Diffusion of Sucrose and α,α-Trehalose in Aqueous Solutions

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Received: January 18, 2002; In Final Form: June 27, 2002

Diffusion coefficients for sucrose and trehalose in aqueous solutions were measured using pulsed-gradient-spin-echo NMR and calculated using molecular dynamics simulations. The diffusion coefficients of sucrose and trehalose are comparable at low disaccharide concentrations but differ from each other with increasing concentration and decreasing temperature. At 400 K and the highest disaccharide concentration considered in this work (80 wt %), the simulated trehalose diffusion coefficient is a factor of 3.5 smaller than that of sucrose (2.6×10^{-8} cm^2/s and 9.3×10^{-8} cm^2/s, respectively). In a 74 wt % disaccharide solution, the experimental diffusion coefficient for trehalose is a factor of 3 smaller than that of sucrose at 358 K. The diffusion coefficients of water are at least 1 order of magnitude larger than those of the disaccharides. This difference increases to 2 orders of magnitude at the highest disaccharide solutions considered here. The decoupling of the water and disaccharide motion is attributed to the different diffusion mechanisms adopted by the two molecules. Simulations reveal that, at high disaccharide concentrations, the diffusion of the disaccharides appears continuous while that of water consists of random jumps followed by rapid vibrations within transient cages created by surrounding molecules.

1. Introduction

Disaccharides are often used in pharmaceutical, food, and biomedical applications to prepare glassy matrices for long-term storage of biological materials. When used in lyophilization processes, these molecules are able to preserve the structure and function of biological entities in reduced-water environments. Understanding the specific interactions that occur between sugars, water, and biological systems would help explain why disaccharides are effective protectants. More importantly, by studying the properties of different sugars, one could try to explain why some are more effective than others, and eventually identify or design molecules (or combinations thereof) capable of providing superior, long-term stabilization of biological materials under anhydrous conditions.

Sucrose and α,α-trehalose are perhaps the most widely used disaccharides for stabilization of biological materials (e.g., proteins,[1] cells,[2] and membranes[3]). As shown in Figures 1 and 2, they have the same molecular formula ($C_{12}O_{11}H_{22}$), but different structures. The most notable difference between the two structures is the presence of a rigid fructose ring in sucrose, as opposed to the two symmetrically linked glucose rings in trehalose. Numerous studies have demonstrated the benefits achieved by the addition of these disaccharides to biological systems during freezing and freeze-drying. Comparisons of their relative efficacy have often found trehalose to be the more effective protectant.[1,2,4]

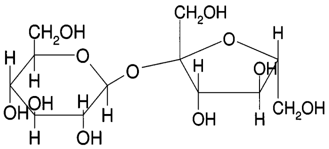

Figure 1. Sucrose−α-D-glucopyranosyl β-D-fructofuranoside. The asymmetric molecule is composed of one rigid fructose ring and one glucose ring.

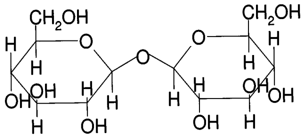

Figure 2. α,α-Trehalose−α-D-Glucopyranosyl α-D-glucopyranoside. The symmetric molecule is composed of two glucose rings bound through a glycosidic bond.

Several recent empirical studies have examined the structure and thermophysical properties of aqueous trehalose and sucrose solutions. Some properties that have been considered include molar volume,[5] viscosity,[6] glass transition temperature,[6−9] and electrical conductivity.[10] Molecular simulations have also been used to examine some of these properties as well as the hydrogen-bonding capabilities of these saccharides in dilute and concentrated solutions.[7,10−14] While simulations have in several cases predicted structures and behaviors that subsequently have been verified experimentally,[7,11,15] systematic comparisons between molecular models and experiments have been difficult due to the scarcity of experimental data. When possible, comparisons between experiments and simulations have been largely limited to equilibrium thermodynamic properties (e.g., density) and structural information (e.g., hydration number).[7,11−14]

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10.1021/jp020187b CCC: $25.00

The ability of available force fields to predict dynamic quantities has not been fully assessed.

Transport properties, particulary diffusion coefficients, provide a direct measure of molecular mobility, an important factor in the preservation of biological materials in disaccharide matrices. In addition, diffusion coefficients can be determined directly from both experiments and theory, thereby providing a useful reference to establish the validity or appropriateness of force fields and simulation models. Unfortunately, experiments and simulations of transport properties in disaccharide solutions have been limited to the dilute regime, which is not as relevant as the concentrated regime for preservation applications.

In this work, we study the dynamics of trehalose−water and sucrose−water systems over a wide range of concentrations using both molecular dynamics simulations and nuclear magnetic resonance (NMR). The translational diffusion coefficients of the solution components are measured using the pulsed-gradient spin-echo NMR technique and are compared to results of molecular simulations. We find that simulations yield results in good agreement with NMR data at low to intermediate disaccharide concentrations. We also find that, despite their chemical and structural similarities, the diffusion coefficients of sucrose are considerably higher than those of trehalose.

