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Note

Comparisons of rigid and relaxed conformational maps for cellobiose and maltose*

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Despite past statements on the importance of flexible monomeric residues in modeling studies[1–4], such residues have only recently been used to make ϕ,ψ conformational maps for disaccharides. Articles on (*a*) mannose dimers by Homans *et al.*[5], (*b*) cellobiose[6,7], and (*c*) maltose by Tran *et al.*[8] and Ha *et al.*[9] pioneered the use of flexible residues for this purpose. A map based on models having strongly restrained values of ϕ and ψ, but optimized in all other respects, is termed a "relaxed map" or an "adiabatic map", in contrast to "rigid maps" produced with retention of initial internal structures.

Now presented are relaxed ϕ-ψ maps of cellobiose and maltose, calculated in the same way with the same force field[10], MM2, so that the overall effects of the axial linkage bond in maltose can be compared with those arising from the analogous equatorial bond in cellobiose. At the energy limits considered reasonable by their authors, previous ϕ-ψ maps for the various disaccharide linkages with only two bonds were often roughly similar, regardless of linkage type[11]. Rigid maps for the two disaccharides are also presented to show the differences that arise from the use of flexible residues. Also shown are ϕ-ψ combinations found in single-crystal diffraction studies of maltose oligomers, plotted on rigid and relaxed energy maps. Those plots permit comparison of the use of the two types of map as bases for rationalizing observed conformations and for predicting structures that might be observed.

The relaxed maps herein are not completely adiabatic, because different starting orientations of rotating groups were not used. Although still computationally expensive, these maps required relatively less human intervention than the maps in references 8 and 9. Those workers[8,9] considered various orientations of the primary alcohol and secondary hydroxyl groups near all minima. Thus, other combinations

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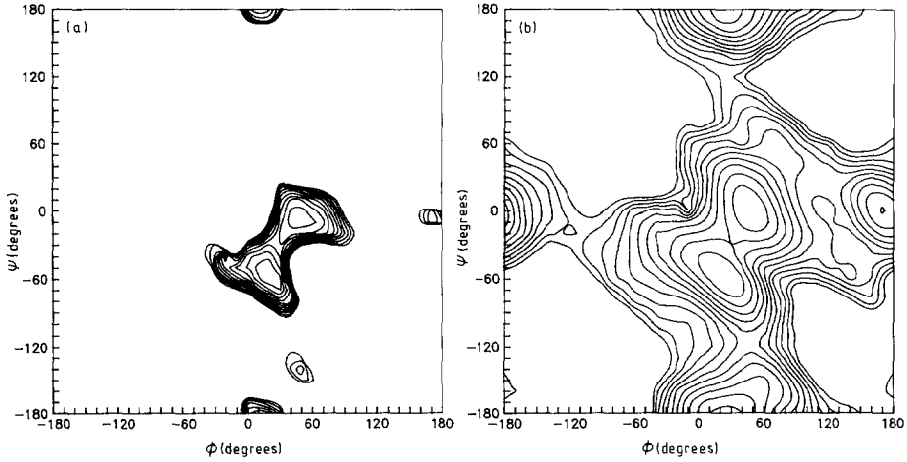

Fig. 1. Rigid (a) and relaxed (b) conformational maps for cellobiose, based on a single starting conformation with O-6 atoms in *gt* orientations. Both maps use a residue optimized with MM2 for the starting geometry. The outer contours are 11 kcal/mol above the minima. ϕ and ψ are the torsion angles H-1–C-1–O-4′–C-4′ and C-1–O-4′–C-4′–H-4′. The two smallest minima on the previously published rigid map[6,7] are artifacts of the contouring algorithm, and result from the irregularly spaced and rapidly changing energy values in this region.

of rotating-group positions will give energy values a few kcal/mol lower in some map locations than are reported on the present maps[6]. Since different force fields yield different energy values, even the most carefully constructed map is not likely to be absolutely accurate.

