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Carbohydrate Research 282 (1996) 13–23

CARBOHYDRATE RESEARCH

Molecular dynamics simulations of glycosides in aqueous solution [1]

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Received 27 April 1995; accepted in revised form 16 October 1995

Abstract

Molecular dynamics simulations for aq solutions of methyl-α- and -β-D-glucopyranoside, galactopyranoside and talopyranoside have been carried out. A single molecule surrounded by 252 SPC water molecules was used under periodic boundary conditions. Preference for the *gt* and *gg* orientations for the hydroxymethyl groups in solution has been confirmed. Examination of the extent and pattern of hydrogen bonding between water and the sugar molecule hydroxyl groups showed that the galactose and glucose derivatives were substantially better hydrogen-bond donors at OH-2 and OH-4 than the respective ones of talose. Intramolecular hydrogen bonding involving OH-2 and OH-4 of the talosides persisted for some 40% of the 100 ps simulation time in water. The remarkably long retention times (on reversed-phase HPLC in pure water) of the talosides relative to the galactosides and glucosides were rationalised in terms of differing extents of hydration, intramolecular hydrogen bonding, and compatibility with the structure of water.

Keywords: Molecular dynamics; Solution structure; Hydrogen bonding; Methyl glycosides; RP-HPLC; Talose

1. Introduction

The solution properties of carbohydrates have been the subject of much study [1–8]. Data obtained from crystallographic studies or in other solvents cannot readily be extrapolated to aq solution [9]. Since Kabayama and Patterson [10] proposed that β-D-glucose matches the tridymite structure of water, a number of attempts have been made to elucidate the relationship between hydration patterns and the conformation of carbohydrates in aq solution [11]. Carbohydrate hydration has been discussed in terms of

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[1] Hydration studies of carbohydrates, Part III. For Part II, see ref. [50].

SSDI 0008-6215(95)00363-0

hydration numbers [12–14], dynamic hydration numbers [15], the ratio of axial vs. equatorial hydroxyl groups [16,17], hydrophobic/hydrophilic index [18], kinetic medium effects of carbohydrates on a water-catalysed hydrolysis reaction [8,19–22], the hydrophilic volume of the carbohydrate [23], and the extent of compatibility with water structure [24,25]. The experimental techniques used in these studies include measurements of compressibility, density, ultrasound, kinetics of reaction, hydrophobic and hydrophilic areas of carbohydrates, natural abundance oxygen-17 spin-lattice relaxation times of water, and near-infrared spectrometry.

Despite this range of studies, no comprehensive theory of carbohydrate solvation has been developed, although the systematic approach of Galema et al. [8,19–22] has led to some valuable insights. These authors propose a modified stereospecific hydration model. This assumes that the "fit" of a carbohydrate into the three-dimensional hydrogen-bond network of water depends on the relative distances between the next-nearest-neighbour oxygen atoms in the carbohydrate, compared with the oxygen–oxygen distances in water [19–21]. The choice of the appropriate thermodynamic property used to study the interaction between the carbohydrate and water is important. Thus partial molar isentropic compressibilities and isothermal compressibilities are sensitive to stereochemical aspects of carbohydrate hydration, while partial molar volumes, partial molar expansibilities and partial molar heat capacities are much less so [21]. Solute–solute interactions [8,19] and dielectric measurements [26,27] are also useful.

It is apparent then, that only a few experimental methods are sensitive enough to measure the differences in specific hydration characteristics between carbohydrates such as the eight D-aldohexoses. Further complexities may arise also in the case of the free sugars, as they occur in aq solution as a temperature-dependent mixture of anomeric and ring forms.

We have attempted to demonstrate [28] that another experimental parameter of a monosaccharide, viz. the capacity factor (K') on reversed-phase high performance liquid chromatography (RP-HPLC), is related to its specific hydration pattern. In order to extend the above HPLC approach, we have decided to use molecular mechanics and dynamics to study hydration patterns of (initially) a series of hexose methyl glycopyranosides, and to relate these to the respective K' values. Essentially, the better a molecule fits into the three-dimensional tridymite structure of water, with minimal disruption of that structure, the more hydrophobic it will behave. In the case of RP-HPLC with pure water as eluent, this implies longer retention times for the more hydrophobic molecules. We herein use the results of MD simulations to help explain the large apparent hydrophobicities of the talosides relative to those of the glucosides and galactosides. Simulations of carbohydrate hydration may thus reveal aspects which are not accessible to current experimental techniques.

