Published in final edited form as:

Biopolymers. 2012 May ; 97(5): 289–302. doi:10.1002/bip.22004.

Sucrose in Aqueous Solution Revisited: 2. Adaptively Biased Molecular Dynamics Simulations and Computational Analysis of NMR Relaxation

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Abstract

We report 100 ns molecular dynamics simulations, at various temperatures, of sucrose in water (with concentrations of sucrose ranging from 0.02 to 4 M), and in a 7:3 water-DMSO mixture. Convergence of the resulting conformational ensembles was checked using adaptive-biased simulations along the glycosidic ϕ and ψ torsion angles. NMR relaxation parameters, including longitudinal (R_1) and transverse (R_2) relaxation rates, nuclear Overhauser enhancements (NOE), and generalized order parameter (S^2) were computed from the resulting time-correlation functions. The amplitude and time scales of molecular motions change with temperature and concentration in ways that track closely with experimental results, and are consistent with a model in which sucrose conformational fluctuations are limited (with 80–90% of the conformations having $\phi - \psi$ values within 20° of an average conformation), but with some important differences in conformation between pure water and DMSO-water mixtures.

1. Introduction

Static pictures of biological molecules from crystallography provide important information relating structures to biological function. Dynamical information on flexibility or conformation changes in solution also provide information of relevance to enzymatic activity and molecular recognition. The flexibility of carbohydrates in solution has been studied for a long time^{1–3} and categorized by Bush and coworkers as flexibility due to single free energy minima and changes due to the transitions among different minima.^{4–8}

For investigating conformations of biological molecules in solution, the most useful experimental tools include NMR longitudinal and transverse spin relaxation (R_1/R_2) and nuclear Overhauser enhancements (NOE),^{9,10} spin-spin coupling constants,¹¹ and residual dipolar couplings (RDCs).¹² For the disaccharide sucrose, early experimental data, such as ¹³C relaxation parameters in pure water or water-dimethyl sulfoxide-*d*₆ (DMSO) mixtures,^{13–17} and residual dipolar couplings (RDCs) in dilute liquid crystal media,¹⁸ support a fairly rigid structure (dominated by structures near the crystal conformation); other data, including steady state nuclear Overhauser effects (NOE),^{7,19} J coupling constants^{20–22} and RDCs⁶ point to considerable flexibility in its glycosidic linkage. Moreover, structural data of sucrose in complex with other macromolecules⁶ show considerable variations in the glycosidic linkage. This suggests that the conformation of sucrose might be subject to environment effects such as temperature and solvent type, as well as binding state.

The interpretation of NMR quantities generally requires structural models and is simplified for models with less flexibility. The available experimental data were performed in different solvation environments such as solvent type (pure water,^{14,15} water-DMSO mixture,^{13,16,17} or dilute liquid crystal media⁶), concentration,^{14,15,23} and temperature.^{6,14,15,17} For a nearly

rigid molecule, all these experimental data should provide a consistent result. As flexibility increases, the derivation of structure information from NMR values becomes much more difficult. The information derived from different experiments might be not in obvious agreement with each other.

The analysis of NMR relaxation parameters is one attractive way to investigate the conformation flexibility of biological molecules in aqueous solution.^{24,25,26,27} For example, the model-free approach²⁷ assumes that the reorientation time correlation function of a spin-spin vector can be separated into the internal motion part and the overall rotation one. The flexibility of internal motions is represented by the general order parameter derived from the correlation function of internal motions. NMR relaxation experiments have been reported for many carbohydrates^{8,28,30,31–34} including sucrose.^{13–17,23} Low temperature cryosolvents are generally needed to avoid the extreme narrowing regime.³⁵

Molecular dynamics simulation is an alternative for studying conformation flexibility. In principle NMR relaxation parameters can be calculated directly by analyzing snapshots from MD trajectories although significantly long simulations are required.^{36,37} Recent advances in computing power have made the direct calculations of relaxation parameters from MD trajectories possible.^{36,38,24} Older MD simulations^{19,39–46} are not fully consistent with each other. These simulations have trajectories varying from 20 picoseconds to 1 nanosecond. Our companion study⁴⁷ extends the simulations to 100 nanoseconds with five different water models. In a separate paper,⁴⁷ we have shown that residual dipolar couplings and indirect spin-spin couplings computed from trajectory snapshots best support a model where the crystal conformation is the dominant one in dilute aqueous solution.

In dilute solution at room temperature, sucrose interconverts among its allowed conformations on sub-nanosecond time scales, so that the sampling seen in 100 ns simulations is nearly converged. This is no longer true at lower temperatures, or at higher concentrations of sugar, or when solvated in liquids with high viscosity. To tackle such sampling problems^{48–54} more advanced MD techniques have been developed including replica exchange,⁵⁵ hyperdynamics,⁵⁶ conformation flooding,⁵⁷ accelerated molecular dynamics,⁵⁸ metadynamics,⁵⁹ adaptive biasing force method⁶⁰ as well as adaptive biasing potential method (ABMD).⁶¹ Although the time scales of conformational transitions can not be directly obtained from these methods, free energy minima, surface and reaction paths can be constructed in a much shorter simulation time scale than with “brute-force” MD methods. The adaptively biased molecular dynamics⁶¹ was developed recently and has been implemented in Amber. In the report, we present ABMD simulations for sucrose in explicit water and water-DMSO mixtures, examining the free energy landscape of sucrose in a water-DMSO mixture at low temperatures.

