Molecular Modeling of Two Disaccharides Containing Fructopyranose Linked to Glucopyranose

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CONTAINING FRUCTOPYRANOSE LINKED TO GLUCOPYRANOSE[1]

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ABSTRACT

Ramachandran energy surfaces for the disaccharides leucrose and turanose were computed using MM3. For each molecule, thirty-two combinations of hydroxyl and primary alcohol group orientations were considered. The calculations used a dielectric constant of 4.0 and the maps were generated on a 20° grid-spacing. The models compared well with the corresponding crystal structures except for significant deviations in some of the anomeric C–O bond lengths.

INTRODUCTION

Together with experimental techniques such as NMR spectroscopy and diffraction crystallography, molecular modeling is a useful tool for studying chemical structure. Improved molecular mechanics modeling software and advances in computer hardware over the past decade have enabled extensive and mostly successful computational studies on carbohydrate compounds.[2-5] In the successful studies, the observed crystal structures were found within the regions of lowest energy, as calculated with the molecular mechanics program, MM3. Good agreement has been found for α,α-trehalose,[2] maltose,[3] sophorose,[4] laminarabiose,[4]

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cellobiose,[4] and lactose (work in progress). In those studies, the energies of models having the same linkage conformations as the relevant disaccharide crystal structures are within 2 to 3 kcal/mol of the global computational minima. It seems reasonable that such differences can be caused by crystal packing effects and differences in substituent orientations, as well as errors in the modeling program. A dielectric constant (ϵ) of 4.0 was used for all of this work, as it gave good results for the glucopyranose ring,[6] as well as for protein related structures.[7] For sucrose (α-D-glucopyranose-(1↔2)-β-D-fructofuranose) at $\epsilon = 4.0$, however, the agreement was not as good. Of 12 sucrosyl linkages in the crystallographic literature, three have conformational energies more than 3 kcal/mol above the global minimum.[5,8] Of those three structures, raffinose[9] has the greatest difference, 5.5 kcal/mol. Although this seems high, an energy surface computed by other workers[10] with CHARMM-based software and a dielectric constant of 1.0 also gave an energy for the sucrosyl linkage in crystalline raffinose high above the global minimum for sucrose. One possible explanation for these high energies is that intermolecular forces within the crystal are large enough to change the torsional angles found for isolated or solvated molecules. Optical rotation and NMR spectroscopy suggest that such modifications occur for maltose at a magnitude of 1-3 kcal/mol.[11] However, recent work with *ab initio* calculations indicated that the 12 crystal structures all fall within 2.5 kcal/mol of the global minimum for the sucrose analogue, tetrahydro-2-[(tetrahydro-2-furanyl)oxy]-2*H*-pyran.[12] Although MM3 has special functions to accommodate anomeric sequences, the effects of overlapping anomeric sequences, present in sucrose, are not well understood and have not been included in the parameterization of MM3. Therefore, another explanation for the high energies of some of the sucrose linkage geometries is that the molecular mechanics calculations are not as accurate as they might be.

In the present work, computational energy maps are generated for β-leucrose and β-turanose (fructopyranosyl form). Our goal was to see if the modeling of other glucose-fructose disaccharides linked through a secondary hydroxyl had problems similar to those of sucrose, bearing in mind that they lack the overlapping anomeric sequences of sucrose. Because both crystal structures have been solved,[13-15] experimental and computed structures can be compared, and we should gain insight on whether the high calculated energies of crystallographically determined disaccharide linkages are peculiar to sucrose.

Leucrose (5-O-α-D-glucopyranosyl-D-fructopyranose) has been isolated as a by-product from the bacterial production of dextran by *Leuconostoc mesenteroides* (NRRL B-512F)[16] and *Streptococcus bovis* [17,18] and has been chemically synthesized.[13,19] Leucrose is also a sweetener.[20] Turanose (3-O-α-D-glucopyranosyl-D-fructose) has been produced by hydrolysis of melezitose.[21] Both carbohydrates have been studied by NMR spectroscopy to determine ring equilibria and anomeric ratios in different solvents and at different temperatures.[22] For turanose, significant concentrations of fructofuranosyl and fructopyranosyl forms are found in water and deuterated dimethyl sulfoxide, while the fructopyranosyl form is dominant in pyridine

(> 80%). A similar equilibrium is not possible for leucrose because the C-5' hydroxyl group forms part of the glycosidic linkage, preventing the formation of furanose rings. In the crystal structures of both compounds, the fructosyl ring is in the $^2C_5$ β-pyranosyl form.