2. Methods and Calculations

2.1. NMR Sample Preparation. Sucrose and α,α-trehalose were obtained from Sigma Chemicals. Milli-Q water (18 MΩ·cm purity) was used in all solutions. The samples were prepared in 10 mm flat-bottomed glass tubes. The sugar and water were added separately into the sample tubes. The undissolved mixtures were then frozen in liquid nitrogen to avoid vaporization, and the glass tubes were flame sealed. The sample tubes were subsequently heated to facilitate dissolution of the sugar. The tubes were reheated in a convection oven whenever crystallization occurred.

2.2. NMR Measurement Technique. Self-diffusion measurements in the sucrose and trehalose solutions were performed using the pulsed-gradient spin-echo (PGSE) NMR method. The implementation of the high-gradient, nonspectroscopic variant of this method has been described in its early form in previous publications,[16−20] and has since been continuously refined and upgraded.[21] The equipment is composed of a stable current-regulated, high-impedance, iron-core magnet and a 33 MHz modified Spin-Lock CPS-2 spectrometer, adjusted to produce a principal spin-echo after a 90°−τ−180° pulse sequence. In this work, the time delay between gradient pulses (τ) was typically set to 15 ms. Measurements of proton spin-echo amplitude were performed at 303 K, 323 K, and 358 K. The temperature was controlled to within 0.2 °C with a steady stream of heated air. A pair of gradient pulses was applied in conjunction with the radio frequency (rf) pulses using gradient coils in the high-uniformity Zupančič-Pirš configuration.[22] The gradient pulses had a magnitude (G) ranging from 711 Gauss/cm at 303 K to 609 Gauss/cm at 358 K. The duration (δ) of the gradient pulses was varied in 8 to 20 increments from zero until the echo was attenuated to below 3% of the free induction decay; the maximal value of δ was 6 ms. A steady gradient $G_0 \approx 0.3$ Gauss/cm was applied to increase the stability of the echo. The spectrometer was operated in single-sideband mode, +3 kHz off resonance, and the signal was demodulated using rf phase-sensitive detection. The echo amplitude recorded was the magnitude of the Fourier transform integrated across the echo peak with an rms correction for the noise integral. Between 5 and 15 signals were acquired and averaged for each setting of δ.

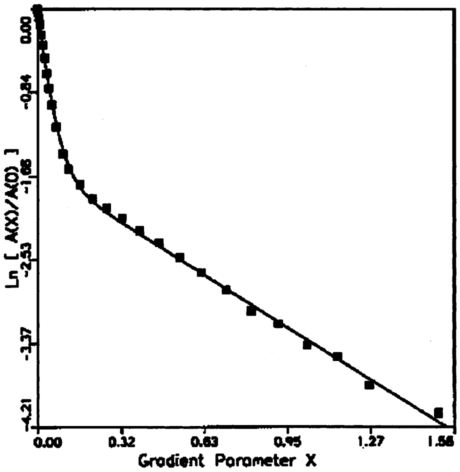

Figure 3. Logarithmic diffusional NMR spin−echo attenuation at 303 K in a sample of 55 wt % trehalose in aqueous solution. Symbols denote measured echo attenuation data; line represents successful three-parameter fit of eq 1. Diffusion rates of trehalose ($\mathscr{D}_{slow}$), and water ($\mathscr{D}_{fast}$) differ by a factor of 15.

TABLE 1: Molecular Distribution in Various Simulated Aqueous Disaccharide Systems

concentration (wt %)	water molecules	sucrose molecules
6	300	1
20	152	2
50	76	4
72.4	29	4
80	29	6

The echo amplitude (A) vs δ was analyzed using an off-line computer code (DIFUS5[23] in its current PC version). As shown in Figure 3, the data was fit to the following expression, which is appropriate for two components diffusing at different rates:

$$\mathscr{A}(2\tau,\delta)/\mathscr{A}(2,\delta=0) = f_{fast}e^{-\gamma^2 D_{fast}\mathscr{X}} + (1-f_{fast})e^{-\gamma^2 D_{slow}\mathscr{X}} \quad (1)$$

where $\mathscr{X} = G^2\delta^2(\tau - \delta/3)$ with additional minor corrections in (G·G_0), and where γ represents the proton gyromagnetic ratio. The three adjustable parameters are f_{fast}, $\mathscr{D}_{fast}$, and $\mathscr{D}_{slow}$; f_{fast} is the fraction of the echo attributed to the faster-diffusing component.

2.3. Simulation Models and Methods. The simulations employed in this work are based on a fully flexible atomistic model. A flexible SPC[24] water potential was used along with an OPLS force field[25] optimized for carbohydrates. The OPLS force field model accounted for stretching, bending, and torsional forces. Intermolecular Lennard-Jones 6-12 and Coulombic interactions were also included in the force field. The Lennard-Jones cutoff radius was set at 7 Å. Long-range corrections were implemented to avoid truncation errors. A particle mesh Ewald (PME)[26] algorithm was used to calculate long-range Coulombic interactions. The number and distribution of molecules are given in Table 1.

