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Effect of stereochemistry on the anti-freeze characteristics of carbohydrates. A thermal study of aqueous monosaccharides at subzero temperatures

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Abstract

Thermal behavior at subzero temperatures has been investigated for aqueous solutions of various monosaccharides. The heat of fusion of ice measured with differential scanning calorimetry has given linear plots against sugar concentration (wt.%), from which the amount of unfrozen water, U_w, has been determined for each monosaccharide. The results for U_w are analyzed by employing, as a measure of hydration characteristics, known physico–chemical properties of aqueous monosaccharides, such as partial molar compressibilities, etc. It was revealed that the anti-freeze characteristics of carbohydrates depend on their stereochemistry. More water remains unfrozen in the aqueous solutions of carbohydrates having poorer compatibility with the three-dimensional hydrogen-bond network of water. Monosaccharides studied can be subdivided into three groups according to the extent of the anti-freeze effect. These results are rationalized in terms of a modified stereospecific hydration model. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Anti-freeze characteristics; Unfrozen water; Monosaccharides; Stereochemistry; Modified stereospecific hydration model

1. Introduction

A noteworthy function of carbohydrates is their protective activity against deterioration of biological molecules at low temperatures [1–3]. This is of significance in terms of using sugar for biological material preservation, food processing and storage. Another facet that arouses interest is the fact that sugar residues play a key role in exerting the inherent effect of the so-called anti-freeze glycoproteins [4]. To elucidate molecular mechanisms behind these phenomena, one needs to obtain deep insight into the anti-freeze effects carbohydrates have on water. To this end, it is imperative that we investigate the behavior of aqueous carbohydrates by way of a freeze–thaw process. In an earlier study [5], the heat of fusion of ice was measured for aqueous solutions of several carbohydrates using differential scanning calorimetry (DSC), and polysaccharides were found to include a larger amount of unfrozen water (U_w) than smaller carbohydrates. With a similar technique, U_w values have been determined for glucose, mannose, and galactose, respectively [6]. Daoukaki-Diamanti et al. [7] have investigated aqueous solutions of several carbohydrates at very low temperatures (down

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to 80 K) using the depolarization thermocurrent method, and they have shown that glucose, mannose, and galactose are distinguishable from both arabinose and ribose on the basis of water behavior in their individual frozen solutions. Such classification of carbohydrates has been rationalized in terms of the number of equatorial hydroxyl groups within a sugar molecule [7]. Kawai et al. [8] have reported that for some di- and oligosaccharides U_w values correspond well with the number of equatorial hydroxyl groups, using the DSC technique. Recently, Wang and Haymet [9] have compared thermal behavior at subzero temperatures among aqueous glucose, fructose, sucrose, and trehalose, and have suggested that sugar effectiveness in cryopreservation may have a certain relationship with the intrinsic property and/or molecular structure for each carbohydrate. In spite of increasing knowledge with respect to the cryoproperties of carbohydrates, the relevant information provided so far is still fragmentary. It remains obscure what relationship exists comprehensively between the anti-freeze characteristics and the details of stereochemistry for carbohydrates.

In this paper, low-temperature thermal properties were investigated with DSC measurements for aqueous solutions of monosaccharides, six aldohexoses, five aldopentoses, and four ketohexoses. Our aim was to present the relative anti-freeze characteristics of these sugars studied as a function of stereochemistry. Performing this work, we should remember that the measured heat of melting of ice is probably a nonequilibrium value [10]. That is, a series of frozen aqueous solutions examined is most likely to be in a slow relaxation process, for example, toward further ice crystal growth, in which water and solute molecules are subject to a considerable diffusion limit, because of the high viscosity of the freeze-concentrated solutions, which may be in the glassy state. Thus, in each run we would merely see one scene in the relaxation process, just like taking a snapshot of the process. However, the significant point to be emphasized is to extract information on the relative ability of carbohydrates with regard to the anti-freeze effectiveness, throughout the experiment under an identical protocol, i.e., on the same time scale. The anti-freeze characteristics of carbohydrates will be evaluated in terms of U_w. These phenomena are discussed from the standpoint of stereochemical compatibilities of carbohydrates with the pre-existing water structure formed by a three-dimensional hydrogen-bond network.

2. Experimental

All sugars studied were purchased from Fluka, except for D-psicose (Sigma Chemical Co.), in the best quality obtainable. All reagents were used as received; their moisture content was checked by use of a Karl–Fischer coulometric titrimeter (Mitsubishi). Aqueous 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 in order to achieve complete dissolution. Carbohydrate concentration was varied in 5 wt.% increments from 10 wt.% up to a maximum of 50 wt.%. The series of aqueous solutions prepared were sealed separately in vials and kept at 25 °C for 24 h in an incubator to allow them to reach steady-state. Aqueous samples obtained were hermetically sealed in aluminum pans for DSC measurements.