COMPUTATIONAL METHODS

The glycosidic torsional angles are defined as ϕ = H(C-1)–C-1–O-1–C-i and ψ = C-1–O-1–C-i–H(C-i) where i is 5' for leucrose and 3' for turanose. MM3 (Jan. 22, 1990 update)[23,24] was obtained from Technical Utilization Corp., Inc., Powell, OH. The procedure for generating relaxed-residue maps using MM3 has been described elsewhere[2-4] and will only be briefly reviewed here. The non-reducing glucopyranosyl rings were given $^4C_1$ conformations, while the reducing fructopyranosyl rings were placed in $^2C_5$ conformations. Thirty-two combinations of starting orientations of exo-cyclic groups were considered for each map. Included were *gauche-gauche* and *gauche-trans* orientations about the glucopyranosyl C-5–C-6 bond, and the equivalent orientations for the C-1'–C-2' bond of the fructopyranose ring. The secondary hydroxyl groups had either clockwise or reverse-clockwise orientations so as to best form partial crowns of weak hydrogen bonds around each ring. In addition, two orientations of the O-2' hydrogen atom were considered— either *trans* to O-6' or *trans* to C-3'. Initial struc-tures were generated using PC-Model (Serena Software, Bloomington, IN). A dielectric con-stant of 4.0 and the default convergence criterion of 3.6 cal/mol were used for all calculations. The lowest energy value from the 32 optimized points at each ϕ-ψ combination was used to construct the maps. Each map was constructed on a 20° grid-spacing. Isoenergy contour lines were generated using SURFER (Golden Software, Golden, CO). The global minimum for each map was located by unrestrained minimization from the lowest energy grid-point. All starting conformers that contributed a map point within the local region were considered, and the structure with the lowest energy was taken as the global minimum. For comparison with crystal structures, we also reoptimized the global minima, restraining them to have the O-5–C-1–O-1–Ci, ϕ(O-5), and C-1–O-1–C-i–C-$i+1$, ψ(C-$i+1$), values of the crystal structures. This was done because bond lengths of anomeric sequences depend on these torsional angles.

