Disaccharide Conformational Flexibility. I. An Adiabatic Potential Energy Map for Sucrose

V. H. TRAN* and J. W. BRADY†

Department of Food Science, Cornell University, Ithaca, New York 14853

SYNOPSIS

Constrained conformational energy minimizations have been used to calculate an adiabatic (ϕ, ψ) potential energy surface for the disaccharide sucrose. The inclusion of molecular flexibility in the conformational energy analysis of this disaccharide was found to have a significant effect upon the allowed conformational space of the molecule. Three low-energy regions were identified on the adiabatic energy surface, and two of these regions were found to contain two related local minimum-energy conformations, with similar energies, differing only in the directionality of the intra-residue hydrogen bonds of the glucose portion of the molecule. The known crystal structures of seven molecules containing the sucrose moiety all fall within the region of the primary allowed minimum and are consistent with the relaxed energy map, while these crystal conformations could not be rationalized using energy maps for rigid residue geometries. The greater flexibility of the furanoid ring relative to that of the pyranoid ring contributed significantly to the enlargement of the low-energy region on the adiabatic map. However, in spite of the importance of limited flexibility in understanding the conformation and fluctuations of sucrose, this molecule was found to be considerably more rigid than some other disaccharides, such as maltose and cellobiose, in accord with experimental studies.

INTRODUCTION

The conformational preferences of the various disaccharides have long been the subject of theoretical study due to the role that these molecules play as simple models for polysaccharide linkages.¹⁻³ Most such studies have employed molecular mechanics conformational energy calculations, the analysis of structures in terms of empirical force fields, to identify low-energy conformations. In most previous studies, the individual monomer units of polysaccharides have been treated as rigid, so that the search for low-energy structures reduces to the identification of the combinations of

glycosidic torsion angles ϕ and ψ , which give low energies of interaction between the two monomer rings. By examining all possible combinations of ϕ and ψ , it is possible to produce a conformational energy map for the disaccharide within the rigid geometry assumption and locate the global energy minimum.

A number of problems complicate the use of such conformational energy maps to understand polysaccharide conformation. For example, while pyranoid rings are reasonably stiff, they are not completely rigid.⁴⁻⁸ As a disaccharide molecule undergoes rotations about the glycosidic torsional angles, the other degrees of internal freedom in the molecule, such as valence bond angles, ring torsional angles, and hydroxyl orientations, may "relax" or adjust themselves in a continuous fashion to the new conformation, lowering the total conformational energy in the process.⁹ Because the accumulation of several small changes in these internal coordinates may often avoid significant steric conflicts, these "relaxed" maps generally lead to non-

© 1990 John Wiley & Sons, Inc.

CCC 0006-3525/90/050961-16 \$04.00

Biopolymers, Vol. 29, 961-976 (1990)

* Permanent address: Laboratoire de Physicochimie des Macromolécules, Institut National de la Recherche Agronomique, B. P. 527, 44026 Nantes Cedex 03, France.

† To whom correspondence should be addressed.

isomorphic enlargements of the thermally accessible conformational space.^{10,11} Another problem of the static, rigid geometry approach is that it ignores the possible role of conformational fluctuations and equilibria between two or more structures of similar energy. Physical properties such as structure, of course, are actually determined by the system free energy, rather than vacuum potential energy, and this will include conformational averaging of structural fluctuations and equilibria between multiple structures,¹² as well as contributions from the solvent.

In order to include flexibility in conformational energy calculations, a relaxed or adiabatic conformational energy map for a disaccharide can be prepared in which all of the strains in the other degrees of freedom of the molecule are relaxed by energy minimization at each point in the (ϕ, ψ) space while keeping these torsional angles fixed. Such an adiabatic map would be a more realistic approximation to the actual energy surface that the molecule experienced during the course of a conformational fluctuation as the internal coordinates relaxed dynamically in response to the changes in ϕ and ψ . The effects of conformational entropy and major conformational transitions can also be treated through the use of molecular dynamics simulations of the actual trajectories followed by disaccharide molecules during the course of normal structural fluctuations, and the solvent contribution can be included in simulations through the explicit inclusion of water molecules. Molecular dynamics simulations have already been applied to several carbohydrate systems^{7,8,13,14} and their interpretation will require more refined potential en-

ergy surfaces than are produced by treatment of all other internal variables in the carbohydrate as fixed.

