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Glucose in Aqueous Solution by First Principles Molecular Dynamics

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Abstract: We present the results of the first *ab initio* molecular dynamics simulations, based on the Car−Parrinello method, of glucose in water, performed to investigate structural aspects of the anomeric equilibrium in aqueous solution. The analysis of the hydrogen bonds around the crucial anomeric oxygen shows a distinct solvation behavior for the β and α anomers. While the β anomer, which is the most abundant in water, allows the water molecules to flow in a disorderly manner around its anomeric site, the α anomer tends to bind them more tightly.

Carbohydrates are an important class of biomolecules involved in a variety of processes such as energy storage, structural support, molecular recognition, and water control in cold and drought-resistant organisms. Many problems related to carbohydrates are still far from properly understood, such as the behavior of glucopyranose (glucose) in aqueous solution.[1]

A sketch of the glucose molecule ($C_6H_{12}O_6$), with the conventional numbering of the heavy atoms, is shown in Figure 1. Its main structure is a puckered six-membered ring in the $^4C_1$ chair conformation, which carries a hydroxymethyl and four hydroxyl groups. Glucose can be found in two different anomeric forms, α and β, which differ by the orientation (axial or equatorial with respect to the ring) of the $C_1{-}O_1$ bond. Moreover, the hydroxymethyl group can adopt three possible orientations associated with the $C_5{-}C_6$ internal rotation, characterized by the dihedral angle $O_5{-}C_5{-}C_6{-}O_6$ that can be gauche clockwise ($\approx$60°, G$^+$), anti ($\approx$180°, T), or gauche counterclockwise ($\approx -$60°, G$^-$).

Experiments show that the abundance ratio of the axial α anomer with respect to the equatorial β anomer in aqueous solution at room temperature is 36:64, corresponding to a free energy difference of only 0.35 kcal/mol.[2] The effect, which is solvent dependent,[3] is therefore subtle, and it is still a matter of debate whether the α:β equilibrium is due to solvation or to effects that also exist in the isolated molecule. According to NMR results,[4] in water the G$^-$ and G$^+$ rotamers are almost equally populated, while the population of the T rotamer appears to be negligible.

There have been many theoretical attempts to tackle the problem.

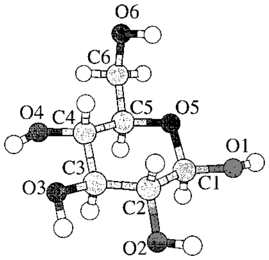

Figure 1. The glucose molecule with the conventional numbering of the heavy atoms.

For the isolated molecule many force-field[5−8] and quantum-chemistry[9−15] calculations have been performed as well as a few calculations of the crystalline structures.[9,16−18] While these studies provide a better insight into the structure of glucose and represent a first step toward the investigation of more complex polysaccharides, they fail to give information about the behavior in aqueous solution. In particular, they do not account for the dynamic hydrogen bond network created by the water molecules around the glucose.

The glucose−water solution has been studied by both force-field molecular dynamics methods[19−24] and quantum-chemical solvation models, which approximate the water surrounding the

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solute by a polarizable continuous medium.[13,15] Recent force-field simulations have investigated xylose (which differs from glucose by the absence of the hydroxymethyl group and shows a similar behavior in aqueous solution) and other pentose sugars.[25,26]

A popular view is that the β anomer is favored in water because of solvation.[19,24,25] Since the α anomer has been traditionally believed to be more stable than the β one in the gas phase due to the so-called "anomeric effect",[27,28] the experimentally observed abundance of the β anomer in water could only be explained by strong solvation effects that counterbalance and overcome the preference for the α anomer. The β anomer might be more favorably solvated because it fits nicely into a trydimite ice lattice, with glucose−water hydrogen bonds replacing the water−water hydrogen bonds.[29,30] However, recent *ab initio* investigations have cast serious doubts on the alleged gas phase preference for the α anomer:[12−14,16] in fact the α-β energy difference is strongly basis-set dependent and decreases as the quality of the calculation is increased.[12−14] This somehow reduces the necessity to call upon strong solvation effects in order to explain the anomeric equilibrium ratio.

According to other authors solvation effects contribute to the hydroxymethyl group rotamer population, rather than to the α:β equilibrium.[13,23]

However, up to now no calculation that treats from first principles both solute and solvent has been performed. Such a calculation would bring substantial progress, since it would accurately describe the hydrogen bonds which determine the water structure around the glucose and, ultimately, its properties.[1] Moreover, it would overcome the difficulties and artifacts linked to the choice of force-field parametrizations. Encouraged by the excellent results recently obtained for liquid water[31] and for crystalline glucose,[16] we have therefore performed density functional molecular dynamics simulations based on the Car−Parrinello method[32] to investigate the glucose−water solution. Reviews of the Car−Parrinello method can be found in ref 33.