RESULTS AND DISCUSSION

The MM3-generated energy map for leucrose is shown in Fig. 1 with contour lines in increments of 1 kcal/mol. Three low-energy regions are found on this map, two of which are similar in energy and are separated by an energy barrier of less than 2 kcal/mol. The third region is quite high in energy and is separated by an energy barrier greater than 8 kcal/mol. All of these regions are centered around ϕ = -30°. As noted before, this is partly an expression of the exo-anomeric effect.[2-4] The global minimum is located at ϕ and ψ values of -27.6 and
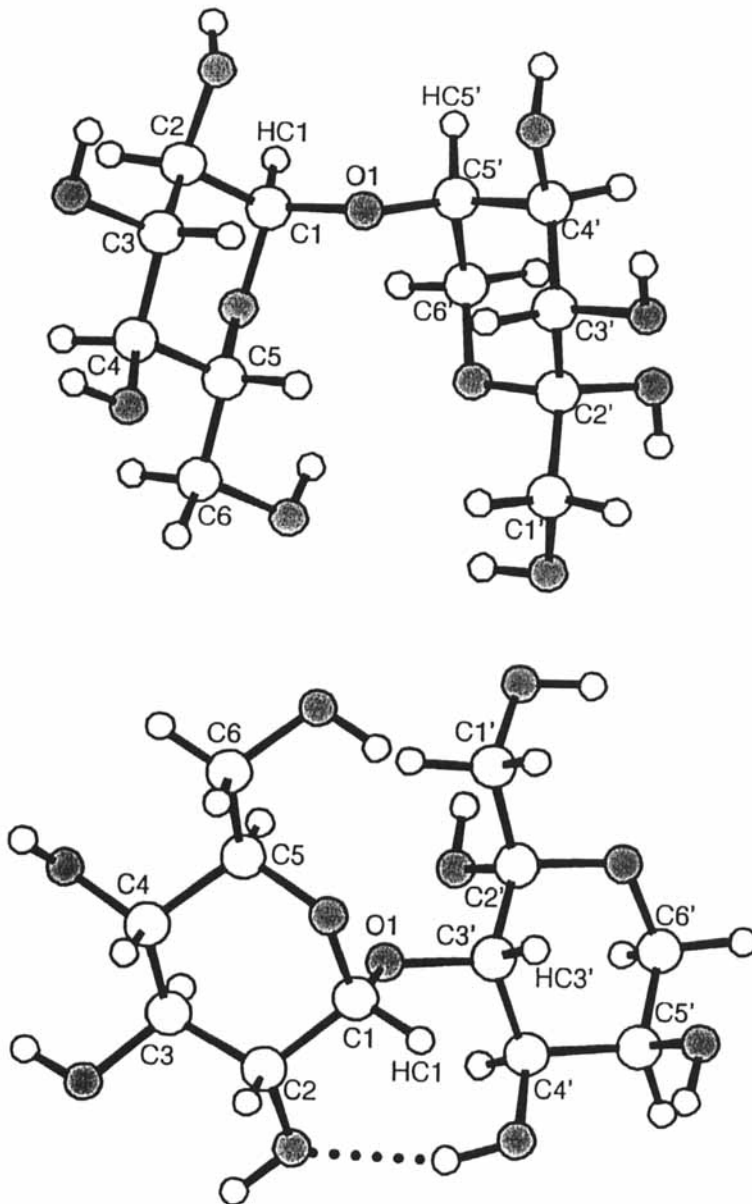
Fig. 1. MM3 - optimized structures of leucrose (upper) and turanose, lower. Oxygen atoms are darkened. The O4'-H--O2 hydrogen bond of turanose is shown, and the carbon and glycosidic linkage atoms are labeled.

Table 1. Selected bond lengths, valence angles, and torsional angles for leucrose.

Bond	MM3 global minimum	MM3 adjusted structure	Crystal structure[a]
	Bond lengths (Å)		
C-1–O-1	1.419	1.418	1.400
C-1–O-5	1.424	1.421	1.430
C-5–O-5	1.427	1.428	1.426
C-2'–O-2'	1.431	1.432	1.392
C-2'–O-6'	1.422	1.423	1.428
C-5'–O-1	1.426	1.428	1.431
C-6'–O-6'	1.422	1.422	1.440
	Valence angles (degrees)		
C-1–O-1–C-5'	116.0	116.5	115.4
C-1–O-5–C-5	114.5	115.3	113.9
O-1–C-1–O-5	110.0	113.7	113.0
C-2'–O-6'–C-6'	114.4	114.4	113.9
	Torsional angles (degrees)		
C-1–O-1–C-5'–H(C-5') (ψ)	60.3	28.8	27.2
C-1–O-1–C-5'–C-6'	-61.6	-93.9	-93.9
C-1–O-1–C-5'–C-4'	179.6	148.6	148.1
C-2–C-1–O-1–C-5'	-147.3	-168.8	-171.1
H(C-1)–C-1–O-1–C-5' (ϕ)	-27.6	-51.6	-51.2
O-5–C-1–O-1–C-5'	93.3	68.8	68.8

[a]Ref. 13.

60.3°, respectively. Also shown in Fig. 1 is the ϕ,ψ pair for crystalline leucrose mono-hydrate.[13] This point is within the central energy well, approximately 2 kcal/mol above the global minimum. Selected geometric properties of the modeled global minimum and the crystal structure are compared in Table 1. The modeled global minimum is depicted in Fig. 3.

