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On the Temperature Dependence of Hydroxymethyl Group Rotamer Populations in Cellooligomers

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ABSTRACT: Empirical force fields for computer simulations of carbohydrates are often implicitly assumed to be valid also at temperatures different from room temperature for which they were optimized. Herein, the temperature dependence of the hydroxymethyl group rotamer populations in short oligosaccharides is investigated using molecular dynamics simulations and NMR spectroscopy. Two oligosaccharides, viz., methyl β -cellobioside and β -cellotetraose were simulated using three different carbohydrate force fields (CHARMM C35, GLYCAM06 and GROMOS 56A_{carbo}) in combination with different water models (SPC, SPC/E and TIP3P) using replica exchange molecular dynamics simulations. For comparison, hydroxymethyl group rotamer populations were investigated for methyl β -cellobioside and cellopentaose based on measured NMR $^3J_{H5,H6}$ coupling constants, in the latter case by using a chemical shift selective NMR-filter. Molecular dynamics simulations in combination with NMR spectroscopy show that the temperature dependence of the hydroxymethyl rotamer population in these short cellooligomers, in the range 263 K to 344 K, generally becomes exaggerated in simulations when compared to experimental

data, but also that it is dependent on simulation conditions, and most notably properties of the water model.

KEYWORDS Molecular Dynamics, NMR, Carbohydrate Structure, Primary Alcohol

INTRODUCTION

With the hydroxymethyl side chain being the bulkiest side group of cellulose and several other carbohydrates, its conformation plays a major role in physical chemistry of carbohydrates in general and of cellulose in particular. Its conformation and dynamics affects hydrogen bond pattern in crystals and solution,^{1,2,3} reactivity,^{4,5,6} and aqueous solubility.^{7,8} The three-dimensional structure of carbohydrates is also a key feature for biological carbohydrate recognition.⁹ For this reason, the hydroxymethyl group has been extensively studied using both experimental^{10,11} and theoretical^{12,13,14,9} approaches. Its orientation is determined by the rotation around the C5-C6 bond (see Figure 1), defined by an angle commonly referred to as ω , which primarily is found in three staggered conformations denoted *tg*, *gt*, and *gg*, where the first letter refers to the conformation of the O5-C5-C6-O6 torsion: *trans* (180°) or *gauche* ($\pm 65^\circ$), and the second to the torsion defined by the sequence C4-C5-C6-O6. In glucopyranosides in solution at ambient conditions, ω is found predominantly in *gg* and *gt*,¹¹ whereas in the native crystal state of cellulose it is exclusively adopting the *tg* conformation.² At elevated temperatures though, the cellulose crystal undergoes a structural transition where the change in ω conformation is assumed to be of great importance.¹⁵

Figure 1. Structure of methyl β -cellobioside with pertaining notations.

Molecular dynamics (MD) computer simulation has during the past decade acquired an increasingly prominent position among the tools available to carbohydrate and cellulose chemists. The predictive power of this technique depends on the quality of the empirical force fields on which it relies. Specifically, numerous studies use MD simulations to study the influence of temperature on, e.g., structure and properties of cellulose,^{16,17,18} and shorter oligosaccharides,^{19,20,21} the interactions between carbohydrates and other molecular species,^{22,23,24} and, not the least, dissolution of cellulose in, e.g., ionic liquids,^{25,26} and water.²⁷

Recently, Shen et al.²⁸ published an extensive simulation study with the GLYCAM04 parameter set (available for download at www.glycam.org) in which the conformational flexibility of short-chain cellooligomers was investigated as a function of temperature. Specifically, they found that the ω torsions in the oligosaccharides methyl β -cellobioside (Figure 1), methyl β -cellotetraose, and methyl β -cellohexaose exhibited clear temperature dependences in the investigated temperature interval (297 K to 557 K): as temperature rises, populations of the *gg* state decreases, while populations in *gt* and *tg* both increase. Recently, Mostofian et al.²⁹ showed that the trend holds qualitatively also for longer cellooligomers, using a more recent version of the GLYCAM force field (GLYCAM06), in the temperature interval 375 K to 450 K.

