Anisotropic Solvent Structuring in Aqueous Sugar Solutions

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Abstract: The details of solution structure have proven difficult to probe experimentally, but computer simulations allow solvent structuring to be examined directly. We report here molecular dynamics (MD) simulations of a series of pentose sugars in solution, describe in detail the three-dimensional structuring which these molecules impose on water, and relate this structuring to the solute molecular topologies. Well-defined first and second solvation shells are observed around the sugar molecules with specific locations determined by the arrangement of functional groups within each solute. Calculated molecular internal energies and solvent interaction energies generally correspond qualitatively with earlier models of sugar energetics based on stability factors. However, pairs of axial hydroxyl groups on the same side of a sugar ring were found to be disfavored by solvation energy, not by internal energy, which is actually more favorable due to intramolecular hydrogen bonding, in accord with both quantum mechanical calculations and a crystal structure for a related molecule determined by diffraction. A single axial hydroxyl group could be favored or disfavored energetically by hydration, depending on the particular circumstance.

Introduction

Water molecules are extensively hydrogen bonded in condensed phases, and the geometric requirements of these hydrogen bonds impart considerable structure to liquid water and aqueous solutions.[1] When a solute molecule is in an aqueous environment, its functional groups must interact with the inherent structural requirements of the solvent water, and its presence can impose an alternate structuring pattern on the adjacent solvent molecules. Such solvent structuring is often invoked to explain the properties of aqueous solutions.[1,2] Liquid structuring and its associated entropy are generally responsible for many of the anomalous properties of bulk water[3] and much of the behavior of biopolymer systems, such as the folding of globular proteins and the formation of lipid bilayers and micelles. The organization of solvent water molecules around a particular solute will in general involve both positional and orientational correlations with the specific chemical architecture of the solute, and thus will vary in its details from one solute to the next. For biological solutes, the actual structuring imposed on water could be quite complex, due to the complicated mix of chemical functionalities found in typical biopolymers, which frequently have polar or hydrogen-bonding functional groups in close proximity to nonpolar groups.

While the general principles involved in hydration are now well understood, detailed information about solvent distributions around particular solutes is usually not available due to experimental limitations. Raman scattering experiments permit some solutes such as sugars to be identified as net "structure makers", but cannot give specific three-dimensional details.[4] Such detail can be obtained from studies of X-ray or neutron diffraction by liquids, which can be used to determine pair distribution functions for pure water[5] and solutions of simple solutes such as argon[6] or ions.[7] The advantage of these experiments is that they are a direct probe of liquid structure, but they usually have the disadvantage of being spherically averaged; for complex, asymmetric solutes, the anisotropic details are lost in radial averaging. The atomic detail available in X-ray diffraction from single crystals allows water molecules incorporated into macromolecular crystals to be located to high precision.[8,9] Unfortunately, crystal environments may not be representative of dilute liquid solutions, and often only a fraction of neighboring water molecules have sufficient electronic densities to be placed in refinement procedures. Computer simulations, however, have proven to be very useful in modeling structure in liquids,[1,2,10] and have been extensively applied to the study of aqueous solutions.[11−21] Only recently have simulations been used to examine fully spatially-resolved structuring in liquids[22−24] and solutions,[21,25−28] providing a direct picture of the nonuniform distribution of molecules in three dimensions.

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Recently we reported the use of molecular dynamics (MD) simulations to model the arrangement of water around a typical carbohydrate.[21] The carbohydrates comprise a particularly important class of biological solutes. Not only are they the most abundant biological molecules, involved in numerous structural, energy storage, and molecular recognition roles, but they also exhibit interesting solution properties that are exploited in nature for a variety of purposes, including the control of water in cold- and drought-resistant organisms.[29] Sugars which are stereoisomers, perhaps differing in configuration at only one asymmetric carbon, can have distinct solution properties,[30,31] presumably due in part to the different structuring which they impose upon solvent water molecules. In aqueous solution, reducing sugars exist in an equilibrium between several different forms, including the open chain aldehyde or ketone and the α and β anomers of both five-membered (furanoid) and six-membered (pyranoid) ring structures, with each of these tautomers having a variety of possible conformations.[32] However, these various forms have different free energies, and in general the tautomeric equilibrium is dominated by a small number of forms; the most common situation for the aldohexoses is for the equilibrium to be primarily between the α and β anomers of the $^4C_1$ conformer of the pyranoid ring.[33−35]

In our previous set of MD simulations of D-xylose in aqueous solution,[21] we calculated the structuring imposed on the adjacent solvent by each of the two anomers of the pyranose ring form of the sugar. The highly anisotropic solvent structuring was found to be configuration dependent, and the differences in hydration between the two forms were found to play a role in determining the anomeric equilibrium concentrations. Because of the clear differences in hydration found for these two anomers and because of the importance of this difference for their solution properties, it would be instructive to examine how solvent water structuring around other similar sugar isomers varies as a function of the molecular configuration. We report here a series of new simulations of various pentose sugars and use these simulations to examine the differences in hydration of these configurational and conformational isomers.

Methods

In the present study, MD simulations were conducted for a series of seven pentose sugar molecules, all in their pyranoid ring forms. As in our previous study, the pentapyranoses were chosen over the hexoses since for these smaller molecules the solvent structuring is not perturbed by the torsional angle rotations of exocyclic hydroxymethyl groups. The sugars studied included α- and β-D-xylopyranose, β-D-lyxopyranose, α-L-arabinopyranose, α-D-arabinopyranose, and β-L-xylopyranose, all in the $^4C_1$ conformation, and β-D-xylopyranose in the $^1C_4$ conformation. These sugars are depicted in Figure 1. The β-D-lyxopyranose and α-L-arabinopyranose molecules were chosen because they are the pentose analogs of the biologically important hexoses β-D-mannopyranose and β-D-galactopyranose, in the same way that β-D-xylopyranose is the pentose analog of β-D-glucopyranose. Specifically, these pentose sugars would result from replacing the exocyclic primary alcohol group of the corresponding hexose with a proton. Both of these pentose sugars have one axial hydroxyl group, at the C2 and C4 positions, respectively. The D-xylose anomeric pair was repeated to confirm the results of the previous study.[21] The β-L-xylopyranose molecule and the $^1C_4$ conformer of β-D-xylopyranose were chosen because they have all of their hydroxyl groups in axial positions, essentially the opposite case from that of the $^4C_1$ β-D-xylopyranose, where all of the hydroxyl groups are equatorial.