Fig. 2 shows the relaxed-residue map of turanose, and Table 2 compares selected geo-metric properties of the best model and the known crystal structures. Only two low-energy regions are found for this disaccharide. Both are again centered near $\phi = -30°$. The global minimum is located at ϕ and ψ values of -19.0 and -23.3°, respectively. The other relatively low-energy minimum is found near $\psi = -160°$ and is approximately 2.7 kcal/mol above the global minimum. The crystal structure has ϕ and ψ values of -20.2 and -8.3°, respectively.[14,15] On the relaxed map these values are within 0.6 kcal/mol of the global minimum. Both the crystal structure and the modeled global minimum have an inter-ring hydrogen bond between O-2 and H(O-4'). The oxygen-to-oxygen distance is 2.93 Å for the modeled structure and 2.88 Å for the crystal. As noted by Angyal,[25] this hydrogen-bond may also stabilize the fructo-pyranose form of turanose in low dielectric constant solvents, which may explain the dominance
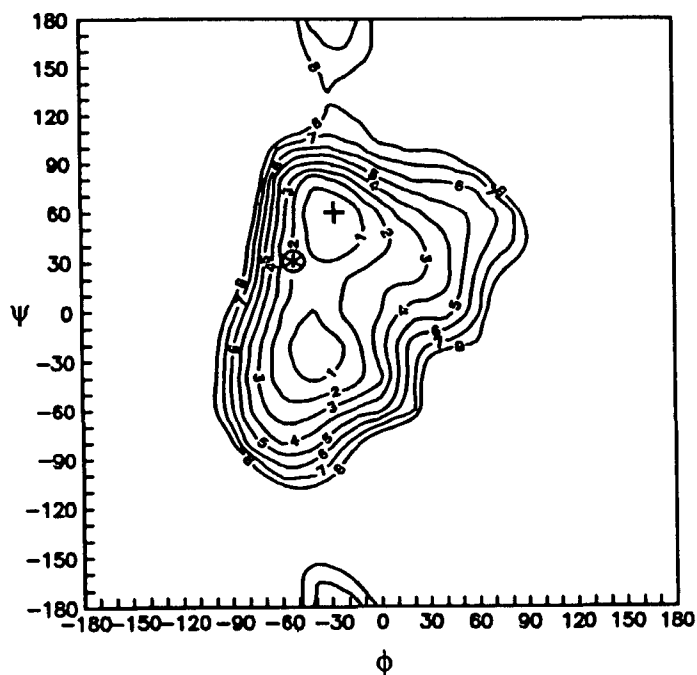
Fig. 2. MM3 Energy surface for leucrose with a dielectric constant of 4. The crystallographically determined conformation is circled and + shows the global minimum..

of this ring form in pyridine (ε = 12.4) but not in dimethyl sulfoxide (ε = 46.7) or water (ε = 78.3).[22] The global minimum structure is shown in Fig. 3.

Both bonds of the α-(1→5) linkage of leucrose are axial to the planes of the respective pyranose rings, and Fig. 2 is similar to the map for the 5-carbo analog of α,α-trehalose, which also has an axial-axial linkage.[2] α,α-Trehalose yields a more restricted MM3 map, possibly because of the overlapping anomeric sequences in the trehaloses.

In general, bond lengths are well reproduced, although the lengths of the anomeric C–O bonds have significant differences (Table 1). For leucrose, the largest difference is the MM3 C-2'–O-2' bond length, which is 0.04 Å too long. The MM3 C-1–O-1 bond length is also long by 0.018 Å. Similar deviations have been observed before for MM3-modeled disaccharides.[3,4] The modeled bond lengths were not substantially affected by reoptimization of the global minima with the observed glycosidic torsional angles. Valence angles were less problematic. The glycosidic valence angle differed by only 1.1°. Wide ranges of glycosidic torsional angles are found for disaccharide crystal structures, and similar deviations between models and experimental results have been reported before.[2-4]

Table 2. Selected bond lengths, valence angles, torsional angles, and intramolecular distances for turanose.