A Mac science DSC3100 apparatus was used for thermal measurements. Samples of interest were cooled down to −70 °C from room temperature at 8 °C/min, and then a heating run was immediately carried out at 5 °C/min to 30 °C. Dry nitrogen gas was streamed in at 130 mL/min to prevent condensation of moisture within the DSC furnace. For all carbohydrates studied, DSC measurements were made for at least triplicate samples at each concentration.

3. Results

The heat of fusion of ice, H_f, was measured for aqueous monosaccharides as a function of sugar concentration (wt.%). An excellent linear relationship was found between H_f and the concentration, with the correlation coefficient

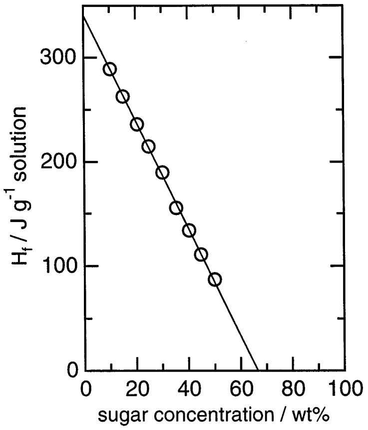

Fig. 1. Plot of H_f vs. concentration of D-glucose.

>0.99 for all carbohydrates studied. As an example, the result for D-glucose aqueous solutions is shown in Fig. 1. The linearity of H_f against the concentration indicates the invariance of the amount of unfrozen water, U_w, at least within the concentration range examined. In other words, the freeze-concentrated concentration reached is independent of the initial concentration of the aqueous solution. The freeze-concentrated concentration can be estimated by extrapolation of the straight line to $H_f = 0$. The concentration of the intercept corresponds to the minimum concentration of the aqueous carbohydrate with $H_f = 0$, from which we evaluate U_w in terms of moles of water per mole of carbohydrate. Numerical data of U_w for the monosaccharides studied are summarized in the third column of Table 1, together with the literature data available in the fourth column. The U_w values obtained here for D-glucose, D-mannose, and D-galactose are obviously different from the corresponding ones reported in Ref. [6]. For U_w of D-glucose, our value is relatively close to the numerical data of Biswas et al. [5]. According to a recent study [9], for both D-glucose and D-fructose aqueous solutions, their minimum

Table 1
Comparison of amount of unfrozen water for monosaccharides with their total number of hydroxyl groups and with the averaged number of equatorial hydroxyl groups

		U_w [a]			
No.	Aldohexose	This work	Literature [b]	N_{OH} [c]	Ne_{OH} [d]
a1	D-galactose	6.3(0.4)	9.2 [e]	5	3.5
a2	D-glucose	5.0(0.3)	9.5 [e], 4.3 [f]	5	4.6
a3	D-mannose	4.9(0.3)	8.2 [e]	5	3.3
a4	D-allose	4.8(0.3)		5	3.6
a5	D-altrose	4.7(0.2)		5	2.1
a6	D-talose	5.6(0.3)		5	2.0
No.	Aldopentose				
b1	D-arabinose	5.6(0.3)		4	2.6
b2	L-arabinose	4.9(0.3)		4	2.6
b3	D-xylose	4.2(0.2)		4	3.6
b4	D-lyxose	4.4(0.2)		4	2.3
b5	D-ribose	3.7(0.2)		4	2.4
No.	Ketohexose				
c1	D-fructose	5.0(0.3)	4.8 [g]	5	2.5
c2	L-sorbose	5.7(0.3)		5	3.9
c3	D-psicose	6.2(0.4)		5	1.7
c4	D-tagatose	5.9(0.4)		5	3.1

[a] Unfrozen water in terms of moles of water per mole carbohydrate. Standard errors in numerical data are given in parentheses.

[b] All literature values cited have been estimated in a similar way to the procedure employed in this study.

[c] Total number of hydroxyl groups within each sugar molecule.

[d] Averaged number of equatorial hydroxyl groups for the anomeric mixture of a carbohydrate in aqueous solution.

[e] Cited from Ref. [6].

[f] Cited from Ref. [5]. The numerical value 4.3 for U_w was calculated from the literature data 70 wt.% obtained by extrapolation to $H_f = 0$.

