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A REVISED POTENTIAL-ENERGY SURFACE FOR MOLECULAR MECHANICS STUDIES OF CARBOHYDRATES

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ABSTRACT

A revised CHARMM-type molecular mechanics potential-energy function has been developed for use in the dynamical simulation of simple carbohydrates in aqueous solution. Atomic charges used in this parameterization were taken to be those previously determined to be appropriate for hydrogen-bonded systems, and the various force-constants were selected by the nonlinear least-squares matching of the calculated normal-mode frequencies and minimum-energy structure to experiment as a function of the parameter set. The new function was found to represent the vibrational spectrum and ring pucker of α-D-glucopyranose as well as previously studied potentials, while incorporating the charges necessary for the simulation of condensed phases. Molecular dynamics simulations of the motions of α-D-glucopyranose *in vacuo* in both the $^1C_4$ and $^4C_1$ conformation were conducted, and compared to the results of previous simulations using another potential-energy function. The revised potential function was found to produce a D-glucose molecule less flexible *in vacuo* than had been previously observed.

INTRODUCTION

Molecular dynamics simulations have proved to be a powerful technique for the study of molecular motions on a microscopic scale. Such simulations, which involve numerical integration of Newton's equations of motion for the atoms in a system subject to the forces arising from empirical energy functions, now have a long history of successful application to various physical problems[1–4], and, in recent years, have been increasingly used to study biopolymer dynamics[5–11]. The information gained from dynamics simulations frequently cannot be obtained by any other means, either experimental or theoretical. However, for such simulations to be useful in deducing the behavior of physical systems, the potential-energy functions used must be an adequate approximation of the actual molecular energy. This is particularly true of the atomic sizes and partial charges in simulations of solutions

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of hydrogen-bonding solutes in dipolar solvents, such as carbohydrates in water. Unfortunately, the selection of parameters is also most difficult for these types of systems, because, for practical computational reasons, the dynamically changing, specific polarization of each hydroxyl group of these molecules, resulting from its instantaneous local environment, must usually be represented in an average way by a single, fixed, partial atomic-charge.

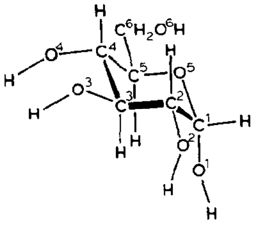
Whereas the molecular dynamics of proteins and nucleic acids have been extensively investigated, and several useful potential-energy surfaces for the modeling of such molecules have been developed[12–16], dynamical simulations of carbohydrates, the most abundant of biological molecules, have been uncommon, apart from the ribosyl and deoxyribosyl components of the nucleic acids. This situation is changing, however, as studies of pyranoid systems, using two different potential functions, have now been reported. In one recent molecular-dynamics study of the enzyme lysozyme[10], an *N*-acetyl-D-glucosamine hexasaccharide substrate was included using a potential-energy surface that represented the pyranose rings of the oligosaccharide in a united atom approximation[12]. In such calculations, hydrogen atoms that do not participate in hydrogen bonds, such as the carbon-bound hydrogen atoms, are not explicitly included in the simulation, but are represented in an average way by increasing the radius of the carbon atom to which the hydrogen atom is bonded, and by modifying its mass and nonbonded parameters. The correct stereochemistry about such carbon atoms is maintained through the use of improper dihedral terms[12]. Simulations have also been reported recently of the vacuum motions of α- and β-D-glucopyranose[17,18], using the most developed all-atom carbohydrate potential-energy surface available, the PEF 422 surface of Rasmussen[19]. This potential function gives good thermodynamic data for small carbohydrate analogs, a reasonable normal-mode spectrum for D-glucose, and an excellent structural representation as judged by the Cremer–Pople pucker parameters[20] describing pyranose-ring deformation. This function was also found to be generally adequate for the description of monosaccharide dynamics in vacuum, to the limited extent that such simulations could be compared to the results of experiment[17,18].