Solvents, which can influence the conformation at the level of a few kcal/mol, are not included. In one approach for dealing with solvent effects, however, solvation energies are added to the solute energies calculated in isolation[12], and a flexible-residue approach provides an attractive basis for the study of conformations in various solvents.

The rigid and relaxed maps for cellobiose are shown in Fig. 1, contoured at increments of 1 kcal/mol above the global minima. The global minima have calculated energies of 31.3 kcal/mol, while the minimum for maltose is 30.3 kcal/mol. Four major minima on the flexible cellobiose map are within a cross-shaped area wherein possible interconversions among minima are indicated. The two central minima are separated by a barrier of 2 kcal/mol, while the outer minima, with energies 0.5 to 1.5 kcal/mol above the central minima, must be accessed over barriers of ~5 and 6 kcal/mol.

Despite the use of a single starting geometry in these calculations, the overall shape of the relaxed map for maltose, depicted in Fig. 2, is similar to those in references 8 and 9. Fig. 2b has one major and one minor central minimum plus a minimum located 150° away, nearly 4 kcal/mol above the central minimum. The minimum at $\psi = -170°$ is separated from the central minimum by a 9-kcal barrier. Surmounting this barrier corresponds to rotation about the equatorial bond of the maltose linkage.

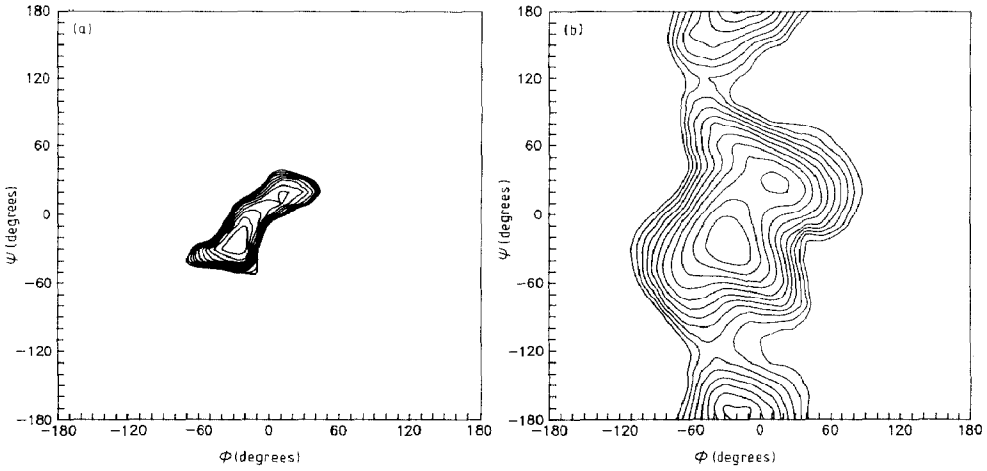

Fig. 2. Rigid (a) and relaxed (b) conformational maps for maltose with O-6 atoms in *gg* orientations. Other details are the same as in Fig. 1. All maps were produced with SURFER for IBM-PC compatibles.

These relaxed maps indicate substantial differences inherent in the axial and equatorial configurations of the linkages. There is greater freedom of motion associated with the diequatorial linkage of cellobiose, as 240° of rotation is available to both bonds of the linkage at a maximum cost of ~5 kcal/mol. This energy is comparable to the values computed for barriers to rotation about the central bond of *n*-butane or for the primary alcohol group of D-glucose. The transition to the second maltose minimum *via* rotation about the equatorial ψ bond would require 4 kcal more than the cellobiose transitions, while rotations about the axial bond comparable to the cellobiose transition encounter barriers of >14 kcal. A small minimum at $\phi = \pm 180°$, $\psi = -30°$ for maltose (not shown) has an energy of ~13 kcal/mol above the global minimum.

