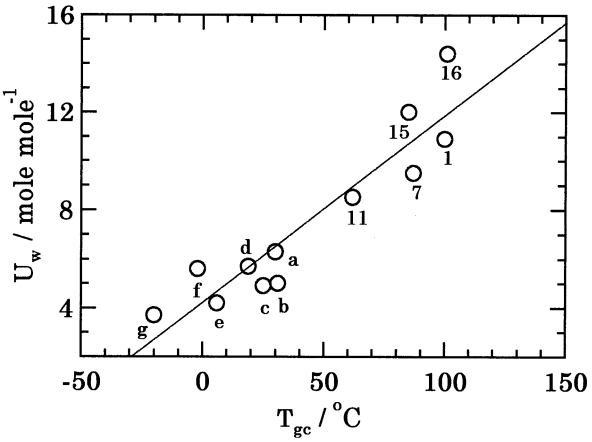

Fig. 2. Plot of U_w vs. T_{gc}. (a) D-Galactose; (b) D-glucose; (c) D-mannose; (d) L-sorbose; (e) D-xylose; (f) D-arabinose; and (g) D-ribose. For these carbohydrates, U_w data in our previous study[41] were used. For others, U_w data and numbering are given in Table 1. Numerical data in Ref. 32 were used for T_{gc} given as onset values. The linear regression line was obtained with the linear squares method, exhibiting a correlation coefficient $r^2 = 0.86$.

follows,[19e,32,34] which is application of the equation originally developed for binary polymer mixtures by Gordon and Taylor.[46]

$$T_g = (w_c T_{gc} + k w_w T_{gw})/(w_c + k w_w) \quad (1)$$

where T_{gc} and T_{gw} represent the glass transition temperatures for the anhydrous carbohydrate and water, respectively; w_c and w_w, the weight fractions for them, respectively, and k represents a constant, the numerical value of which is determined to be specific to the binary system of interest. T_{gw} is -131 °C.[47] T_{gc} values vary largely dependent on the carbohydrates. In the case of disaccharides studied recently,[48] for example, T_{gc} varies from 68.9 °C for palatinose to 116.9 °C for trehalose. On addition of water to an anhydrous carbohydrate, the glass transition temperature of the system is greatly depressed relative to the water-free state by the plasticizing effects of added water. This results in a small range of T_g values of different aqueous carbohydrates as compared with their anhydrous cases, T_{gc}.[32]

So far, especially in view of comparative characterization of trehalose and sucrose, a correlation between T'_g and U_w (or C'_g) has been noted.[38,39] The relatively higher T'_g of aqueous trehalose leads to inducing more amount of unfrozen water as compared with aqueous solution of sucrose. Although the current experiment provided a finding in agreement with this for the two carbohydrates, there is seemingly, as a whole, an ambiguous correlation between U_w and T'_g because of almost similar values of T'_g observed for a large part of the carbohydrates studied. Such a situation was encountered also for aqueous monosaccharides in our previous research,[41] where T'_g values for a series of carbohydrates belonging to the same family, such as aldohexoses, were almost equal, whereas they differed significantly in the magnitude of U_w. It has been recently found that U_w is related closely to the glass transition temperatures of anhydrous carbohydrates, T_{gc}, as shown in Figs. 2–4, illustrating that a linear correlation exists between the two physical amounts. These results indicate that carbohydrate dependence of aqueous unfrozen phenomena arises from the difference in inherent properties of glassy carbohydrates. It is possible that the reason why U_w exhibits a clearer correlation with T_{gc} rather than with T'_g is merely a problem of the present experimental precision with which T'_g measurements did not have an accuracy of better than ± 0.5–1.0 °C. If it were possible to locate T'_g more precisely on the DSC thermograms, the correlation of U_w and T'_g could be clarified.