Although the potential-energy functions used in these two studies are useful, it would be desirable to be able to apply molecular-dynamics simulations to carbohydrate problems in which intermolecular interactions and hydrogen bonding are important, as in polysaccharides, glycoproteins, aqueous solutions, and the interaction of enzymes (lysozyme, for example) with carbohydrate substrates. Such simulations require potential functions in which all atoms are explicitly included and in which the van der Waals radii and charges are appropriate for hydrogen bonding or other solvent interactions. In the past, hydrogen bonding in molecular-mechanics calculations has often been represented through the use of an additional special function[12]. More recently, however, extensive *ab initio* calculations have shown that such functions are unnecessary, and that the interactions of a hydrogen-bonded atomic pair can be adequately represented by an appropriate adjustment of atomic charges[21].

We report here an attempt to develop a potential-energy function to describe carbohydrate pyranose rings in an all-atom representation that uses the atomic partial-charges determined by *ab initio* calculations to give reasonable descriptions of hydrogen bonding, and which uses the functional forms of the general molecular mechanics program CHARMM, developed by M. Karplus and co-workers[12]. The purpose of this effort was to develop a set of parameters for use in solution simulations which employed these newly determined charges and which also described the structural and vibrational properties of the D-glucose molecule as well as does the PEF 422 function. This report describes the resulting surface for a conventional CHARMM functional form[12]. We also report the results of molecular-dynamics simulations employing this new parameter set, and compare these results to those reported previously[17,18].

PROCEDURES

In developing new potential-energy parameters for carbohydrate molecules, the procedure used was a systematic optimization of the force constants by a non-linear least-squares fitting of calculated vibrational and structural properties to experimentally observed values for the prototypical carbohydrate molecule α-D-glucopyranose (**1**). This refinement was implemented by using the parameter-optimization capability of the CHARMM program[12,22]. As experimental input, both spectral and structural data were employed. The crystal structure of α-D-glucose is known to high precision from neutron-diffraction studies[23,24], and there have been a number of studies of the infrared and Raman spectra of D-glucose[25–28]. For the calculations reported herein, the spectrum of α-D-glucose reported by Vasko *et al.*[25] was used. Although well-assigned spectra are available for several small-molecular analogs of some the functional components of D-glucose (*e.g.*, methanol, dimethyl ether, and ethyl methyl ether), the sugars themselves are the only common, stable examples of hemiacetals which can be used for extracting such terms as O–C–O angle parameters. Furthermore, the force constants for such motions as O–H and C–O bond-stretches are a function of environment, and will be different in the sugars and in their smaller molecular analogs. For this reason, it is inappropriate to use the force constants for these molecules to represent D-



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glucose, and *vice versa*, although in the present work, constants determined by preliminary studies of these alcohols and ethers were used as starting points to ensure that the minimization–optimization procedure began using reasonable values.

Following a procedure previously employed for the parameterization of nucleic acid potential-functions[22], the normal modes of D-glucose were calculated by diagonalization of the mass-weighted matrix of second derivatives of the energy, using the program CHARMM, and these were matched to the standard valence force-field modes based on the force constants used to assign the experimental vibrational spectrum[25].

In general, the normal modes calculated from an initial parameter set will be out of order with respect to the experimental spectrum. Thus, a mechanism is needed by which the calculated modes are correctly assigned to the experimental frequencies. Using a standard valence force-field program, namely, NORMCOR[29], and the force constants used to assign the experimental vibrational spectrum[25], a set of normal mode eigenvectors was calculated. Each CHARMM mode was then assigned to the valence force-field mode with which it had the greatest overlap. Overlap was determined by dot products taken between the eigenvectors. With a correspondence between the modes established, the sum of the squares of the deviations could be computed, and systematically minimized as a function of the potential parameters as described in ref. 22.