Molecular mechanics and dynamics studies of carbohydrates with specific inclusion of water molecules have been responsible for a number of important insights into carbohydrate properties, including solvation [29–34]. Thus molecular dynamics studies by Ha et al. [35] satisfactorily reproduce the energy difference in water between α- and β-D-glucopyranose. The authors reported a large difference in the solvation free energies for the two anomers, and concluded that the preference for the equatorial β-anomer is primarily a hydration effect. Homans [36] concluded that for an adequate representation

of the available conformational space in oligosaccharides, inclusion of solvent water is necessary. Kroon-Batenburg and Kroon [34] proposed that the preference for the *gt* and *gg* hydroxymethyl rotamers of glucose in aq solution is due to solvation effects. Cramer and Truhlar [37] reached the same conclusion, using a quantum-chemical solvation model, based on a continuum treatment of dielectric polarisation and solvent-accessible surface area.

It has been pointed out by a number of authors (Brady [38]; Homans [36]; Kroon-Batenburg and Kroon [34]; Kohler [39]) that partial atomic charges, forcefields, atom types, water models, and hydrogen-bonding parameters need careful consideration for accurate modelling. The Amber forcefield [40,41] was originally parameterised against a limited number of compounds related to proteins and nucleic acids. It has been extended by Homans to make it suitable for the simulation of oligosaccharides [36]. This Amber–Homans forcefield (Amber–H) uses the same functional form as Amber and extends its applications to include polysaccharides and glycoproteins [42,43]. The Amber–H forcefield utilises charges and van der Waals parameters derived by Ha et al. [42], such that hydrogen bonding can be simulated without a hydrogen-bonding term in the Hamiltonian, i.e. it does not contain explicit hydrogen-bonding parameters.

2. Experimental

A Silicon Graphics Personal Iris Workstation was set up with the Insight II molecular modelling program (version 2.2) and molecular mechanics/dynamics package Discover (version 2.9) (Biosym Technologies, San Diego, CA, USA). The Amber–Homans forcefield (Amber–H) has been incorporated into the Amber forcefield available in Discover, version 2.9 and later. For the formation of methyl glycosides, it was decided to balance charges local to the atoms in the linkage (a suggestion for which we thank D. Renouf). Geometric criteria were employed for the definition of intramolecular hydrogen bonding; such a bond is considered to exist if the distance between the hydrogen atom and the acceptor oxygen is < 2.5 Å, the angle about the O–H $\cdots$ O is greater than 120°, and these conditions persist for at least 0.5 ps. The SPC-type model for water [44] exhibits more "structuring" than, for example, the TIP3P model [48] which is also commonly used [33]. Thus the oxygen–oxygen pair distribution functions calculated from simulations of pure SPC water display considerable structuring, showing a primary solvation shell peak at 2.85 Å and smaller peaks at ≈ 5.1 and ≈ 7.4 Å, presumably due to the second and third shell water molecules, respectively.

Molecular parameters.—Torsion angles:

ϕ: CMe–O-1–C-1–O-5

ω: O-6–C-6–C-5–O-5

Starting conformations:

gg: $\phi = -60°$, $\omega = -60°$

gt: $\phi = -60°$, $\omega = 60°$

tg: $\phi = -60°$, $\omega = 180°$

Table 1
Atom types and partial charges used for molecular dynamics simulations of methyl pyranosides

Atom name	Atom type [a]	Description	Charge (e)
C	CS	methyl carbon	0.151
H	HT	methyl hydrogen	0.083
C-1	AC, BC	α/β anomeric carbon	0.350
H-1	AH, BH	α/β anomeric hydrogen	0.100
O-1	OA, OB	α/β anomeric oxygen	−0.65
C-2, C-3, C-4	CS	α/β carbon in sugar ring	0.150
C-5	CS	carbon in sugar ring	0.100
H-2, H-3, H-4, H-5	HT	sp^3 hydrogen	0.100
O-5	OE	ring oxygen	−0.40
O-2, O-3, O-4	OT	hydroxyl oxygen	−0.65
HO-2, HO-3, HO-4	HY	hydroxyl hydrogen	0.400
C-6	CS	hydroxymethyl carbon	0.050
H-6	HT	sp^3 hydrogen	0.100
O-6	OT	hydroxyl oxygen	−0.65
HO-6	HY	hydroxyl hydrogen	0.400
O_w	OH	water oxygen	−0.82
H_w	HO	water hydrogen	0.410

[a] From Homans [36].