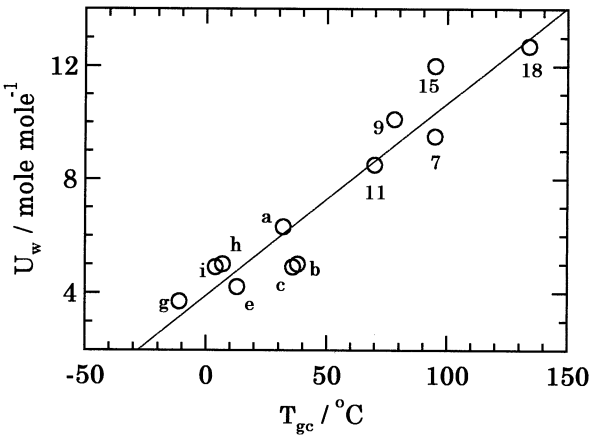

Fig. 3. Plot of U_w vs. T_{gc}. (h) D-Fructose; (i) L-arabinose. Other alphabets denote the same as in Fig. 2. For these carbohydrates, U_w data were used from Ref. 41. For others, U_w data and numbering are given in Table 1. Numerical data in Ref. 49 were used for T_{gc} given as mid point values. The linear regression line was obtained with the linear squares method, exhibiting a correlation coefficient $r^2 = 0.91$.

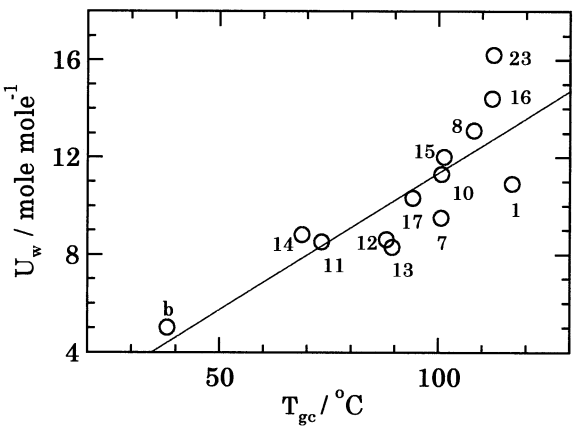

Fig. 4. Plot of U_w vs. T_{gc}. Numerical data in Ref. 48 were used for T_{gc} given as mid point values. The linear regression line was obtained with the linear squares method, exhibiting a correlation coefficient $r^2 = 0.70$.

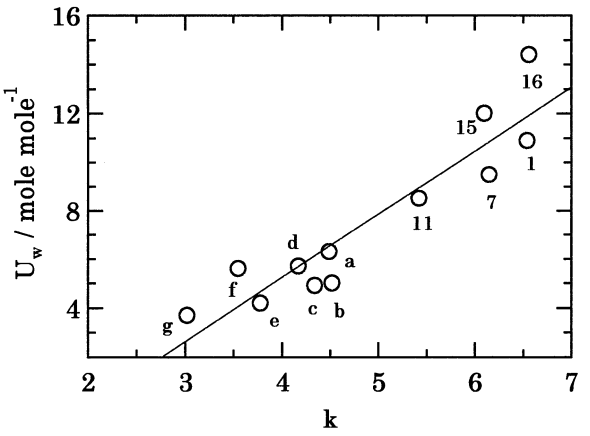

Fig. 5. Plot of U_w vs. k. Numerical data in Ref. 32 were used for k. The linear regression line was obtained with the linear squares method, exhibiting a correlation coefficient $r^2 = 0.86$.

For several carbohydrates, values of the parameter k in Eq. (1) have been estimated empirically with T_{gc} values.[32] Using the numerical data of k, we plot it against U_w in Fig. 5, which demonstrates the presence of the close relationship between unfrozen water and the location of the glass transition curve in a state diagram.

Good correlations shown in Figs. 2–5 tempt us to predict T_{gc} and k values for kojibiose, sophorose, nigerose, and laminaribiose, for none of which the corresponding numerical data have been reported anywhere elsewhere to our knowledge. These predicted values are given in the second to fifth columns in Table 2. Now we must bear in mind that T_{gc} values are very sensitive to preparation and residual moisture of amorphous carbohydrates of interest. Thus it should be recognized that these predicted values correspond to those for carbohydrates prepared under the same experimental conditions as those in the literature data that were used for the linear regression line in each figure.