While the cellobiose linkage is apparently more flexible than the maltose linkage, contrary to an earlier suggestion[13], cellulose might well be said to be less flexible than amylose[14]. Changes in ϕ and ψ yield small changes in the helical parameters *n* and *h* (number of residues per turn and rise per residue) for cellulose. Larger changes result for amylose, because of the large angles between the C-1–O-1 and C-4–O-4 bond vectors in the α-D-glucose residue.

Fig. 3 shows observed conformations of maltose, related linear oligomers, and two cycloamyloses[13,15–24], plotted on magnified segments of the rigid and relaxed maltose maps. On the rigid map, some energies corresponding to the observed points are quite high, while the maximum energy of any observed point is just over 4 kcal/mol on the relaxed segment. The high energy values on the rigid maps arise in part from the presence of hydroxylic hydrogen atoms, which are usually disregarded in conformational analyses using rigid residues. However, in order to compare the two techniques, it was necessary to include these atoms. (They were also used in the rigid map in ref. 9.) When plotted on a rigid map of

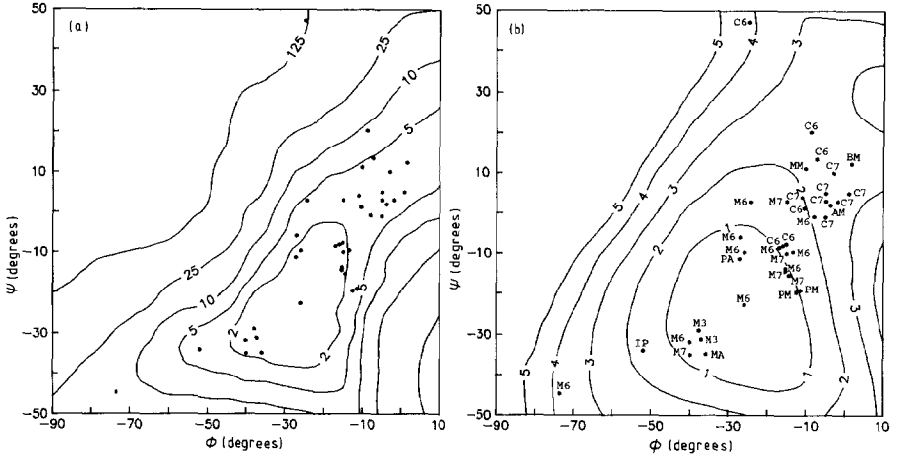

Fig. 3. Orientations of maltose linkages observed in single-crystal studies, superimposed on segments of the rigid (a) and relaxed (b) maps. The energy contours show the differences from the optimal value. Codes for the observed values are: AM, α-maltose[17]; BM, β-maltose[15]; C6, cyclohexaamylose[23]; C7, cycloheptaamylose[24]; IP, 6-iodophenyl α-maltoside[13]; MA, β-maltose octaacetate[18]; MM, methyl β-maltoside[16]; M3, methyl α-maltotrioside[19]; M6, p-nitrophenyl maltohexaoside[22]; M7, maltoheptaose[21]; PA, α-panose[20]; and PM, phenyl α-maltoside[13]. The hydrogen-based torsion angles ϕ and ψ, if not given in the original articles, were computed by subtracting or adding 120° to the analogous torsion angles based on O-5 and C-5′.

maltose, calculated with the PFOS method and no hydroxylic hydrogen atoms[8], one observed structure is positioned >20 kcal/mol above the minimum. According to Fig. 3b, the crystal packing forces appear to affect linkage torsion angles at energies that are comparable in magnitude to hydrogen bonding and van der Waals forces that can be readily expected. Conformations of cellobiose, both from diffraction studies and from modeling studies by other workers, are shown on a relaxed map in ref. 7.