Initial values for the force constants in these optimization calculations were estimates based upon studies of such small-molecular analogs of carbohydrates as methanol and dimethyl ether. Equilibrium bond-lengths and angles were taken to be the average values observed in the α-D-glucose crystal, and were not changed during the optimization. The functional form selected is a typical CHARMM molecular-mechanics potential-energy function of the internal coordinates q, representing bond lengths b, angles θ, torsion angles ϕ, and interatomic, nonbonded separations r, as shown in Eq. *1*.

$$V(q) = \Sigma\, k_b\,(b - b_o)^2 + \Sigma\, k_\theta\,(\theta - \theta_o)^2 + \Sigma\, k_\phi\,[1 + \cos(n\phi - \delta)] + \sum_{i \neq j} (A_{ij}/r_{ij}^{12} - B_{ij}/r_{ij}^{6} + q_i q_j/\varepsilon\, r_{ij}), \tag{1}$$

where the values of b_o are the equilibrium bond-lengths, and those of θ_o are the equilibrium bond-angles, n is a periodicity number, δ is a phase factor, r_{ij} is the interatomic separation between atoms i and j, q_i and q_j are atomic partial charges, and k_b, k_θ, and k_ϕ are the force constants to be determined. Only one torsional term was included for each bond, regardless of the number of substituents on the atoms defining the bond. No explicit hydrogen-bond function was used[12]. The partial charges used in this study, listed in Table I, were based on standard CHARMM values resulting from *ab initio* calculations[21] for hydrogen-bonding atoms, and are closely related to those determined independently by Jorgensen[30], in Monte Carlo

TABLE I

ATOMIC PARTIAL CHARGES FOR α-D-GLUCOPYRANOSE USED IN THE PRESENT STUDY[a]

Atom	*Charge*	*Atom*	*Charge*
C-1	0.350	C-4	0.150
H-1	0.100	H-4	0.100
O-1	−0.650	O-4	−0.650
OH-1	0.400	OH-4	0.400
C-2	0.150	C-5	0.100
H-2	0.100	O-5	−0.400
O-2	−0.650	H-5	0.100
OH-2	0.400	C-6	0.050
C-3	0.150	H-6a	0.100
H-3	0.100	H-6b	0.100
O-3	−0.650	O-6	−0.650
OH-3	0.400	OH-6	0.400

[a] H-X refers to the carbon-bound hydrogen atom of the corresponding number X, and OH-X refers to the hydroxyl hydrogen atom.

calculations, to be appropriate for hydroxyl groups in condensed phases. Previous studies had demonstrated that the choice of charges and the general treatment of electrostatics have only small effects on equilibrium vacuum structures, while being quite important for aqueous solvation behavior. In the present refinement, a dielectric constant of unity was used, whereas the PEF 422 function uses a value of 3.

During the optimization calculations, all force constants were varied, in order to achieve the best fit to the experimental data for D-glucose. However, not all force constants were varied simultaneously, but were grouped into subsets of coupled parameters in order to increase the optimization efficiency[22]. The grouping of force constants into sets was changed from one optimization cycle to another in order to avoid artifacts resulting from the selection of those force constants being varied. Eventually, the complete set of force constants converged to consistent values. This parameter set was then fine-tuned to fit available structural data. For this purpose, the geometry of the ring pucker as defined by the positions of the six ring-atoms was used. In this portion of the optimization, the sum of the squares of the deviations of the positions of the six ring-atoms in the energy-minimized structure from their positions in the experimental crystal structure was minimized as a function of the force constants in the potential energy. During this process, only the torsional constants and the bond-stretching and -bending constants associated with the ring atoms were varied, using the values previously arrived at by spectral optimization as a starting point. In general, the changes in the force constants resulting from this procedure were small.