Force fields for MD simulations of carbohydrates are usually optimized for use at room temperature. This includes fitting the potentials associated with the rotations of hydroxyl and hydroxymethyl groups to reproduce *ab initio* calculations and/or experimental data.^{30,31,32,33} This means that, based on the common approaches for force field optimization, there is no guarantee that the optimized parameters perform well also at temperatures that deviate from room temperature, although, as noted above, this kind of transferability has often been implicitly assumed. Despite the obvious importance of the temperature dependence of hydroxymethyl group conformational preferences, there are, to the best of our knowledge, no studies where both advanced structural determination experiments and MD simulations are applied under similar conditions, where temperature is being varied. To this end, in the present study, results from NMR experiments are

combined with MD simulations in order to evaluate the ability of computer simulations to capture changes in populations of the ω torsion angle due to temperature alterations, using three different carbohydrate force fields.

MATERIALS AND METHODS

Compounds

The compounds studied were the disaccharide methyl β -cellobioside (Figure 1), i.e., the shortest cellooligomer in the study by Shen et al., Ref. 28 as well as longer cellooligomers, namely β -cellotetraose in the MD simulations, and cellopentaose in the NMR experiments. Oligosaccharides were available at the Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University.³⁴

Molecular Dynamics Simulations

All MD simulations were performed using GROMACS 4.5 (ref. 35) using three different carbohydrate force fields: CHARMM C35^{31,36} (simply referred to as CHARMM for the remainder of this manuscript) and GLYCAM06³², both of which were initially used together with the TIP3P³⁷ water model, and GROMOS 56A_{carbo},³⁰ (referred to as GROMOS) with the SPC³⁸ model for water.

All bonds were held constant at their equilibrium values using LINCS³⁹ for the carbohydrates, and SETTLE⁴⁰ for water. The simulations used a leapfrog integrator with a basic time step of 2 fs. Lennard-Jones (LJ) interactions were made to go smoothly to zero between 0.8 and 1.2 nm using a switching function, and electrostatic interactions were handled with Particle Mesh Ewald (PME) summation,^{41,42} with a 1.0 nm cutoff for the real space part. The non-bonded interactions between pairs of atoms separated by three consecutive covalent bonds, commonly referred to as 1-4 interactions, are of specific importance for rotamer conformations. Both GLYCAM06 and CHARMM make no distinction between 1-4 interactions and ordinary non-bonded interactions, whereas GROMOS 56A_{carbo} explicitly lists 1-4 interactions parameters, as well as specific

parameters for some 1-5 interactions. In the optimization of the GROMOS parameters, Reaction Field (RF) electrostatics together with a straight 1.4-nm cut-off for the LJ part were used for the non-bonded interactions instead of PME and a switched LJ potential, which was used here. To test the influence of these parameters, simulations were run using the prescribed non-bonded potential functions. The result showed that for the properties studied herein, namely the temperature dependence of ω , the difference was negligible, justifying the use of the same simulation conditions for all three force fields.

The disaccharide was solvated in a cubic, fully periodic, box of dimensions $3 \times 3 \times 3$ nm, containing 877 water molecules. The tetrasaccharide was simulated in a box of $4 \times 4 \times 4$ nm, containing 2147 waters. Temperature was maintained using a stochastic velocity-rescale algorithm⁴³, and pressure was held constant at 1 atm with a Parrinello-Rahman barostat.⁴⁴ Simulations were performed using Replica Exchange Molecular Dynamics,⁴⁵ with 12 replicas running simultaneously at 12 different temperatures ranging from 263 to 329 K, in 6 K increments, for 50 ns each. Exchange between neighboring replicas was attempted every 10 steps.

NMR Spectroscopy

Oligosaccharide samples were prepared in 5 mm NMR tubes in D₂O, pD $\approx$ 6, with a sample concentration of 27 and 22 mM for methyl β -cellobioside and cellopentaose, respectively. NMR experiments were carried out on a 600 MHz Bruker AVANCE III spectrometer equipped with an inverse detection probe and on a 700 MHz Bruker AVANCE III spectrometer equipped with a TCI Z-Gradient CryoProbe. ¹H chemical shifts were referenced to internal sodium 3-trimethylsilyl-(2,2,3,3-²H₄)-propanoate (TSP, δ_{H} 0.00) and predicted for the oligosaccharides using the CASPER software.^{46,47} For the disaccharide, chemical shifts and coupling constants were refined iteratively from ¹H NMR spectra both with the integral transform fitting mode and the total line-shape mode of the PERCH NMR iterator PERCHit.^{48,49} Starting values for the iteration were extracted from the experimental 1D spectra directly. Due to the fact that the line-widths and line-shapes were part of

the iterative refinement process of the spectral parameters, the accuracy of the parameters are on the order of the digital resolution.^{50,51}

