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Note

Molecular dynamics simulations of β-D-glucopyranose

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An understanding of the conformational and mutarotational equilibria of simple sugars has long been an important goal of carbohydrate chemistry[1,2]. Much effort has been directed toward the use of molecular mechanics techniques to examine the energy differences between different forms, with a considerable degree of success[3–6]. These techniques generally find, based primarily on vacuum models (that is, without solvent explicitly present), that for D-glucopyranose the $^4C_1$ conformer and the β anomer are favored, in qualitative accord with experiment. Recently, molecular dynamics simulations of the vacuum motions of α-D-glucopyranose in the $^1C_4$ and $^4C_1$ conformations were reported[7]. These studies employed the potential energy surface developed by Rasmussen and co-workers[8], and were undertaken in part to determine the suitability of this potential function for use in molecular mechanics simulations of the aqueous solvation of carbohydrates, and to examine the motions and structural fluctuations of pyranose rings at normal temperatures. Molecular dynamics calculations have the advantage of providing explicit information about the flexibility of molecules for a given potential function. Dunfield and Whittington[6], and Joshi and Rao[9], have pointed out the possible importance of molecular flexibility in conformational equilibria, and of the coupling of various types of deformations, such as that of substituent bond angles with ring torsional fluctuations. A similar series of dynamics calculations has now been completed for β-D-glucopyranose-$^4C_1$ *in vacuo* using this same potential, and the results are reported here.

As in the α-D-glucopyranose study[7], the equations of motion for an ensemble of 12 trajectories were integrated by using a Verlet algorithm, with the molecular bond lengths being constrained to fixed values[10]. As a result of the constraints, any contribution to the anomeric ratio arising from differences in average bond lengths will not be seen in these simulations. Although an experimentally determined geometry is available for β-D-glucopyranose from X-ray crystallography[11], this was not used for the starting structure in the present study owing to the lack of precision in determining the hydrogen positions (such errors in bond length are potentially

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important because the bonds were constrained to remain at these initial values). Instead, the atomic coordinates for α-D-glucopyranose determined by neutron diffraction[12] were used, with the CH and CO bonds on the anomeric carbon atom interchanged, maintaining the correct bond lengths and appropriate bond angles determined for β-D-glucopyranose[11]. The difference in the O-5–C-1 bond length between the α and β anomers from these two crystal structures[11,12] is only 0.3 pm, and no attempt was made to incorporate this small difference into the starting structures built from the α-D-glucose coordinates. As before, each trajectory was equilibrated for 40 ps of dynamics after assigning initial velocities from a Boltzmann distribution at 300 K, and an additional 20 ps was then integrated for analysis. The mean ensemble temperature averaged over all twelve trajectories was 299.7 K; for further details of the calculation procedure, see ref. 7.

Table I contains the dynamically averaged structure, in terms of selected internal coordinates, for the ensemble of β-D-glucose trajectories, and compares these values to those found experimentally[11,12] and to those calculated[7] from simulations of α-D-glucose. Fluctuations in these internal coordinates are also

TABLE I

MEAN STRUCTURES FOR β-D-GLUCOPYRANOSE IN THE $^4C_1$ CONFORMATION AS COMPUTED FROM MOLECULAR-DYNAMICS SIMULATIONS, COMPARED WITH EXPERIMENT AND THE DYNAMICALLY AVERAGED STRUCTURE OF α-D-GLUCOPYRANOSE IN THE $^4C_1$ CONFORMATION