For the first 100 iterations in order to shorten running time, the steepest descents algorithm (with mbdglu in the $^4C_1$ conformation) was used. Minimisation was continued by the use of the conjugate gradients algorithm, until the maximum derivative (rms) was < 0.001 kcal/Å. A cutoff radius of 10.0 Å was used, with a switching distance of 1.5 Å. During minimisation there was no transition to other ring conformations. In water, energy minimisations were performed with explicit inclusion of solvent. The final geometries obtained by energy minimisation of the *gg*, *gt* and *tg* conformers of methyl-β-D-glucopyranoside in the Amber–H forcefield were used as the starting conformations for the molecular dynamics (MD) runs. Atom types and partial atomic charges used for MD runs are listed in Table 1.

Molecular dynamics.—The starting conformers were placed in the centre of a periodic-boundary-conditions (PBC) water box of dimensions 20 Å × 20 Å × 20 Å. MD simulations with explicit inclusion of solvent, using an orthogonal space group PBC containing 252 water molecules, were performed at a constant pressure of 1 bar and relaxation time of 0.1 ps, and a loose coupling to a thermal bath at 300 K with a coupling constant of 0.1 ps. This was minimised with the Amber–H forcefield with a cutoff value for the unbonded pair interactions of 10 Å, and a switching distance of 1.5 Å. After 10 ps equilibration, a further 90 or 100 ps of dynamics was run with a time step of 0.1 ps and analysed. The whole system was well equilibrated and the energy adequately conserved during the simulations, with no overall drift in temperature or energy.

3. Results and discussion

Methyl-β-D-glucopyranoside was chosen for development of suitable molecular modelling parameters. Table 2 summarises the results for energy minimisation in water of methyl-β-D-glucopyranoside, with the Amber–H and Amber forcefields. For molecular dynamics in water the *tg* conformation was chosen, as it was known not to contribute to any extent in the crystal [45,34] or aq [46,47] environments, and its rapid disappearance would lend support to the realism of the simulation. In the event the *tg* conformation was lost during the 10 ps equilibration period, and did not reappear (Fig. 1). Fig. 1 also shows that the Amber–H parameters predict a *gg*:*gt*:*tg* distribution of approximately 55:45:0 (after the equilibration period), in close agreement with crystal structures (60:40:0) for the *gluco*-configuration [45], and with NMR (60–50:40–50:0) [46,47]. The Amber parameters were less successful, predicting an approximately 15:85:0 distribution.

The Amber–H forcefield and the *tg* starting conformation for the hydroxymethyl group were also chosen for the MD simulations of the methyl-β-D-galactosides and talosides. In all cases the *tg* conformation disappeared during the equilibration period, and did not reappear, the distributions being:

	gg	*gt*	*tg*
β − gal	40	60	0
β − tal	65	35	0

This is not in agreement with experimental work on the *galacto*-configuration, which indicates the presence of the *tg* conformation in amounts up to 34% [48]. Considerable simulation time was spent in the *gg* conformation, within an intramolecular hydrogen-bonding distance for OH-4 and OH-6. It is possible that the molecular and forcefield parameters used tend to overemphasise intramolecular hydrogen bonding between suitably configured hydroxyl groups.

The radial distribution functions (RDF) for O-1–O_w for the methyl-α- and -β-anomers of all three monosaccharides display typical nonpolar solvation behaviour [38] with no

Table 2
Energy minimisation in water. The effect of forcefield on the dihedral angles ϕ and ω, and on the total energies for methyl-β-D-glucopyranoside

Starting conformation [a]	Forcefield	Dihedral angle [b] (deg)				Total energy (minimised) (Kcal)
		ϕ_i	ϕ_f	ω_i	ω_f	
gg	Amber–H	−60	−55.3	−60	−41	−3787
gg	Amber	−60	−69.1	−60	−26.5	−3848
gt	Amber–H	−60	−57.3	60	−20.4	−3818
gt	Amber	−60	−77.9	60	48.1	−3831
tg	Amber–H	−60	−84.5	180	178.2	−3755
tg	Amber	−60	−130.8	180	170	−3770

[a] For definitions of *gg*, *gt*, and *tg* conformations, see Section 2.
[b] i = initial dihedral angle; f = final (energy-minimised) dihedral angle.