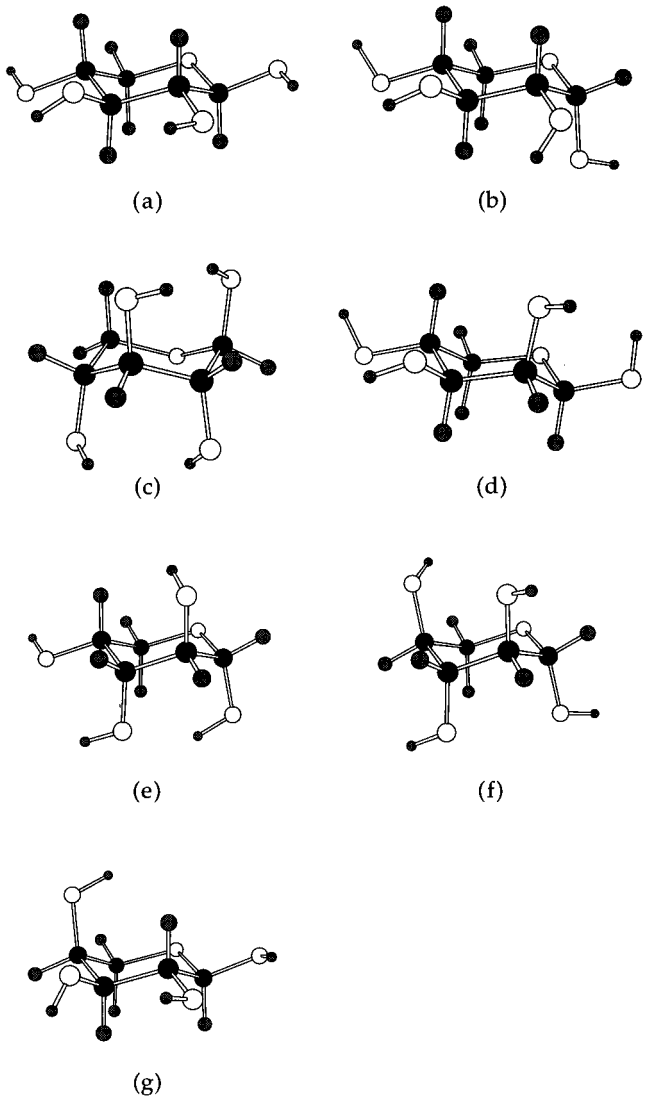

Figure 1. Trajectory-averaged structures for the several pentoses modeled in the present study: (a) β-D-xylopyranose; (b) α-D-xylopyranose; (c) β-D-xylopyranose in the $^1C_4$ conformation; (d) β-D-lyxopyranose; (e) α-D-arabinopyranose; (f) β-L-xylopyranose; (g) α-L-arabinopyranose.

Each of the sugars modeled in this study was simulated at 300 K in a periodic cubic box 24.7711 Å in length containing 502 TIP3P solvent water molecules,[36] corresponding to more than three full solvation "shells" around the sugar solute in the primary box in every direction. Group-based switching functions[37,38] were used to make nonbonded interactions go smoothly to zero between 10.0 and 11.0 Å. These systems were thus significantly larger than those used in our previous

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Table 1. Average Potential Energy Components for Each Sugar Simulation and the Average Number of Hydrogen Bonds to Solvent Made by Each Sugar[a]

	energy components (kcal/mol)				
sugar	solute internal	solvent−solute interaction	sum	difference in free energy estimated from stability factors	total number of hydrogen bonds to solvent
β-D-xylopyranose	0.00	0.00	0.00	0.00	11.70
α-D-xylopyranose	−0.89	2.34	1.45	0.35	11.41
β-D-xylopyranose ($^1C_4$)	−5.76	6.93	1.17	2.30	10.47
β-D-lyxopyranose	4.31	0.06	4.37	0.90	11.62
α-D-arabinopyranose	−2.37	8.50	6.13	1.60	10.52
β-L-xylopyranose	−2.82	7.22	4.40		10.50
α-L-arabinopyranose	3.80	−6.94	−3.14	0.01	12.04

[a] All energies are given in kilocalories per mole, relative to the corresponding value for β-D-xylopyranose.

simulations of D-xylopyranose,[21] which modeled the sugar in an 18.4987 Å cubic box of 205 water molecules, and cut nonbonded interactions at 8.0 Å. Each sugar was modeled in its pyranoid ring form, with the initial structures being built from the standard equilibrium bond lengths and angles for the chosen force field, and with the torsional angles appropriate for such rings. For each sugar, this initial geometry was superimposed without minimization of the center of a previously equilibrated box of water molecules, and those solvent molecules which overlapped with the heavy atoms of the sugar were removed. Initial velocities were randomly assigned from a Boltzmann distribution, and each system was equilibrated for 30 ps using a Verlet integration scheme[39] with a step size of 1 fs, followed by an additional 150 ps of simulation used for data collection. The lengths of bonds involving hydrogen atoms were kept fixed using the SHAKE constraint algorithm,[40] which was also used to keep the water molecules rigid. All calculations were performed using the program CHARMM[37] and the same CHARMM-type force field for carbohydrates[41] used in our previous simulations.

No transitions away from the initial ring geometry occurred in any of the simulations, whether in the $^1C_4$ or $^4C_1$ conformation. Thus, on this time scale, the only thermally-accessible conformational freedom consisted of hydroxyl proton rotations; in these pentoses, with four hydroxyl groups, there are $3^4 = 81$ possible staggered conformational substates for these groups. These conformational substates were repeatedly sampled through frequent rotational transitions in the hydroxyl groups.[42] The relatively rigid rings of pyranoid sugars[43,44] kept the hydroxyl oxygens and aliphatic protons in these molecules at approximately fixed relative orientations throughout the simulations. This rigidity of the sugar rings was exploited for the mapping of the anisotropic long-range structuring in the solution induced by the asymmetric arrangement of sugar functional groups. Each stored coordinate set from the MD simulations was translated and rotated so as to achieve the best least-squares overlap of the instantaneous positions of the six ring atoms with their initial positions, to remove the effects of solute diffusion and allow the definition of a coordinate frame fixed on the sugar. This same coordinate transformation was applied to all solvent molecules and their periodic images. A Gaussian function centered on each water oxygen atom was used to approximate the distribution of electrons for each molecule, and the spatially-resolved average solvent electronic density relative to a frame fixed on the sugar ring was calculated and contoured in three dimensions with the program CHAIN.[45]

Results and Discussion

The new simulations of α- and β-D-xylopyranose produced results for the first hydration shell qualitatively the same as those found in our previous study of these molecules using a smaller primary box and shorter nonbond cutoff distances;[21] no evidence was found to indicate that the nearest-neighbor structuring in the smaller simulation was significantly perturbed by edge or concentration effects. In particular, the β anomer made more hydrogen bonds to the solvent than did the α form (see Table 1), and this difference in average number of hydrogen bonds came almost entirely from the anomeric hydroxyl group in the two anomers. Table 1 also lists the relative average energies of these two forms and the component contributions to this total. As before, the α anomer was found to have a lower internal energy, in accord with the expectation of a stabilization of this form by the anomeric effect, and this stabilization was found to be counterbalanced by a solvent interaction term which favors the β anomer, again as seen previously. However, in contrast to the earlier results, the sum of these two components in the present case favors the β anomer by 1.45 kcal/mol. From calorimetry, the β anomer is lower in enthalpy by 0.54 kcal/mol,[46] about one-third of this calculated value. Because the α anomer is preferred entropically, the experimental free energy difference between the two forms is only 0.38 kcal/mol, favoring the β isomer. These experimental numbers are not directly comparable to the calculated energy difference just cited since this number does not include the water−water enthalpy, which cannot be reliably calculated from these simulations (see below), and does not include any entropic contributions.