Both molecular dynamics (MD) and Monte Carlo (MC) simulations were employed in our study. Monte Carlo simulations were used to prepare the initial equilibrated configurations for the systems of interest. For concentrated disaccharide systems (80 wt %), a parallel tempering algorithm described elsewhere[11] was used to generate equilibrated initial configurations. An isothermal isobaric hybrid Monte Carlo[27] (NPTMC) technique was employed in our simulations. Our hybrid algorithm used 5 MD steps to move all the molecules in the system; after these MD steps, the resulting trial configuration was accepted according to appropriate probability criteria.[7,11] A multiple time scales algorithm was implemented to increase the efficiency of our calculations. The reversible reference system propagator algorithm (r-RESPA)[28] separates the force into short- and long-range components. Short-range forces are calculated more frequently than long-range forces. In our simulations, the long-time step was set at 5 times the short-time step ($\Delta t = 0.10$ fs). The densities of the systems at a specified temperature and pressure were determined from isobaric simulations; all NPTMC simulations were run for at least 1 million steps.

Isothermal molecular dynamics (NVTMD) simulations were subsequently used to calculate the diffusion coefficients of the disaccharides and water. All simulations were run for at least 5 ns at a step size of 0.25 fs. More dense systems required longer times. Five Nosé-Hoover thermostats[29,30] were implemented to control the simulation temperature. Whereas a single r-RESPA was used in the Monte Carlo simulations, a double r-RESPA algorithm[28] was employed in the MD simulations to improve their efficiency. A modification of the r-RESPA described above, the double r-RESPA factorizes the Verlet algorithm into intramolecular (i.e. bonds, bending, and torsion), short-range (i.e., nonbonded interactions within the Lennard-Jones cutoff radius), and long-range (i.e., Coulombic) interactions. Bonded interactions were calculated every time step; short-range and long-range interactions were calculated every 2 and 4 MD steps, respectively.

Diffusion coefficients were calculated from the mean-square displacement ($\langle r(t)^2 - r(0)^2 \rangle$) of the center of mass of the molecules. All of our simulations were run long enough to guarantee the linearity of a log−log plot of $\langle r(t)^2 - r(0)^2 \rangle$ versus time. As shown in Figures 7 and 8, the diffusive regime was attained for the systems of interest.

3. Results

The composition and diffusion coefficients (D) of the samples analyzed using NMR are given in Table 2. Diffusion coefficients for water (D_W) and trehalose (D_T) or sucrose (D_S) were determined for each sample at 303 K, 323 K, and 358 K (Table 2). Figures 4 and 5 show experimental diffusion coefficients for water and the disaccharides, respectively, at 303 K and 358 K, as a function of concentration.

As expected, measured diffusion coefficients for both water and the disaccharides decrease with increasing disaccharide concentration. Raising the concentration of sucrose from about 16 wt % to 74 wt % decreases D_S by almost 2 orders of magnitude at all temperatures. Water in both systems also exhibits a decrease in molecular mobility with increasing disaccharide concentration; D_W changes by approximately 1.5 orders of magnitude over the range of sugar concentration considered in this work. As can be seen from the shapes of the curves in Figures 4 and 5, the rate of decrease in the diffusion coefficient with increasing disaccharide concentration is more pronounced between 40 wt % and 74 wt % than in more dilute systems. This rapid decrease in D is consistent with the rapid increase in viscosity with increasing disaccharide concentration observed by Miller and co-workers.[6] The rapid change in the diffusion coefficients reflects the proximity of the transition from the liquid to the glassy state; as the solution approaches the glass transition, molecular mobility is expected to decrease by several orders of magnitude.

In previous work,[11] we showed that at concentrations of approximately 50 wt % or higher, trehalose and sucrose assume a structure that is analogous to that adopted in the crystalline state. For sucrose, the structure involves the formation of two intramolecular hydrogen bonds; for trehalose, only one intramolecular hydrogen bond is formed. In both cases, the conformation at high concentrations is more compact and allows an increase in density, which could contribute to the observed pronounced decrease in the diffusion coefficients of the disaccharides.

The temperature of the solution also has a significant effect on the measured diffusion coefficients of the disaccharides as shown in Figures 4 and 5. Increasing the temperature from 303 K to 358 K causes an increase of 1 order of magnitude in the diffusion coefficient of both disaccharides. A similar increase is observed for D_W in both systems. Previously reported NMR diffusion data for sucrose and trehalose[31] solutions are consistent with our measurements.

The results of our molecular simulations exhibit trends similar to those observed in the NMR data. Figure 6 shows the diffusion coefficients for water, sucrose, and trehalose in 80 wt %