Sucrose, although not a component of larger polysaccharides, is a particularly interesting example of this class of disaccharide molecules. Sucrose (Fig. 1) is a dimer with an $\alpha[1 \rightarrow 2]$ -linkage between a pyranoid glucose ring and a β -furanoid fructose ring. This important molecule is well characterized experimentally, with its crystal structure determined by both neutron and x-ray diffraction,^{15,16} and there have been several nmr studies of its conformational behavior in aqueous solution¹⁷⁻¹⁹ and in $[^2\text{H}_6]\text{Me}_2\text{SO}$ (DMSO) solution.^{20,21} Raman and diffraction experiments have been interpreted as indicating that sucrose contains no intramolecular hydrogen bonds in dilute aqueous solution,²²⁻²⁴ but more recent nmr work has indicated that sucrose is fairly rigid and maintains a conformation in aqueous solution close to that in the crystal, with an intramolecular hydrogen bond between the two rings,^{17,18} while in DMSO solution it may adopt another hydrogen-bond network.²⁰ As the five-membered ring is considered more flexible than the six-membered one,¹⁷ this molecule is also an interesting test for the interplay of conformational flexibility within and between two ring systems. In this paper we report the calculation of an adiabatic conformational energy map for sucrose, and in an accompanying paper²⁵ we discuss the results of vacuum molecular dynamics simulations of sucrose and illustrate the relationship between the adiabatic energy surface and the dynamic fluctuations and possible conformational transitions of the molecule.

Figure 1. Schematic representation of the sucrose molecule, indicating the labeling of the atoms and selected torsional angles.

PROCEDURES

In the calculations reported here, the potential energy function used was that of the general molecular mechanics program CHARMM,²⁶ which has the form

$$V(q) = \sum k_b (b - b_0)^2 + \sum k_\theta (\theta - \theta_0)^2 \\ + \sum k_\phi [1 + \cos(n\phi - \delta)] \quad (1) \\ + \sum (A_{ij}/r_{ij}^{12} - B_{ij}/r_{ij}^6 + q_i q_j / r_{ij})$$

containing terms to describe bond stretching, valence angle bending, hindered torsional rotations, van der Waals interactions, and coulombic interactions between the partial charges of the individual atoms. The force constants, equilibrium bond lengths and angles, and partial atomic charges were those developed recently for use in carbohydrates,²⁷ with the charges selected to be appropriate for condensed phase (solution and crystal) systems. It has been shown that with appropriate charges and van der Waals radii, hydrogen bonding can be represented adequately by coulombic interactions,²⁸ and thus no special hydrogen-bonding term was included in the potential energy function. These larger charges may tend to overemphasize hydrogen bonding when used in vacuum calculations, as in the present study.

Using this potential energy function, contour maps were prepared for the variation of the total molecular energy with the glycosidic torsion angles ϕ and ψ , defined as H1g-C1g-O1-C2f and C1g-O1-C2f-O5f (see Fig. 1 for the definitions of the atomic numbering scheme). Such a map could be prepared by rotating the rigid residues from the crystal geometry of sucrose to each combination of ϕ and ψ on a suitable grid and evaluating the energy,²⁹⁻³¹ which would consist only of van der Waals and electrostatic terms and the two torsional contributions from the hindered rotations about the glycosidic torsion angles. However, for sucrose, the low-energy region in (ϕ, ψ) space is quite small if flexibility is not taken into account, with the only allowed conformations lying very close to the crystallographic position.^{17,32} Sucrose is of course not completely rigid, and in the actual molecule, larger regions of conformation space are dynamically sampled by the molecule. In the present study, in order to examine this flexibility, an adiabatic map⁹ for sucrose was produced by allowing the molecular structure to relax to give the lowest energy confor-

mation corresponding to each combination of glycosidic angles.¹⁰

In preparing this map, points were selected at regular 20° intervals on a grid in (ϕ, ψ) space, and all other internal parameters were allowed to relax to convergence by application of energy minimization while the glycosidic torsion angles were constrained to remain at the selected grid point.^{9,26} All the points were minimized in the same way, using the CHARMM program, by first performing five steps of steepest descent minimization, followed by conjugate gradient minimization until the rms gradient for the molecule had fallen to below 0.1 kcal/mole-Å.²⁶ To reduce the possibility of the initial configuration for each minimization produced by rigidly rotating the glycosidic torsions to the selected grid point being a spurious, artificial geometry, each point has been minimized from the closest neighboring one already determined by minimization, 20° distant in either ϕ or ψ in a stepped, radial-like path from the nearest local minimum. This procedure avoids excessively high starting energies that could lead to spurious results, but cannot be totally automated and must be in some sense iterative, since an initial search must be made for likely positions of the major minima.