2. Computational Methods

1. Molecular Dynamics Simulation

Explicit MD simulations with different water models were performed using the same procedure as described in the previous report.⁴⁷ We continue to choose the latest GLYCAM 06⁶² for sucrose in SPC,⁶³ TIP3P,⁶⁴ TIP4P,⁶⁵ and TIP4P/Ew⁶⁶ waters, and its lone-pair counterpart GLYCAM04EP⁶⁷ for TIP5P waters.⁶⁸

For the simulations of sucrose at different concentrations, we used the PACKMOL program⁶⁹ to produce the initial configurations with different number of sucrose molecules mixed with the same number of waters. The number of TIP4P/Ew water molecules was fixed at 2000 and the number of sucrose molecules was set to 4, 18, 36, 72, 108 and 144, roughly corresponding to the concentrations of 0.1, 0.5, 1.0, 2.0, 3.0, and 4.0 mol/L. In

addition to the same equilibration runs in NVT (0.5 ns) and NPT (0.5 ns) as the study of different water models, 10 ns NPT equilibration run was done for each concentration before the formal 100 ns NVE production run in order to obtain a well-equilibrated system.

We also used PACKMOL⁶⁹ to build the system for simulations of sucrose in the 7:3 water-DMSO mixture. The system contains 1 sucrose, 857 DMSOs, and 2000 TIP4P/Ew waters. In general the atomic models of DMSO⁷⁰ are not so well-developed as water. We used the one compatible with AMBER force field, the all-atom model developed by Kollman and coworkers.⁷¹ We performed equilibration runs in 0.5 ns NVT and 10.5 ns NPT before the 100 ns production NVE, and saved the snapshots of trajectories every picosecond.

2. Construction of Free Energy Landscape from Adaptively Biased MD

In a previous paper⁴⁷ we built free energy landscapes (potential of mean force) via the probability distributions ($P(\varphi, \psi)$) from unbiased molecular dynamics trajectories:

$$F(\varphi, \psi) = -k_B T \ln P(\varphi, \psi), \quad (1)$$

where k_B is Boltzmann's constant and T is temperature. For convenience we also shifted all surfaces to make the values of global minima equal to 0.

The assumption of Eq. 1 is that the MD trajectories are long enough to fully sample the relevant phase space, which is a good approximation for systems with low free energy barriers. In a water-DMSO mixture, the sucrose molecule exhibits much slower dynamics and the barriers between free energy minima are much higher. Due to these high barriers, transitions between minima are difficult to observe during the 100 ns normal MD simulations. The adaptively biased molecular dynamics⁶¹ is a recent addition to the advanced molecular dynamics techniques for accelerating simulations and is capable of overcoming these high free energy barriers and estimating the potential of mean force in the relevant space.

In the ABMD method, the free energy associated with a reaction coordinate $\sigma(\vec{r}_1, \dots, \vec{r}_N)$, a smooth function of the atomic positions, $\vec{r}_1, \dots, \vec{r}_N$, is defined as

$$f(\xi) = -k_B T \ln \langle \delta[\xi - \sigma(\vec{r}_1, \dots, \vec{r}_N)] \rangle, \quad (2)$$

which is a formal version of Eq. 1.

In order to obtain this free energy surface, ABMD adds a time-dependent potential, which is a function of reaction coordinates such as glycosidic dihedrals in our case, to the normal equations of motions. This biasing potential accelerates the system to escape deep free energy valleys:

$$m_a \frac{d^2 \vec{r}_a}{dt^2} = \vec{F}_a - \frac{\partial}{\partial \vec{r}_a} U[t|\sigma(\vec{r}_1, \dots, \vec{r}_N)], \quad (3)$$

where $\vec{F}_a$ represents forces from ordinary MD, the second term on the right are the force derived from the biasing potential $U[t|\sigma(\vec{r}_1, \dots, \vec{r}_N)]$, and σ is the value of the reaction coordinate at a given conformation. The biasing potential evolves as

$$\frac{\partial U(t|\xi)}{\partial t} = \frac{k_b T}{\tau_F} G[\xi - \sigma(\vec{r}_1, \dots, \vec{r}_N)], \quad (4)$$

with the initial condition $U(t=0|\xi) = 0$. $G(\xi)$ is a positive definite and symmetric kernel, like a smoothed Dirac delta function. τ_F defines a flooding timescale. For large enough τ_F and small enough kernel width, the biasing potential $U(t|\xi)$ converges towards $-f(\xi)$ as $t \rightarrow \infty$. In real simulations, the choice of τ_F value is a trade-off between accuracy and simulation time. A large value will improve the accuracy of the estimation but slow down the simulation time to convergence. For our simple disaccharide, $\tau_F = 100$ ps, the same value used for two prototypical peptides in reference.⁶¹ Other simulations of $\tau_F = 500$ ps and $\tau_F = 50$ ps produced similar results. In Figures 2S and 3S we show the free energy surface (the negative value of biasing potential) at different times of ABMD flooding simulation. The probabilities within the circle region of 20 degree of four free energy minima are plotted in Figure 3S as the increase of flooding time. We can see that the biasing potential roughly converges at around 12 ns for pure water and 40 ns for water-DMSO mixture.

To improve the accuracy of the free energy surface, the ABMD simulations can be followed by additional simulations that use the final ABMD result as an approximate bias. Barriers between conformations are thus much reduced, allowing estimates to again be made based on Eq. 1. After the biasing potentials become converged (around 12 ns for pure water and 40 ns for water-DMSO mixture, as discussed below), we sampled the ABMD bias on a $2^\circ \times 2^\circ$ grid, and interpolated from this to run a final 100 ns simulation.

