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Hydration of glucose in the rubbery and glassy states studied by molecular dynamics simulation

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Abstract

We have studied the hydration properties of an 85% (w/w) aqueous solution of glucose using molecular dynamics. The experimental values of the relative populations of α and β anomers were introduced into the description of the system. We computed the radial distribution function, hydrogen bond residence times, hydration number and mean lifetimes, as well the mean glucose and water cluster sizes. The simulated glass transition temperature (T_g) of the solution was computed to evaluate the quality of the model; the computed value of 241 K was in fair agreement with the experimental value of 232 K. It was concluded that most of the water molecules are connected to more than one glucose molecule by hydrogen bonds. The residence time of the water molecules in hydration sites changes from one site to another, but for the anomeric and chain-oxygen atoms, the residence time is greater than for the rest. The average residence time goes from 2.00 ps for the rubbery state at 280 K to 5.75 ps for the glassy state at 200 K. The mean value of the cluster size of glucose is very close to the corresponding to full connectivity and does not vary much from the rubbery to the glassy state.

Keywords: Glass transitions in carbohydrates; Glucose hydration; Carbohydrate percolation; Molecular dynamics simulation of hydration

1. Introduction

Glasses of various carbohydrates are found in nature as protecting systems for biological polymers under conditions of drought or severe cold [1]. The practical applications of this property are mainly associated with the food and pharmaceutical industries [2–6]. To maintain the quality and stability of products, advantage is taken of the fact that a high concentration of carbohydrate can prevent the damaging effects produced by water crystallisation at low temperatures [7,8]. Preservation and encapsulation of compounds are the main applications of this protective effect of saccharides. Generally, preservation is related to the shelf life of unstable compounds such as medicines and enzymes, whereas encapsulation usually has to do with the role of matrix in retaining flavours in processed foods. Disaccharides and oligosaccarides are often used for these purposes [9]. A possible mechanism to explain this effect is the partial replacement of water by carbohydrates as a solvating agent [10]. Nowadays, the glass transition is recognised as a physicochemical event (i.e., a

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change of state, but not a change of phase [11]) that can govern food processing, product properties, quality, safety and stability [12]. In developing a basis for the preservative and encapsulating actions of saccharides, it is important to understand how molecular structure and water content affect molecular mobility and potentially damaging chemical reactions. When the concentration of water decreases, the glass transition temperature (T_g) increases, and glasses can be obtained more easily at temperatures near room temperature [13]. This fact presents an advantage when related to the protective effect of these kinds of glasses.

As far as we know, little is known about the structure of the H-bond network between saccharides units and solvent molecules and hydration properties of glasses of small carbohydrates at very low water contents. In previous work [14,15], we have been able to successfully reproduce the glass transition temperatures of pure glucose and of aqueous solutions of glucose at several concentrations. Although the molecular dynamics simulation was done only on β-D-glucose, which is unrealistic, we could satisfactorily reproduce the glucose–water state diagram [15]. In this work, we use molecular dynamics to describe the hydration characteristics of an 85% (w/w) aqueous solution of α- and β-D-glucose, using parameters such as the residence time of water close to the oxygen atoms of glucose units, hydration number for solute, mean lifetime of hydrogen bonds between solute and solvent, and the percolation state in the aqueous mixture. Consistent with earlier reports, the composition at the solution concentration studied turns out to be close to 50% for each anomer [9,16]. The establishment of T_g (241 K) and the analysis of hydration were done on the trajectories corresponding to 200 and 280 K, i.e., about 40 K below and above T_g of the simulated system in the Gibbs ensemble.

2. Computational methods

Molecular dynamics simulations were done using the GROMOS package (Biomos n.v. Groningen) [17], in which equations of motion are solved using the Leap Frog algorithm. The system was linked by weak coupling to thermal and hydrostatic baths to maintain the temperature and pressure at constant values (Berendsen's thermostat [18]). Under those conditions, we were working in the Gibbs ensemble.

An integration time step of 2 fs was used for all the simulations. The simulations were performed with in an IBM RS/6000 computer, and the analysis of the results was done with a personal computer.

The force field of GROMOS was used to describe the interactions between glucose molecules. That force field has been successfully tested and used previously for simulations of carbohydrates [19,20]. Interactions between non-bonded atoms were represented with a 6–12 Lennard–Jones potential (GROMOS force field values), and the electrostatic interaction between the atomic partial charges [21] was taken into account by adding a Coulombic term to the expression for the effective potential.