The 1D $^1\text{H}, ^1\text{H}$ -VT-CSSF-TOCSY experiments⁵² were performed at 700 MHz with an increment delay $\Delta = 0.39$ ms and 256 increments resulting in a maximum chemical shift evolution interval $t_{\text{max}} = 100$ ms. Experiments with a mixing time of 120 ms were carried out in which the transmitter frequency offset was set to the chemical shifts of the H1 resonances of glucosyl residues 2, and 3 and 4 at 4.422 ppm. A selective r-SNOB.1000 180° pulse⁵³ with duration of 120 ms and a bandwidth of 15 Hz at 80% height of the excitation profile was used to single out a narrow spectral region. A DIPSI-2 spin-lock was applied with strength of 10 kHz during the isotropic mixing period.

Analysis of Scalar Coupling Constants

The Karplus-type relationships for the conformational dependence of the ω torsion angle developed by Stenutz et al.¹¹ are given by:

$$^3J_{\text{H5,H6R}} = 5.08 + 0.47\cos(\omega) + 0.90\sin(\omega) - 0.12\cos(2\omega) + 4.86\sin(2\omega) \quad (1)$$

$$^3J_{\text{H5,H6S}} = 4.92 - 1.29\cos(\omega) + 0.05\sin(\omega) + 4.58\cos(2\omega) + 0.07\sin(2\omega) \quad (2)$$

Although they are similar to those given by Haasnot et al.⁵⁴ for which some applications result in unphysical models with negative populations⁵⁵ the study by Stenutz et al. was the first one to put forward a suitable molecular model for the canonical *gt* conformation, for which experimental NMR data subsequently were measured. This parameterization was therefore chosen for the evaluation of the hydroxymethyl populations of the *gt*, *gg* and *tg* states and their conformational preferences in the oligosaccharides presented herein. In the analysis the *J* values were either directly calculated from the MD simulations employing equations 1 and 2 or used in deriving a population distribution using limiting *J* values corresponding to torsion angles of $+65^\circ$, -65° or 180° with respect to the $^3J_{\text{H5,H6R}}$ and $^3J_{\text{H5,H6S}}$ coupling constants⁵⁶ of the three conformational states and the fact that the populations add up to unity, i.e., $p_{gt} + p_{gg} + p_{tg} = 1$, for this three-state model.

RESULTS AND DISCUSSION

Validation of Simulations

MD simulations of methyl β -cellobioside and β -cellotetraose in water were carried out at 12 different temperatures between 263 and 329 K for the purpose of investigating the temperature dependence of the orientation of the hydroxymethyl group, i.e., the torsion angle ω . Replica exchange MD was used in order to enhance the sampling of the configurational space at low temperatures. Even if the focus of this investigation is on a single degree of freedom, ω is likely coupled to other internal degrees of freedom, for instance rotations of hydroxyl groups, and the conformation of the glycosidic linkages. This means that a reliable estimate of the equilibrium distribution of ω is dependent on a sufficient sampling of those degrees of freedom as well. Rotations of hydroxyl groups occur on a shorter time scale than hydroxymethyl rotations. Specifically, the free energy barriers separating low energy conformations of the OH6-O6-C6-C5 torsion angle are at least a factor of two lower than for the rotations around ω , meaning that they will not pose any problem in this respect.⁵⁷ On the other hand, conformations of the glycosidic linkage, which are determined by the two torsion angles ϕ and ψ (see Figure 1), exhibit slower dynamics.^{58,59} Peric-Hassler et al.⁶⁰ have shown that even on a 50 ns time scale enhanced sampling through, e.g., steered MD, is needed in order to obtain converged distributions of ϕ and ψ in water, at room temperature. From both ϕ/ψ distributions and time evolutions (see Figure S1 and S2 in the Supporting Information) the replica-exchange scheme employed here was judged to be sufficient for reaching convergence in these degrees of freedom. Incidentally, free energy barriers that separate stable minima in ϕ/ψ space are of about the same height as for ω , ~ 5 kcal mol⁻¹, indicating that the conformational dynamics takes place on comparable time scales.⁶⁰

Furthermore, the conformation of the pyranose rings, the ring pucker, was monitored by calculation of the three pseudo-dihedral angles α_1 , α_2 , and α_3 described by Strauss and Picket.^{61,62} They were predominantly adopting values in the range -28° to -38° (using the convention of the

original publication) showing that, at room temperature, the pyranose rings were almost exclusively found in the 4C_1 chair conformation (see Figures S3 - S6 in the Supporting Information).