3. Calculation of Relaxation Parameters

From the simulation trajectories the rotational time correlation function of a ^{13}C - ^1H pair is defined as^{37,36}

$$C(\tau) = \left\langle \frac{1}{r^6(t)} \right\rangle^{-1} \left\langle \frac{P_2(\cos \chi_{t,t+\tau})}{r^3(t)r^3(t+\tau)} \right\rangle, \quad (5)$$

where P_2 is the second-order Legendre polynomial, χ is the angle between the inter-spin vector in the laboratory reference frame at time t and $t+\tau$, $r(t)$ is the instantaneous pair distance, and the angle brackets represent a thermal average. Each resulting correlation function can be fit to double-exponential or multi-exponential function. The spectral density function is then obtained by Fourier-transforming the fitted time correlation function:

$$J(\omega) = \int_{-\infty}^{\infty} C(\tau) e^{i\omega\tau} d\tau, \quad (6)$$

If the correlation function is fitted to sums of exponentials, then the spectral density becomes a sum of Lorentzian functions:

$$\int_{-\infty}^{\infty} e^{-\tau/b} e^{i\omega\tau} d\tau = \frac{2b}{1+\omega^2 b^2}. \quad (7)$$

Relaxation parameters depend on the spectral density functions at the relevant ^1H and ^{13}C Larmor frequencies.^{24,25} The spin-lattice relaxation rate is defined as

$$R_1 = \frac{1}{T_1} = R_1^{DD} + R_1^{CSA}, \quad (8)$$

where R_1^{DD} and R_1^{CSA} are the dipolar and chemical shift anisotropy (CSA) components of the spin-lattice relaxation-rate constants, respectively. Similarly, the spin-spin relaxation rate is defined as

$$R_2 = \frac{1}{T_2} = R_2^{DD} + R_2^{CSA}, \quad (9)$$

where R_2^{DD} and R_2^{CSA} are the dipolar and CSA components of the spin-spin relaxation-rate constants, respectively.

The dipolar relaxation rates

$$R_1^{DD} = (1/20)K^2 [J(\omega_H - \omega_C) + 3J(\omega_C) + 6J(\omega_H + \omega_C)], \quad (10)$$

$$R_2^{DD} = (1/40)K^2 [4J(0) + J(\omega_H - \omega_C) + 3J(\omega_C) + 6J(\omega_H) + 6J(\omega_H + \omega_C)], \quad (11)$$

are the main contributors to the overall relaxation. The constant factor K has the form as

$$K = \frac{\mu_0}{4\pi r_{\text{eff}}^3} \hbar \gamma_H \gamma_C, \quad (12)$$

where μ_0 is the permeability of free space, $\hbar$ is Planck's constant, and γ_H and γ_C are the gyromagnetic ratios for the nuclei of carbon and hydrogen, and r_{eff} is

$$\gamma_{\text{eff}} = \left\langle \frac{1}{r^6(t)} \right\rangle^{-1/6}, \quad (13)$$

the appropriately averaged internuclear distance between atoms. We used $r_{\text{eff}} = 1.11 \text{ \AA}$ for C-H vectors in our calculations; this is slightly larger than the equilibrium distance of about $1.09 \text{ \AA}$ to account for quantum vibrational effects not present in these classical simulations.⁷²

Chemical-shift anisotropy contributions to R_1 and R_2 are incorporated as below

$$R_1^{CSA} = (1/5)\Delta\delta^2\omega_C^2 J(\omega_C), \quad (14)$$

$$R_2^{CSA} = (1/30)\Delta\delta^2\omega_C^2 [4J(0) + J(\omega_C)], \quad (15)$$

where $\Delta\delta$ is the chemical shift tensor difference between parallel and perpendicular components, assuming an axially symmetric chemical shift tensor. For sucrose in single-crystal state,⁷³ $\Delta\delta$ varies from 26.8 to 50.5 for different Carbon atoms with a mean of 38.6. In general the chemical-shift anisotropy contributions are very small and can be neglected in our study. For example, the dipolar contribution of C-H_{4g} to R_1 is 1.31 sec^{-1} (Table 2),

whereas the CSA contribution (using 25 ppm) is $6.2 \times 10^{-5} \text{ sec}^{-1}$. If we used a larger value of the CSA (such as 40 ppm) the CSA contribution would only increase to $2.48 \times 10^{-4} \text{ sec}^{-1}$ still much smaller than the dipolar contribution.

We fit the correlation function for a given atom pair to double-exponential or triple-exponential function using a perl script invoking the fit routine in Gnuplot which minimizes the residual error using the nonlinear least squares Marquardt-Levenberg algorithm. The typical fitting range varies for different systems: 0 to 500 ps for sucrose in pure water and low concentrations, 0 to 5 ns for sucrose in water-DMSO mixture and high concentrations. Adjusting the fitting ranges did not induce obvious changes to the fitting parameters since we have obtained the trajectories a couple of orders longer than the characteristic time constants of overall rotations. For the sucrose studied two-exponential fittings from Gnuplot usually resulted in smaller variances of fitting parameters (less than 5% in general) than triple-exponential form except for systems at high concentrations of sucrose or water-DMSO mixture. In this report, all correlation functions are fit to a double-exponential form as in the model free formalism,²⁷

$$C(t) = e^{-t/\tau_m} (S^2 + (1 - S^2)e^{-t/\tau_e}) = S^2 e^{-t/\tau_m} + (1 - S^2)e^{-t/\tau}, \quad (16)$$

where τ_m and τ_e define characteristic times of the exponential decays, and $\frac{1}{\tau} = \frac{1}{\tau_m} + \frac{1}{\tau_e}$, and S^2 is a measure of the amplitude of internal motion.