RESULTS

The set of force constants obtained from the optimization procedure is listed in

TABLE II

FINAL POTENTIAL-ENERGY PARAMETERS[a]

Bond stretches

Bond type	k_b *(kcal/mol·Å)*	b_o *(Å)*
O–H	460.5	0.972
C–H	337.3	1.099
C–C	214.8	1.523
C–O	334.3	1.411
C–OE	296.7	1.427

Bond angles

Angle type	k_θ *(kcal/rad²)*	θ_o *(degrees)*
C–O–H	53.6	109.35
H–C–H	33.6	107.85
H–C–C	43.0	108.72
H–C–O	45.9	109.89
H–C–OE	45.2	107.24
C–C–C	38.0	110.70
C–C–O	75.7	110.10
C–C–OE	81.0	109.40
C–OE–C	90.7	113.80
OE–C–O	92.6	111.55

Torsions

Angle type	k_ϕ *(kcal/mol)*	n	δ
X–C–C–X	1.021	3	0
X–C–O–X	0.443	3	0
X–C–OE–X	0.928	3	0

Van der Waals parameters

Atom type	E_{min} *(kcal/mol)*	R_{min} *(Å)*
H (hydroxyl)	−0.0498	0.800
H	−0.0045	1.468
C	−0.0903	1.800
O	−0.1591	1.600
OE	−0.1591	1.600

[a] See text following Eq. *1* for definition of symbols. OE refers to the ring-oxygen atom.

Table II. Most of the constants are similar to values previously used in CHARMM[31], and, as might be expected, are close to the valence force-field parameters of Vasko *et al.*[25] from which they are derived. The terms involving the sugar carbon atoms were generally found to have somewhat lower force constants than those derived for the aliphatic carbon atoms in cyclohexane[31], indicating that the chemical environment of the carbon atoms in these polyfunctional alcohols is sufficiently different to warrant treating them as a distinct atom-type. The force constants for the angle bending terms involving the ring ether oxygen atom are significantly larger than those in the PEF 422 potential-energy function, but are similar to those used, in the valence force-field for α-D-glucose, by Vasko *et al.*[25]. The C–C–C bending-constant is also significantly larger in this potential function than in PEF 422. Although a study of cyclohexane that used the previous CHARMM values found that a value of 113° for θ_0 for this C–C–C bond-angle gave the best structural and energetic properties[31], in the case of the pyranose carbohydrates reported here, acceptable ring-geometries were obtained only when this equilibrium bond-angle was set closer to the experimentally determined values for pyranoid sugars (see Table II).

The larger force-constants for ring-angle deformations should have the net effect of making the ring less flexible than in the PEF 422 model, as was observed in molecular dynamics simulations (see later). The torsional barrier, k_ϕ, for rotations about C–O bonds was found to be of approximately the same magnitude as that for torsions about C–C bonds, even though each carbon atom is attached to three other substituents, while the ether oxygen atom is bonded to only one other atom. When only one torsional term is being considered per bond, as in the present force-field, it might be expected that the barrier for C–C torsions would be roughly three times that for C–O torsions. This larger value of k_ϕ was found to be necessary in order to achieve a reasonable match to structural data (pucker parameters). The spectral data were less sensitive to this parameter, particularly as the spectra of Vasko *et al.*[25] did not directly measure the frequencies below 150 cm^{-1}, which are presumably most sensitive to this parameter. Although the PEF 422 force-field effectively does not use torsional terms (they are present, but the barriers are set to negligible values), in a related previous potential-function developed by the same group[32], the barriers for rotations about these two types of bonds were both of approximately the same magnitude as found here.

The normal-mode frequencies for α-D-glucose obtained by using the parameter set listed in Table II are given in Table III, along with the valence force-field frequencies used for the refinement and those for the PEF 422 potential-energy function of Rasmussen[19]. As may be seen from Table III, the new potential-energy function produces a vibrational spectrum that is reasonably close to experiment, as does the PEF 422 function. In the somewhat less biologically interesting high-frequency region, corresponding to modes primarily involving C-H and O-H stretches, the new function is closer to that found by experiment, due to a substantially lower force-constant for O-H stretches. The Cremer–Pople pucker parameters[20] for the minimum-energy structure in vacuum for α-D-glucose obtained by using the new potential parameters are listed in Table IV, and are compared to those for the crystal structure and for the PEF 422 function. Again, the new potential function gives values quite acceptably close to those from experiment, preserving this feature of the PEF 422 function. The large error in ϕ_2 for the PEF 422