Accumulated experimental data such as those included in Fig. 3 show that a variety of conformations must be anticipated in response to various environments. A similar conformational range has been found for the sucrose linkage[25]. Since any attempt to learn the ranges of allowed conformations will have limits, it is important to set priorities regarding which influences on conformation should be included in the study. Based on the magnitudes of the energy values, monomeric-residue flexibility should be given high priority. Even near minima, residue flexibility is apparently important, since contours 1 kcal above the minima on both relaxed maps contain parts of the 10-kcal contours on their rigid counterparts.

In modeling studies, allowing the atomic coordinates to adjust in response to interactions with their neighbors appears to have the advantages of avoiding high energy values for observed structures and of visualizing feasible transitions. Another advantage is that a choice of internal residue geometry is not required. Tvaroška and Perez[26] have shown that there are substantial variations in rigid conformational maps when coordinates from different crystal structures are used.

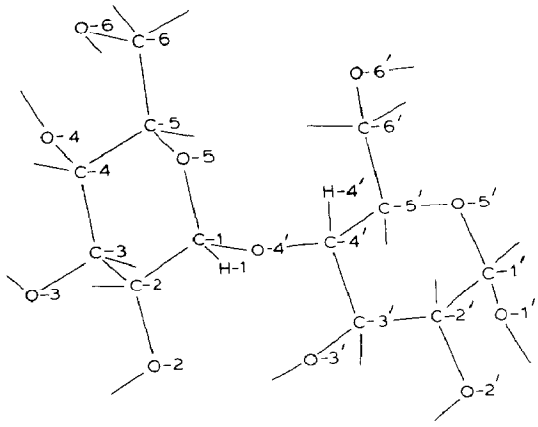

Fig. 4. The starting (and final, optimal) conformation of maltose. The hydroxyl groups have "clockwise" orientations[9]; $\phi = -28.3°$ and $\psi = -28.6°$.

COMPUTATIONAL DETAILS

These calculations were made with the program MM2 (specifically the 1985 version of MMP2, which includes anomeric effects) which was developed by Allinger[10]. The MM2 program minimizes the total potential energy of a computer model of a molecule by allowing all of the details of the internal structure of the model to change, subject to an assumed force field. Previous work[27] had shown the ability of the predecessor of MM2, namely, MMI, to model flexible D-glucose residues. In the present study, however, the forces distorting the D-glucose residue came not from imposed internal constraints but from a second residue, linked through bonds to a common oxygen atom. Details of an optimization of an initially high-energy conformation are given in refs. 6 and 7.

The starting models had minimal energy according to brief preliminary studies using MM2 (Fig. 4 shows the maltose structure, and the cellobiose structure is shown in refs. 6 and 7). The O-6 atoms had *gt* positions (the C-4–C-5–C-6–O-6 torsion angles were 180°) on cellobiose, and *gg* positions (the C-4–C-5–C-6–O-6 torsion angles were +60°) on maltose. Lower energy values can be obtained with O-6 in the *tg* position[9], but, because it is not generally observed, that conformation was not used in these calculations. Single conformations of O-6 and the hydroxyl groups were used in order to save computer time. Hydrogen bonding is not explicitly treated in MM2, but dipole interactions show attractive forces up to 2 kcal when the hydroxyl groups are aligned suitably for hydrogen bonding. The hydroxyl groups formed intraresidue hydrogen bonding networks in the starting residues but, along with all other parts of the structure, were free to assume other orientations when energies were high enough to surmount barriers.

The initial residue coordinates were retained for optimization at each ϕ,ψ combination by preparing a separate input file for each conformation to be optimized. The calculated energies can be considered to result from assembly at each specified conformation rather than from progressive rotations about the linkage bonds. When the starting structure for each optimization was the result from the previous optimization, the energy depended on the origin of the map. This is especially problematic with maltose, as substantial distortion occurs at high-energy conformations. With the approach herein, computational effort can be concentrated in the interesting, low-energy areas. Step sizes were as large as 40° and as small as 10°. For cellobiose, 497 conformations were optimized; 297 conformations were optimized for maltose.

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