TABLE 2: NMR Measurements of the Diffusion Coefficients (D) for Water and Disaccharides in Various Aqueous Solutions[a]

concentration	temperature (K)			temperature (K)		
wt % disaccharide	303	323	358	303	323	358
	Trehalose			Water		
0				22.4	34.7	64.6
16	4.17	3.16	7.41	16.2	24.0	45.7
28	1.41	3.72	7.94	11.2	18.6	37.2
37	1.12	2.19	5.62	8.91	13.2	28.2
44	0.708	1.51	3.89	6.17	10.5	21.9
55	0.234	0.603	2.00	3.47	5.75	13.8
60	0.107	0.347	1.32	2.09	3.98	10.0
64	0.0468	0.158	0.724	1.74	2.63	6.61
71	0.0135	0.0603	0.339	0.724	1.45	4.47
74		0.0191	0.182		1.12	2.95
	Sucrose			Water		
0				21.9	33.9	61.7
16	1.86	5.25	12.9	15.8	24.5	49.0
32	1.62	3.24	7.41	10.2	16.6	33.1
43	1.02	1.86	4.79	6.92	11.5	24.0
55	0.380	0.832	2.34	3.63	6.17	13.8
60	0.224	0.537	1.70	2.63	4.79	11.0
66	0.0933	0.269	1.00	1.66	3.09	7.59
68	0.0490	0.182	0.776	1.29	2.45	6.46
72	0.0219	0.0851	0.490	0.537	1.35	4.17
74	0.0151	0.0589	0.355	0.427	1.15	3.55

[a] All values are reported as $D \times 10^6$ cm^2/s. The error in the $D \times 10^6$ values is ±11% for the disaccharides and ±7% for water.

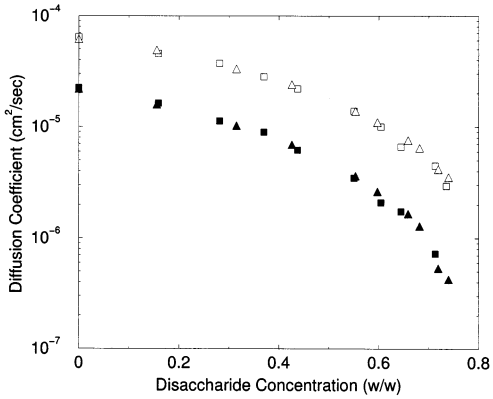

Figure 4. NMR measurements of the diffusion coefficients of water in trehalose and sucrose solutions as a function of concentration. (Closed square = trehalose at 303 K, open square = trehalose at 358 K, closed triangle = sucrose at 303 K, open triangle = sucrose at 358 K).

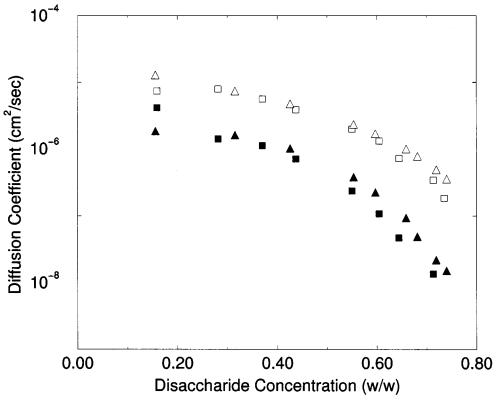

Figure 5. Effect of concentration on the diffusion coefficients of the disaccharides trehalose and sucrose in aqueous solution as measured by NMR. (Closed square = trehalose at 303 K, open square = trehalose at 358 K, closed triangle = sucrose at 303 K, open triangle = sucrose at 358 K).

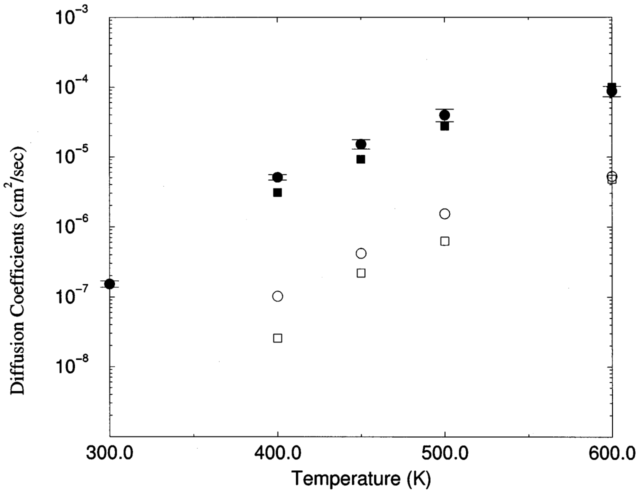

Figure 6. Effect of temperature on the diffusion coefficients of water, sucrose, and trehalose[7] in an 80 wt % aqueous solution as calculated through simulations. (Closed circle = water in sucrose, closed square = water in trehalose, open circle = sucrose, open square = trehalose).

disaccharide solutions at different temperatures. Again, we observe an increase in D with increasing temperature.

To compare the NMR and simulation results under identical conditions, simulations were run at concentrations of 6, 50, and 72.4 wt % disaccharide at 358 K. The calculated diffusion coefficients are shown in Table 3. The experimental data were interpolated to the appropriate concentration for comparison. In all cases, the diffusion coefficients calculated for water are in good agreement (within 30%) with NMR data. For the disaccharides, the coefficients calculated for the 6 and 50 wt % solutions are within a factor of 2 to 4 of the experimental data. At the higher sucrose and trehalose concentrations, however, the difference between the two sets of data is larger. For the 72.4 wt % trehalose and sucrose solutions, our simulations predict diffusion coefficients within a factor of 10 of the experimental value.