Also as was seen previously, the solvent structuring around the two anomers of D-xylose was distinct. For both anomers, the sugar molecule imposes considerable local structure upon the water in a complex pattern which is a specific function of the solute topology. Figure 2a displays contours enclosing regions of both positive and negative deviations from bulk solvent density around the β-D-xylopyranose molecule in the $^4C_1$ conformation. The long, tube-shaped red "clouds" in this figure, nearly identical to those seen previously, represent regions of high water density, and are located primarily between adjacent hydroxyl groups. Many of the water molecules which occupy these regions are hydrogen bonded to both adjacent hydroxyl groups. The geometric requirements for simultaneously interacting with two hydroxyl groups are severe, which is the reason for these concentrations of solvent density, and result in many of these hydrogen bonds being significantly distorted away from an ideal linear geometry.[21] The high-density clouds converge above and below the ring, where the water molecules solvate the hydrophobic C−H surfaces and generally make no hydrogen bonds to the sugar at all. Note that the empty regions between the lobes are not actually empty, but are simply regions with densities closer to the average bulk density. The regions enclosed within the yellow contours have solvent densities lower than the bulk value and correspond to the "first minima" in the solvent distribution. These shallow density minima generally occur directly behind the clouds of

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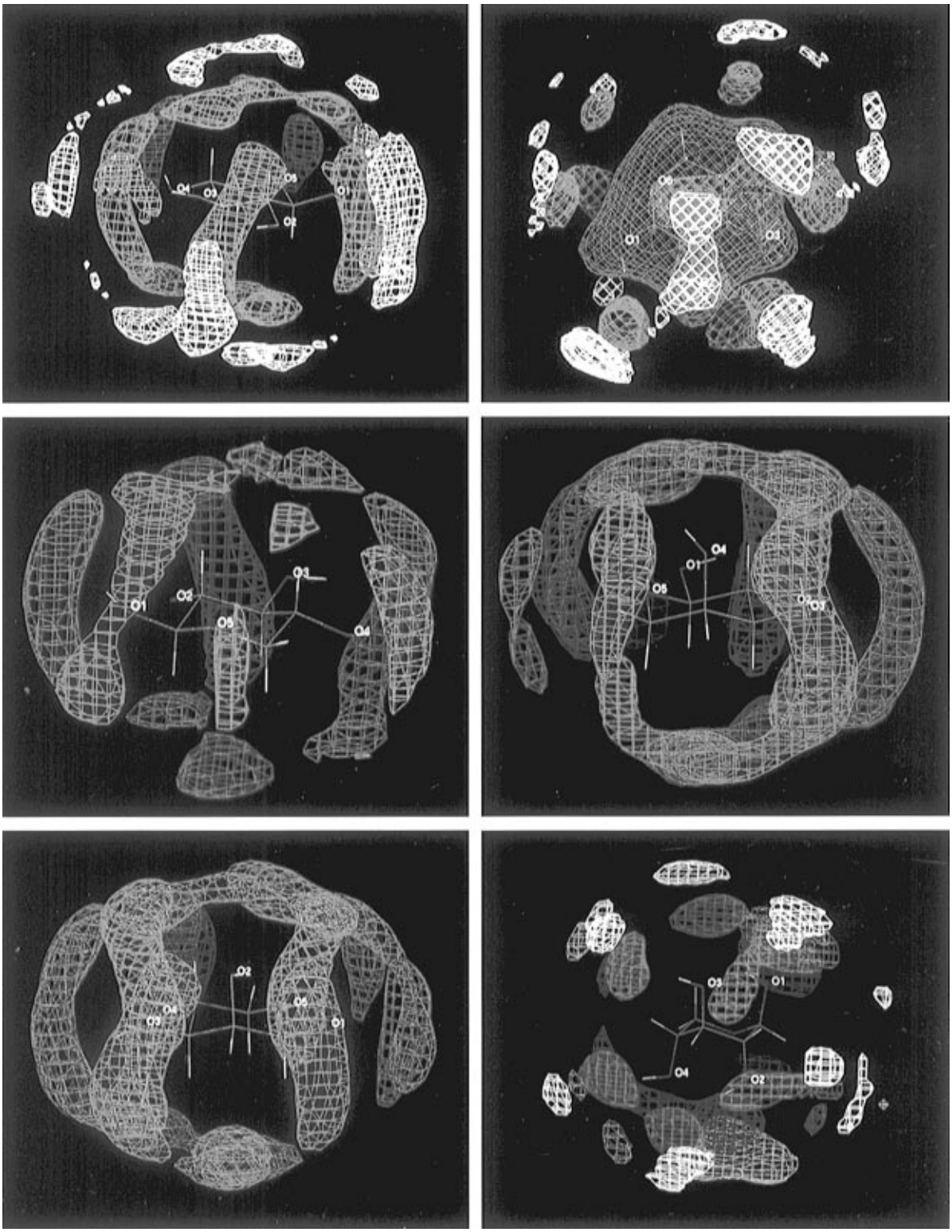

Figure 2. (a, top left) Contours of solvent density around a β-D-xylopyranose molecule as calculated from an MD simulation. The red contour encloses those regions with a density 50% greater than the bulk density, and the yellow contour encloses those regions more than 2.5 Å from any solute atom which have a density 20% below the bulk density. This view is approximately in the orientation used in conventional Haworth formulas; the sugar structure is indicated, and the oxygen atoms are labeled for reference. (b, top right) View from below, looking up at the hydrophobic surface of aliphatic protons. A third blue contour is included which encloses the regions close to the solute with a density below 90% of the bulk density, and essentially parallels the solvent accessible surface of the sugar. (c, middle left) Density contours approximately 50% greater than the bulk density, color coded to indicate the average number of hydrogen bonds to the solvent made by water molecules in the regions enclosed by the contours. Blue indicates 1.5 hydrogen bonds to the solute or more, purple indicates between 0.8 and 1.2 hydrogen bonds to the solute, and red indicates 0.5 hydrogen bond to the solute or less. (d, middle right) Contours of solvent electronic density around α-L-arabinopyranose, compared to the density for β-D-xylopyranose. The blue contours are the 50% density contours for the xylose, and the red contours are the same contour level for the arabinose. (e, bottom left) Contours of solvent electronic density around β-D-lyxopyranose, compared to the density for β-D-xylopyranose. The blue contours are the 50% density contours for the xylose, and the red contours are the same contour level for the lyxose. (f, bottom right) Contours of positive and negative deviations in density around the $^1C_4$ β-D-xylopyranose molecule.

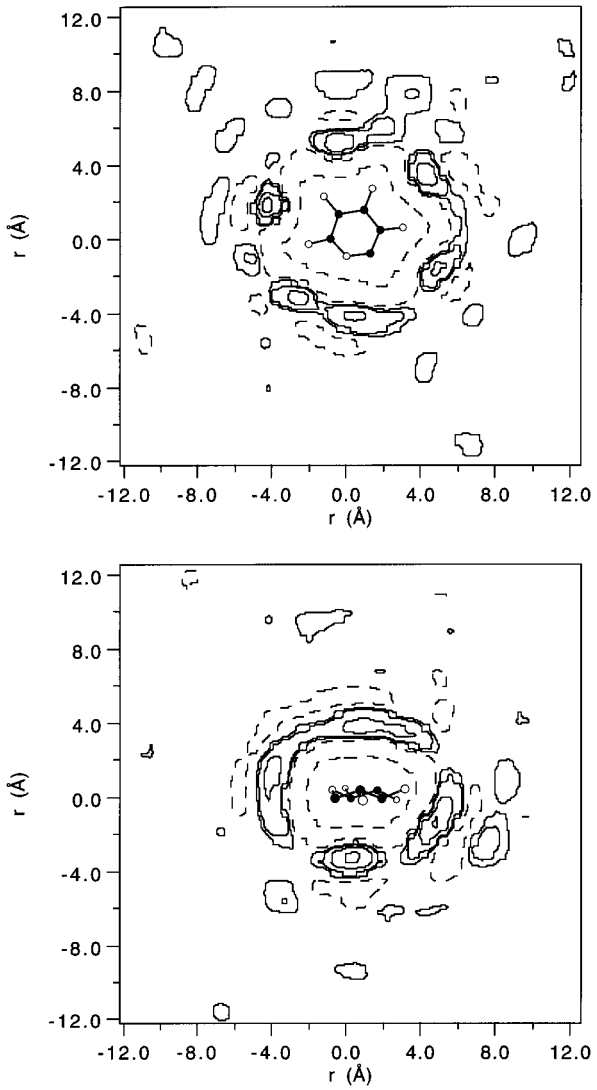

Figure 3. (a, top) Conventional contour map of the density in a two-dimensional cross-section through the approximate plane of the ring. Positive deviations from the bulk density are contoured with solid lines, while negative deviations are indicated by dashed lines; contours are shown at 90% and 10% below the bulk density and 10%, 20%, 50%, and 90% above the bulk density. (b, bottom) Cross-section in a plane perpendicular to the ring.

highest density, since the presence of those water molecules excludes the close approach of others. Figure 2b displays these density contours as seen from "below", which allows a clearer view of how these clouds of higher density tend to wrap around the molecule with the lobes located between the adjacent hydroxyl groups at roughly constant azimuthal angles and distances from the center of the ring. As in our earlier calculation, the first-shell density around the α anomer of this sugar differed significantly from this pattern only around the anomeric center, where there was less solvent structuring.