Results for Methyl β -cellobioside

Populations of the torsion angles ω , defined by the atom sequence O5-C5-C6-O6, in methyl β -cellobioside were calculated for the reducing (denoted G) and non-reducing (denoted G') ends separately, at each temperature. The distributions at three different temperatures are shown in Figure 2. They differ somewhat between force fields, but they all show a clear dependence on temperature. The populations found in each conformation were obtained by integrating the distributions between 0 to 120°, 120° to 240°, and 240° to 360° separately, for populations of *gt*, *tg*, and *gg*, respectively. Populations as a function of temperature are shown in Figure 3. Changes in populations over the whole temperature interval, given as averages of the two hexopyranose units are presented in Table 1, together with data from Shen et al.,²⁸ for comparison. From Figure 3 and Table 1 we see that all simulations agree with the trend found in Shen et al.²⁸: *gg* decreases with temperature, and *tg* and *gt* increases, but to different extent when using different force fields. CHARMM and GLYCAM06 give the overall strongest temperature dependence, whereas it is significantly weaker in both GROMOS and GLYCAM04. There is also a clear difference between the reducing and non-reducing ends, where the temperature dependence generally is weaker in the latter.

Figure 2. Distributions of ω defining the conformation (gt : +65, gg : -65, and tg : ± 180) of the hydroxymethyl group in methyl β -cellobioside, for the non-reducing (G') and reducing (G) ends separately, at three different temperatures.

Figure 3. Populations of the hydroxymethyl groups and their temperature dependence from both modeling and experiments for the non-reducing (G') and reducing (G) ends of methyl β -cellobioside.

Table 1. Population changes (%) of the hydroxymethyl group of methyl β -cellobioside in water over the temperature interval 263 K to 329 K given as averages over both residues

	<i>gt</i>	<i>gg</i>	<i>tg</i>
CHARMM C35 ^a	+10 (+6/+11) ^c	-19 (-19/-18) ^c	+9 (+6/+11) ^c
GLYCAM06 ^a	+20 (+12/+28) ^c	-25 (-15/-34) ^c	+5 (+3/+6) ^c
GROMOS A56 _{carbo} ^a	+4 (+1/+6) ^c	-8 (-4/-11) ^c	+5 (+3/+6) ^c
GLYCAM04 ^b	+5	-8	+3
NMR ^a	-1	-2	+3

^aThis work. ^bFrom Shen et al.²⁸ ^cNumbers in parenthesis refer to G' and G respectively.

In addition, an NMR temperature study was carried out consisting of seven different temperatures ranging from 281 K to 344 K (Figure 4). The ¹H NMR chemical shifts of methyl β -cellobioside in D₂O solution were resolved for the methylene protons of the hydroxymethyl groups. The conformation-dependent ³*J*_{H5,H6pro-R} and ³*J*_{H5,H6pro-S} coupling constants reporting on the populations at the ω torsion angles¹¹ were obtained by an iterative NMR spin-simulation procedure⁶³ resulting in highly accurate data, for the reducing and non-reducing ends separately. In contrast to both published simulation results²⁸, and the ones obtained herein, the temperature dependence of the ³*J*_{H5,H6pro-R} and ³*J*_{H5,H6pro-S} coupling constants is small (Table 2) and consequently the conformational changes between the three conformational states *gt*, *gg* and *tg* of the hydroxymethyl groups upon increasing temperature are also very small, or even negligible (Table 1).

When the homonuclear three-bond coupling constants ³*J*_{H5-H6pro-R} and ³*J*_{H5-H6pro-S} are experimentally accessible they can readily be used to identify whether an MD simulation produces a reasonable model, with respect to a population equilibrium, by direct comparison of calculated and experimentally observed ³*J*_{HH} values or from the population distribution derived therefrom.^{64,56} It is evident that the *J* coupling data differ between experiment and simulation (Table 2 vs. Tables S1 - S3), in particular for ³*J*_{H5-H6pro-R} when the CHARMM and GLYCAM06 force fields are used (Figure

5), whereas the differences are small when the GROMOS force field is employed. It is instructive that all three force fields investigated herein give reasonable agreement with experiments around 300 K, at least within $\pm 10\%$ of the experimental population distribution, with a notable exception of the non-reducing residue with GLYCAM06 (see Figure 3), but the fact that they all show stronger temperature dependencies than the experiments is noteworthy.