4. Discussion

The trends observed in the diffusion coefficients calculated from the simulation are in agreement with those arrived at experimentally. For pure water, simulated and experimental diffusion coefficients at 303 K are in quantitative agreement at 2.77×10^{-5} cm²/s and 2.24×10^{-5} cm²/s, respectively. These D_W values are also consistent with previously published simulation data for rigid SPC/E water[12] (2.58×10^{-5} cm²/s) and flexible SPC water[32] (3.6×10^{-5} cm²/s).

TABLE 3: Simulated and Experimental (NMR) Diffusion Coefficients (*D*) for Water and Disaccharides in Various Aqueous Solutions at 358 K[a]

concentration	trehalose		water (T)	
wt %	sim	exp	sim	exp
6	3.16	8.26	50.4	57.5
50	0.620	2.86	18.6	17.5
72.4	0.0308	0.266	2.94	3.76

concentration	sucrose		water (S)	
wt %	sim	exp	sim	exp
6	8.34	16.3	68.1	56.9
50	1.02	3.36	18.6	18.0
72.4	0.0377	0.463	3.06	4.05

[a] The experimental values are extrapolated from Table 2. All concentration and diffusion coefficient values are reported in wt % disaccharide and $D \times 10^6$ cm²/s, respectively.

For the disaccharide solutions, simulated and experimental diffusion coefficients for water and the sugars are in reasonable agreement in the low to intermediate concentration regime (less than 50 wt % disaccharide). As mentioned above, the diffusion coefficients calculated for water are within 30% of the NMR results. A similar level of agreement was reported by Roberts and co-workers[12] for the diffusion of water in concentrated glucose solutions. At higher concentrations (72.4 wt % disaccharide), the simulated and experimental diffusion coefficients for water agree reasonably well with each other, but those for the disaccharides differ by a factor of 10. Note that the results of simulations at high concentrations are prone to significant statistical errors. The discrepancy between experiment and simulation results observed at high concentrations could be partly due to insufficient sampling and finite size effects. Also, note that the PGSE NMR method employed in this work becomes less reliable as the diffusion coefficients become smaller and relaxation times increase. In those regions where both simulations and experiments are more reliable, the agreement between simulations and experiments is highly satisfactory. The agreement between experimental and simulated diffusion coefficients for water at all concentrations indicates that the combination of force fields used for water (SPC/E[24]) and the disaccharides (OPLS[25]) is reliable for determining dynamic quantities pertaining to water in dense systems. However, the lack of agreement observed for the diffusion coefficients of the disaccharides at high concentrations suggests that the disaccharide force field (OPLS[25]), while reliable for calculating structural information,[11] may not be entirely adequate for calculating dynamic properties of concentrated disaccharide solutions. Despite the lack of quantitative agreement, however, the results at high concentrations concur qualitatively and are sufficient to establish a number of interesting trends.

A comparison of the experimental and simulated diffusion coefficients of sucrose and trehalose reveals that, at lower disaccharide concentrations, trehalose and sucrose have similar values of D; at higher concentrations, however, D_S is consistently larger than D_T. The difference between D_S and D_T also becomes more pronounced at lower temperatures, consistent with the results of Ramp and co-workers.[31] At the highest concentration (74 wt %) and 323 K, the experimental diffusion coefficient for sucrose is three times that of trehalose (5.89×10^{-8} cm²/s and 1.91×10^{-8} cm²/s, respectively). Similarly, the simulated results for an 80 wt % solution at 400 K indicate that the sucrose diffusion coefficient is 3.5-fold larger than that of trehalose (9.3×10^{-8} cm^2/s versus 2.6×10^{-8} cm^2/s). The lower trehalose diffusion coefficient is consistent with the fact that trehalose systems have higher glass transition temperatures (the T_g values for pure trehalose and sucrose are 115 °C[6] and 77 °C,[33] respectively).

The faster motion observed for sucrose can be partly attributed to its smaller hydration number. In previous work,[11] the hydration number was defined as the average number of water molecules hydrogen bonded to a disaccharide molecule at a given point in time. By defining the geometry for a hydrogen bond as having an O···O distance of 3.4 Å or less and an O−H···O angle of 120° or greater, the hydration numbers for sucrose and trehalose in 80 wt % solutions were calculated from Monte Carlo simulations to be 5.4 and 6.7, respectively.[11] It is proposed that the sucrose−water complex is less bulky than the more hydrated trehalose complex and can therefore diffuse more rapidly.

The measured water diffusion coefficients are similar in the sucrose and trehalose systems below 72 wt %. A possible explanation could be that water molecules not directly involved in the hydration shell of a sugar are less affected by the identity of the disaccharide and more likely to diffuse at the same rate in both solutions. At higher disaccharide concentrations, water diffuses faster in sucrose than in trehalose. Simulations of more concentrated systems, reveal clear quantitative differences between D_W in trehalose and sucrose. In 80 wt % solutions at 400 K, D_W is a factor of 2 larger in sucrose than in trehalose. Similar distinctions in water diffusion were reported by Roberts and Debenedetti[12] for different monosaccharide solutions. In their systems, the differences in simulated D_W are appreciable even for intermediate sugar concentrations (e.g., 29 wt %).