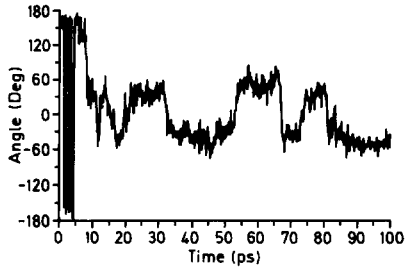

Fig. 1. Methyl-α-D-glucopyranoside in water. History of ω torsion angle (O-6–C-6–C-5–O-5) (with initial *tg* conformation of the hydroxymethyl group) during 100 ps molecular dynamics simulation including the 10 ps equilibration period.

sharp maximum in the function, indicative of shielding from water. The ring oxygen (O-5) is similarly shielded. In all cases the α-anomer has the lower RDF in the first hydration shell, and the (slightly) more structured second and third shells. In the light of the observation that α- and β-anomers of methyl glycopyranosides are in general resolved on RP-HPLC [48] this could be significant. It is also consistent with a stereospecific model of hydration for carbohydrates.

The RDFs for water molecules around OH-2 and OH-4 (Fig. 2) show sharp narrow maxima around 2.85 Å typical a strong hydration. As will be demonstrated later, detailed behaviour at OH-2 and OH-4 depends on the stereochemistry at these positions.

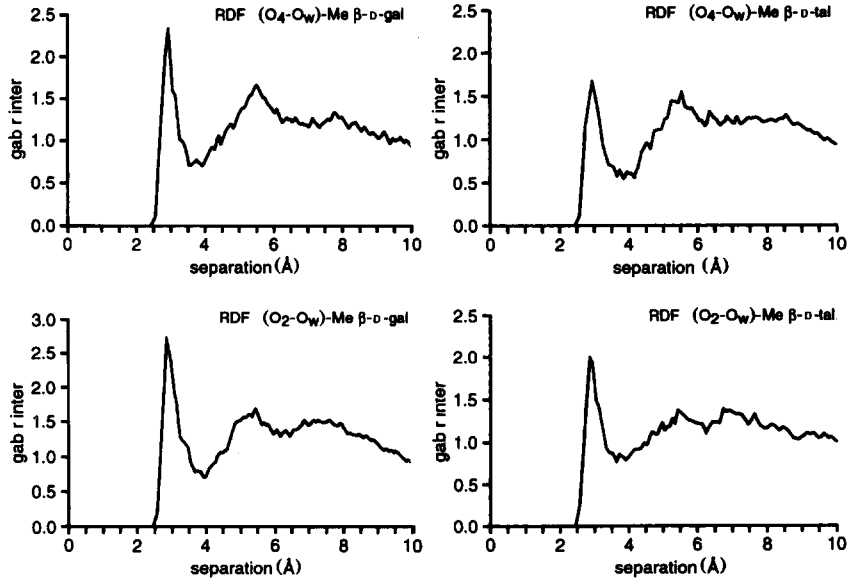

Fig. 2. Radial distribution functions (O-2,4–O_w) for methyl-β-D-galactopyranoside and methyl-β-D-talopyranoside.

Table 3
Integrals (out to the first minimum) of the average coordination numbers $N(O_s-O_w)$ of water oxygen atoms around solute oxygen atoms for methyl galactosides, glucosides, and talosides

Carbohydrate	O_s-O_w						
	O-1	O-2	O-3	O-4	O-5	O-6	Total
Methyl-α-D-galactopyranoside	0.77	1.88	2.0	1.46	0.53	1.81	8.45
Methyl-β-D-galactopyranoside	0.86	1.91	1.77	1.31	0.60	2.01	8.46
Methyl-α-D-glucopyranoside	0.82	1.68	1.58	1.58	0.52	1.90	8.08
Methyl-β-D-glucopyranoside	0.77	1.88	1.85	1.53	0.58	1.78	8.39
Methyl-α-D-talopyranoside	0.71	1.51	1.63	1.33	0.61	1.86	7.65
Methyl-β-D-talopyranoside	0.80	1.35	1.43	1.23	0.57	1.91	7.29

The results of the MD simulations for methyl-α- and β-D-glucopyranosides showed that the RDFs for water oxygen atoms around the glucose oxygen atoms display a sharp narrow peak near 2.85 Å, indicative of the significant first hydration shell expected. The sums of the average coordination numbers, N, (out to the first minimum) for solute oxygen–water oxygen over the period of simulation are shown in Table 3. If one takes the integrals of these as being proportional to the average degree of hydration of a carbohydrate molecule, the α-anomer (sum of $N = 8.08$) appears "less hydrated" (or more hydrophobic) than the β-anomer (sum of $N = 8.45$) and would be expected to elute later. However, the α-anomer emerges earlier on RP-HPLC (Table 4) but not substantially so. The question arises as to whether statistical variations due to inadequate sampling, or inappropriate forcefield atom types, are outweighing other factors.