TABLE III

CALCULATED VIBRATIONAL FREQUENCIES FOR α-D-GLUCOPYRANOSE COMPARED TO THOSE FOR THE REFERENCE VALENCE FORCE-FIELD

Mode no.	*Vibrational frequencies*		
	Valence force-field	*Calculated*	
		Present work	*PEF 422*
1	59	54	65
2	79	74	97
3	95	78	113
4	133	107	150
5	186	139	212
6	223	211	243
7	224	226	245
8	229	245	262
9	229	259	270
10	236	270	297
11	240	279	313
12	386	329	322
13	301	341	345
14	328	346	352
15	348	369	377
16	383	393	391
17	424	403	417
18	472	430	438
19	494	470	491
20	553	518	514
21	558	576	537
22	610	580	550
23	725	649	582
24	894	801	830
25	922	842	856
26	940	903	925
27	993	917	953
28	1008	941	977
29	1034	957	1008
30	1060	965	1036
31	1074	1007	1068
32	1085	1017	1071
33	1111	1021	1123
34	1124	1061	1141
35	1143	1068	1166
36	1169	1096	1194
37	1181	1200	1207
38	1211	1205	1235
39	1224	1210	1252
40	1243	1235	1264
41	1253	1245	1279
42	1258	1264	1293
43	1271	1270	1305
44	1276	1287	1317
45	1288	1298	1339
46	1306	1329	1369

Table III (continued)

Mode no.	*Vibrational frequencies*		
	Valence force-field	*Calculated*	
		Present work	*PEF 422*
47	1317	1345	1407
48	1326	1397	1459
49	1331	1405	1486
50	1336	1426	1496
51	1344	1434	1510
52	1357	1449	1524
53	1398	1489	1545
54	1466	1496	1563
55	2884	2897	2904
56	2929	2931	2931
57	2933	2934	2938
58	2937	2937	2945
59	2939	2939	2951
60	2943	2943	2961
61	2983	2969	2972
62	3397	3384	3655
63	3397	3386	3663
64	3397	3387	3663
65	3397	3390	3663
66	3397	3391	3665

potential function is of little significance, because, in the polar-coordinate system described[20] by (Q, θ, ϕ_2), the minimum-energy conformation is a chair form close to the pole defined by $\theta = 0°$, where variations in ϕ_2 are least important.

To evaluate the dynamical behavior of α-D-glucose by using this newly developed function, an ensemble of 12 vacuum molecular-dynamics trajectories was computed, and compared to those calculated by using the PEF 422 function. These trajectories were started from the crystal structures, with random assignments of atomic velocities selected from a Boltzmann distribution at 300 K for each trajectory, as in previous studies[17,18], and bond lengths were again kept fixed, using the constraint procedure SHAKE[33]. Each trajectory was equilibrated for 20 ps at 300 K, and was then integrated for an additional 20 ps of data collection, as before[17]. The total system-energy was well conserved in these trajectories, with no systematic drift and with the ratio of the r.m.s. fluctuation in total energy divided by the r.m.s. fluctuation in the system kinetic energy being less than 0.01 throughout.

The ensemble-averaged, dynamical structure for these D-glucose molecules, as summarized by selected internal coordinates, is presented in Table V, along with the crystal values and the average values computed from the previous simulations, and the fluctuations in the dynamical quantities. As may be seen from Table V, the

TABLE IV

PUCKER PARAMETERS FOR α-D-GLUCOPYRANOSE ENERGY-MINIMIZED *in vacuo*

	q_2	ϕ_2	q_3	Q	θ
Experiment	0.0347	−36.9	0.5657	0.5668	3.51
Present work	0.0249	−37.0	0.5662	0.5667	2.51
PEF 422	0.0348	+51.1	0.5607	0.5617	3.55

mean dynamical structure with the new potential surface is acceptably close to the crystal structure, as is the mean structure for the PEF 422 function. The fluctuations in these internal coordinates are considerably smaller, however, for the new function, indicating less molecular flexibility in the pyranose ring than was seen on using the PEF 422 function. For the torsional coordinates, the fluctuations are 30–40% smaller using the new function, and the decrease in fluctuations for most of the bond angles is ∼20–30%.