[g] Cited from Ref. [11], where the amount of unfrozen water is given in g H_2O/g solute. The numerical value 4.8 for U_w was calculated from the literature data 0.481 g H_2O/g solute (fructose).

concentrations with $H_f = 0$ lie between 50 and 70 wt.%, respectively. The corresponding concentrations estimated here for the two carbohydrates are consistent with the literature findings. Validity of the extrapolation method to estimate U_w has been described in Ref. [11], where either the frozen or unfrozen phenomenon has been visually demonstrated for several aqueous solutions of fructose with different concentrations. It was confirmed that ice formation occurs at fructose concentrations up to at least 65 wt.%, while no frozen water exists at a concentration of 70 wt.% or higher [11]. It has reported [11] that the U_w value for fructose, 4.8, estimated from the linear regression analysis of the H_f plot similar to Fig. 1, is consistent with such behavior actually observed for the aqueous solutions of interest. The present U_w value for fructose, 5.0, is in agreement with the literature value within experimental error.

4. Discussion

At first sight, an outstanding feature of the result for U_w is that its numerical value for each carbohydrate is comparable to the total number of hydroxyl groups within the sugar molecule, N_{OH} (Table 1). This strongly suggests that the occurrence of the unfrozen water has a close relationship with the hydroxyl groups of carbohydrates (hereafter hydroxyl groups will be represented as OH groups). Now our main interest is in the carbohydrate dependence of unfrozen water. We will focus more on the U_w differences from sugar to sugar rather than on the absolute values for U_w.

As described in Section 1, the relative behavior of unfrozen water in aqueous carbohydrates has often been interpreted from a viewpoint of the number of equatorial OH groups, Ne_{OH}, the importance of which comes from the higher hydration ability of equatorial OH groups relative to axial ones [7,8]. For each monosaccharide studied in the current paper, Ne_{OH} was evaluated as a weighted average in accordance with the relative abundance of anomeric isomers given in Ref. [12]. Numerical data of Ne_{OH} are summarized in the last column of Table 1, being plotted against U_w in Fig. 2. Apparently, there is no good correlation between Ne_{OH} and U_w, which indicates that the relative behavior of the unfrozen water is not predominantly determined by Ne_{OH}.

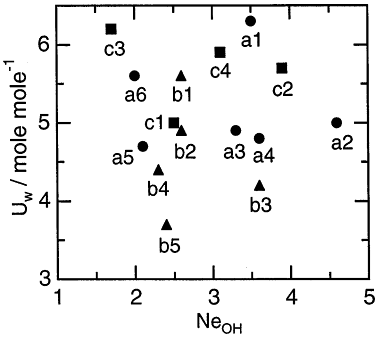

Fig. 2. Plot of U_w vs. the number of equatorial hydroxyl groups, Ne_{OH}: aldohexoses (●); aldopentoses (▲); ketohexoses (■). Numbering of each carbohydrate is given in the first column of Table 1.

For several di- and maltooligosaccharides, the amount of unfrozen water has been reported to correspond well to Ne_{OH} [8]. This principle, however, does not hold for the monosaccharides studied here. Daoukaki-Diamanti et al. [7] have described that glucose, mannose, and galactose belong to the same group, in terms of water behavior in frozen aqueous carbohydrates, while arabinose and ribose belong to another group. Such classification has also been explained on the basis of the relative magnitude of Ne_{OH} [7]. Our data for U_w show, however, clearly different trends from these earlier results. For example, U_w for galactose is far different from those for glucose and mannose, respectively. According to this, galactose seems to fall into a different group from that which consists of glucose and mannose. For arabinose and ribose, a new finding is seen similar to the case of the three aldohexoses.

The carbohydrate dependence of the antifreeze effectiveness observed can be rationalized in terms of a modified stereospecific hydration model [13–16]. The hydration model states that hydration properties of carbohydrates depend on their compatibilities with the pre-existing three-dimensional hydrogen-bond network of water, and that the compatibilities are preferentially governed by the relative distance of next-nearest-neighbor oxygens of OH groups within a given carbohydrate molecule. The hydration model has been proposed by Galema et al. [13–16] throughout studies of kinetic medium effects G(c)[1] [13,14] and volumetric properties, such as partial molar compressibilties $K^0_{S,2}$[2] [15], of carbohydrates. As shown in Figs. 3 and 4, there is a good relationship between U_w and G(c), except for D-talose (a6), which shows an upward trend of U_w with G(c). U_w also correlates well with $K^0_{S,2}$, again except for D-talose, and decreases with increasing $K^0_{S,2}$ (Fig. 5). These results indicate that in general the unfrozen water in the aqueous solutions of monosaccharides is closely related to the compatibilities of carbohydrates with water. More water remains unfrozen in the case of the poorer fitness of carbohydrates into the three-dimensional water structure.