Amount of unfrozen water versus molecular structure of carbohydrates (hydration model).—Our previous paper,[41] where U_w was estimated for various monosaccharides, reported that when we interpreted their different degree of anti-freeze effects in view of molecular structure of the carbohydrates, it was possible to apply a modified stereospecific hydration model,[50–52] according to which the hydration features of carbohydrates depend on their compatibilities with three-dimensional water structure, i.e., its tetrahedral hydrogen-bond network, and the compatibilities are largely dictated by the relative configuration of next-nearest-neighbor hydroxyl groups. The hydration model focuses on, in particular the relative positions of OH-2 and OH-4. The monosaccharides are grouped into three classes based upon the following stereochemistry about predominant anomeric isomer in water. Group A: OH-2 is equatorial and OH-4 is axial; Group B: OH-2 is either axial or equatorial, and OH-4 is equatorial; and Group C: both OH-2 and OH-4 are axial. For D-aldohexoses, D-galactose belongs to Group A, D-glucose and D-mannose to Group B, and D-talose to Group C. For D-aldopentoses, D-arabinose belongs to Group A, and D-xylose, D-ribose, and D-lyxose belong to Group B.

Such a hydration model has been advanced by Galema et al.[50–52] through the observation of, for example, partial molar compressibility $K^{\circ}_{S,2}$ of aqueous carbohydrate solution.[52] This physical amount sencitively reflects the characteristics of the hydration shell structure. When a dissolved solute disturbs or breaks the three-dimensional hydrogen-bond network of water surrounding the solute, the water in the hydration shell is more dense and less compressible than bulk water. This results in a negative value for $K^{\circ}_{S,2}$. We have previously demonstrated that U_w for monosaccharides increased linearly with decreasing $K^{\circ}_{S,2}$, and that the relative magnitude of U_w follows the above classification in cases of both aldohexoses and aldopentoses, being larger for Group A than for Group B.[41] Such

Table 2
Representative prediction with the use of a linear regression line drawn in each figure

Carbohydrate	T_{gc} [a]			k	$10^4 K^{\circ}_{s,2}$ [d]	ΔH_{sol} [e]
	Fig. 2 [b]	Fig. 3 [c]	Fig. 4 [c]	Fig. 5	Fig. 6	Fig. 7
Kojibiose	64.1	76.9	80.0	5.48	−23.4	−18.8
Sophorose	71.9	85.7	85.3	5.71	−24.9	−20.5
Nigerose	73.2	87.2	86.2	5.74	−25.1	−20.8
Laminaribiose	96.7	113.8	102.2	6.43	−29.5	−26.0

[a] Given in °C.
[b] Given as onset values.
[c] Given as midpoint values.
[d] Given in cm^3/mol/bar.
[e] Given in kJ/mol.

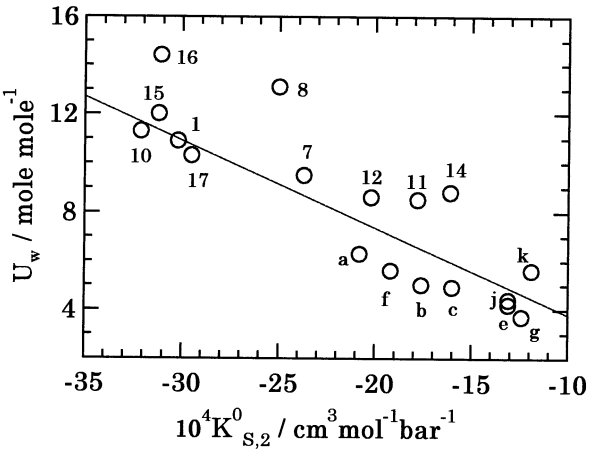

Fig. 6. Plot of U_w vs. $K^\circ_{S,2}$. (j) D-Lyxose; (k) D-talose. Other alphabets denote the same as in Fig. 2. For these carbohydrates, U_w data were used from Ref. 41. For others, U_w data and numbering are given in Table 1. Numerical data in Ref. 52 were used for $K^\circ_{S,2}$. The linear regression line was obtained with the linear squares method, exhibiting a correlation coefficient $r^2 = 0.77$.