In the simulations previously reported that used the PEF 422 function, significant populations were observed for the exocyclic CH_2OH group in all three minima of the local bond-torsional function, including the *gauche–trans* orientation at −60°

TABLE V

COMPUTED MEAN STRUCTURE OF α-D-GLUCOPYRANOSE IN THE $^4C_1$ CONFORMATION[17]

Angle	*Exptl. value (degrees)*	*Computed values (degrees)*			
		Present work		*PEF 422*	
		Mean	*R.m.s. fluc.*	*Mean*	*R.m.s. fluc.*
C-1–C-2–C-3–C-4	−51.3	−53.0	5.74	−51.6	7.89
C-2–C-3–C-4–C-5	53.3	53.6	5.86	49.7	8.13
C-3–C-4–C-5–C-6	−176.6	−174.8	7.18	−171.1	9.85
C-3–C-4–C-5–O-5	−57.5	−52.9	5.96	50.3	10.15
C-4–C-5–O-5–C-1	62.2	57.2	5.92	56.3	10.58
C-5–O-5–C-1–C-2	−60.9	−59.2	5.52	−58.7	9.36
O-5–C-1–C-2–C-3	54.1	54.4	5.61	54.6	8.88
C-1–C-2–C-3–O-3	−172.0	−175.4	6.49	−174.0	8.97
C-2–C-3–C-4–O-4	175.3	175.3	6.56	172.9	9.05
O-1–C-1–C-2–O-2	56.9	55.2	7.49	56.7	10.85
O-1–C-1–C-2–C-3	−68.7	−67.6	6.66	−68.4	10.00
C-1–C-2–C-3	111.1	109.6	3.11	110.4	4.32
C-2–C-3–C-4	109.8	111.3	3.29	110.9	4.15
C-3–C-4–C-5	111.1	110.5	3.28	111.9	4.33
C-4–C-5–C-6	111.5	113.4	3.69	114.0	4.62
C-4–C-5–O-5	108.7	110.0	2.82	110.5	4.54
C-5–O-5–C-1	113.7	115.5	2.58	115.4	4.35
O-5–C-1–O-1	111.5	109.8	3.00	110.7	5.83
C-1–C-2–O-2	110.9	111.9	3.00	111.3	4.76
C-2–C-3–O-3	108.1	110.0	3.20	110.6	4.83
C-3–C-4–O-4	108.2	109.5	3.15	111.2	4.77
C-5–C-6–O-6	109.9	111.5	3.18	112.7	4.75

TABLE VI

COMPUTED MEAN STRUCTURE OF α-D-GLUCOPYRANOSE IN THE $^1C_4$ CONFORMATION[17]

Angle	*Computed values (degrees)*			
	Present work		*PEF 422*	
	Mean	*R.m.s. fluc.*	*Mean*	*R.m.s. fluc.*
C-1–C-2–C-3–C-4	54.5	5.0	50.7	7.5
C-2–C-3–C-4–C-5	−51.3	4.7	−44.7	8.3
C-3–C-4–C-5–C-6	−79.8	6.7	−86.7	9.7
C-3–C-4–C-5–O-5	48.1	5.9	42.4	10.0
C-4–C-5–O-5–C-1	−51.5	5.9	−50.2	10.0
C-5–O-5–C-1–C-2	56.2	5.9	58.1	9.2
O-5–C-1–C-2–C-3	−56.1	6.1	−56.4	8.6
C-1–C-2–C-3–O-3	−69.4	6.3	−71.1	9.2
C-2–C-3–C-4–O-4	72.9	5.9	81.1	9.8
O-1–C-1–C-2–O-2	−51.1	8.2	−54.2	11.1
O-1–C-1–C-2–C-3	−175.3	6.7	−177.6	9.5
C-1–C-2–C-3	109.2	3.4	109.4	4.5
C-2–C-3–C-4	110.5	3.1	111.9	4.4
C-3–C-4–C-5	112.5	3.1	114.1	4.3
C-4–C-5–C-6	114.7	3.6	116.5	4.5
C-4–C-5–O-5	111.7	2.8	111.9	4.4
C-5–O-5–C-1	116.4	2.7	116.4	4.4
O-5–C-1–O-1	107.3	3.0	109.6	5.7
C-1–C-2–O-2	112.9	3.0	113.1	4.7
C-2–C-3–O-3	110.7	3.1	109.3	5.0
C-3–C-4–O-4	110.5	3.1	110.2	4.8
C-5–C-6–O-6	110.9	3.2	112.4	4.9