Angle	*Mean value (β-D-glucose)*	*RMS fluct.*	*Expt. value*[a]	*Mean value (α-D-glucose)*	*RMS fluct.*[b]	*Expt. value*[c]
C-1–C-2–C-3–C-4	49.7	8.53	−50.8	−51.6	7.89	−51.3
C-2–C-3–C-4–C-5	49.4	8.32	53.4	49.7	8.13	53.3
C-3–C-4–C-5–C-6	−172.1	9.74	−179.0	−171.1	9.85	−176.6
C-3–C-4–C-5–O-5	−51.9	9.93	−59.8	−50.3	10.15	−57.5
C-4–C-5–O-5–C-1	57.3	10.39	66.3	56.3	10.58	62.2
C-5–O-5–C-1–C-2	−57.4	9.91	−62.8	−58.7	9.36	−60.9
O-5–C-1–C-2–C-3	51.2	9.63	53.7	54.6	8.88	54.1
C-1–C-2–C-3–O-3	−171.6	9.30	−170.5	−174.0	8.97	−172.0
C-2–C-3–C-4–O-4	172.2	9.18	173.1	172.9	9.05	175.3
O-1–C-1–C-2–O-2	−65.3	11.14	−69.2	56.7	10.85	56.9
O-1–C-1–C-2–C-3	171.6	10.06	169.5	−68.4	10.00	−68.7
C-1–C-2–C-3	110.9	4.43	112.1	110.4	4.32	111.1
C-2–C-3–C-4	111.5	4.23	110.5	110.9	4.15	109.8
C-3–C-4–C-5	111.8	4.20	109.8	111.9	4.33	111.1
C-4–C-5–C-6	113.9	4.55	115.0	114.0	4.62	111.5
C-4–C-5–O-5	110.0	4.61	107.6	110.5	4.54	108.7
C-5–O-5–C-1	115.4	4.22	112.7	115.4	4.35	113.7
O-5–C-1–O-1	108.6	5.67	107.0	110.7	5.83	111.5
C-1–C-2–O-2	110.8	4.96	108.5	111.3	4.76	110.9
C-2–C-3–O-3	110.5	4.90	108.7	110.6	4.83	108.1
C-3–C-4–O-4	111.0	4.77	111.1	111.2	4.77	108.2
C-5–C-6–O-6	112.6	4.79	111.9	112.7	4.75	109.9

[a]Values taken from ref. 11. [b]Values taken from ref. 7. [c]Values taken from ref. 12.

listed. As may be seen, both structures are close to those found experimentally. The largest discrepancies, on the order of 5–9°, lie in the ring torsion angles. Both calculated ring structures are found to be too flat on using this potential, and the difference between the two dynamical structures, particularly in these ring torsions, is in general less than that by which each differs from its corresponding crystal structure. There is some shifting of the dynamical averages away from the minimum-energy values, as was seen for the α anomer[7]; C-5–O-5–C-1, for example, was found to have a mean value of 115.4° for both anomers, whereas the minimum-energy angles of 113.4° for the α anomer and 113.7° for the β anomer[8] are closer to the values for the crystal[10,11] of 113.7 and 112.7°. The fluctuations in the internal coordinates are also quite similar to those seen for the α anomer, with the result that there is little configurational entropy difference between the structures (see later). Averaged over the entire ensemble of trajectories, the van der Waals energy of the α anomer was slightly (0.2 ±0.2 kcal/mol) more favorable, and the electrostatic energy was slightly (0.1 ±0.2 kcal/mol) less favorable, although it should be noted that the magnitudes of these numbers are within the uncertainty level. Also averaged over the entire ensembles, the difference in the dynamically averaged potential energies for the two configurations favored the α form by 0.1 kcal/mol (that is, there was no difference in the mean angle energies).