The glucose−water system was modeled in a cubic supercell of lattice parameter 12.42 Å with periodic boundary conditions, containing 58 water molecules and one glucose molecule for a total of 198 atoms. No constraints were applied on the bond lengths or bond angles. We used the Becke, Lee, Yang, and Parr (BLYP) gradient corrected functional,[34,35] which proved to be very efficient in describing liquid water,[31] and Troullier−Martins norm-conserving pseudopotentials.[36] The electronic wave functions were expanded in a plane wave basis set with a kinetic energy cutoff of 70 Ry. The time step for the molecular dynamics was 0.145 fs, and the electronic mass was set to 900 au; in order to avoid too small time steps without affecting the structural properties of the solution, the isotopic mass of deuterium was used for hydrogen. The simulations were performed on a CRAY T3D (128 nodes), where a single time step required a CPU time of about 47.4 s.

We performed three distinct simulations, the first one for the β anomer starting with the G^- hydroxymethyl rotamer (as in the crystalline structure[37]), the second one for the α anomer starting with the G^- hydroxymethyl rotamer (as in the monohydrate crystalline structure[38]), and the third one for the α anomer starting with the G^+ hydroxymethyl rotamer (as in the anhydrate crystalline structure[39]): in the following, we refer to these simulations respectively as β, α_1, and α_2. Constant energy molecular dynamics simulations were performed at room temperature for about 6 ps, and statistics were collected in the last 3 ps: all the average values that will be cited in the following were calculated during these last 3 ps. Such a time scale certainly does not allow us to explore the different conformational changes that occur in solution nor to give a quantitative estimate of the free energy difference between the two anomers; nevertheless it provides a reliable characterization of the water structuring around the solute and qualitatively assesses the reliability of the proposed models. The average temperatures of the three samples were 312 ± 17 K for β, 331 ± 14 K for α_1, and 338 ± 15 for α_2.

The conformers β and α_2 maintained the original hydroxymethyl group orientation throughout the simulations; the $O_5-C_5-C_6-O_6$ dihedral angle was measured as having the average values of $-67° \pm 7°$ and $78° \pm 21°$ for β and α_2, respectively. After about 3 ps, α_1 underwent a transition from G^- to T; the T orientation was then maintained during the 3 ps of data collection, where the average value of the $O_5-C_5-C_6-O_6$ dihedral angle was $177° \pm 9°$. Similarly, a transition to the T hydroxymethyl rotamer was observed in previous force-field simulations.[21] However this result is in contrast with NMR data, which are usually interpreted as reflecting a negligible population of the T rotamer. The reasons for this apparent discrepancy are not clear. On the one hand, our simulation time is not long enough for the system to explore all possible configurations in a statistically meaningful way. On the other hand, there is always the possibility that our level of theory is not accurate enough. It is unlikely that the transition has been caused by an unfortunate choice of the initial conditions. In fact, the conformational change took place after about 3 ps, which should be enough for the system to lose memory of the initial state. Energy barriers for the transition between the three hydroxymethyl rotamers in vacuum have been calculated and found to be fairly large.[11] However, in solution the potential energy surface can be significantly different. Some indication that this might be the case can be found in ref 23.

We have analyzed the distribution of hydrogen bonds in our three samples. The criteria for the existence of a hydrogen bond $O_D-H\cdots O_A$, where O_D and O_A are respectively the donor and acceptor oxygens, were $O_D-H \leq 1.5$ Å; $H\cdots O_A \leq 2.4$ Å; and

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Table 1. Average Number of Hydrogen Bonds for the β, α_1, and α_2 Simulations[a]

	β		α_1		α_2	
total	109.1 ± 3.3	[110.5]	108.4 ± 2.8	[110.1]	106.0 ± 2.9	[107.9]
water−water	98.9 ± 3.2	[99.9]	97.1 ± 2.9	[98.4]	95.5 ± 2.8	[97.0]
water−glucose	10.2 ± 1.4	[10.4]	11.1 ± 1.5	[11.3]	10.5 ± 1.4	[10.6]
glucose−glucose		[0.17]	0.17 ± 0.38	[0.37]	0.03 ± 0.16	[0.31]

[a] The criteria for the existence of a hydrogen bond $O_D{-}H\cdots O_A$, where O_D and O_A are, respectively, the donor and acceptor oxygens, are $O_D{-}H \leq 1.5$ Å; $H\cdots O_A \leq 2.4$ Å; and $O_DHO_A > 120°$. The average numbers obtained for $O_DHO_A > 100°$ are shown in square brackets.