results revealed that a larger amount of unfrozen water was produced in the presence of a monosaccharide, for example D-galactose, which has poorer compatibility with the pre-existing three-dimensional hydrogen-bond network of water. The linear correlation between U_w and $K^\circ_{S,2}$ is now extended to disaccharides, as shown in Fig. 6, although a few of them largely deviate from the straight line.

In what follows, the present results of U_w will be discussed in terms of molecular structures of the carbohydrates studied, together with $K^\circ_{S,2}$ data, if available. At first we will consider the disaccharides **1**–**10**, where the numbering corresponds to that in the first column in Table 1, all of which are composed of two glucose units. For disaccharides **1**, **3**, **5**, **7**, and **9**, their numerical data for U_w differ from each other, indicating that the aqueous unfrozen behavior induced by the presence of disaccharides depends on the position of the glycosidic linkage between the two constituent units. A similar statement can be made from comparison of the U_w values for disaccharides **2**, **4**, **6**, **8**, and **10**. Disaccharides, such as **3** and **4**, where the glycosidic linkage occupies the same position, exhibit to different extents of the yield of unfrozen water. Our results show that in general the presence of a β-type of linkage results in a larger amount of water unfrozen than does the presence of the α-type. The unfrozen water content depends, not only on the position, but also the type of linkage between the constituent monosaccharides.

Trehalose **1** has a more negative $K^\circ_{S,2}$ as compared with maltose **7**, which means the former exhibits a poorer compatibility with the three-dimensional hydrogen-bonding structure of water. It was observed here that aqueous trehalose had a larger amount of unfrozen water content in comparison with aqueous maltose. Conjunction of the two findings supports the recent argument from the Raman scattering study of aqueous solution of trehalose, which states that trehalose has structure-breaking effects on the tetrahedral hydrogen-bonding network of water and thereby reduces the amount of freezable water.[53]

Another component type of disaccharides, melibiose **15** and lactulose **17**, have $K^\circ_{S,2}$ values as negative as that of trehalose, exhibiting poor compatibility with the water structure, which would relate to the presence of a galactose unit that has a bad fit into the three-dimensional hydrogen-bond network of water. At present the two carbohydrates **15** and **17** are found to remain large amounts of unfrozen water comparable to the case of trehalose. In view of unfrozen water content, as well as partial molar compressibility, trehalose is not special, at least not anomalous, among the disaccharides studied, although trehalose has the relative characteristics as described in the above paragraph.

It is of interest that an almost equal amount of unfrozen water is induced in the aqueous solutions of disaccharides **11**–**14**, all of which consist of a glucose and a fructose unit. These carbohydrates have different values of $K^\circ_{S,2}$ from each other. Of these disaccharides, turanose **12** conforms most the linear correlation between U_w and $K^\circ_{S,2}$ shown in Fig. 6. This finding may be associated with the molecular structure of turanose, in which the fructose unit is in the pyranose form. This aspect distinguishes turanose from both sucrose and palatinose. A large deviation found for palatinose **14** from the correlation line could be due to the existence of the (1→6)-type linkage between the constituent glucose and fructose units.