(see formula *1*), even though this rotamer has not been observed to occur in any of the known crystal structures of related carbohydrate polymers[34]. This orientation of the CH_2OH group can, in principle, allow the formation of an internal hydrogen-bond, but it has also been postulated to lead to an unfavorable alignment of the dipoles on the 6- and 4-hydroxyl groups (*peri*-interactions). Using the PEF 422 function, the *trans–gauche* orientation was found to be 0.25 kcal/mol more stable than the *gauche–trans* orientation[17], with the small energy difference allowing significant populations of all three orientations at normal temperatures. With the larger charges of the present model, and with its unit dielectric constant instead of the value of 3 used in PEF 422, the intramolecular hydrogen-bond becomes much more important for the vacuum molecule, producing a dynamically averaged energy-difference between the *trans–gauche* and *gauche–trans* orientations of 2.9 kcal/mol. Transitions between orientations of the CH_2OH group are correspondingly less frequent, with the *trans–gauche* orientation by far the most populated at 300 K. Spontaneous transitions were nevertheless observed to occur, but the large energy-difference in the conformers resulted in significant temperature changes following such transitions, frustrating efforts to quantitate the transition frequency.

The decreased flexibility of the ring also affects the frequency of ring conformational inversions, as might be expected. A number of trajectories were computed for α-D-glucopyranose in the $^1C_4$ conformation, as in the previous study[17]. In these new simulations, no spontaneous transitions to the more favorable $^4C_1$ conformer were observed. Table VI compares the ensemble-averaged, dynamical structure for an ensemble of six such trajectories to the values observed previously for the PEF 422 function. As in the $^4C_1$ case, the average structure using the new potential function is similar to that using the PEF 422 function, but the fluctuations in the internal coordinates are again much smaller. For this ring conformation, the most favored orientation of the CH_2OH group is +60°, which is ~2.1 kcal/mol more stable than the orientations at −60° and +180°, due to the formation of an intramolecular hydrogen-bond to the 3-hydroxyl group. In spite of the lower energy of this conformation, the CH_2OH group frequently undergoes brief transitions out of this geometry as it is knocked out of position by free rotations of the 3-hydroxyl group in these vacuum simulations. Because such conformational transitions involve a substantial change in potential energy, the average structure reported in Table VI is for an ensemble of six trajectories for which the exocyclic dihedral angle C-4–C-5–C-6–O-6 remains at 180° without transitions. The energy difference between the $^4C_1$ and the $^1C_4$ conformer with this orientation of the exocyclic group, averaged over the respective ensembles of trajectories, is 3.1 kcal/mol. The difference in energy for these two ring conformations, using the PEF 422 function, in which the molecular energy is not as sensitive to exocyclic orientation, was found previously[17] to be 2.1 kcal/mol. Thus, the conformational energy difference between the two ring conformers is lower in the most populated exocyclic orientation using the new potential-energy function, and somewhat higher than the energy difference using PEF 422 for the other two exocyclic arrangements. Because spontaneous transitions in ring conformation are not observed to occur for $^1C_4$ trajectories using the new potential, it might be inferred that the barriers to such transitions are higher using the new function.