The exocyclic CH_2OH groups underwent 30 transitions during the 12 trajectories, or approximately 0.125 transition/p; that is, 1 transition every 8.0 ps. This is somewhat slower than was found for the α anomer, where the average transition frequency was ~1 transition every 4.8 ps. The population distribution for the three main orientations of this hydroxymethyl group was found to be 0.40 (−60°) ⇄ 0.39 (180°) ⇄ 0.21 (+60°), where the conformation at 180° is the one with O-6 *gauche* to O-5 and *anti* to C-4, the conformation at 60° has O-6 minus-*gauche* to O-5 and *gauche* to C-4, and the conformation at −60° has O-6 *anti* to O-5 and minus-*gauche* to C-4. This distribution, with its equal populations of rotamers at −60° and 180°, differs from that observed for the dynamically averaged α anomer, where the "extended" conformer with C-4–C-5–C-6–O-6 in the *gauche-anti* arrangement preponderates. When averaged for both anomers (with equal weighting; see later), these relative populations are almost exactly the proportions for these two forms that were estimated by Rasmussen[8] from analyzing the potential energy wells for this same potential function. The important difference in these dynamical simulations is that the conformation at −60°, not considered by Rasmussen, is dynamically significant; in fact, it is a dominant conformation for the β anomer. This result also differs from the distribution found by surveying known crystal structures of carbohydrates, where the conformer with the hydroxymethyl group at −60° was not observed to occur[13], and it may indicate inaccuracy in the PEF 422 potential energy surface. For both anomers, the conformer with the exocyclic group at +60° was significantly less probable dynamically.

In two additional trajectories not included in the ensemble averaging, spontaneous transitions to different conformations occurred. In both such cases,

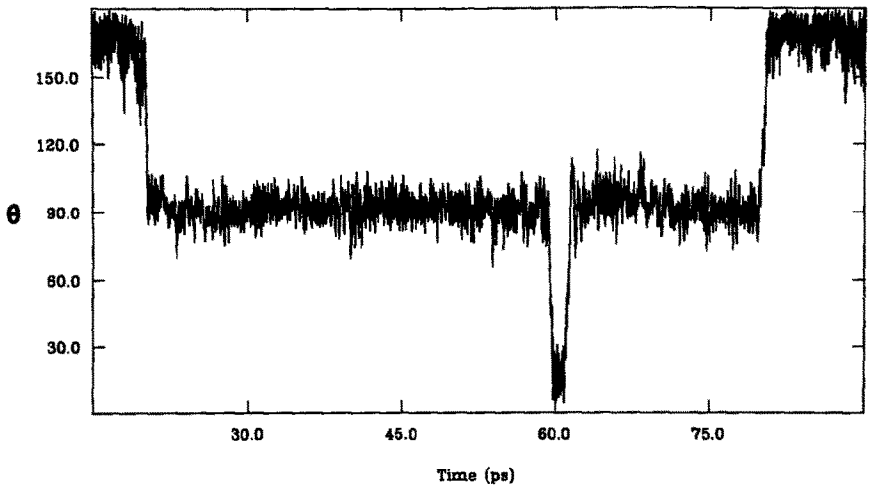

Fig. 1. History of the Cremer–Pople pucker parameter for a β-D-glucose-$^4C_1$ trajectory which underwent four transitions in conformation.

the initial transition was from the starting $^4C_1$ conformation to boat-like conformations. The time course of the Cremer–Pople pucker parameter[14] θ for one of these trajectories (which was integrated for a longer period than for those in the standard ensemble in order to observe the conformational evolution) is shown in Fig. 1. The initial, random velocity selection for this trajectory resulted in a molecular temperature of 309 K, a value that is too high to be acceptable for the thermal ensemble, but just low enough to escape the automatic scalings which took place during the first 20 ps of these trajectories if the system temperature deviated from 300 K by more than 10 K. Perhaps as a result of this somewhat higher temperature, the trajectory illustrated in Fig. 1 underwent a spontaneous transition, at ~21 ps into the simulation, to a higher potential energy skew conformation. This transition resulted in a substantial drop in temperature, to 291 K, as the thermal kinetic energy was converted into potential energy. In spite of being scaled back to 300 K during the second round of automatic equilibration scalings, since its temperature differed from 300 K by more than the 5 K allowed during the second 20 ps, the molecule nevertheless remained in higher-energy, boat shapes for almost 40 ps, and even underwent a brief transition to the $^1C_4$ conformer before returning to a boat conformation. Eventually, after a subsequent 19 ps of oscillating about various skew and boat conformations, the molecule underwent another transition, to the favored $^4C_1$ shape. It should be noted that all of the transitions occurred fairly directly, and quite rapidly on the molecular time-scale. Stereo-drawings of this molecule at the end of 15, 40, 60, 70, and 85 ps are displayed in Fig. 2. Attempts to simulate the dynamics for an ensemble of β-D-glucose-$^1C_4$ molecules failed, because none of them remained in this starting conformation long enough to be equilibrated; all underwent spontaneous transitions to boats and subsequently, to