Molecular conformations of both anomers, α- and β-glucopyranoses, were kept in the $^4C_1$ conformation, and transitions between other conformational states were avoided during the simulation by applying improper torsional potentials. These kinds of potentials were also used to maintain the tetrahedral character of the carbon atoms. Periodic boundary conditions were applied. To keep bond lengths rigid, we used the SHAKE procedure [22]. Bond angles and bond lengths were treated in a similar way using harmonic potential terms. Solvent molecules were simulated using the SPC/E model [23].

To achieve a concentration of 15% water (w/w), it was necessary to put 85 molecules of water and 48 molecules of glucose within an orthorhombic box of dimensions 1.9 × 2.75 × 2.16 nm. Half of the total amount of solute corresponded to the α anomer and the rest to the β anomer.

3. Results

Glass transition temperature.—To characterise the T_g of the system, several simulations

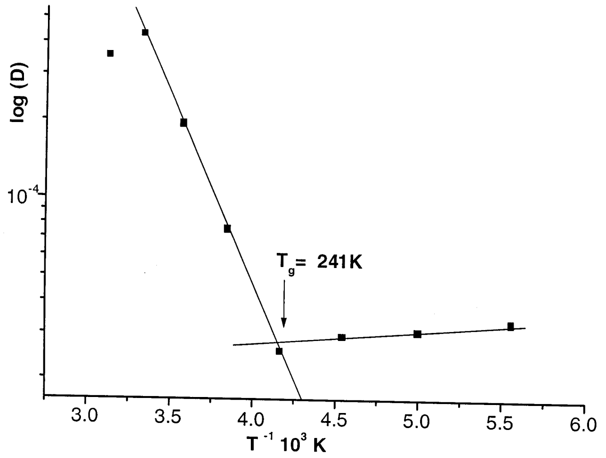

Fig. 1. Determination of the glass transition temperature for a mixture of α- and β-D-glucose at 15% (w/w) water content.

were done at different temperatures, starting at $T = 120$ K and slowly increasing the value in steps of $\Delta T = 20$ K, ending at $T = 320$ K. In each case after the equilibrium was attained (20 ps), we collected the trajectories for 30 ps to make the analysis.

Since mobility is the key principle underlying the definition of the glass transition, T_g was calculated by analysing the behaviour of the diffusion coefficient of water [15]. In Fig. 1, we show the values in a logarithmic scale of the self-diffusion coefficient for water versus inverse temperature. We drew straight lines, using for fitting the points that corresponded to temperatures at which the system was equilibrated. The intersection of the lines coincided with the value of T_g, equal to 241 K. Dielectric measurements have shown that the experimental value of T_g is close to 232 K for a concentration of 15% of water [24]. The fair agreement between the experimental and simulated values of T_g is a good indication of the validity of the topological model used to describe the system.