At any rate, the correlation illustrated in Fig. 6 indicates that the relative unfrozen phenomena observed for different aqueous carbohydrates reflect their hydration properties, especially the compatibility of the dissolved solute with the three-dimensional hydrogen-bond structure of water. In other words, carbohydrate dependence of unfrozen water content would be associated with the extent of forming hydrogen bonds between a dissolved carbohydrate and its surrounding water molecules, and with the extent of breaking hydrogen bonds between water molecules surrounding the solute. This is also strongly suggested in Fig. 7 that shows a good correlation between U_w and the heat of solution of amorphous carbohydrates, ΔH_{sol}, which is a measure of hydrogen-bonding ability of solutes under the assumption that molecular interactions of solutes in an amorphous state are comparable among different solutes to be compared.[48] The straight line that is drawn covers the wide range of carbohydrates, although it must be recognized that the plot includes only one example for mono- and oligosaccharides, respectively. What we should note is that the linear correla-

tion holds even in a small area, the middle region in the figure, which covers disaccharides. This suggests that the correlation found has its origin in the delicate aspects of stereochemistry that characterizes the hydration behavior of carbohydrates.

An interesting comparison is given for four representative disaccharides in Table 3. For each aqueous carbohydrate, the unfrozen water content observed is in good agreement with hydration numbers determined by different methods. In addition it is noteworthy that the amount of unfrozen water and hydration numbers follow the same order of lactose > trehalose > maltose > sucrose. A series of numerical data as measured with various techniques may reflect in a similar manner stereochemical features of each carbohydrate.

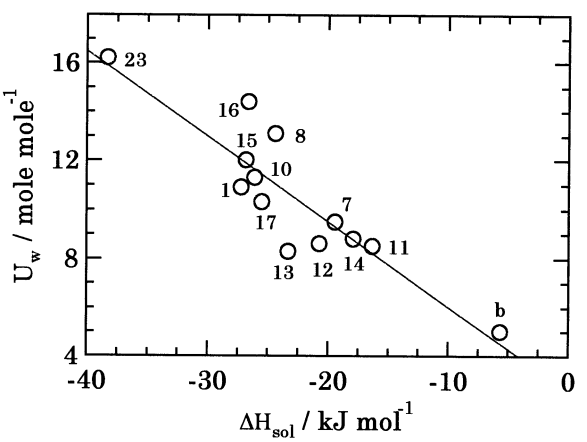

Fig. 7. Plot of U_w vs. ΔH_{sol}. Numerical data in Ref. 48 were used for ΔH_{sol}. The linear regression line was obtained with the linear squares method, exhibiting a correlation coefficient $r^2 = 0.80$.

Table 3
Comparison of numerical data of U_w with hydration numbers as determined by various techniques

Carbohydrate/ method	U_w [a]	Hydration number [a]		
	DSC [b]	Viscosity and density [c]	QENS [d]	MD [e]
Lactose	14.4	8.3		
Trehalose	10.9	8.0	9.0	7.8 [f]
Maltose	9.5	7.45	8.4	
Sucrose	8.5	6.8	7.5	7.0 [g]

[a] Given in moles of water per mole carbohydrate.
[b] Present work.
[c] Cited from Ref. 54.
[d] Quasielastic neutron scattering. Cited from Ref. 23.
[e] Molecular dynamics simulations with the same force field (CHARMM) and water potential (TIP3P). The hydration numbers cited have been estimated as the averaged number of water molecules existing within 2.8 Å of the carbohydrate oxygen atoms throughout the simulation.
[f] Cited from Ref. 55.
[g] Cited from Ref. 56.

A common key of stereochemistry behind different thermodynamic parameters.—When a given aqueous solution of carbohydrate starts to freeze, water molecules move from the liquid phase to the solid one, i.e., ice, one after another. The residual liquid fraction becomes gradually concentrated with the dissolved carbohydrate. The freeze concentration elevates the viscosity of the residual liquid fraction and limits the ability of water molecules to diffuse, leading to a situation unfavorable for further ice formation. When the viscosity reaches values as high as 10^{12}–10^{14} Pa's, the unfrozen fraction undergoes the glass transition, and ice crystal growth is halted on an experimental time scale.[57] A higher T'_g allows less water to freeze, resulting in production of more unfrozen water. Thus various amounts of unfrozen water are associated with differences in the glass transition temperatures. In the present study, as described above, almost equal glass transition temperatures were measured for many aqueous carbohydrates, whereas their unfrozen water differed in amount from each other. However, unfrozen water induced by the presence of a carbohydrate is definitely related to the vitreous properties of the carbohydrate, increasing linearly with T_{gc}, as shown in Figs. 2–4. In addition, at the same time, there is such a correlation as that U_w increases linearly with decreasing partial molar compressibility, i.e., with lowering the extent of fit into the water structure (Fig. 6). A combination of these results enables us to suggest that a carbohydrate with higher T_{gc} has a poorer compatibility with the three-dimensional hydrogen-bond network of water.