When a molecule is small the overall rotational constant τ_m is also small. If $\tau_m \gg \tau_e$ and $(\omega_c + \omega_H)^2 \tau_m^2 \ll 1$ are satisfied, one has the extreme narrowing regime³⁵

$$J(0) \approx J(\omega_H - \omega_c) \approx J(\omega_c) \approx J(\omega_H) \approx J(\omega_H + \omega_c) \approx 2\tau_m. \quad (17)$$

From equations 10 and 11 it is easy to see that $R1 \approx R2 \propto \tau_m$, and nothing can be learned about internal motions from such data. It is for this reason that low temperatures or viscous solvents are used for relaxation studies on small molecules like sucrose.

3. Results and Discussion

1. Simulations of sucrose in pure water at varying sucrose concentrations

1. Free Energy Landscapes—In Figure 1, we show the free energy landscapes in $\varphi - \psi$ glycosidic space for sucrose in TIP4P/Ew waters at different concentrations, 0.1, 0.5, 1.0, 2.0, 3.0, and 4.0 mol/L. The free energy surfaces were constructed via the Equation 1 from the probability distributions averaged over all sucrose molecules in solution. As in our previous study,⁴⁷ we found four major minima, shown in Table 1. There is no significant concentration effect compared to simulations of one sucrose molecule case found earlier.⁴⁷ The global minimum M1 is also close to the crystal conformation ($108^\circ, 305^\circ$).⁷⁴ All F1s are global minima and the free energy differences between F1 and F2 are small. The probabilities (P1) of the sucrose molecule staying around the global minima (M1) are close to that of M2. Namely, there two conformations are almost equally important. Our analysis⁴⁷ of experimental RDC and J-coupling data suggested that M2 should be much less populated than is found with the force fields used here.

2. Relaxation Parameters—Early NMR studies^{14–17,23,35} on sucrose in solution usually resorted to the measurement of relaxation parameters R1 and R2. For direct comparison we calculated these values from the MD simulation trajectories. Figure 2 displays the reorientation correlation functions for a typical vector (C-H1g) of the glucose ring in sucrose

for different water models and concentrations of sucrose. All curves decay smoothly to zero as the time is increased. In Figure 2(c), as an example, we also show the model-free expression for the correlation function at 0.1 mol/L and $T = 298\text{K}$. The calculated data is fitted very well with small uncertainties in the resulting parameters.

Table 2 lists the $R1$ and $R2$ values of sucrose in various explicit water models: SPC/E,⁶³ TIP3P,⁶⁴ TIP4P,⁶⁵ TIP4P/Ew,⁶⁶ and TIP5P.⁶⁸ There is an obvious model dependence, even though the free energy surfaces are very similar.⁴⁷ $R1$ and $R2$ for TIP4P/Ew and TIP5P have largest values, and are almost twice those of TIP3P. This effect of water models is due to the fact that the rotational diffusion of sucrose depends on the water model.^{75,36} For example the self diffusion coefficients of SPC, TIP3P, TIP4P, TIP4P/Ew and TIP5P at $T = 298\text{K}$ are 3.85, 5.19, 3.29, 2.4 and $2.62 (\times 10^{-5} \text{ cm}^2/\text{s})$ respectively, and all these values are larger than the experimental value of 2.30.⁷⁵ This is probably the main reason for the wide distribution of rotational time constants (τ_m in Table 2) for different water models, ranging from 30 to 70 ps. From Table 2, it is apparent that TIP4P/Ew and TIP5P have the slowest dynamics among the studied water models. These trends closely resemble those reported earlier for peptides and proteins.^{24,36} Furthermore, the simulations are carried out in H_2O whereas the experiments used nearly pure D_2O . There is no rigorous way to “correct” the simulations, since both the mass of the hydrogen and the importance of nuclear quantum effects lead to differences between light and heavy water. As a simple approximation, one can scale τ_m (and $R1$ in the extreme narrowing limit) by 1.23, which is the ratio of the viscosities of heavy and light water. But this is only a rough correction, and this plus the strong dependence of the computed results on water model suggest that the main error in the calculations (at least for these sorts of relaxation properties) lies in the values of molecular tumbling times, although errors in the populations of different internal conformers might also play a role.

In addition to determining a τ_m value for each CH vector, we also calculated the average τ_m for the 8 CH vectors in two sugar rings as shown in Table 2. We also directly computed the overall tumbling from the single exponential fitting for the correlation function of the vector (C4g-C4f) crossing two rings. All values agree well with each other and are close to the values from each CH vector in the rings. Hence, (nearly) isotropic rotation of an approximately rigid molecule seems to be a good approximation. The observed relaxation rates for carbons bonded to a single hydrogen²³ have only a small scatter, and the details of their relative values change are a strong function of concentration (see Fig. 4 of Ref. ²³), suggesting that attempts to interpret molecular shape from an anisotropic tumbling model is probably beyond the expected accuracy of the current results.

Figure 3 shows $R1$ at different concentrations.²³ For a direct comparison, $R1$ has been averaged over all the C atoms from the two sugar rings and three hydroxymethyl groups as in the experimental analysis.²³ Generally the calculated values are in good agreement with experiment, although the difference becomes larger as the concentration is increased.