Bond	MM3 global minimum	MM3 adjusted structure	Crystal structure[a]
	Bond lengths (Å)		
C-1—O-1	1.424	1.422	1.416
C-1—O-5	1.422	1.422	1.410
C-5—O-5	1.427	1.427	1.441
C-2'—O-2'	1.431	1.431	1.400
C-2'—O-6'	1.423	1.423	1.422
C-3'—O-1	1.428	1.429	1.441
C-6'—O-6'	1.422	1.421	1.439
	Valence angles (degrees)		
C-1-O-1-C-3'	114.3	114.7	116.0
C-1-O-5-C-5	114.9	115.2	116.1
O-1-C-1-O-5	109.7	111.1	111.0
C-2'-O-6'-C-6'	114.8	115.0	113.8
	Torsional angles (degrees)		
C-1-O-1-C-3'-H(C-3') (ψ)	-23.3	-10.1	-8.3
C-1-O-1-C-3'-C-4'	98.5	111.1	111.2
C-1-O-1-C-3'-C-2'	-142.6	-130.7	-127.8
C-2-C-1-O-1-C-3'	-138.8	-140.3	-139.6
H(C-1)-C-1-O-1-C-3' (ϕ)	-19.0	-21.8	-20.2
O-5-C-1-O-1-C-3'	101.0	99.2	99.2
	Intramolecular distance (Å)		
O-2 ···· O-4'	2.926	2.945	2.884

[a]Refs. 14 and 15.

Turanose has glycosidic linkage bonds that are equatorial relative to the reducing fructo-pyranose ring and axial relative to the nonreducing glucopyranose ring. Maltose has the same linkage characteristics, and its MM3 map[3] is very similar to the turanose map.

Table 2 gives some selected geometric properties of the modeled global minimum, the adjusted (torsional angle) model, and the crystal structure for turanose. As with leucrose, bond lengths are well reproduced except for the anomeric sequences, with the C-2'—O-2' length having the greatest deviation (0.031 Å). MM3 does not treat anomeric C—O—H sequences individually as it does anomeric C—O—C sequences, which may account for the long anomeric carbon-oxygen bond lengths of the reducing residues. Valence angles were well modeled, al-though the glycosidic valence angle was 1.7° less than the crystal value.
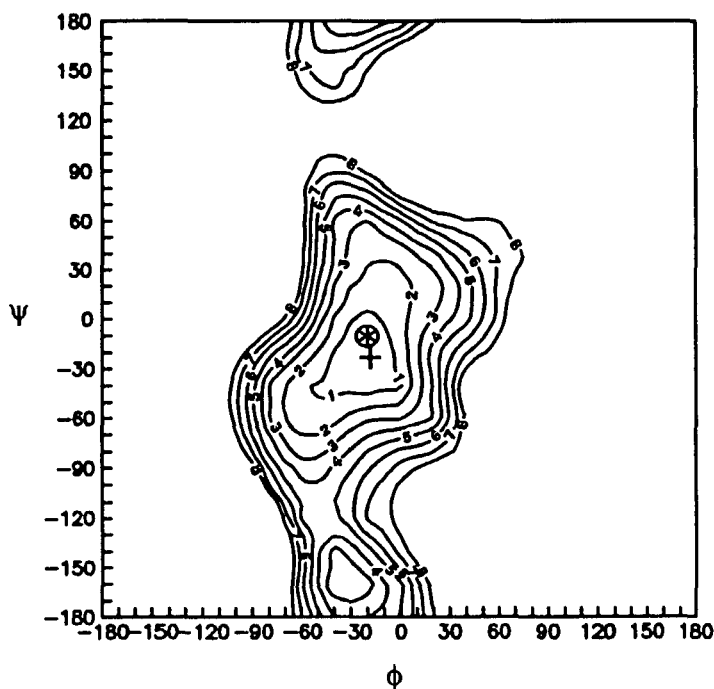
Fig. 3. MM3 Energy surface for turanose with a dielectric constant of 4. The crystallographically determined conformation is circled and + shows the global minimum..

CONCLUSIONS

The molecular mechanics program MM3 has been used to model leucrose and turanose. Relaxed-residue maps for these disaccharides are similar to previously reported maps for disaccharides that have similar types of glycosidic linkages. Crystal structures of these disaccharides are located within the global low-energy wells of the maps. Our results do not contradict the hypothesis that problems with the MM3 energies for sucrosyl linkages arise because of the overlapped anomeric sequences. The systematic lengthening of modeled carbon-oxygen bonds in anomeric C–O–H sequences compared to crystal structures suggests that anomeric correction terms for these sequences should be added to MM3.

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