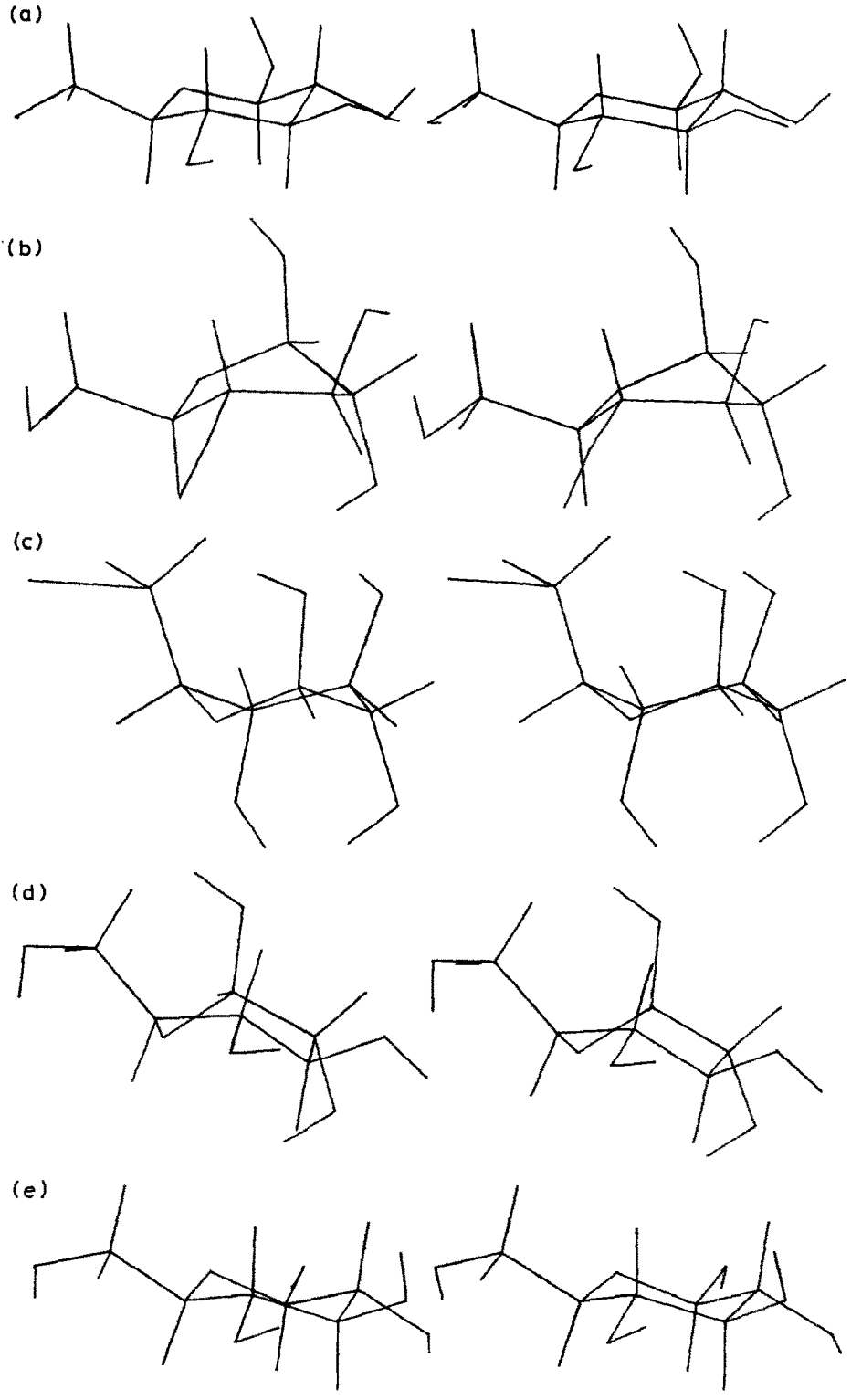

Fig. 2. Stereo-views of the instantaneous molecular conformation for the trajectory illustrated in Fig. 1 at various points in the trajectory; (a) 15; (b) 40; (c) 60; (d) 70; and (e) 85 ps.

the energetically more favorable $^4C_1$ conformer. Although no spontaneous transitions were observed for $^4C_1$ trajectories at the desired 300 K, these simulations would tend to indicate that, for this potential energy function, boat conformations *in vacuo* might exist with a non-negligible frequency at higher temperatures, and that transitions from one chair conformation to the other seem to take place *via* reasonably long-lived, boat intermediates.

As in the previous study[7], the method of Karplus and Kushick[15] for estimating configurational entropy differences was applied to the entropy difference between the β and α anomers. Employing the same choice of "important" coordinates as before (see Table IV of ref. 7), the entropy difference between β and α, with both in the $^4C_1$ conformation, was estimated to be only -0.33 cal/mol–K, or -0.1 kcal/mol at 300 K, a value indistinguishable from 0, given the uncertainties in this approximation. This balances (probably fortuitously) the -0.1 kcal/mol energy change for this process, giving a free energy difference of 0. While the uncertainty in this number must be larger than the $\sim\pm 0.3$ kcal/mol uncertainty in the energy difference, it is clear that the free energy difference between the two anomers is very small for this potential function. This result is not in accord with the experimentally measured anomeric ratio[6], where only 37% of the molecules exist in the α form in aqueous solution. It is in excellent agreement, however, with the prediction, from Monte Carlo calculations by Dunfield and Whittington[6], of 47% of α for D-glucose *in vacuo,* and with the ratio estimated by Rasmussen[8] for this potential function. Although in neither study can inadequacies in the potential energy functions used be precluded as the cause of the discrepancy between theory and experiment, this result nevertheless further reinforces the observation by Dunfield and Whittington[6] that the main contribution to the anomeric equilibrium may come from differences in solvation for the two structures.