NMR measurements and simulations both demonstrate that, at low disaccharide concentrations, water diffusion is at least 1 order of magnitude faster than sugar diffusion (see Figure 13 and Table 3). As the concentration of the disaccharide increases, the difference in the diffusion coefficients increases to almost 2.5 orders of magnitude at the highest concentration considered in this work. This decoupling of water and disaccharide motion becomes particularly apparent as the concentration reaches 40−50 wt %, which is also the concentration range at which the hydration numbers of the sugars start to change more rapidly with concentration.[7,11]

It is interesting to consider the decoupling of the diffusion coefficients in the context of a recent mode-coupling analysis of diffusion in binary systems of small solute particles and large solvent molecules.[34] In that work, deviations from a Stokes−Einstein diffusion regime were explained in terms of a friction coefficient ζ of the form

$$\frac{1}{\zeta} = \frac{1}{\zeta_D + R_{\rho\rho}} + R_H \tag{2}$$

where ζ_D is the friction contribution arising from direct collision of the solute and the solvent particles, $R_{\rho\rho}$ describes the coupling of solute motion to the density modes of the solvent, and R_H is the coupling to the transverse current function. Bhattacharyya and Bagchi investigated the diffusion behavior of Lennard-Jones systems at elevated densities and relatively low temperatures ($T/T_c \approx 0.6$, where T_c is the critical temperature[35]). They showed that for a solvent-to-solute size ratio of 1.5 to 15, the friction coefficient is dominated by the direct collision term ζ_D, $R_{\rho\rho}$ becomes essentially zero, and the hydrodynamic contribution R_H is relatively minor. Under these conditions, the ratio of the solute to solvent diffusion coefficients is higher than the Stokes−Einstein prediction. This mode-coupling analysis of a simple Lennard-Jones system is consistent with the trends observed in our diffusion coefficient measurements and calculations for more realistic, hydrogen-bonding mixtures. In the concentrated solutions investigated here (72.4 and 80 wt % disaccharide), the size ratio of the disaccharide to water is approximately 4. The concentration is such that water becomes a solute diffusing in a disaccharide solvent. For the size ratio of 4, the ratio of the solute to solvent diffusion coefficients is ≈7 for the Lennard-Jones system.[35] For our systems, the ratio of the simulated diffusion coefficients of water to the disaccharides is about 100. As indicated by Bhattacharyya and Bagchi, the formalism presented in their study may not apply to supercooled liquids where activated hopping or jumping mechanisms occur. However, their analysis does offer a starting point for the study of more complex fluids such as those simulated here. Part of the decoupling between water and disaccharide diffusion coefficients may be a reflection of the non-Stokesion diffusion behavior that results from the dominant non-hydrodynamic element of friction.

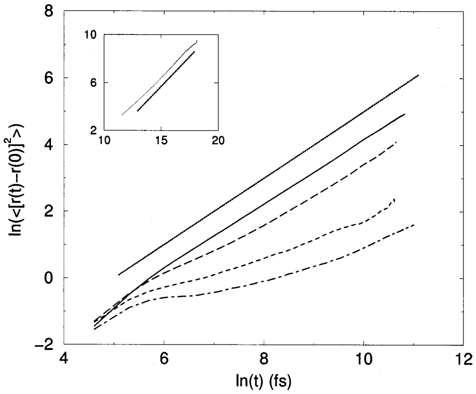

Figure 7. Effect of concentration on the initial mean-squared displacement of water in various sucrose solutions. A slope of unity is represented by the heavy dotted line for comparison. All concentrations were simulated at 358 K, except the 80 wt % solution which was simulated at 300 K. The inset shows the mean-squared displacement of water in the 72.4 wt % solution as it reaches the diffusive regime after 1.2 ns. (Solid line = 6 wt %, dashed line = 50 wt %, dotted line = 72.4 wt %, dot−dash line = 80 wt %).

The decoupling of the molecular mobility of water and disaccharide molecules can be further investigated by studying the simulated mean-squared displacement as a function of time at relatively short time scales. Figures 7 and 8 show the effect of disaccharide concentration on the rate of water and disaccharide displacement, respectively. The rates at which the slopes of the curves in Figures 7 and 8 approach unity provides an indication of how fast the molecules reach the diffusive regime (or how long they are trapped in transient “cages”). At lower concentrations, the slope in the case of water approaches unity relatively rapidly (Figure 7). As the disaccharide concentration increases, the rate at which the slope approaches unity decreases. At the highest concentration, 72.4 wt %, the slope of the mean-squared displacement as a function of time for water reaches unity after 1.2 ns (shown in the insert of Figure 7) as opposed to 7.4×10^{-4} ns in the 6 wt % solution. Similarly, the mean-squared displacement of sucrose (Figure 8) approaches unity after about 1.5 ns in the dilute solution (6%), and after about 15 ns in the more concentrated system (72.4%). Similar results were observed for trehalose, where a slope of unity is reached after 1.6 ns and 15 ns for the 6 and 72.4 wt % solutions, respectively. At all concentrations, the rate of approach to unity is much slower for the disaccharides than for water.