Figure 4. ^1H NMR spectra (blue) at 600 MHz of methyl β -cellobioside in D_2O at 8 $^\circ\text{C}$ (a), 34 $^\circ\text{C}$ (b) and 72 $^\circ\text{C}$ (c) and simulated spectra (red) at 8 $^\circ\text{C}$ (a'), 34 $^\circ\text{C}$ (b') and 72 $^\circ\text{C}$ (c') by total-lineshape analysis using the PERCH NMR software.

Table 2. Experimentally determined $^3J_{\text{HH}}$ coupling constants and populations at the ω torsion angles in methyl β -cellobioside in D_2O as a function of temperature

T (K)	$^3J_{\text{HH}}$ Terminal End (Hz)		$^3J_{\text{HH}}$ Reducing End (Hz)		Pop. ω Terminal end (%)			Pop. ω Reducing end (%)		
	H5'-H6' _{pro-R}	H5'-H6' _{pro-S}	H5-H6 _{pro-R}	H5-H6 _{pro-S}	<i>gt</i>	<i>gg</i>	<i>tg</i>	<i>gt</i>	<i>gg</i>	<i>tg</i>
281.6	5.9057	2.1791	5.2477	2.1585	52.80	39.06	8.14	45.49	46.33	8.08
290.9	5.9160	2.2491	5.2763	2.2130	52.31	38.51	8.88	45.67	45.68	8.65
298.6	5.9140	2.2833	5.2704	2.2341	52.44	38.32	9.25	45.52	45.61	8.87
307.4	5.9144	2.2906	5.2911	2.2781	52.41	38.26	9.32	45.55	45.10	9.35
317.7	5.9331	2.3141	5.2968	2.2910	52.52	37.91	9.57	45.56	44.96	9.47
330.7	5.9081	2.3433	5.3200	2.3389	52.11	38.00	9.89	45.61	44.41	9.98
344.7	5.9177	2.4089	5.2943	2.4024	51.94	37.48	10.58	45.04	44.29	10.66

Figure 5. Simulated and experimental $^3J_{\text{HH}}$ coupling constants in methyl β -cellobioside as a function of temperature.

Results for Longer Cellooligomers

Many computational studies use oligosaccharides as model compounds for longer polysaccharides, e.g., cellulose. This is the case in studies performed by, for instance, both Shen et al.,²⁸ and Mostofian et al.²⁹ Here, to investigate how the hydroxymethyl conformation is affected by increased chain length, simulations of cellotetraose in water were also employed with the CHARMM C35 parameter set, using the same basic setup as for the disaccharide. Rotamer

populations were collected for the outer groups and for the two inner groups separately and are presented in Figure 6 and Table 3. A similar temperature dependence as was seen for the disaccharide was present also here, for both the end segments and the inner segments, with a notably stronger temperature dependence for the inner residues. This result is in line with Shen et al.²⁸, who noted a similar temperature dependence for ω in the two innermost segments of methyl β -cellotetraose and methyl β -cellohexaose, respectively, as in methyl β -cellobioside.

To compare these simulations with experimental data, an NMR investigation was performed on cellopentaose in solution. However, in cellopentaose the spectral overlap is severe and the three central residues show essentially non-distinguishable chemical shifts as observed in the ^1H NMR spectrum, and also as deduced by NMR chemical shift predictions using the CASPER program.⁴⁷ The chemical shift difference of H1 at the reducing end in the β -anomeric form of the pentasaccharide and the anomeric protons for the three central residues differ at 700 MHz by only 18 Hz and this difference facilitates the differentiation of the central spin systems from those at the ends. We therefore performed a 1D ^1H , ^1H -VT-CSSF-TOCSY experiment⁵² at two different temperatures, 293 K and 335 K, which results in selective observation of the proton resonances from the methylene protons of the three central hydroxymethyl groups (Figure 7). The coupling constants are closely similar at both temperatures, with $^3J_{\text{H5},\text{H6pro-R}} \approx 5.2$ Hz and $^3J_{\text{H5},\text{H6pro-S}} \approx 2.2$ Hz. Although the resonances for each residue are not resolved, the minor spectral changes indicate that the conformational changes of the ω torsion angles in the temperature region studied are indeed small, and on the order of 1%. Thus, even if it is possible to experimentally detect a temperature dependence also at the pentasaccharide level, it is significantly smaller than what is observed in the MD simulations.