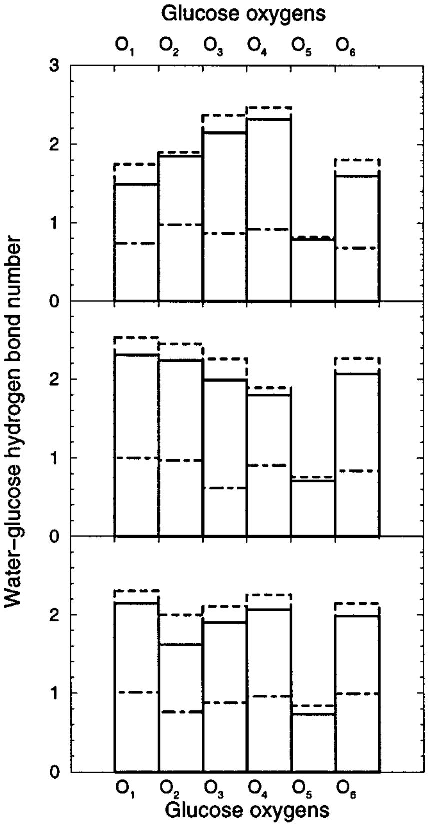

Figure 2. Solid line: the average numbers of hydrogen bonds between the glucose oxygens and the water molecules for β (top panel), α_1 (central panel), and α_2 (bottom panel). Dashed-dotted line: bonds in which the glucose atoms act as donors. Dashed line: results obtained using the less strict hydrogen bond criteria of ref 25.

$O_DHO_A > 120°$. The total, water−water, water−glucose and glucose−glucose average numbers of hydrogen bonds per simulation cell are shown in Table 1. According to the chosen selection criteria, no intramolecular hydrogen bond was found for β, while very short-lived ones were found for α_1 and α_2; among the intramolecular hydrogen bonds, it is the one between O_4 and O_6, the oxygen of the hydroxymethyl group for α_1 in the T rotamer configuration, that had the longest life. While in the gas phase the energetically favored conformations are those whose hydroxyl groups generate an ordered pattern of intramolecular strained hydrogen bonds,[9,12] intramolecular hydrogen bonds do not seem to be favored in aqueous solution where no evidence of ordered patterns was found. The average number of intramolecular hydrogen bonds remained low, even if the criteria for selecting hydrogen bonds were changed, either by lowering the threshold for the O_DHO_A angle to 100° (see Table 1) or by both lowering the threshold for the O_DHO_A angle to 100° and increasing the $H\cdots O_A$ threshold to 2.7 Å. In the latter case the average numbers of intramolecular hydrogen bonds were found to be 0.59 for β, 0.61 for α_1, and 0.55 for α_2.

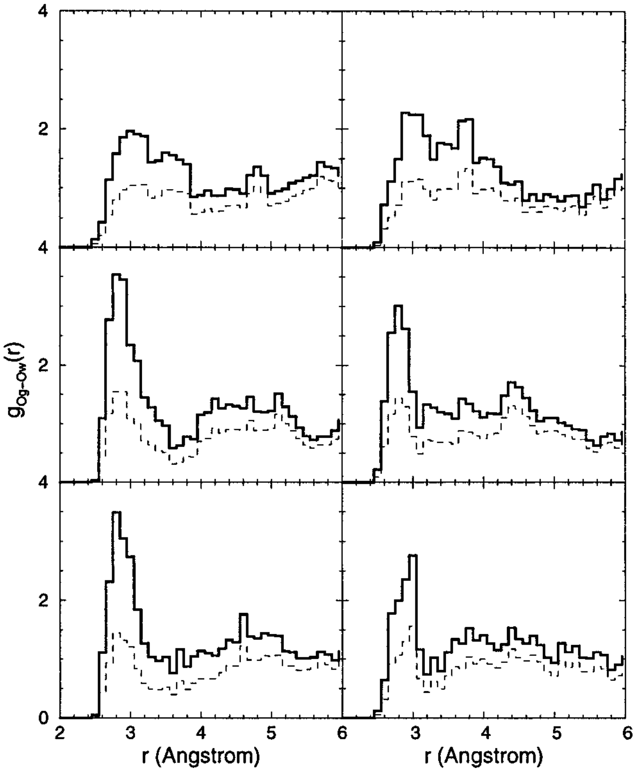

Figure 3. Anomeric oxygen−water oxygens pair correlation functions (left) and hydroxymethyl oxygen−water oxygens pair correlation functions (right) for β (top panel), α_1 (central panel), and α_2 (bottom panel). The volume normalization for these correlation functions (solid line) takes into account only the so-called "accessible volume", according to the prescription of ref 24, in order to eliminate artifacts resulting from spherical averaging; at variance to ref 24, the volume here was calculated with a Monte Carlo sampling of the space around the glucose. The correlation functions obtained with the conventional spherical volume normalization are also shown (dashed line).