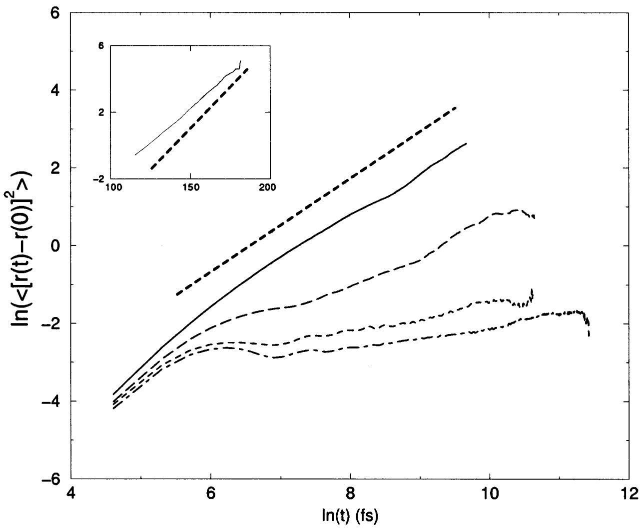

Figure 8. Effect of concentration on the initial mean-squared displacement of sucrose in various aqueous solutions. A slope of unity is represented by the heavy dotted line for comparison. All concentrations were simulated at 358 K except the 80 wt % which was simulated at 300 K. The inset shows the mean-squared displacement of sucrose in the 72.4 wt % solution as it reaches the diffusive regime after 15 ns. (Solid = 6 wt %, dashed = 50 wt %, dotted = 72.4 wt %, dot−dash = 80 wt %).

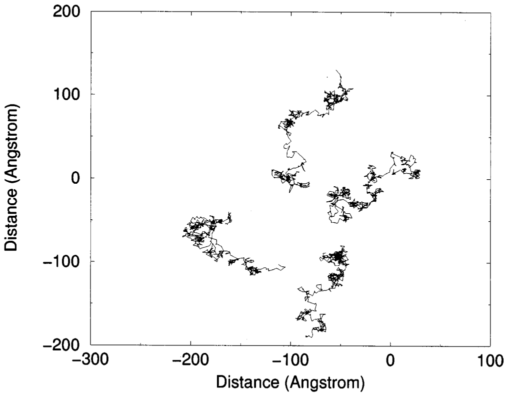

Figure 9. Trajectories of several water molecules at 358 K in a 50 wt % sucrose solution.

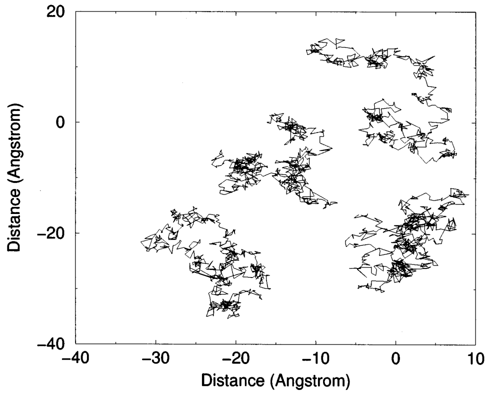

Figure 10. Trajectories of several sucrose molecules at 358 K in a 50 wt % sucrose solution.

A close inspection of the simulated trajectory of the molecules reveals several interesting features. Figures 9 and 10 show typical traces of paths of individual sucrose and water molecules, respectively, in 50 wt % sucrose solutions at 358 K. In these solutions, the trajectories of water molecules and those of sucrose molecules appear continuous. These trajectories change significantly as the temperature is decreased or the concentration of sucrose is increased. Figures 11 and 12 show the paths followed by several water and sucrose molecules, respectively, in an 80 wt % solution at 300 K. At this high concentration, water molecules are trapped in transient "cages" created by surrounding molecules.[7,12,36] These caged water molecules appear to vibrate for some time in a "cages" before making a jump to a new cage. If a jump is defined as a move that is larger than the average move size plus the standard deviation, then water molecules jump about 2 Å between cages. Note that the jump is commensurate with a water molecule's size. If the cage size is defined as the distance between the two farthest locations within the cage, then the average size of the water cage is about 3.0 Å. In contrast, within the time scales of our

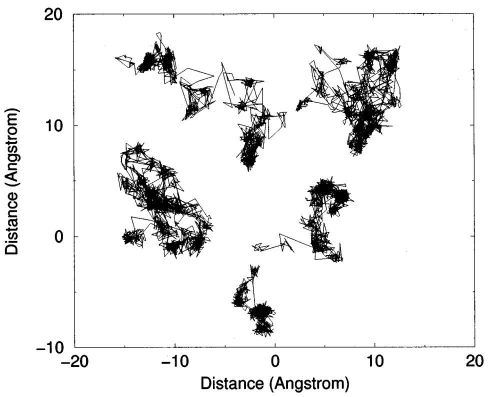

Figure 11. Trajectories of several water molecules at 300 K in a 80 wt % sucrose solution.