Galema et al. [13–16] have emphasized, throughout the above hydration model, the importance of the relative positions for OH-2 and OH-4. Monosaccharides have been divided into three groups based upon the following stereochemical aspects in regard to their predominant anomeric isomer in water: (a) OH-2 is equatorial and OH-4 is axial; (b) OH-2 is either axial or equatorial, and OH-4 is equatorial, and (c) both OH-2 and OH-4 are axial. According to this classification, the

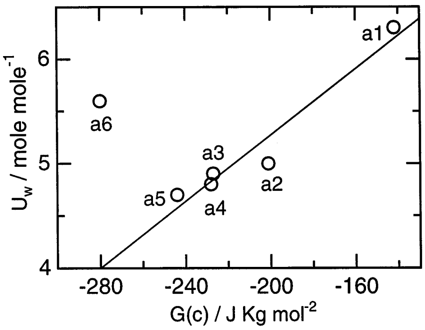

Fig. 3. Plot of U_w vs. kinetic medium effects, G(c), for aldohexoses. Numbering of each carbohydrate is given in the first column of Table 1. Numerical data in Ref. [14] were used for G(c).

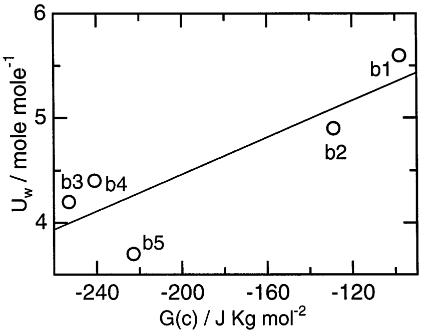

Fig. 4. Plot of U_w vs. kinetic medium effects, G(c), for aldopentoses. Numbering of each carbohydrate is given in the first column of Table 1. Numerical data in Ref. [14] were used for G(c).

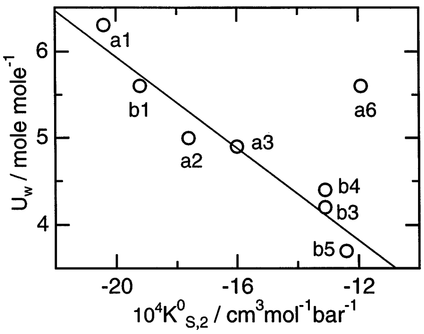

Fig. 5. Plot of U_w vs. partial molar isentropic compressibility, $K^0_{S,2}$, for aldoses. Numerical data in Ref. [15] were used for $K^0_{S,2}$.

aldohexoses of interest are grouped as (a) D-galactose[6.3]; (b) D-glucose[5.0], D-mannose[4.9], D-allose[4.8], D-altrose[4.7], and (c) D-talose[5.6], where numerical data in each bracket represent the value of U_w. This classification corresponds well with the relative behavior of the unfrozen water. For the aldopentoses b1–b5, a similar rule holds for the

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[1] G(c) is defined in the following equation, which describes kinetic aspects of the water-catalyzed hydrolysis of 1-benzyl-3-phenyl-1,2,4-triazole:

$$\ln (k/k_w) = (2/RT)G(c)m - Cm,$$

where k and k_w represent the pseudo-first-order rate constants in a given aqueous carbohydrate and pure water, respectively, and m, R, T, C, and G(c)m represent the sugar concentration (in mol/kg), Boltzmann constant, absolute temperature, constant with respect to osmotic coefficient of water, and the activation Gibbs free energy of the hydrolysis at the sugar concentration m, respectively. Details have been described in Refs. [13] and [14]; we simply emphasize the following two points: (1) the presence of carbohydrates in water brings about a rate retardation of the hydrolysis, leading to the negative value of G(c). (2) This kinetic parameter reflects the carbohydrate-induced perturbation of the three-dimensional hydrogen-bond structure of water, becoming less negative with decreasing carbohydrate fitness into water.

[2] $K^0_{S,2}$ reflects the feature of the hydration shell structure. When a dissolved solute disturbs or breaks the three-dimensional hydrogen-bond network of water around the solute, the water in the hydration layer is more dense and less compressible than bulk water. In this case, $K^0_{S,2}$ has negative values.

case of the aldohexoses in characterizing the relative behavior of U_w. These results show that remaining water unfrozen by dissolved monosaccharides can be related to details of their stereochemistry, especially the relative positions of OH-2 and OH-4.

The aforementioned anomalous behavior of D-talose (a6) in comparison with other carbohydrates (Figs. 3 and 5) may be due to its characteristic molecular structure, where both OH-2 and OH-4 occupy axial configurations in the preferential anomeric isomer in water. This relative position of the two OH groups allows them to form an intramolecular hydrogen bond [16], making D-talose behave like a hydrophobic solute. This situation is quite different from the cases of the other carbohydrates studied.