In contrast, there is little dependence on water model for the generalized order parameters. We found that the S^2 values of C atoms in the glucose ring (C-H1g, C-H2g, C-H3g, C-H4g, and C-H5g) are close to 0.90 which is consistent with the assumption that the glucose residue is rather rigid. The relatively larger variations and slightly smaller values of S^2 from C-H3f, C-H4f, and C-H5f in the fructose ring indicate that this ring has larger fluctuations than the glucose ring. The order parameters from hydroxymethyl carbon-13 nuclei (C-H6g, C-H1f, and C-H6f) are in turn smaller than those of atoms in the rings, which originates from the larger degree of mobility of hydroxymethyl groups.¹⁷ These simulation results are consistent with the experimental data where averaged S^2 are 0.92 for ring atoms and 0.77 for hydroxymethyl carbons at $T=273\text{K}$.¹⁷ We also notice that the S^2 values from both ring

atoms are still large >0.80 although significant transitions exist between $M1$ and $M2$ as shown in Figure 1S. The transition times from $M1$ to $M2$ are about 50 ps at $T = 300\text{K}$, which are close to overall rotational time constants (τ_m in Table 2). Hence the transitions between $M1$ and $M2$ are internal motions have the time scale close to that of overall rotation motions and will not greatly affect S^2 . But this also means that errors in our estimated populations of free-energy minima will not be evident in this sort of analysis.

The temperature effects on relaxation data are displayed in Table 3 and Figure 3, showing that τ_m and relaxation rates increase significantly as the temperature is lowered. The average τ_m values (around 50 ps at 315 K) are somewhat smaller than experimental values at low concentrations (70 ps in 0.5M solution at 315K³⁵ and 50 ps in 0.1M solution at 328K²³). As might be expected, values of S^2 increase slightly at lower temperature.

Table 2S shows the relaxation data for Carbon-13 nuclei in sucrose calculated from MD simulations at different concentrations. At low concentrations (0.1M and 0.5M), $R1$ is almost equal to $R2$ as the case of without sucrose-sucrose interactions in Table 2. As the concentration is increased, $R1$, $R2$ and the ratio $R2/R1$ becomes larger as we leave the extreme narrowing regime. The increase of $R1$ and $R2$ values stems from the slower decay of reorientation correlation function as shown by the large values of τ_m . In Table 2S, we list all other calculated relaxation data from the fitting as model-free formula in Equation 16. We also fit the data with the three-exponential form. The resulting $R1/R2$ values (Table 3S), however, show no pronounced changes.

2. Molecular Dynamics Simulations with a water-DMSO Mixture

1. Free Energy Landscapes—Figure 4 shows the free energy landscapes in $\phi - \psi$ glycosidic space in pure water and 7:3 water-DMSO mixture at two different temperatures $T = 273$ and 283 K . These free energy surfaces were constructed by Equation 1 from 100ns normal MD simulations with the initial crystal conformation of sucrose. It is evident that during the 100ns simulations the sucrose molecule in water-DMSO mixture is trapped in the minimum $M1$ or $M2$ and only a local equilibrium state was approached. Hence the PMFs in Figure 4(c) and (d) are not converged as transitions to $M3$ and $M4$ could not be observed within 100 ns as in pure water. This implies that the free energy barriers between $M1$ and $M4$ or $M2$ and $M3$ could be higher than that in pure water at the same temperature. To overcome these high barriers and obtain more information in other high-energy regions, we performed ABMD simulations both in pure water and water-DMSO mixture. Figures 2S and 3S show the free energy surface (the negative value of biasing potential) at different times of ABMD flooding simulation. The probabilities within the circle region of 20° of all four minima are plotted in Figure 4S as a function of flooding time. The biasing potential roughly converges at 12 ns for pure water and 40 ns for water-DMSO mixture. Following the ABMD flooding simulations we performed 100 ns simulations with the fixed biasing potential from the ABMD procedure. The resulting surfaces are displayed in Figures 5 and 6 for $T = 273\text{ K}$ and 283 K respectively. A large portion of $\phi - \psi$ space is sterically disallowed due to the overlap of atoms from two sugar rings.⁷⁶ These free energy maps cover most of the sterically allowed regions for the disaccharide, but most of the conformations are in the $M1$ or $M2$ regions.

From the final free energy maps in Figures 5 and 6, we collected important information pertaining to the free energy minima as listed in Table 4. Although the four minima found pure water are also present in water-DMSO mixture, there are significant differences in their relative energies. The global minimum is dependent on temperature. Namely, $M1$ is the global minimum at 283 K but $M2$ at 273 K . The positions of minima are also shifted slightly. The free energy barriers $B23$ (from $M2$ to $M3$) and $B14$ (from $M1$ to $M4$) are much higher (at least 2–3 KCal/mol) than that in pure water. These changes on potential of mean

force have resulted in the significant probability differences between M1 and M2 as shown in Table 4.

2. Relaxation Parameters—We chose our temperature and DMSO content to match experimental conditions reported by Effemey and Kowalewski.¹⁷ We also calculated the R1 and R2 values for 11 Carbon-13 nuclei from the 100 ns trajectories with normal MD simulations at both temperatures. The resulting values along with corresponding experimental data¹⁷ are listed in Table 5 and plotted in Figure 7. Calculated values of R1 agree well with experiment. In contrast, the values of R2 are generally overestimated although the numbers are reasonable. This is mostly due to the fact that the global rotational time is slightly longer in the simulations ($\tau_m \approx 2$ ns at $T = 273$ K) than experiments¹⁷ ($\tau_m \approx 1.5$ ns from the ring atoms at $T = 273$ K). Note that this is opposite to the behavior seen in pure water, where values of τ_m extracted from the simulation were smaller than those extracted from experiment.