DISCUSSION

Whereas molecular dynamics simulations of carbohydrates have only recently been attempted, molecular mechanics studies of carbohydrates have a long and reasonably successful history, and several useful potential functions have been advanced for use in studying such molecules[35–38]. Most of these potentials do not include provisions for internal motions, or were not specifically derived from experimental data on carbohydrates. However, this was not the case for the PEF 422 function, which was extensively revised and tested against carbohydrate data, and which has force constants for all bond stretches and bends and includes electrostatic interactions between nonbonded atoms, thus implicitly including some intra- and inter-molecular hydrogen-bonding. The primary goal of the present work was to develop a comparable surface which incorporates the newly determined partial charges appropriate for condensed phases, in order to allow simulations of aqueous solutions.

Because the refinement procedure employed here involves minimization in a complex multidimensional space, it is potentially limited by the multiple minimum problem, and the results produced can depend upon the initial choices of parameters and the way in which the optimization was carried out. For this reason, independent experimental input, such as crystal geometries or thermodynamic data, can be useful in selecting between various parameter sets that produce vibrational spectra of comparable quality.

Apart from these formal difficulties, there are additional problems involved in selecting optimum potential parameters. The first of these is that the simple functional forms selected are only rough approximations to the true functional behavior of the molecular energy. Such simplifying approximations may result in different parameter sets giving the best representation of different molecular properties, as has been found to be the case for water[39]. Other difficulties involve experimental uncertainty. In the present problem, for example, the greatest experimental uncertainties are in the low frequency modes, particularly in those below 100 cm^{-1}, which are the most useful in determining weaker force constants. In the assigned valence force-field spectrum used in this refinement[25], these low frequencies are absent, but, for many interesting processes, as in conformational inversion, such low-frequency motions could be quite important. The structural data from neutron diffraction experiments used in these optimizations has uncertainties of negligible importance for these applications.

A possibly more significant problem lies in the nature and appropriateness of the system selected to serve as the theoretical model for the experimental data. For practical reasons, this theoretical model was the D-glucopyranose molecule in vacuum, as was the case in previous parameterizations[19], while both the spectral and structural data used in the refinement applied to crystalline or solvated D-glucose. It is probable that the lattice neighbors of a sugar molecule in a crystalline environment affect both its structure and vibrational motions. By forcing the vacuum molecule to match crystalline properties, these effects have been "built in" to the behavior of the molecule in a somewhat artificial way, and there is no guarantee that this would be necessary if the refinement could be done for the molecule in a full crystal. While currently unavoidable, this problem could produce the undesirable result of masking any differences between crystalline and solution behavior.

As already discussed, the exocyclic torsional conformational preference using the PEF 422 function differs from that found experimentally for D-glucose and a variety of D-glucose analogs and carbohydrate polymers. The *trans-gauche* rotamer, never observed experimentally in crystals, is found to be significantly populated in the ensemble of thermal vacuum trajectories, and using the newly determined parameterization reported herein, it is by far the dominant form due to the much lower energy of this geometry resulting from the internal hydrogen-bond. This result is not necessarily in contradiction to the experimental data, however; in crystals, the exocyclic group is invariably involved in intermolecular hydrogen-bonds with crystal neighbors, with the expected concomitant gain in energy. Such a geometry would not necessarily be retained in the absence of a hydrogen-bonding partner, however, and the formation of an internal hydrogen-bond under such circumstances hardly seems surprising.

In general, the decreased flexibility of the pyranose ring, resulting from the stiffer force constants and larger charges in this refinement, has the effect of making the ring structure more rigid than is the case using the PEF 422 function. While conformational flexibility is still important in this model, the somewhat lessened flexibility is more in accord with past models of pyranose rings in polysaccharide molecules, which have traditionally been treated as completely rigid units. Using the new function, transitions in structure still occur, and would presumably be of significance in determining the properties of polysaccharides, but would occur with a significantly diminished frequency requiring a greater activation energy.

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