When contoured at levels closer to the average bulk density, additional structure is observed extending much further out into the solvent. Although too complex to depict in Figure 2, this structuring exhibits well-defined second peaks. Figure 3 displays two-dimensional cross sections of the total density around the β anomer of D-xylose in the average plane of the ring and in a plane perpendicular to the ring. In the equatorial plane there are six positions of very high density in the "first shell" of solvent, corresponding to cross-sections of the lobes of high density visible in Figure 2a,b. There are two such peaks for each hydroxyl oxygen atom, both located at distances of

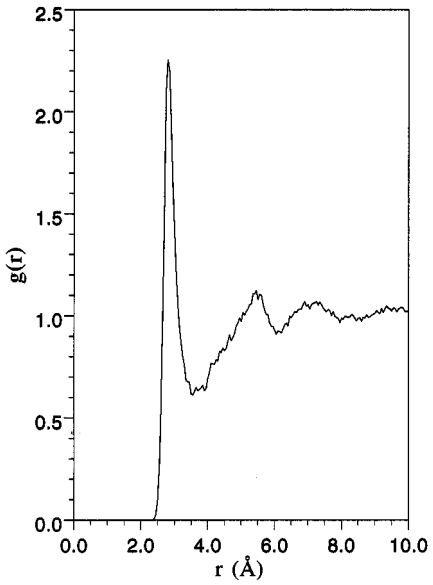

Figure 4. Conventional spherically-averaged pair distribution function for water oxygen atoms as a function of distance from the O3 atom of β-D-xylopyranose.

approximately 2.8 Å. Because of the structure of β-D-xylopyranose, these peaks overlap with those for the adjacent oxygen atoms, with the water molecules in these regions hydrogen bonded to both groups, as already noted. At the C4 hydroxyl oxygen and the O5 ring oxygen atom, the intervening CH_2 group and the absence of a proton on O5 disrupt the pattern. Beyond the density peaks are regions of lower than bulk density corresponding to the yellow lobes in Figure 2. There is also a clear indication of a ring of second hydration shell peaks generally located 5.2−6.0 Å from the sugar oxygen atoms. The still more distant peaks could represent even more extended structure, but could also be statistical noise. It should be noted in particular that these hydration "shells" are not uniform, but consist of well-defined preferred regions.

Figure 4 displays a conventional, spherically-averaged pair distribution function[10,16] for the water oxygen density around the hydroxyl oxygen of the C3 carbon, calculated over a full 4π solid angle. This distribution exhibits a typical form for such simulations,[18,20] with a first peak at the hydrogen-bonding distance around 2.8 Å, a pronounced first minimum centered around 3.6 Å, a broad second peak at 5.5 Å, and a third peak around 7.4 Å. All of the hydroxyl groups in this simulation had similar solvent pair distribution functions; only the ring oxygen exhibited a different profile due to its reduced hydrogen-bonding capacity. In Figure 2, the regions enclosed by the red contours correspond roughly to the first peaks in these radial distributions, while areas indicated by yellow contours contribute to the first minima.

However, Figure 4 gives a somewhat misleading impression of the nature of solvent structuring in the β-D-xylopyranose solution since much of the structure observed in this function is an artifact of the full radial averaging and spherical normalization. Because of the relatively regular ring structure of the sugar, when spherically averaged for any particular hydroxyl oxygen atom, the second peaks of water density out in the solution, in the second hydration shell, are at approximately the same distance as the distal first peak of the adjacent hydroxyl groups on either side of the hydroxyl being considered (see Figure 3a). These unrelated peaks thus contribute strongly to the second peak height, and the absence of solvent looking across the sugar to this other peak causes the unusual depth of the first minimum. Similarly, the distinct

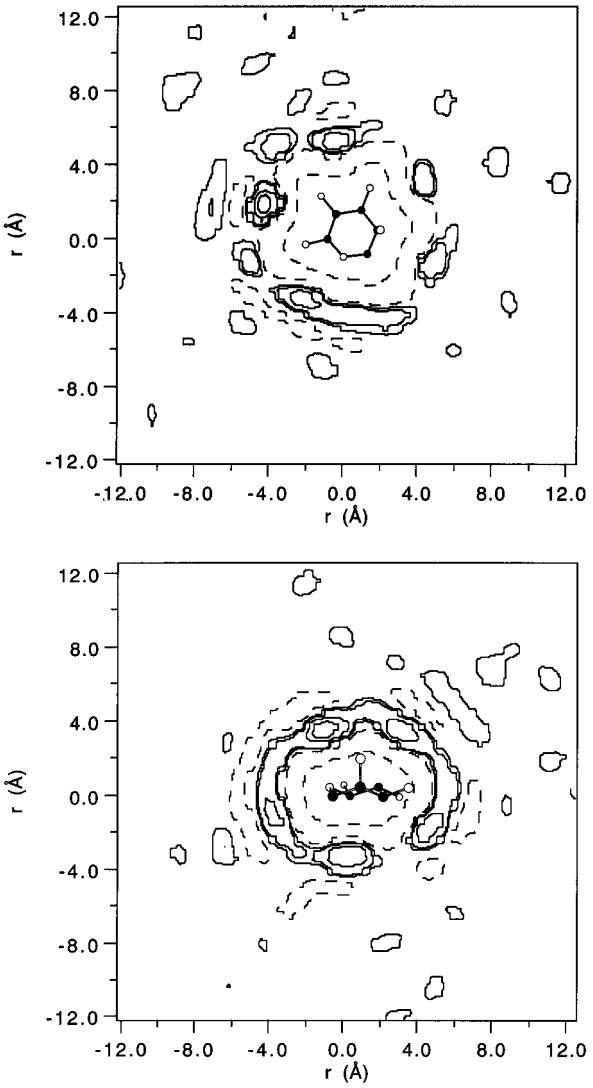

Figure 5. Two-dimensional solvent density contour maps, as in Figure 3, for α-L-arabinopyranose.