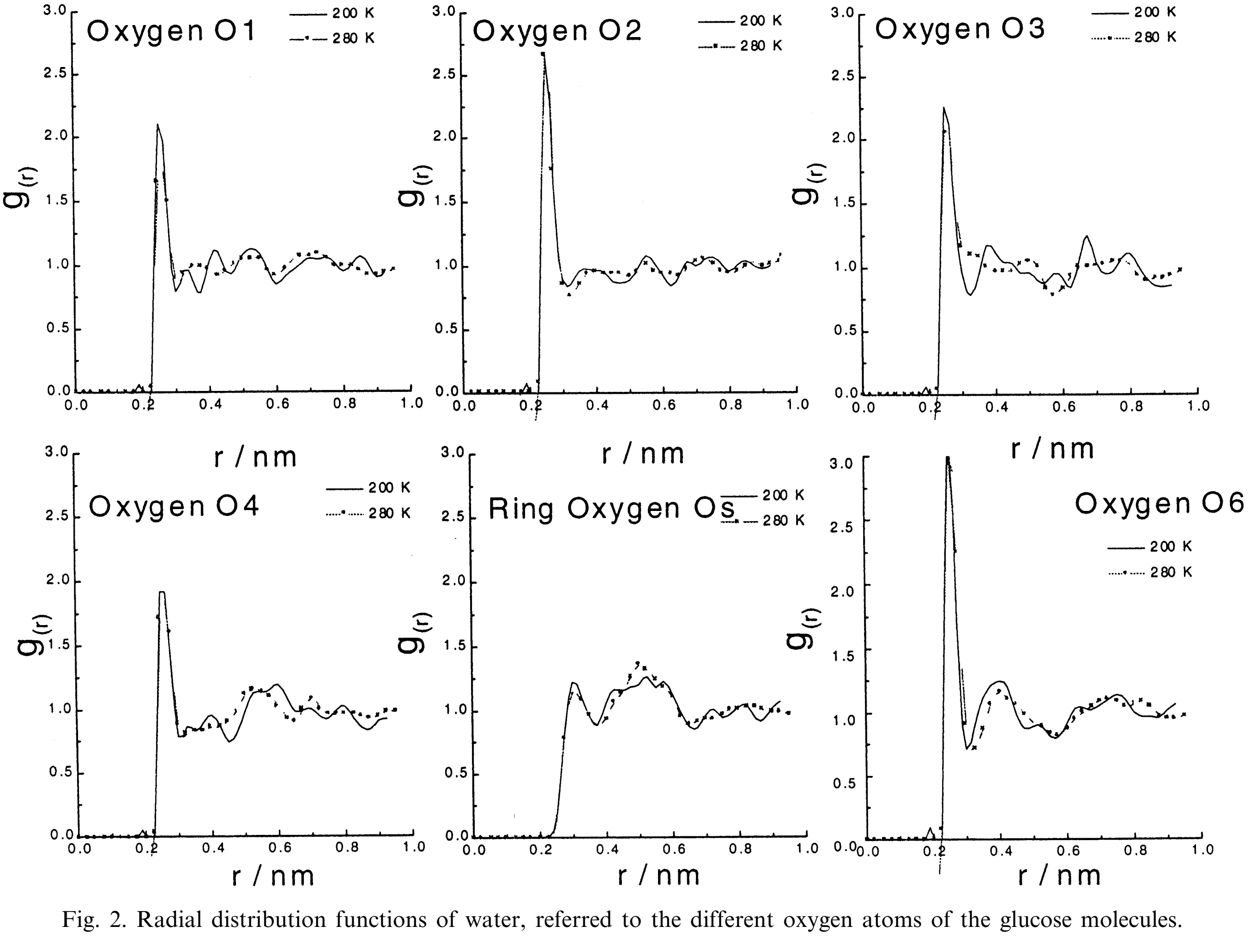

Fig. 2. Radial distribution functions of water, referred to the different oxygen atoms of the glucose molecules.

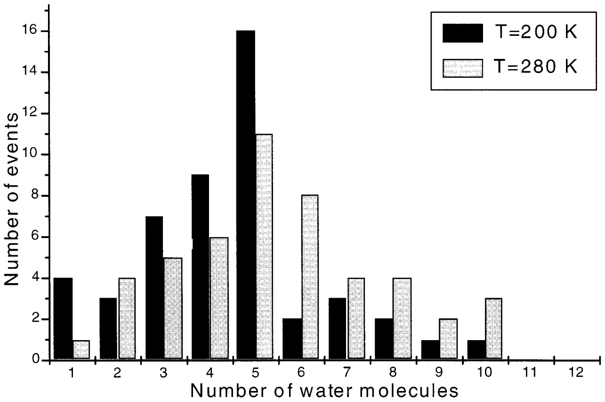

Fig. 3. Distribution of hydration numbers for glucose molecules. All molecules within the 'first hydration shell', as defined by the first peak of the radial distribution, were considered.

Hydration number and radial distribution functions.—Fig. 2 shows the radial distribution functions for all the oxygen atoms of glucose. All functions look quite similar, having one main peak at the characteristic H-bond distance (0.25 nm), except in the case of the ring oxygen, in which the first and second peaks are of similar values and much lower than the first peak of the other oxygen atoms. The average distance of the first minimum in the functions, which indicates the limit of the first hydration shell, is 0.32 nm for $T = 200$ K and 0.337 nm for $T = 280$ K. These values were used to calculate the hydration number for glucose at this concentration. The hydration pattern, emerging from $g_{(r)}$, shows that the hydration is not homogeneous around the entire glucose molecule. Moreover, we can see that, although some 'fine structure' can be seen in the system at 200 K which is absent at 280 K, the hydration, as seen from the radial distribution functions, does not differ much between the rubbery state ($T = 280$ K) and the glassy state ($T = 200$ K).