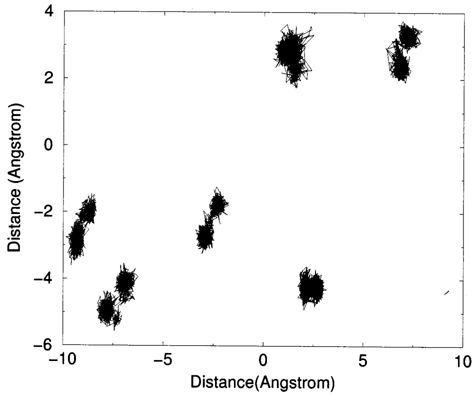

Figure 12. Trajectories of several sucrose molecules at 300 K in a 80 wt % sucrose solution.

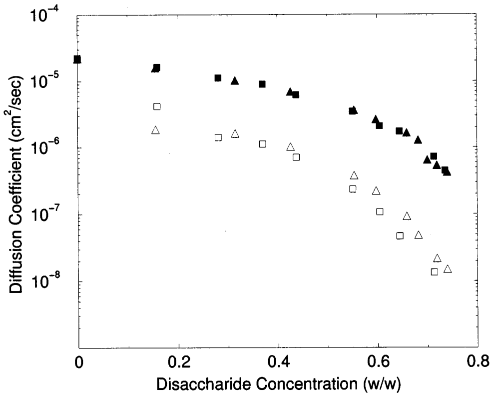

Figure 13. NMR measurements of the diffusion coefficients for water, sucrose, and trehalose at 303 K as a function of disaccharide concentration.

simulations, sucrose molecules perform smaller jumps or tight steps of about 0.7 Å, and their motion is much more localized.

The ability of water to diffuse in highly viscous solutions by jumping between cages raises an interesting issue concerning the immobilization of water by carbohydrate glasses. At 303 K, pure sucrose and trehalose are glasses well below T_g. A simple extrapolation of our data for water to the pure sucrose or trehalose case (infinitely dilute water) indicates that D_W at 303 K would be approximately 10^{-9} cm^2/s. This value is consistent with experimental data for water diffusion coefficients below T_g in similar systems, namely ficoll[37] and glucose syrup[38] glasses where D_W is of the order 10^{-9} cm^2/s. Conversely, experimental data for tracer molecules in glassy polymers[39] indicate that the diffusion coefficients of these small molecules are on the order of 10^{-13} cm^2/s or lower near T_g. These results indicate that water in carbohydrate glasses is considerably more mobile than tracer molecules in polymers near T_g, implying that glass formation is not sufficient to arrest water mobility in these systems. For the purposes of stabilization of biological materials, reducing water mobility corresponds to reducing degradative processes involving water. Given that, near T_g, glass formation alone is not sufficient, other means of immobilizing water would lend increased stability to preserved biological materials. In previous work,[11] we showed that trehalose is more hydrated than sucrose at concentrations as high as 90 wt %, consistent with the fact that trehalose forms dihydrate crystals whereas sucrose does not. Aldous et al.[40] suggested that the ability of trehalose molecules to form crystalline dihydrates could provide a trap for water molecules. Data reported by Crowe et al.[41] have further confirmed this idea by showing that some dihydrate crystallization does occur upon exposure of trehalose glasses to 58% relative humidity. The T_g of the glass was not lowered by the absorption of as much as 0.08 g of water/g of dry sample because the formation of dihydrate crystals prevented water from plasticizing the amorphous glass. These results suggest that trehalose may be a superior protectant not only because it forms a glass readily, but also because it is able to further reduce water mobility through the formation of dihydrate crystals.

5. Conclusions

The goal of this study was to compare the diffusive behavior of two disaccharides in aqueous solutions. The simulated and experimental diffusion results for water are consistent in the range of temperatures and concentrations where they overlap. In the case of the disaccharides, there is less agreement between experimental and simulated results. At low to intermediate concentrations (less than 50 wt %), the experimental diffusion coefficients are 2 to 4 times larger than the simulated values. At high disaccharide concentrations, the reliability of the OPLS force field to calculate transport quantities decreases, as evidenced by the factor of 10 difference between the calculated and measured diffusion coefficients of the disaccharides. While this disagreement is significant, it is important to note that the force field is based on structural thermodynamic data, not transport properties. The fact that the force field can predict the experimental trends in the diffusion coefficients to the extent shown here is gratifying. We have found that sucrose and trehalose exhibit different mobilities when dissolved in water. The higher mobility observed in the sucrose system is attributed to its small hydration number and more compact shape. At concentrations below 72 wt %, the diffusion of water appears to be largely independent of the type of sugar. In 80 wt % disaccharide solutions, water diffuses twice as fast in sucrose solutions than in trehalose solutions. In addition, the mechanism of water diffusion changes from a continuous trajectory to a hopping mechanism with increasing disaccharide concentration.

The results obtained here help shed light on the performance of the two disaccharides as protectants for biological materials under anhydrous conditions. Both sucrose and trehalose have been shown to protect biological materials during freezing and freeze-drying. By reducing water mobility and forming dihydrate crystals, the results presented here suggest that trehalose can be more effective than sucrose at immobilizing water and preserving biological materials during long-term storage.

Acknowledgment. This work was supported by the National Science Foundation (CTS-0218357).

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