The RDFs for water oxygen atoms show a similar pattern for methyl-α- and -β-D-galactopyranosides to those of the glucosides. The sum of the average coordination numbers (Table 3) for the α-anomer (8.45) is identical to that of the β-anomer (8.46) in agreement with the fact that the anomers co-elute on HPLC (Table 4). The most obvious differences between the glucosides and galactosides on one hand and the talosides on the other is the lower apparent degree of hydration of the latter (Fig. 2; Table 3). In nine out of the twelve cases, the average coordination numbers of the talosides are significantly lower than those for the respective glucosides, while the number is ten out of twelve for the galactosides vs. the talosides. This is in keeping with the more hydrophobic elution

Table 4
RP-HPLC retention times of methyl glycosides of D-galactose, D-glucose and D-talose. Column: Dextropak. Eluent: water. Flowrate: 2.0 mL/min

Glycoside	Retention time (min)
Methyl-α-D-galactopyranoside	2.21
Methyl-β-D-galactopyranoside	2.21
Methyl-α-D-glucopyranoside	2.70
Methyl-β-D-glucopyranoside	2.84
Methyl-α-D-talopyranoside	4.97
Methyl-β-D-talopyranoside	3.15

behaviour of the talosides (Table 4). Again, the sum of the coordination numbers for the α-anomer (7.65) compared with that of the β-anomer (7.29) would suggest that the α-anomer is more highly hydrated and should elute first. As with the glucosides, some other explanation than apparent hydration must be sought—inadequate sampling or inappropriate forcefield aside. Another feature of the talosides is the presence of OH-2–OH-4 intramolecular hydrogen bonds, which persist for some 40% of the simulation time for the β-anomer, and 70% for the α-anomer. However, these values need to be considered in the light of possible overemphasis on intramolecular hydrogen bonding, as previously mentioned.

The presence of these intramolecular hydrogen bonds would help to explain the hydrophobicity of the talosides, as it would need to compete successfully with hydrogen-bond formation to the solvent. Galema et al. [20] have already proposed the existence of an intramolecular OH-2–OH-4 hydrogen-bonding talose, in which the hydroxyl groups share the role of H-bond donor and acceptor. However, these values need to be considered in the light of possible overemphasis on intramolecular hydrogen bonding, as previously mentioned.

Examination of the extent and patterns of intermolecular hydrogen bonding between solute hydroxyl groups and water, showed that the galactose and glucose methyl glycosides were substantially better hydrogen-bond donors to water at OH-2 and OH-4 than were the talosides.

Overall, the data derived from radial distribution functions (Table 3) are consistent with the elution order on RP-HPLC (Table 4), i.e. galactosides before glucosides before talosides, although the individual elution times are not correctly predicted. The methyl glycosides with next-nearest-neighbour oxygen distances similar to those in water should "fit" into the water structure (i.e. disrupt it minimally) and thus appear more hydrophobic than those glycosides having markedly different next-nearest-neighbour oxygen distances from those of water. Table 5 compares the next-nearest-neighbour oxygen distances (determined by minimisation after the MD run) in the methyl glycopyranosides studied, with those of the nearest (2.85 Å) and next-nearest (5.1 Å) distances in SPC water. The layout of Table 5 allows ready comparison between the fit of each methyl glycoside into the SPC water model. The oxygen atoms of the hexoses have been divided into the O-2–O-4–O-5 plane and the O-1–O-3–O-6 plane. The latter plane appears to play a minor role in determining the compatibility of a carbohydrate with the three-dimensional structure of water [20]. The rationale proposed is that the anomeric position in the free sugars, and the hydroxymethyl group, can adjust themselves to fit better into the structure of water, while the O-2–O-4–O-5 plane, being the more rigid one, plays the most important role in determining the fit into water. In Table 5, the difference between the next-nearest-neighbour oxygen–oxygen atoms in a carbohydrate and the nearest or next-nearest oxygen–oxygen atoms of SPC water is designated δ_{O-O}. The smaller the value of δ_{O-O}, the closer is the fit of the two oxygen atoms involved, to the structure of water. The sum of the δ_{O-O} values for each sugar should be proportional to its degree of hydration, and inversely proportional to its HPLC elution time. The predicted order of HPLC elution using this approach and the data from Table 5 is methyl-α-D-galactopyranoside, methyl-α-D-glucopyranoside, methyl-β-D-galactopyranoside, methyl-β-D-glucopyranoside, methyl-β-D-talopyranoside, and methyl-α-