On cooling of a given aqueous carbohydrate, ice nucleation occurs below a certain temperature, and then each nucleus starts to grow with water molecules migrating one after another from the liquid phase to the solid one, i.e., ice. The residual liquid phase becomes increasingly concentrated with the dissolved carbohydrate and consequently, more viscous. As a result the diffusion of water molecules is considerably limited due to the high viscosity. This situation results in apparent cessation of freezing of the residual part of water. In this context, the reason for the occurrence of unfrozen water is that the water diffusion is kinetically retarded because of the high viscosity of the freeze-concentrated liquid phase [10]. Interestingly, however, the relative behavior of the unfrozen water in the current study was found to reflect clearly hydration properties characteristic of individual carbohydrates. Moreover, the absolute amounts of unfrozen water are not greatly different from the total number of hydroxyl groups within each sugar molecule (Table 1). Thus, the unfrozen water observed seems, as a result, to correspond to the water in the first hydration shell area of the sugar molecules.

It must be kept in mind, however, that even if the observed unfrozen water has hydrogen bonds with the adjacent solute carbohydrates, such bonds may not be permanent [10]. A series of the freeze-concentrated aqueous carbohydrates obtained in the current study must have been in a slow relaxation process, for example toward further growth of ice crystals. Probably we have merely observed one scene of the relaxation process, just like taking a snapshot of the process. The important point we would like to emphasize, however, is that one has been able to provide deep insight into the relative ability of carbohydrates as anti-freeze agents through experiments on the same time scale. According to Ref. [17], at the final stage of such a relaxation process the concentration of freeze-concentrated aqueous carbohydrates reaches an almost equivalent plateau value (ca. 80 wt.%) regardless of the kinds of small carbohydrates. In other words, the amount of virtually 'unfreezable' water in terms of H_2O moles per mole of sugar is determined by the molecular weight of dissolved carbohydrates. In conjunction with the present observations of U_w, an implication can be extracted that in an absolute sense the anti-freeze characteristics of carbohydrates are little or not dependent on details of their stereochemistry, while in terms of slowing down the rate of ice crystallization the anti-freeze characteristics of carbohydrates are governed by their detailed stereochemistry.

The unfrozen water of carbohydrates may be subject to influence by the glass transition, which aqueous carbohydrates go through on cooling to low temperatures [9]. Caution is hence required when one compares amounts of the unfrozen water for carbohydrates that have largely different glass transition temperatures. In the current study, however, this aspect would not be a serious problem, since glass transition temperatures observed have fallen within a very narrow range at least for carbohydrates belonging to the same family among monosaccharides: -64 ± 1 °C for aldopentoses, -57 ± 1 °C for aldohexoses, and -58 ± 1 °C for ketohexoses. Such trends of glass transition temperatures for aqueous carbohydrates are consistent with ones reported previously [17].

Results obtained in this paper suggest an abundance of implications for roles of carbohydrates in cryobiology. A relevant example to be noted is the so-called anti-freeze glycoproteins (AFGPs), which have been found in blood serum of Antarctic fishes [4]. The glyco moiety of AFGP molecules plays a key role in the functional behavior of the glycoproteins. The glyco unit of AFGPs includes a D-galactopyranosyl residue[3]. This fact seems likely to correlate with the present observation that the presence of D-galactose has resulted in more water remaining unfrozen than have any other of the aldohexoses investigated.

5. Conclusions

This study has shed light, at the molecular level, on the aqueous unfrozen phenomena due to dissolved carbohydrates. These phenomena depend on the stereochemistry of carbohydrates, and the relative behavior of the unfrozen water is rationalized in terms of the modified stereospecific hydration model. Those carbohydrates that have a poorer fit into the three-dimensional hydrogen-bond network of water retain more amounts of water unfrozen. The anti-freeze characteristics of monosaccharides can be related to the relative positions of the sugar hydroxyl groups OH-2 and OH-4. The results obtained would be of great value for the molecular-level elucidation of roles of sugar in cold-resistance organisms. The current study is furthermore very informative from a viewpoint of molecular design and development of new cryoprotectants and/or cryostabilizers. Further studies extended to another carbohydrates are currently under way in our laboratory.

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[3] The backbone for most AFGP molecules is composed of repeating units of the tripeptide Ala-Ala-Thr, with a disaccharide β-D-galactopyranosyl-(1 → 3)-2-acetomido-2-deoxy-α-D-galactose glycosidically linked to the side chain of each threonine residue. See Ref. [4] and references therein for details.