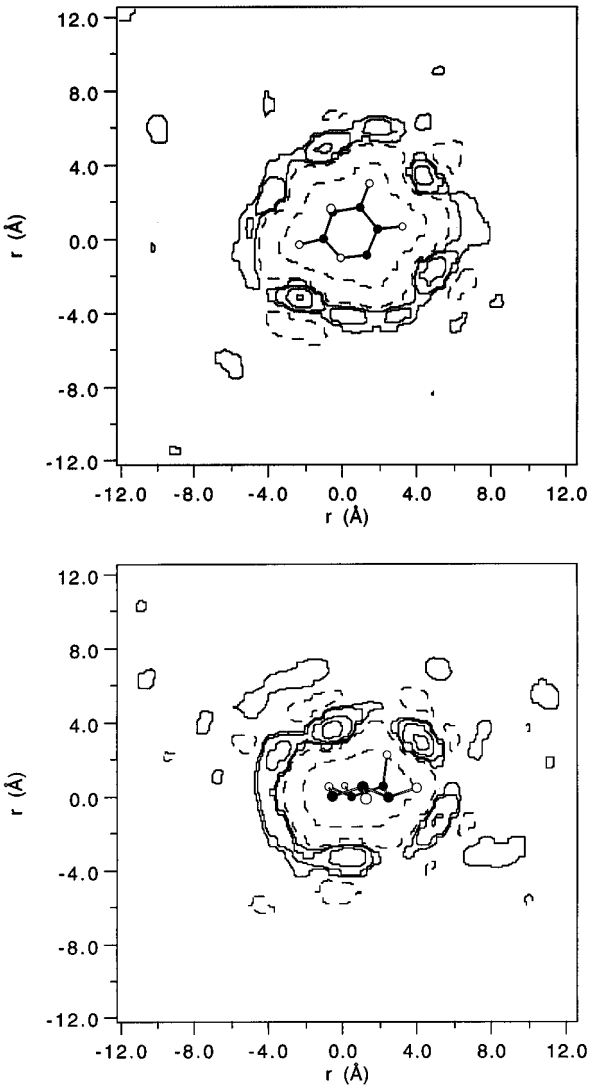

Figure 6. Two-dimensional solvent density contour maps, as in Figure 3, for β-D-lyxopyranose.

third peak in density results almost entirely from first peaks for oxygen atoms on the opposite side of the sugar ring. The spherical averaging is thus deceptive when used in analyzing the long-range solution structure since the features of greatest interest are being "swamped" by spurious structuring due to other solute functional groups. This limitation of spherically-averaged pair distribution functions has long been recognized, and methods have been developed to deal with this difficulty.[47] However, even these methods would obscure the discrete nature of the preferred positions in the second hydration shell revealed in Figure 3, and it would be difficult to deduce the complex patterns of solvent structuring seen in Figure 2 from any type of radial distribution.

As noted, the clouds representing high densities of water molecules in Figure 2a,b merge above and below the ring to form caps of density almost completely encaging the molecule at the displayed contour level. These density caps, which can be clearly seen in cross-section in Figure 3b, arise from water molecules primarily solvating the nonpolar aliphatic protons of the ring, all of which are in axial positions except for the second hydrogen on C5. Since there are three axial protons "below" the ring and only two "above", the cloud below the ring is larger. These axial protons constitute extended regions of hydrophobic surface, and the β-D-xylopyranose molecule has an approximately sandwich-shaped character, with the upper and lower faces being hydrophobic and the periphery of the ring, where the equatorial hydroxyl groups are located, being hydrophilic. In solution the water molecules in the polar caps are structured differently from those around the periphery of the ring.[17,18] These molecules are located at a greater distance from the nearest heavy atom, and generally make no hydrogen bonds to the solute, and are consequently oriented so that their hydrogen atoms point either away from the sugar or at a tetrahedral angle relative to the normal (since only one of the two protons can point directly away from the sugar). Figure 2c displays contours of greater than bulk density as illustrated in Figure 2a,b, but color coded to indicate the average number of hydrogen bonds made to the solute by water molecules within these regions, using standard geometric criteria.[20] Note that, in addition to caps of hydrophobic hydration above and below the rings, there is also such a lobe along the side of the ring adjacent to the CH_2 group where the water molecules do not hydrogen bond to the sugar. The purple cloud of density adjacent to the O4 hydroxyl group is a special case in which the water molecules on average make approximately one hydrogen bond to the sugar because of the special position of the O4 group.

The solvent structuring observed in these simulations is highly dependent upon the molecular topology of the solute. Our

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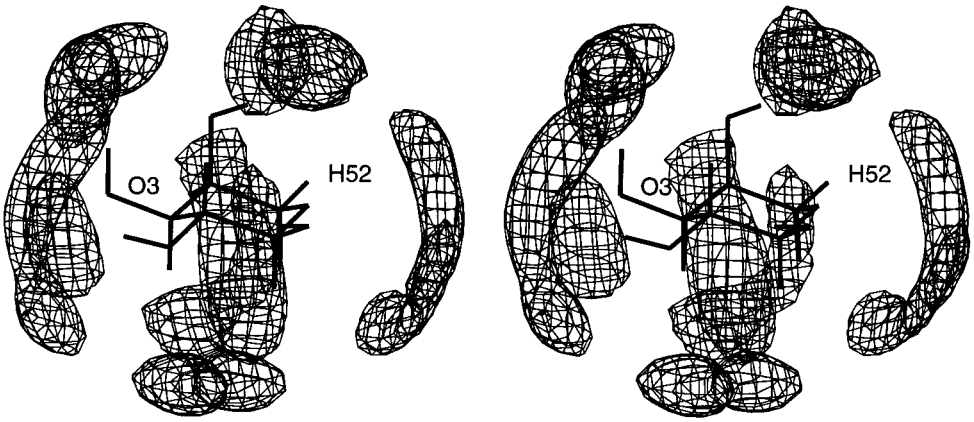

Figure 7. Stereo pair illustrating contours of solvent density around α-L-arabinopyranose as calculated from MD simulation. The contour level shown is 50% above the bulk density.

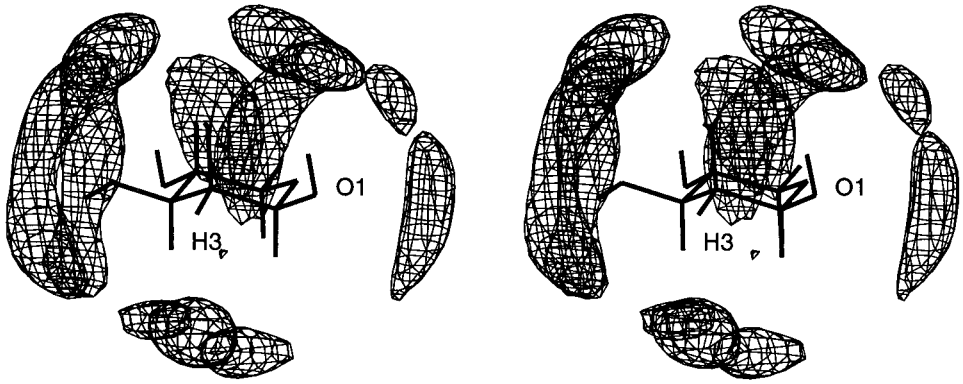

Figure 8. Stereo pair illustrating contours of solvent density around β-D-lyxopyranose as calculated from MD simulation. The contour level is the same as in Figure 7.

previous studies found a significant difference in solvent structuring between the two anomeric forms of D-xylopyranose, and in the present series of simulations each stereochemical form also produced a unique structuring pattern. As can be seen from Figure 2d,e, the distribution of peaks around α-L-arabinopyranose and β-D-lyxopyranose, the analogs of β-D-galactopyranose and β-D-mannopyranose, respectively, differ from those for β-D-xylopyranose in the vicinity of their axial hydroxyl groups. These differences can also be seen in the cross-sectional density plots for these sugars, Figures 5 and 6. In both cases, the axial group disrupts the regular complementary hydration pattern found in D-xylose. Both sugars exhibit the same tube-shaped density clouds between the equatorial hydroxyl groups as were seen in the β-D-xylopyranose, but have an extended density region displaced toward the top of the molecule at the axial hydroxyl group, since the presence of the axial polar group disrupts the upper hydrophobic surface, while slightly increasing the hydrophobic surface area underneath the ring as the equatorial aliphatic proton is directed slightly downward. Figures 7 and 8 present the contours of high solvent density as stereo pairs.