Table 5
Next-nearest-neighbour oxygen–oxygen distances (Å) in some carbohydrates in the crystalline state and in simulated aq solution

Carbohydrate	State	O-2–O-4	O-2–O-5	O-4–O-5	O-1–O-3	O-1–O-6	O-3–O-6	δ_{O-O} sum
β-D-Glucose	crystal [a]	4.87	3.67	3.66	4.75			
	aq solution [c]	4.9	3.7	3.7	4.9	5.7	5.3	
Methyl-β-D-	solution [b]	4.78	3.67	3.68	4.84	4.56	5.57	
glucopyranoside	δ_{O-O} [d]	0.32	0.82	0.83	0.26	0.54	0.47	3.24
Methyl-α-D-	solution [b]	4.71	3.65	3.70	4.18	4.91	5.67	
glucopyranoside	δ_{O-O}	0.39	0.80	0.85	0.92	0.19	0.57	3.72
α-D-Talose	crystal [a]	2.66	2.93	2.87	4.17	4.44	6.19	
	aq solution [c]	2.7	3.1	3.0	4.2	5.1	5.5	
Methyl-α-D-	solution [b]	2.78	2.86	2.85	4.31	5.03	5.20	
talopyranoside	δ_{O-O}	0.07	0.01	0.00	0.79	0.07	0.10	1.04
β-D-Talose	solution [c]	2.70	3.0	3.0	4.9	5.7	5.6	
Methyl-β-D-	solution [b]	3.01	2.93	2.86	4.67	4.57	5.24	
talopyranoside	δ_{O-O}	0.16	0.08	0.01	0.43	0.53	0.14	1.35
β-D-Galactopyranose	crystal [a]	4.28	3.66	2.83	4.82	4.70	6.14	
	solution [c]	4.3	3.7	3.0	4.9	5.7	5.5	
Methyl-β-D-	solution [b]	4.21	3.66	2.89	4.75	4.84	6.12	
galactopyranoside	δ_{O-O}	0.89	0.81	0.04	0.35	0.26	1.02	3.37
Methyl-α-D-	solution [b]	4.21	3.64	2.83	4.30	4.31	6.09	
galactopyranoside	δ_{O-O}	0.79	0.79	0.02	0.80	0.79	0.99	4.18

[a] Galema et al. [8].
[b] This study.
[c] Galema et al. [20].
[d] δ_{O-O} = (next-nearest-neighbour oxygen–oxygen distance (Å) for the carbohydrate) − (nearest (2.85 Å) or next-nearest (5.1 Å) oxygen–oxygen distance in SPC water).

D-talopyranoside. The only anomalous result is that for the β-galactoside, which on the basis of the same elution time should have the same sum of δ_{O-O} values as the α-galactoside.

The overall results are consistent with (a) the relative hydration order being galactosides after glucosides after talosides [19–21] and (b) the elution times on RP-HPLC (Table 5) being galactosides before glucosides before talosides.

The relative elution times of the α- and β-anomers of each pair of glycosides may also be rationalised by the degree of fit approach. Reference to Table 5 shows that the β-glucoside, β-galactoside, and α-taloside are more compatible with the SPC water structure than the α-glucoside, α-galactoside, and β-taloside, respectively. In this respect, the compatibility with the structure of water approach is more predictive ($r = 0.85$) than are the data derived from the atomic pair distribution functions ($r = 0.65$), at least under the conditions studied herein.

Comparative simulation studies are potentially very useful, but must be treated with some caution until the reliability of the methodology has been established, preferably by comparison with experimental results. Another decision to be made is that of which parameters to examine and which data to report. A 110 ps molecular dynamics run of even a monosaccharide in water generates an enormous output file containing a plethora

of information: intramolecular hydrogen bonding, conformation of the sugar ring, hydroxymethyl group conformation, changes in dihedral angles, water–solute atomic pair distribution functions, distribution and orientations of water molecules adjacent to the solute, and doubly hydrogen-bonded water molecules. This wide selection allows one to examine aspects of specific hydrogen bonding in the solvation of monosaccharides which are not accessible when using mean field treatments of solvation [37,49] or in vacuum calculations using a large dielectric constant to model water molecules [33]. We believe that the present results are encouraging enough to warrant extension of the approach to other available carbohydrate derivatives.

Acknowledgements

This research was supported by a grant from the Australian Research Council.

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