In the unit box, there are 48 glucose molecules and 85 water molecules, which give a ratio of 1.77. Nevertheless, the hydration number for each glucose unit is five, which indicates that most of the water molecules are shared with different molecules of solute.

Looking at the histogram of the number of water molecules (shown in Fig. 3) in the vicinity of a solute molecule, we can see that the maximum for the system corresponds to five water molecules per glucose molecule. For the system at $T = 280$ (rubbery state), the tail of the distribution is extended to a larger number of hydration waters, in the sense defined. Some authors have pointed out that in such glasses, the water molecule motions are severely slowed down, but hardly to the extent of complete inhibition [24]. The translational motions are mainly constrained below T_g. Above T_g, the water molecules can migrate obeying Arrhenius kinetics. Consequently, the possibility of connecting a large number of solute molecules is greater.

Hydrogen bonds and residence times.—Most polyhydroxy compounds are similar to high polymers in some of their properties [1], probably due to the formers' capacity to form multiple hydrogen bonds. A geometric criterion was used to characterise the H-bond network in the glucose–water matrix [25]. We say that an H-bond is established when the distance between donor hydrogen and acceptor oxygen is less than 0.24 nm, and the H–O–H angle falls between 145 and 215° [26]. Mean lifetimes of H-bonds were calculated for every oxygen atom of the solute molecule, including

Table 1
Average lifetime of hydrogen bonds between water and oxygen atoms of glucose

Oxygen atom	280 K		200 K	
	Donor (ps)	Acceptor (ps)	Donor (ps)	Acceptor (ps)
O1	1.28	0.23	0.16	0.10
O2	1.23	1.22	0.13	0.17
O3	0.68	0.72	0.14	0.09
O4	0.54	0.27	0.23	0.10
O6	2.02	0.63	0.24	0.14
Os		0.11		0.04

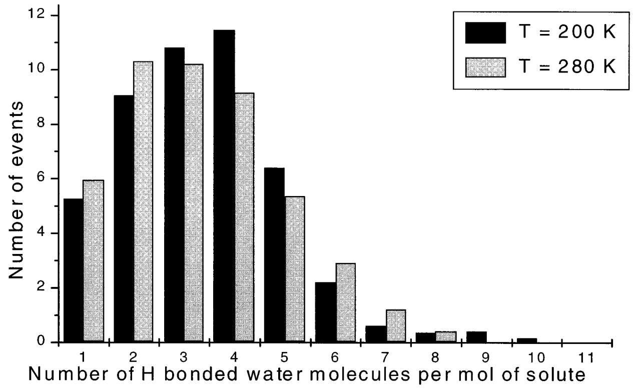

Fig. 4. Distribution of water molecules having hydrogen bonds to glucose molecules.

the one in the pyranosic ring, at both simulation temperatures. Results are presented in Table 1; for the hydrogen bonds in which the glucose oxygen atoms act as a proton donors, the lifetimes are, in most cases, longer. This fact can be understood for the acceptor case at both temperatures, but in the rubbery state (above T_g), the difference between acceptors and donors is not as important. It is worth remarking that H-bonds formed by the oxygen atoms of the anomeric and the side groups have greater lifetimes than the rest. The longer residence time corresponds to better-defined hydration, observed from the radial distribution function.

The histogram of Fig. 4 shows the number of glucose molecules that have N ($N = 1,2,3...$) water molecules in their vicinity forming H-bond linkages.

The longer mean lifetimes of H-bonds below T_g, relative to the values for the rubbery state, suggest that the translational motion of water molecules surrounding the carbohydrates is severely slowed down. As a consequence, chemical processes involving water will also be slowed down. Table 2 shows the values of residence times of water molecules in the vicinity of the oxygen atoms of the glucose molecules (hydration lifetime). To compute residence time, we considered a sphere of radius equal to the average distance of the minimum of the corresponding radial distribution function. The values of the radii were 0.320 and 0.337 nm for $T = 200$ and 280 K, respectively.