The average distributions of hydrogen bonds indicate no striking difference in the solvation of the two anomers. β has the largest average number of hydrogen bonds, followed by α_1 and α_2. In the simulations of the α anomers more hydrogen bonds involving the solute (inter- and intramolecular) were formed, while in the simulation of β, more hydrogen bonds among the water molecules were found. However, the differences are comparable to the statistical errors.

Of course these results depend on the criteria chosen to select hydrogen bonds. We have verified that if the threshold for the O_DHO_A angle is lowered to 100°, all the trends in Table 1 are still maintained. The same occurs if less strict criteria are used, as those of refs 25 and 26 where the requirements were $O_D{-}O_A \leq 3.4$ Å and $O_DHO_A > 120°$.

In Figure 2, the average numbers of glucose−water hydrogen bonds for the three samples are shown. In principle, each hydroxyl group could participate in three hydrogen bonds, one as donor and two as acceptor, while O_5 could participate in two hydrogen bonds as acceptor. According to our analysis, the hydrogen bond capacity of these groups in not saturated on average in any of the studied cases.

We compared the average number of hydrogen bonds that each glucose oxygen forms with the water molecules in the three simulations. We found that β forms 0.82 and 0.66 hydrogen bonds involving O_1 and the water molecules less than α_1 and α_2, respectively. This contrasts with what was recently found with force-field molecular dynamics simulations for xylose (whose anomeric ratio in aqueous solution is very similar to that of glucose), where the anomeric hydroxyl group makes fewer hydrogen bonds in the α than in the β anomer.[25,26] Moreover, the hydrogen bonds involving O_1 have different behaviors in the β and α anomers. In β O_1 participates in hydrogen bonds with several water molecules (eight during the observation time) surviving for short times, while in α_1 and α_2 the O_1 hydrogen bonds live longer and involve fewer water molecules (four in both cases). Therefore, in β there is a higher degree of disorder around the anomeric site than in either α_1 and α_2: hydrogen bonds are easily formed and destroyed in β, while they remain for longer in both α_1 and α_2. This situation is reflected in the anomeric oxygen−water oxygen pair correlation functions, shown in Figure 3 (left panels). In β the first peak of the pair correlation function is broad, indicating a higher degree of disorder, while in α_1 and α_2 it is much better defined: in our simulations, the water molecules that solvate the crucial anomeric site are less tightly bound and have a shorter residence time in β than in α. A similar situation occurs for the distribution of water molecules around the hydroxymethyl group oxygen (Figure 3, right panels).

This distribution of the waters at the anomeric site contrasts with the idea that the solvation shell of β should resemble a trydimite ice lattice, i.e., a fairly ordered structure.[29,30] Similarly, no evidence to support solvation models based on the compatibility of the glucose structure and the icelike lattice was found in the analysis of force-field molecular dynamics simulations.[24] Nevertheless, remarkable differences between our water oxygen−sugar oxygen pair correlation functions and those reported in ref 24 for the α anomer remain. In particular, we did not find such high peaks for the O_2−water and O_3−water correlation functions, even adopting a similar technique for the volume normalization.

In conclusion, we have demonstrated the feasibility of *ab initio* molecular dynamics simulations of glucose in water solution and investigated some structural aspects of the anomeric equilibrium. While all our samples show a similar average number of hydrogen bonds, consistent with the small estimated free energy difference between the two anomers, the behavior of the water molecules around the anomeric oxygen is definitely different for the β and α case. The β anomer, which is the most abundant in water, allows water molecules to flow freely around its anomeric site, while the α anomer tends to bind them more tightly and in a more orderly manner. The difference in the solvation of the two anomers is therefore more qualitative than quantitative.

The discrepancies between our parameter-free results and force-field simulations raise some doubts on the reliability and transferability of such potential parametrizations. While our results are limited to short time scales, they represent a first step toward an accurate treatment of a relatively complex system, such as glucose in water.

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