Figure 6. Temperature dependence of the populations at the ω torsion angles in cellotetraose for G' and G'' (inner residues) and G and G''' (outer residues) from simulations using the CHARMM force field.

Table 3. Population changes of the hydroxymethyl groups of cellotetraose in water over the temperature interval 263 K to 329 K from simulations using CHARMM C35

	% <i>gt</i>	% <i>gg</i>	% <i>tg</i>
Outer residues	+8	-15	+7
Inner residues	+23	-30	+7

Figure 7. Experimental ^1H NMR spectrum of cellopentaose in D_2O at 20 °C (a), 1D ^1H , ^1H -VT-CSSF-TOCSY spectrum at 20 °C in which the H1 resonances of glucosyl residues 2, and 3 and 4 at 4.422 ppm were targeted (b) and correspondingly at 62 °C (c).

Influence of Water on Simulated Rotamer Populations

It is well known that the solvent affects the rotamer equilibrium distributions to a great extent in computer simulations of carbohydrates.^{9,57} For that reason it is reasonable to investigate temperature dependent properties of water to understand the temperature dependence of the population distributions. One candidate, known to be temperature dependent, is the dielectric properties. The relative permittivity, ϵ_r , of water is decreasing with increasing temperature, which is generally true also in simulations. In the SPC model, ϵ_r decreases from 70 to 60 between 300 and 350 K,⁶⁵ with values for TIP3P generally being somewhat higher,⁶⁶ which is reasonably close to the experimental numbers.⁶⁷ One hypothesis is that since the rotamer conformations differ in local electrical dipole moment, this would lead to that less polar conformations are gradually promoted as T increases. From the geometry of the glucose unit, it is evident that the segment O5-C5-C6-O6 would have the highest dipole moment in the gt conformation, followed by gg and tg , in decreasing order. However, recalling that simulations predict that gg populations are decreasing in favor of tg and gt , and that the increase in tg is comparable to (GROMOS and GLYCAM) or much smaller (CHARMM) than for gt , the simulations do not provide any support for this idea.

This can be understood by considering that the self-energy of an electrical dipole located in a spherical cavity of radius R in a dielectric medium can be expressed as⁶⁸

$$E_{dp} = \frac{\mu^2}{4\pi\epsilon_0} \int_R^\infty \frac{1}{\epsilon_r r^4} dr \quad (3)$$

where μ is the dipole moment and ϵ_0 is the permittivity of free space. This means that the electrostatic solvation energy, which is the difference in self-energy between a medium with high dielectric constant and one with $\epsilon_\square = 1$, becomes

$$E_{solv} = \frac{\mu^2}{4\pi\epsilon_0} \frac{3}{R^3} \left(\frac{1}{\epsilon_r} - 1 \right). \quad (4)$$

Thus, changing $\epsilon_\square$ from 70 to 60 leads to a change in solvation energy of approximately 0.2%, which, with a dipole moment of 7.2 D (calculated for a glucose unit with its hydroxymethyl group

in *gt* conformation using the CHARMM force field) and a cavity radius of 0.4 nm (approximately the size of one glucose unit) becomes 0.3 kJ mol^{-1} . Thus, this is too small to have any appreciable effect.

Interestingly, one thing that differentiates the GROMOS simulations, which gives the weakest T dependence in this study, from the GLYCAM06 and CHARMM simulations is that the former uses the SPC model for water, whereas the other two employ the TIP3P model, since these were the models that were used in the force field optimizations. To probe the influence of the water model two additional sets of simulations were performed, one in which the CHARMM force field was combined with the SPC model, and one in which GROMOS parameters were used with TIP3P water. The results show that in the former case, the temperature dependence was significantly reduced, and in the latter case it was slightly enhanced (see Figure 8). In addition it was seen that the effects of changing water model is generally smaller in the reducing residue than in the non-reducing, and close to negligible when using GROMOS parameters.