The Lipari-Szabo order parameters S^2 (≈ 0.9 at $T = 273$ K) for the ring carbons as listed in Table 5 are slightly smaller than experimental values¹⁷ (The averaged S^2 value for ring carbons from experimental model-free fitting¹⁷ is 0.92 although no values were shown for individual atoms). There are no significant differences between ring carbons from glucose and that from fructose. This is opposite to the results for pure water listed in Table 2, where the S^2 values in the fructose ring are up to 10% smaller than in glucose. This difference might be understood by the probability density distribution of pucker phase angles of fructose ring as shown in Figure 5S. The distribution from pure water has wider spread than that from water-DMSO mixture, *i.e.*, the fructose ring is more flexible in pure water than in the mixed solvent. From Table 5 we also can find that the calculated values of S^2 for three hydroxymethyl carbons are slightly smaller than their corresponding values in experiments.¹⁷ Hydrogen-bond analysis shows sucrose at DMSO mixture has larger occupations (25.54% of O2g-H1Of and 15.08% of O5g-H6Of at $T = 283$ K, 0.0% of O2g-H1Of and 83.31% of O5g-H6Of at $T = 273$ K) comparing to that of pure water as shown in the previous paper⁴⁷ (10.51% of O2g-H1Of and 5.86% of O5g-H6Of at $T = 283$ K, 8.18% of O2g-H1Of and 5.45% of O5g-H6Of at $T = 273$ K). The stronger hydrogen-bond connections are consistent with the larger S^2 values and higher free energy barriers in DMSO mixture as discussed above.

4. Conclusions

Continuing our earlier simulations⁴⁷, we performed MD simulations of sucrose to reproduce NMR experimental conditions such as temperature (273 to 315 K), concentration of sucrose (0.1M to 4.0M), and solvent type (pure water and water-DMSO mixture). To avoid the problem of high-free energy barriers in the water-DMSO mixture, we also carried out adaptively biased MD simulations. NMR relaxation parameters such as ^{13}C spin-lattice and spin-spin relaxation rates were directly calculated from the simulations; given the capabilities of modern computers, such comparisons are increasingly straightforward to carry out. Generally the R1 and R2 values are slightly underestimated in pure water and overestimated in water-DMSO mixture due to faster or slower rotational dynamics. Nevertheless, the errors found here are generally smaller than those found in similar calculations on proteins.

The calculations suggest that the structure and internal dynamics of sucrose in water is not much affected by the sucrose concentration, although the overall viscosity of the solution (as monitored by molecular tumbling times) increases with concentration (and with decreasing temperature) in a way that closely matches experiment. However, the differences between water and DMSO-water are not large, and it is not clear how much credence should be given

to such changes, given the uncertainties in the water and DMSO force field models used here.

The calculated order parameters S^2 from ring atoms are large (>0.85) both in pure water and water-DMSO mixture. The S^2 values from fructose ring atoms in pure water general are smaller than that of glucose. In contrast, no such difference is observed for sucrose in the water-DMSO mixture. This indicates that although the crystal conformation still dominates, sucrose might have more conformational flexibility in pure water than in the mixture. This result is consistent with the narrower distribution of pucker phase angles of the fructose ring, the larger occupations of inter-residue hydrogen bonds, and the higher free energy barriers to other metastable states in DMSO/water versus water. Hence the rigidity of sucrose molecule seems to be sensitive to the solvent environment. The order parameters S^2 are also significantly larger than experimental data from other sugars such as heparin sulfate NA-domains (0.30–0.70),²⁸ hyaluronan oligosaccharides (0.30–0.65),³² human milk oligosaccharide (0.30–0.80).⁸ This implies that considerable internal motions might exist for these sugars. We expect that the same procedure can be applied for understand the conformation flexibility of these complex sugars.

Supplementary Material

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Acknowledgments

This research was supported by NIH grant GM45811. We thank Dr. Andreas Dolle for kindly providing R_1 data, and Volodymyr Babin and Celeste Sagui for useful discussions about adaptively-biased simulations.

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Figure 1.

Free energy landscapes in $\phi - \psi$ glycosidic space for sucrose in TIP4P/Ew waters at $T = 298$ K and different concentrations. The contour lines were generated from 0.0 to 5.0 kcal/mol by the difference of 0.1.

Figure 2.

Reorientation correlation functions of C-H1g vector of glucose residue in sucrose. In (c), the fitting curve of model-free formula is also shown with $S^2 = 0.84 \pm 0.01$, $\tau_m = 74.76 \pm 0.45$ and $\tau = 9.56 \pm 0.65$

Figure 3.

R1 values of sucrose in TIP4P/Ew water at B=5.875 T (left) at T=298K as a function of sucrose concentration, and (right) at low concentration (0.02M calc., 0.1M exp) as a function of temperature. R1 values have been averaged over all the C atoms in the two sugar rings and three hydroxymethyl groups. Experimental values are from Reference.²³ Calculated R1 values have been multiplied by 1.23, to approximately account of the greater viscosity of D₂O vs H₂O.

Figure 4.

The free energy landscapes in $\phi - \psi$ glycosidic space for sucrose in pure water and water-DMSO mixture at different temperatures from 100ns normal MD simulations. Note that in (c) and (d) the systems only visit a portion of PMFs as constructed from ABMD simulations in Figures 5 and 6, so the PMFs shown here are not converged.

Figure 5.

The free energy landscapes in $\phi - \psi$ glycosidic space for sucrose in the water-DMSO mixture and the TIP4P/Ew pure water at $T = 273$ K from ABMD simulations. The contour lines were generated from 0.0 to 10.0 kcal/mol in steps of 0.25.

Figure 6.