Vitreous properties of a given system are surely subject to the influence of hydrogen-bond formation, with a large increase in its glass transition temperature.[58] This makes it possible to speculate that a significantly lower T_{gc} for sucrose relative to other disaccharides is due to its less extensive hydrogen-bond network in its amorphous state. We have no clue as to a key point of stereochemistry in forming a hydrogen-bond network between carbohydrate molecules in their amorphous states. An unambiguous elucidation of these points would require the aid of theoretical approaches. At the present stage one possible interpretation about both linear correlations of U_w versus T_{gc} and U_w versus $K^{\circ}_{S,2}$ is that relative hydroxyl arrangements derived from molecular structure of a given carbohydrate largely contribute to T_{gc} determination, being at the same time a crucial factor for the compatibility of the carbohydrate with the three-dimensional hydrogen-bond structure of water. In this context the modified stereospecific hydration model described above provides a tentative frame of grouping carbohydrates in terms of their molecular structures.

Thermodynamic properties and biological protective function.—In nature di- and oligosaccharides are important compounds capable of protecting biological substances and organs against freezing damage. For example, trehalose exists as a cryoprotectant in overwintering insects.[2] Trehalose is also useful as an artificially added protectant in cryopreservation of plant cells.[8] This carbohydrate is also distributed in organisms that experience extreme desiccation stress, in some cases anhydrobiosis.[59] In many species carbohydrates acting as a cryoprotectants also exert protective effects against dehydration stress.[2] The current study demonstrates that glassy properties of a given anhydrous carbohydrate correlate well with its altering effects on the freezing behavior of water, as shown in Figs. 2–4. This result provides a physicochemical background of the diversified protective roles played by carbohydrates in biological systems. The choice of carbohydrates accumulated in living cells determines the efficiency of their dehydration tolerance, as well as of freezing tolerance. Gentibiose **10**, melibiose **15**, and lactulose **17** have considerable structure-breaking effects on the three-dimensional hydrogen-bond network of water, which results in forming unfrozen water in as large quantity as the case of trehalose **1** (Fig. 6). In a dry environment, however, the relatively higher T_{gc} of trehalose[19e,32,36,38,48] would make this carbohydrate more favorable as a protectant than the others.

4. Conclusion

This study has provided insight into the effects of molecular structure of di- and oligosaccharides with respect to their ability to alter the behavior of water at subzero temperatures. Important thermodynamic parameters are the amount of unfrozen water and the glass transition temperature, both of which have been measured by differential scanning calorimetry for 17 di- and eight oligosaccharides in aqueous solution. The amount of unfrozen water increases linearly with the glass transition temperature of anhydrous carbohydrates. In addition, the amount of unfrozen water has a linear correlation with known physicochemical properties of aqueous carbohydrates, such as partial molar compressibility and the heat of solution. The different anti-freeze effectiveness among various di- and oligosaccharides is related to a combination of constitutive monosaccharides and its overall molecular structure features, including the position and type of the glycosidic linkage between the constituent units. More water remains unfrozen by the existence of a carbohydrate with a poorer fit into the three-dimensional hydrogen-bond network of water. A series of these results lead to an implication that the same carbohydrate moiety in a molecular structure of each carbohydrate compounds governs several different thermodynamic parameters of a given system.

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