In the modeling of polysaccharides built from pyranoid sugars, the monomer units have frequently been treated as rigid, although it has been demonstrated that a considerable range of ring geometries occurs in known crystal structures and that it is necessary to consider environmentally induced flexibility in ring conformation in order to build reasonable polymer models[9,16,17]. The importance of furanose flexibility in determining nucleic acid conformation and function has also been noted[18] and potential energy functions have been developed to provide a physically reasonable, theoretical description of this flexibility[19]. The simulations reported here illustrate directly that for pyranose rings, with the potential energy function selected, there is indeed considerable molecular flexibility, and they provide a time scale for geometric fluctuations. Such dynamic flexibility of pyranose rings may have important consequences for polysaccharide flexibility and geometry, and its role in such conformational transitions as those seen here is obvious, although it is interesting that in this case molecular flexibility seems to have been of little significance for the calculated anomeric equilibrium.

Finally, it would be interesting to know the extent to which the results reported here are a function of the potential energy surface used, and how

accurately this function represents physical reality. While there are few direct ways to test such functions, the discrepancy between these simulations and experiment concerning the orientation of the exocyclic CH_2OH group may be indicative of inaccuracy in the force field. The degree of flexibility observed in these trajectories, and the frequency of transitions, may thus be not completely realistic.

The stereo diagrams in Fig. 2 were produced by use of the HYDRA molecular graphics program written by R. E. Hubbard of the University of York, and were plotted by using the PLT2 plotting program developed by D. J. States of the University of California at San Diego. This work was supported by Grant number GM34970 from the National Institutes of Health.

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