Table 2
Residence time of water molecules near glucose oxygen atoms

Oxygen atom	200 K (ps)	280 K (ps)
O1	3.10	1.34
O2	7.72	2.71
O3	4.88	1.56
O4	4.42	1.32
Os	2.17	1.13
O6	6.45	1.98

Note the difference between the lifetime of hydrogen bonds (Table 1) and the hydration lifetime. In the latter, we counted a water molecule within the defined ‘hydration sphere’ irrespective of its bonded state, i.e., it might not have a proper hydrogen bond. Consistent with this definition, the correlation with $g_{(r)}$ shows a larger value.

Again we can see, from residence times, that water mobilities below and above T_g are significantly different.

Percolation.—Small carbohydrates behave in some aspects like high polymers [12]. This is probably due to the capacity to form hydrogen bonds between individual molecules that act as subunits of a larger aggregate. If this is so, the overall connectivity should be large and could be characterised by a percolation parameter. If we define the percolation parameter as the cluster mean size [27],

$$P = \frac{\sum_i^N i^2 f(i)}{\sum_i^N i f(i)} = \frac{\sum_i^N i^2 f(i)}{N}$$

where i is the cluster of occupancy probability $f(i)$, we can compute this value from the simulation, for both the glucose and water. Fig. 5 shows the mean cluster size, as a function of the simulation time, for glucose and for water at the two temperatures studied.

For full connectivity of glucose in the present system the mean cluster size is 48 for the glassy state; this value is close to that obtained. At higher temperatures, not only is the mean cluster size lower, but also the temporal fluctuations are much greater. However, the connectivity in the rubbery state is important and justifies the assumption that the polymer-like behaviour of small carbohydrates is due to the H-bond connectivity of individual molecules. At this relatively high concentra-

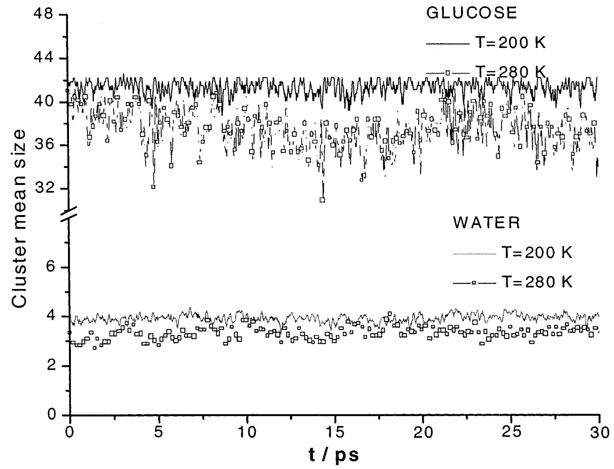

Fig. 5. Temporal evolution of mean cluster size, averaged over a 30 ps simulation time.

tion, the poor connectivity between water molecules (for pure water—defining a cluster as the molecules connected by hydrogen bonds with the criterion used in this work—it is about 20% of that corresponding to full connectivity) is not surprising. The effect of temperature on water is the same as for glucose, decreasing the mean cluster size and increasing the fluctuations as temperature increases.

4. Conclusions

The results of the present molecular dynamics simulations show that the model used was good enough to describe the T_g of the aqueous solution of glucose having the experimental anomeric ratio corresponding to the concentration simulated. Most of the water molecules are connected to more than one glucose molecule by hydrogen bonds. Two different definitions of hydration water were considered: one in which the water molecules are hydrogen-bonded to glucose, and the other in which all water molecules within a prescribed distance of glucose are considered as hydration water. Both definitions, although producing different hydration numbers, are consistent. The residence times of water molecules in hydration sites change from one site to another, but for the anomeric and chain oxygen atoms, the residence time is longer than for the rest. The average residence time ranges from 2.00 ps for the rubbery state at 280 K to 5.75 ps for the glassy states at 200 K. Moreover, the water molecules in the glassy state have much more restricted motions than in the rubbery state. This result is consistent with a lower reactivity of glucose in the glassy state. The behaviour of glucose, as well as other small carbohydrates, like that of high polymers, is believed to be due to the association of molecules through hydrogen bonds. To be effective, this association has to be extended over a relatively large cluster size. The computation of the glucose cluster size, as a function of time, indicates that the mean value is very close to that corresponding to full connectivity and does not vary much from the rubbery to the glassy state. By the use of molecular dynamics simulation, it is possible not only to reproduce the available experimental data, but to gain insight on the molecular mechanisms and microscopic characteristics of the glassy state.

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