The free energy landscapes in $\phi - \psi$ glycosidic space for sucrose in the water-DMSO mixture and the TIP4P/Ew pure water at $T = 283$ K from ABMD simulations. The contour lines were generated from 0.0 to 10.0 kcal/mol in steps of 0.25.

Figure 7. Relaxation data for Carbon-13 nuclei in sucrose with 7:3 water-DMSO mixture at different temperatures and $B = 9.4$ T. Experimental data are from Reference.¹⁷

Table 1

Free energy information of four minima of sucrose in TIP4P/Ew waters at different concentrations with $T = 298$ K. Positions give (ϕ, ψ) value in degrees; energies of the minima barriers between minima are in kcal/mol; populations integrate the Boltzmann populations in a circle of radius 20° about the minimum.

	0.1 mol/L	0.5 mol/L	1.0 mol/L	2.0 mol/L	3.0 mol/L	4.0 mol/L
<i>positions</i>						
M1	(101.4, 302.5)	(104.3, 302.6)	(101.6, 301.83)	(102.0, 301.9)	(103.7, 303.9)	(102.1, 302.2)
M2	(71.0, 286.9)	(71.5, 288.8)	(71.7, 287.9)	(73.4, 288.7)	(72.4, 288.6)	(74.1, 289.0)
M3	(83.9, 193.7)	(85.1, 194.3)	(83.8, 194.9)	(82.9, 193.3)	(84.1, 194.9)	(85.1, 195.4)
M4	(91.1, 51.3)	(89.9, 55.1)	(90.3, 55.4)	(87.2, 55.1)	(89.8, 55.4)	(92.2, 55.2)
<i>energies</i>						
F1	0.0	0.0	0.0	0.0	0.0	0.0
F2	0.2	0.1	0.1	0.1	0.1	0.2
F3	1.6	1.7	1.8	1.8	1.9	1.6
F4	3.1	1.9	1.9	2.3	2.5	2.4
<i>populations</i>						
P1	0.472	0.456	0.478	0.504	0.522	0.502
P2	0.444	0.481	0.475	0.444	0.429	0.399
P3	0.019	0.022	0.017	0.021	0.01	0.024
P4	0.003	0.014	0.013	0.010	0.006	0.008
<i>barriers</i>						
B12	0.3	0.2	0.2	0.3	0.2	0.3
B23	3.6	3.4	3.5	3.4	3.7	3.6
B14	4.2	4.0	4.2	4.0	4.1	4.2

Table 2

Relaxation data for Carbon-13 nuclei in sucrose with different water models at T = 300 K and B = 9.4 T. R1, R2 in s^{-1} , τ_m in ps.

SPC			TIP3P			TIP4P			TIP4P/Ew			TIP5p			
C-H	S ²	τ_m	R ₁	S ²	τ_m	R ₁	S ²	τ_m	R ₁	S ²	τ_m	R ₁	S ²	τ_m	R ₁
C-H1g	0.91	32.6	0.64	0.88	31.6	0.60	0.93	42.4	0.84	0.90	63.3	1.22	0.87	69.4	1.31
C-H2g	0.93	32.3	0.64	0.78	37.1	0.65	0.92	45.9	0.90	0.91	66.3	1.28	0.90	69.0	1.32
C-H3g	0.92	31.9	0.63	0.81	35.6	0.64	0.91	46.0	0.89	0.90	65.6	1.26	0.85	72.1	1.32
C-H4g	0.92	33.2	0.65	0.87	34.9	0.65	0.91	47.4	0.92	0.89	69.4	1.31	0.87	71.5	1.33
C-H5g	0.93	32.6	0.65	0.79	37.3	0.66	0.92	46.0	0.91	0.90	67.4	1.30	0.88	69.8	1.32
C-H3f	0.83	38.8	0.70	0.89	33.0	0.63	0.91	47.3	0.92	0.85	68.1	1.25	0.84	63.8	1.15
C-H4f	0.79	36.9	0.64	0.87	30.8	0.57	0.88	44.6	0.84	0.86	58.7	1.08	0.85	57.4	1.05
C-H5f	0.90	34.7	0.67	0.89	34.1	0.65	0.86	54.2	1.00	0.88	69.6	1.30	0.83	72.9	1.29
C-H6g	0.79	29.	0.50	0.77	29.4	0.49	0.81	40.2	0.70	0.77	58.0	0.96	0.79	66.2	1.12
C-H1f	0.84	28.7	0.52	0.83	27.	0.49	0.83	36.7	0.65	0.84	51.1	0.92	0.80	68.6	1.18
C-H6f	0.77	26.4	0.44	0.78	25.2	0.42	0.77	34.6	0.57	0.75	47.0	0.76	0.82	50.0	0.88
Ring ^a	0.89	34.2	0.65	0.85	34.3	0.63	0.91	46.7	0.90	0.89	66.1	1.25	0.86	68.2	1.26
HDXM ^b	0.80	28.2	0.49	0.79	27.3	0.47	0.80	37.2	0.64	0.79	52.0	0.88	0.80	62.6	1.06
C4g-C4f ^c		36.2			34.6			52.9			71.3			73.9	

^a averaged values from the CH vectors in the two sugar rings (first 8 rows).

^b averaged values from the CH vectors from hydroxymethyl groups (last 3 rows).

^c the overall rotation constant, τ_m , is computed from the single exponential fitting for the correlation function of the vector (C4g-C4f) crossing two rings (with errors < 1%).

Table 3

Calculated relaxation parameters in TIP4P/Ew water at $B = 9.4$ T. R1, R2 in s^{-1} , τ_m in ps.)