From Table 1 it can be seen that the α-L-arabinopyranose molecule had a significantly more favorable interaction energy with the solvent than does β-D-xylopyranose, for reasons which are not clear, while for β-D-lyxopyranose this energy term was the same as in the xylose case. These two sugars are alone among the series studied in not having a much less favorable interaction with solvent than β-D-xylopyranose. Apparently having a single upward pointing axial hydroxyl group results in a geometrically favorable hydrogen-bonding environment, and these two molecules, along with β-D-xylopyranose, make the highest average number of hydrogen bonds to solvent of any in the series (Table 1). Both of these sugars have much higher internal energies, however, as the result of unfavorable internal dipole interactions.[48] This instability is greater for the lyxopyranose with its axial C2 hydroxyl group, in accord with expectations based upon the so-called Δ2 effect.[35,48]

A particularly interesting case is the $^1C_4$ ring conformation of β-D-xylopyranose, which has all of its hydroxyl groups in axial positions. This conformation presents an "inverse sandwich" of polarity, compared with the $^4C_1$ case, in which the top and bottom are polar and the equatorial periphery is hydrophobic. This conformation is not found in NMR studies of this molecule in solution, indicating that it is significantly higher in energy than the $^4C_1$ geometry. It was long thought that this higher energy resulted from unfavorable dipole alignment and steric clashes between bulky axial groups.[34,35] Indeed, the ring in this conformation was found to be somewhat flattened and distorted in the MD simulations, with the hydroxyl groups pushed slightly apart, but the internal energy for this form was the lowest of any of the molecules studied. The reason for this lower energy is that the hydroxyl groups are not so close that the oxygen atoms clash, but are actually in excellent positions to allow intramolecular hydrogen bonds between these groups to form, which more than compensate for the cost of the dipole interactions. One such internal hydrogen bond is formed in the simulations on either side of the ring, between O1−O3 and O2−O4, thus substantially lowering the internal energy. Such a situation has been observed experimentally, in the crystal structure of α-D-talopyranose.[49] In addition, semiempirical and high-level *ab initio* quantum mechanical calculations on both the $^4C_1$ and $^1C_4$ ring conformations of β-D-glucopyranose also found the $^1C_4$ conformer to be more stable

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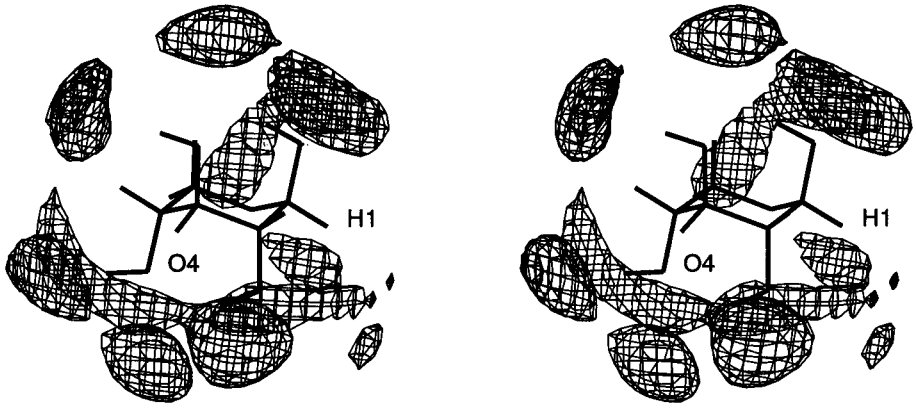

Figure 9. Stereo pair illustrating contours of solvent density around β-D-xylopyranose in the $^1C_4$ conformation. The contour is the same as in Figure 7.

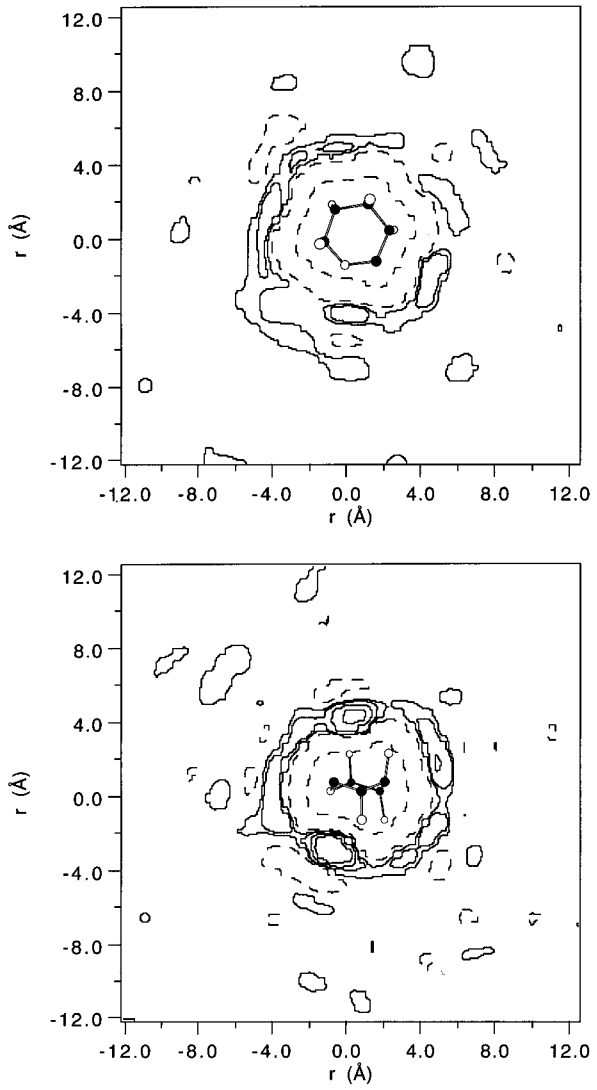

Figure 10. Two-dimensional solvent density contour maps, as in Figure 3, for β-D-xylopyranose in the $^1C_4$ conformation.

for the same reason.[50] With these internal hydrogen bonds, however, the molecule makes fewer hydrogen bonds to solvent (Table 1), so that the interaction energy with the solvent is much less favorable. The sum of the internal and solvent interaction energy terms is thus calculated to be 1.2 kcal/mol higher than for the $^4C_1$ conformation, which would be in reasonable agreement with the experimental observation of no significant population of this conformation in solution. The free energy of this conformation should be even more unfavorable, since presumably its entropy is lower due to the strained internal geometry and restrictions on hydroxyl rotations.

As might be expected, the structuring imposed by this molecular conformation upon the solvent water is quite different from that found for the opposite chair conformer. Figure 2f displays the solvent distribution for this system, which is also shown in stereo in Figure 9, while Figure 10 presents the cross-sectional density maps. Rather than occupying long, tube-shaped regions of higher density, in this conformation the hydrogen-bonded water molecules are confined to much more localized clouds arrayed around the hydroxyl groups. Some of these regions have very high solvent densities and correspond to bridging positions between two hydroxyls, as in the equatorial case, but apparently with a much smaller range of permitted positions, perhaps because of the presence of the hydroxyl proton between the two oxygen positions. Presumably the loss of freedom by the various more localized water molecules results in a lower entropy for this solvent distribution than for the $^4C_1$ case, further destabilizing this conformation in terms of free energy.