Bulk water densities were calculated from the simulations using the un-normalized radial distribution functions between the solute and the solvent molecules (see Figure S7) and the result is presented in Figure 9. Both SPC and TIP3P give bulk densities that are well below the experimental values for the larger part of the investigated temperature interval, with SPC data falling below the TIP3P curve. The decrease with temperature is also much larger than experimental data, for both models. This is in line with what has been observed before,⁶⁹ although the values obtained herein may seem a little too low. Computed density is however, generally, sensitive to simulation details, such as treatment of long-range forces and system size.³⁷ It is reasonable to expect that the temperature dependence of the water density affects also the temperature dependence of rotamer populations.

There are extensions to both SPC and TIP3P that aim to correct the shortcomings of the models. One such extension is the SPC/E⁷⁰ model, which only differs from the original SPC model by a slightly modified partial charge distribution, thereby retaining the computational efficiency of a

three-site model. The SPC/E model does indeed exhibit improved dielectric, thermodynamic, and dynamic properties of bulk water.^{70,71,72} Specifically, the computed density is much improved over the original SPC model in the temperature interval considered here (see Figure 9) which also was noted before.⁷² However, as shown in Figure 8, simulations using SPC/E with the GROMOS parameters for carbohydrates induce a clear, but marginal, effect on ω torsion distributions and its temperature dependence in the reducing residue, whereas the effect on the non-reducing residue is negligible.

In want of a water model that correctly reproduces the bulk density over a large temperature interval, one possibility is to use simulations in the NVT ensemble, with the density restrained to the experimental value at that specific temperature. However, this is not trivial to achieve in the case when there are solutes present. Moreover, such an approach is not directly compatible with a REMD setup. To gain information on the sensitivity of the rotamer populations to water density, a set of simulations were run in which the volumes were locked to the equilibrium volume at room temperature. All 12 replicas were thus simulated at the same water density. This of course means that the densities will still be deviating compared to the experimental values, but in this case they will not vary with temperature. Interestingly, as can be seen in Figure 10, this approach led to a significant reduction of the temperature dependence of the ω torsion angle when the CHARMM and GLYCAM06 force fields were employed, whereas for GROMOS it was not affected.

Figure 8. Temperature dependence of the hydroxymethyl conformations in methyl β -cellobioside: effect of changing water model.

Figure 9. Bulk water density calculated from the simulations compared to the experimental water density at atmospheric pressure.

Figure 10. Temperature dependence of the hydroxymethyl conformations in methyl β -cellobioside: effect of simulating at constant volume.

CONCLUSIONS

We have found a significant overestimation of the temperature dependence of the hydroxymethyl rotamer population in both the disaccharide methyl β -cellobioside and longer cellooligomers in molecular dynamics simulations with three well-known carbohydrate force fields combined with their respective recommended water model, compared to the conformational temperature dependence determined from NMR experiments. The magnitude of the temperature dependence is affected by the choice of carbohydrate force field, and by the water model employed. The overall largest T dependence is found in the GLYCAM06 simulations, followed by the CHARMM simulations. For the CHARMM and GLYCAM06 force fields, which both use the TIP3P water model, a good part of the T dependence can be suppressed by simulating at constant volume, which in the present setup leads to higher water density at elevated temperatures. This does not significantly affect the GROMOS simulations, although in the latter case the T dependence was relatively small to begin with.

At this stage it is difficult to estimate to what extent the observed discrepancy between modeling and experiments has affected results and conclusions reported in the literature, but it certainly reveals a need to re-visit several previous MD studies of carbohydrates at elevated temperatures. It also points to the necessity of re-examining the temperature dependence of other conformational properties as well. We finally conclude that considering temperature dependencies could be an important aspect of future refinements of force fields for molecular dynamics simulations of carbohydrates, especially in aqueous media. In the meantime, when simulations are needed at a different state point than the force field was parameterized for, careful evaluation of the results and, if possible, validation against experimental data are crucial to avoid artificial effects.

ASSOCIATED CONTENT

Supporting Information. Additional figures showing: ϕ/ψ scatter plots and time evolutions; distributions of ring pseudo-dihedral angles; calculated J coupling constants. This material is available free of charge via the Internet at <http://pubs.acs.org>

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Notes

The authors declare no competing financial interests.

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