	273K			283K			300K			315K		
	C-H	S ²	τ_m	R ₁	S ²	τ_m	R ₁	S ²	τ_m	R ₁	S ²	τ_m
C-H1g	0.93	0.93	118	2.28	0.92	105	2.03	0.90	63	1.22	0.79	48
C-H2g	0.93	0.93	125	2.42	0.89	113	2.11	0.91	66	1.28	0.89	46
C-H3g	0.92	0.92	125	2.39	0.86	115	2.10	0.90	65	1.26	0.89	45
C-H4g	0.91	0.91	133	2.51	0.89	112	2.11	0.89	69	1.31	0.88	47
C-H5g	0.93	0.93	127	2.44	0.90	112	2.11	0.90	67	1.30	0.89	46
C-H3f	0.82	0.82	153	2.65	0.90	105	2.00	0.85	68	1.25	0.86	47
C-H4f	0.84	0.84	136	2.38	0.84	107	1.91	0.86	58	1.08	0.82	44
C-H5f	0.87	0.87	150	2.68	0.87	118	2.14	0.88	69	1.30	0.86	50
C-H6g	0.81	0.81	111	1.90	0.81	94	1.61	0.77	58	0.96	0.77	37
C-H1f	0.83	0.83	99	1.75	0.83	83	1.47	0.84	51	0.92	0.81	36
C-H6f	0.79	0.79	92	1.53	0.75	77	1.25	0.75	46	0.76	0.72	34

Free energy information of four minima of sucrose in pure water and water-DMSO mixture at two different temperatures from the ABMD simulations.

Table 4

	Water		Water-DMSO	
	T = 273 K	T = 283 K	T = 300 K	T = 283 K
	positions (φ , ψ) (in degree)			
M1	(107.9, 305.7)	(104.5, 306.1)	(104.8, 305.4)	(108.8, 308.5)
M2	(72.9, 286.6)	(69.7, 283.3)	(69.9, 284.7)	(70.8, 298.6)
M3	(85.2, 198.4)	(88.5, 195.9)	(83.3, 196.4)	(96.2, 199.2)
M4	(92.2, 51.5)	(92.9, 54.3)	(94.8, 54.9)	(80.5, 54.3)
	heights (in kcal/mol)			
F1	0.0	0.0	0.0	1.8
F2	0.2	0.3	0.5	0.0
F3	3.1	2.9	2.9	3.6
F4	1.6	1.4	1.8	2.4
	probabilities within a radius of 20 degree			
P1	0.458	0.557	0.577	0.055
P2	0.351	0.241	0.355	0.889
P3	0.002	0.003	0.005	0.002
P4	0.018	0.018	0.024	0.012
	barriers between minima (in KCal/mol)			
B12	0.3	0.6	0.6	0.0
B23	4.0	3.8	3.4	7.2
B14	2.6	2.5	2.4	4.3

Table 5

Calculated relaxation data for the Carbon-13 nuclei of sucrose in 7:3 water-DMSO mixture at different temperatures and $B = 9.4T$. The averaged S^2 for ring atoms from experimental model-free fitting in Ref.⁴⁷ are 0.92 and 0.86 for $T = 273$ and 283 K respectively. Experimental values from Ref. 17 with S^2 values derived from model-free fits. R1, R2 values in s^{-1} , τ_m in ps.

273K									
C-H	S^2	exp.			calc.				NOE
		R1	R2	NOE	S2	τ_m	R1	R2	
C-H1g		5.48	9.5	1.27	0.89	2006	5.30	10.81	1.25
C-H2g		5.32	8.5	1.27	0.87	2003	5.24	10.64	1.26
C-H3g					0.89	2153	5.17	11.28	1.24
C-H4g	0.92	5.32	8.4	1.30	0.88	2776	4.64	13.25	1.26
C-H5g					0.87	1939	5.30	10.44	1.27
C-H3f		5.17	8.6	1.32	0.90	2296	5.06	11.82	1.23
C-H4f		5.39	8.9	1.30	0.87	2143	5.07	11.02	1.24
C-H5f		5.18	9.6	1.30	0.87	2450	4.81	12.02	1.24
C-H6g	0.78	4.63	6.9	1.35	0.70	706	4.25	5.08	1.70
C-H1f	0.80	4.56	7.7	1.34	0.63	1230	4.60	6.42	1.55
C-H6f	0.74	4.37	6.8	1.45	0.75	1869	4.84	9.03	1.35
283K									
C-H	S^2	exp.			calc.				NOE
		R1	R2	NOE	S2	τ_m	R1	R2	
C-H1g		5.23	7.2	1.54	0.77	1454	5.63	8.58	1.49
C-H2g		4.91	6.9	1.53	0.81	1585	5.55	9.05	1.42
C-H3g					0.85	1398	5.56	8.56	1.37
C-H4g	0.86	4.82	6.9	1.58	0.82	1657	5.43	9.21	1.38
C-H5g					0.82	1602	5.54	9.15	1.40
C-H3f		4.99	7.2	1.41	0.70	2014	5.19	9.59	1.52
C-H4f		4.99	7.0	1.56	0.68	1607	5.21	8.23	1.56
C-H5f		5.21	7.4	1.44	0.68	2083	5.08	9.56	1.55
C-H6g	0.81	4.23	5.6	1.62	0.77	970	4.93	6.44	1.49
C-H1f	0.75	4.41	5.3	1.63	0.65	725	4.38	5.19	1.79

273K									
exp.					calc.				
C-H	S ²	R1	R2	NOE	S2	τ_m	R1	R2	NOE
C-H6f	0.71	3.95	4.8	1.77	0.62	685	3.94	4.65	1.79