The β-L-xylopyranose molecule in the $^4C_1$ conformation is very similar to the $^1C_4$ β-D-xylopyranose in that it has all of its hydroxyl groups in axial positions. Cross-sectional density maps for this molecule are presented in Figure 11. As with the previous sugar, this molecule induces a similar pattern of numerous, very localized solvent positions, some of which are capable of interacting with either or both nearby hydroxyl groups. The ring of water density visible in the cross-section through the average ring plane is due to hydrophobic hydration structuring, and its nearly continuous character is because hydrophobic hydration is much less site-specific and thus does not force the water to be in such restricted positions. Due to internal hydrogen bonding, this form was also found to have a significantly lower internal energy than β-D-xylopyranose ($^4C_1$) but to have a less favorable interaction energy with water, and to make more than one full hydrogen bond less to water on average than does the D isomer.

The final sugar modeled in this series of simulations was α-D-arabinopyranose, which has three axial hydroxyl groups, O1, O2, and O3, and an equatorial O4 hydroxyl group, the exact converse of α-L-arabinopyranose. In the simulations this molecule also formed a persistent intramolecular hydrogen bond between O1 and O3, and consequently also made fewer hydrogen bonds to solvent. As a result, its intramolecular energy was more favorable than that of β-D-xylopyranose and its solvent interaction energy was much less favorable. The configuration of this molecule produces a long hydrophilic region along the

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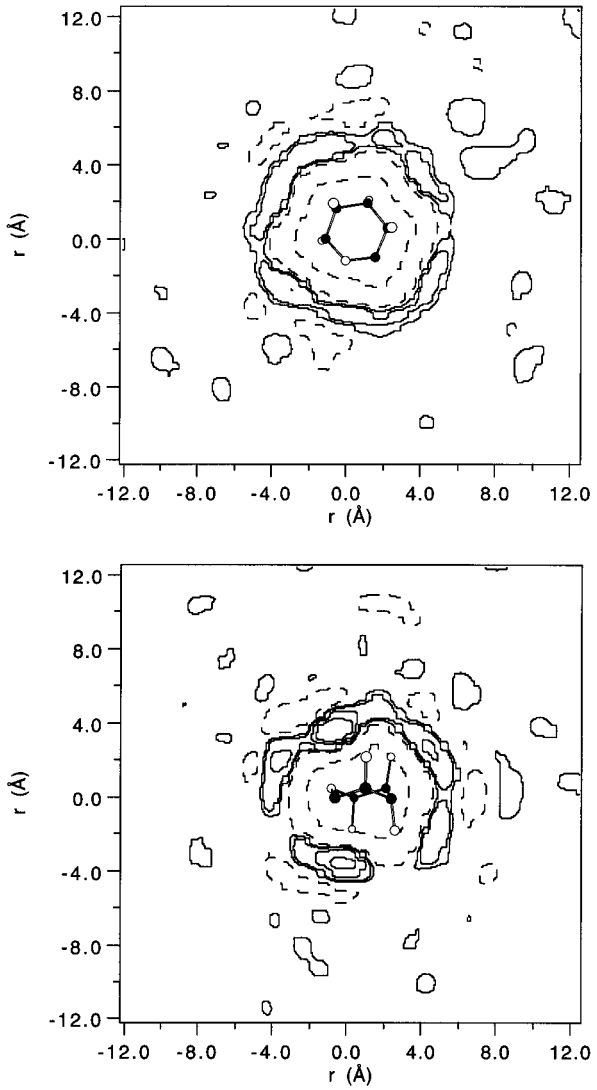

Figure 11. Two-dimensional solvent density contour maps, as in Figure 3, for β-L-xylopyranose.

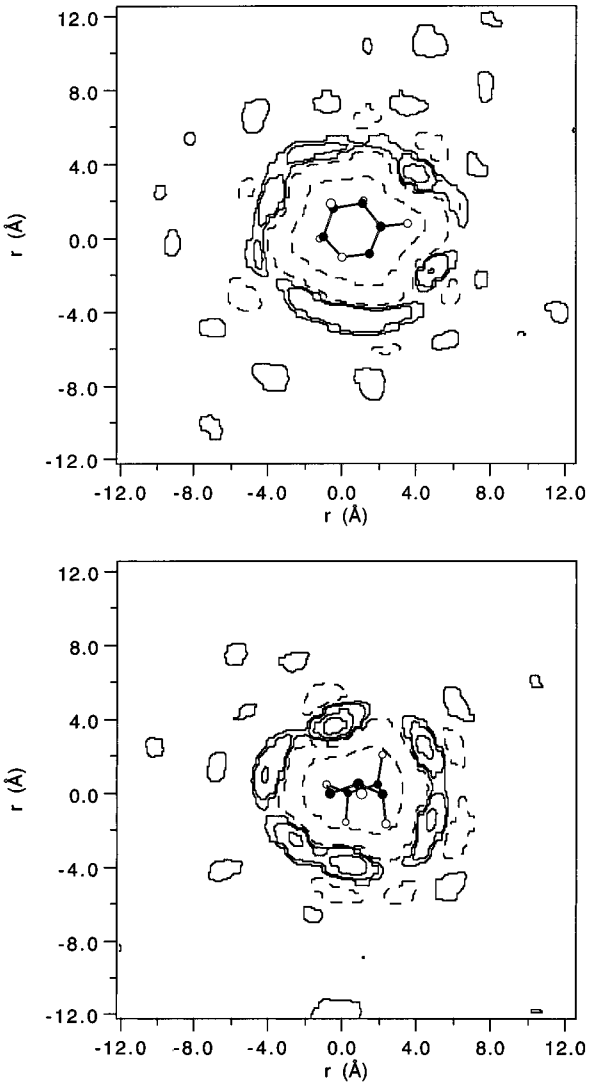

Figure 12. Two-dimensional solvent density contour maps, as in Figure 3, for α-D-arabinopyranose.

lower face of the ring, with three of the hydroxyl groups directed "down", and yet another unique solvent structuring pattern (Figure 12), shown in stereo in Figure 13. From the figure it can be seen that the axial hydroxyl groups again produce small ellipsoidal clouds of density rather than the long channels found when there are adjacent equatorial hydroxyl groups. It is interesting to note that, for this molecule, which is the only example in the series other than α-D-xylopyranose of a $^4C_1$ conformer with an axial O1 group, there is a large region around O1 with little buildup of solvent density, as was also found for α-D-xylopyranose (compare with Figure 3 of ref 19).

In general, the sugar molecules were all well hydrogen bonded to solvent. Those molecules with internal hydrogen bonds made fewer hydrogen bonds to solvent, although β-L-xylopyranose and the $^1C_4$ conformation of β-D-xylopyranose, which possess two such internal hydrogen bonds, had the same number of hydrogen bonds to solvent as α-D-arabinopyranose, which has only one such internal bond. The reason for this difference is not clear. While the average number of hydrogen bonds to solvent made by each sugar may not be statistically converged on the present short time scale, it is clear that there is a strong linear relationship between the number of hydrogen bonds to solvent and the observed solvent interaction energy. This dependence would seem to indicate that the majority of the configuration-dependent solvent interaction energy is due to hydrogen-bonded first neighbor water molecules rather than to the collective effects of more distant solvent or even to nearest-neighbor molecules hydrating only hydrophobic groups.

Conclusions

Although the solvent density mapping approach described here would be more difficult to apply to less rigid solutes, from the present analysis, it can be seen that sugar molecules do indeed impose considerable structuring upon their adjacent solvent water. This structuring can be quite complex, and can depend strongly upon the specific topology of the molecule. For these pentose monosaccharides the solvent structure appears to be enhanced by the relative rigidity of the sugar rings and by overlaps of preferred hydration locations in some of the sugar geometries. Since the observed structuring is stereospecific, it seems probable that the most favorable hydration will occur for those solute molecules whose functional groups are arranged such that their hydration requirements are mutually compatible. The favorable hydration energy of α-L-arabinopyranose may be due to such solvation compatibility; it could be that its configuration produces less interference between the hydration requirements of adjacent hydroxyl groups. Solutes whose functional groups do not have compatible hydration requirements might be expected to experience less favorable interactions with water, and perhaps to be less soluble. Presumably such

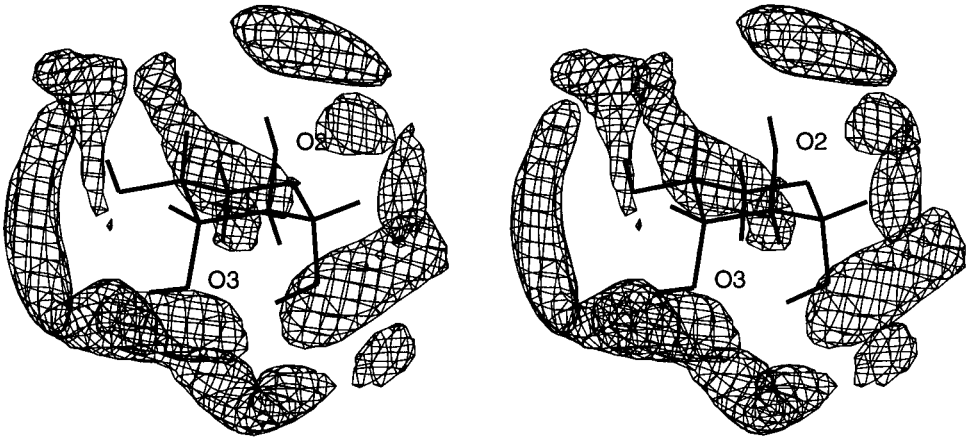

Figure 13. Stereo pair illustrating contours of solvent density around α-D-arabinopyranose as calculated from MD simulation. The contour level is the same as in Figure 7.

structuring could have consequences for solution viscosity, solvation entropy, solute dynamics, and the approach of other molecules to the solute in complex formation or chemical reactions.[2]

Unfortunately, as seen in the diverse solvent distribution patterns of the present series of molecules, guessing which collective stereochemical structures have the most mutually compatible hydration requirements might sometimes be difficult. While it appears clear that the hydrogen-bonding requirements of the hydroxyl groups play a dominant role, few of the preferred positions correspond to ideal hydrogen bond geometries, probably because of the structural compromises needed to also interact favorably with other nearby functional groups and other structured water molecules. In general it appears that the water molecules are making one fairly strong and linear hydrogen bond to one of the nearby hydroxyl groups and interacting as favorably as possible with the other hydroxyl, consistent with the requirements of the first hydrogen bond. The density distributions adjacent to hydrophobic regions could be even more difficult to deduce due to their weaker localization effects and the possibility that these distributions might be substantially distorted by the structuring around the nearby hydroxyl groups.

An early model of sugar hydration[46,51] postulated that β-D-xylopyranose and β-D-glucopyranose experienced the most favorable interactions with liquid water because their hydroxyl oxygen atoms were arrayed in a pattern almost the same as that of the basal plane of the ice I_h lattice, but with the distances slightly expanded. This model apparently assumed that liquid water retained some character of this extended hexagonal symmetry, and that sugars with axial hydroxyl groups were less compatible with this assumed long-range organization. Although it has long been known that this description of liquid water is not correct, no better general models have been advanced for sugar hydration. From the present results it appears that while one important component of the solvation energy is how well the structuring imposed by the various functional groups interacts with the bulk water structure, also at least as important is how much the hydration requirements of one functional group are either compatible with or conflict with the requirements of adjacent functional groups in the solute.[52] In this context, it is interesting to note that from the simulations the single axial hydroxyl groups of β-D-lyxopyranose and α-L-arabinopyranose do not result in an unfavorable solvent interaction energy; indeed, the latter has by far the most favorable interaction energy with water.

Another early attempt to rationalize relative sugar energies in solution ignored the explicit solvent role but included its contribution implicitly through fitting to experiment.[33] This approach assigned specific energy values to various "instability factors", such as an equatorial O1 substituent (the anomeric effect) of bulky axial groups at any other position (the Hassel−Ottar effect, the Δ2 effect, etc.), and treated the total molecular energy as a simple sum of independent terms. By careful parametrization of the numerical values to experiment, a quite satisfying description of the anomeric and ring conformational equilibria was obtained for nearly all of the pentose and hexose sugars.[35] In general the present results are in qualitative accord with that system, as they must be in order to approximate experimental results. For example, as predicted by the system, the $^4C_1$ conformation of β-D-xylopyranose was found to have the lowest sum of internal and solvent interaction energies of the series except for α-L-arabinopyranose, which is predicted from stability factors to be only 0.1 kcal/mol less favorable than β-D-xylopyranose. It should be noted here that the sum of these two energy components is not the complete internal energy change for the system (which can be taken as the approximate enthalpy change) since it does not include the water−water interaction term, which cannot be extracted from simulations as short as these. In addition, the instability factor numbers are estimates of free energies, which also include the entropy change. It is interesting to note from these simulations that the presumed instability of two axial hydroxyl groups on the same side of the ring comes from the interaction with the water, rather than from internal effects, as was supposed by the older approach, as does the instability of the α-D-xylopyranose molecule relative to its β anomer. In general, relative energies between different sugars are not available from experiment. However, it is known from experiment[53] that the free energy difference between β-D-glucopyranose and β-D-mannopyranose is 1.2 kcal/mol, and the enthalpy difference is 2.6 kcal/mol, paralleling the result found here for the analog molecules β-D-xylopyranose and β-D-lyxopyranose, 4.4 kcal/mol, with the glucose/xylose molecules having the lower values.

Finally, it is important to consider the magnitude of the errors which might be expected in these results. Unfortunately, it is difficult to give a meaningful estimate of the uncertainty in the energies calculated from the present simulations without repeating a simulation many times and computing a standard deviation for the resulting numbers. Deviations between the actual and

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calculated values of these energy components come from both systematic errors and statistical errors. The systematic errors would include, for example, any limitations in the sugar force field and water models, as well as simulation protocols such as truncation schemes and periodicity. The statistical errors arise from several sources, including the problems of limited sampling of distributions which fluctuate due to a number of processes with widely different time scales. In our previous study[21] we used statistical inefficiency methods[10] to estimate the degree of local statistical convergence, but long-time-scale contributions are best gauged by extending the duration of the simulation. Experience with our previous simulations indicates that simulations twice as long as the present ones are insufficient to achieve useful convergence in the water−water interaction energy, but that the statistical uncertainty in the internal energy and the sugar−water interaction energy terms is probably on the order of 0.5 kcal/mol.[21]

The statistical uncertainty in the density distributions calculated here is probably small for the first and second peaks. In our previous studies of the D-xylose anomers, the simulation time was 300 ps, twice the length of the present trajectories, but the densities in these statistically-independent simulations were qualitatively identical to the distributions calculated from the present study, strongly implying that the results are statistically converged. This finding would be in accord with the results of previous simulations which found that structural properties such as solvent pair distribution functions converge relatively quickly, and can be qualitatively estimated even from very short simulations.[16] However, the statistical uncertainty is probably too large to allow the reliable identification of third-shell peaks, should they exist. While the pyranoid rings are kinetically stable on the present time scale, on longer time scales significant conformational fluctuations and even ring transitions could occasionally be expected to occur (for example, the unstable $^1C_4$ ring in β-D-xylopyranose would be expected to "flip" at some point). However, the relatively rapid convergence of the solvent structuring means that it should still be useful to study the density distributions of the different ring conformations separately as has been done here.

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