In the complex conformational hyperspace of a large molecule like sucrose, there are for each value of ϕ and ψ many local minima in the total energy surface (as a function of all internal coordinates) corresponding to various arrangements of the hydroxyl groups, furanoid pseudo-rotation angles,³³⁻³⁷ and orientations of the exocyclic hydroxymethyl groups.²⁹ Due to this multiple minimum problem, there is no guarantee that a minimization beginning from any given arbitrary starting point will actually produce the lowest energy minimum for that choice of ϕ and ψ . To be certain that the lowest energy structure had been located, starting points at every possible combination of these various degrees of freedom would need to be minimized, a task that was not practical for sucrose with the computer resources available. However, the topological details of the regions near the minima identified as the study proceeded were verified by minimizing all likely geometries, a procedure that was impractical for the entire map. Unfortunately, in the present study the great number of important internal parameters precluded a systematic study of the entire (ϕ, ψ) space and the final map was obtained in separate stages.

As a first step, using the crystallographic conformation as a starting point,¹⁵ an initial relaxed map was generated using the approximately radial ex-

ploration method described above. This map clearly showed that no realistic low-energy conformations can exist outside a column in conformation space limited by the values of ϕ of $(-100^\circ, +100^\circ)$. Inside this zone three important low-energy regions were found, approximately centered on the ϕ and ψ values $(0^\circ, -60^\circ)$, $(-20^\circ, 180^\circ)$, and $(20^\circ, 60^\circ)$, named A, B, and C respectively in what follows, with the A region including the crystallographic point. Few of the energies at any of the grid points calculated in this initial map subsequently proved to be the actual lowest possible energy at that combination of ϕ and ψ (due to the many other degrees of freedom involved), but in the subsequent refinement of the surface, neither the general topology or the number of principal low-energy regions changed. These low-energy conformers in the two-dimensional (ϕ, ψ) space were further investigated by arbitrarily setting the other important degrees of freedom (exocyclic torsional angles and hydroxyl orientations) to different values to produce new starting points for minimization at the same (ϕ, ψ) grid point. The lowest energy structures at each grid point produced in this fashion were then used as starting points for a new mapping of their adjacent regions using the radial-like search procedure from each newly identified local minimum. In this way the global energy minimum conformation and the other principal low energy conformers were identified.

These minimum energy structures were used to explore the adjacent region of (ϕ, ψ) space based on the assumption that nearby points might be similar in terms of the arrangements of the many other internal coordinates. Unfortunately, due to the complexity of the problem, this assumption of local structural continuity often was not true, and it was frequently necessary to minimize a number of different starting conformations at each (ϕ, ψ) grid point to identify the lowest one. The amount of additional searching done for each point was arbitrary, and in part was determined by how well the first minimized conformation found for a particular grid point satisfied several continuity criteria. The first of these criteria suggested that the energy should increase smoothly and continuously as one moved away from the tentatively identified local minimum energy point; that is, potential barrier regions between wells in (ϕ, ψ) space received particular attention, as did any structure that did not seem to "fit" into its surroundings. Another continuity criterion was based upon the pseudorotation phase^{33,35} of the fructose ring as defined by the Cremer-Pople pucker parameter³⁶ ϕ_2 .

Molecular dynamics simulations performed on α -D-glucose have shown that while the pyranose ring cannot be considered as rigid, it remains, under normal conditions, in the 4C_1 conformation.⁷ However, the furanoid ring is somewhat more flexible,³⁸ and more readily undergoes deformations in response to its environment, necessitating care in the minimizations by introducing still further conformational possibilities that required the investigation of many additional alternate starting structures, particularly in the regions of crossover from one five-membered ring conformation to another. The final continuity criterion involved the hydrogen-bonding pattern, as measured by the hydrogen-bond distances. In particular, when changes in ϕ and ψ resulted in the breaking of a hydrogen bond, a manual search was made for conformers containing possible alternate hydrogen-bond partners. During this rather tedious "manual" stage of the search around each of the principal local minima, a substantial proportion of the initially calculated points were replaced by other conformers subsequently found to be of lower energy.

The directionality of the hydrogen bond considerably increases the risks of false minima in these minimization studies. In the absence of crystal neighbors or solvent hydrogen-bond partners, the hydroxyl groups of the six-membered ring upon minimization tend to form intramolecular hydrogen bonds with the adjacent hydroxyl groups of the same monomer unit. Christofides and Davies have deduced such an intramolecular hydrogen-bond network from their 1H -nmr experiments in DMSO solution.^{20,21} To avoid steric clashes in these networks, the hydrogen bonds must all "point" in the same direction around the circumference of the pyranoid ring, producing another "ring" of hydrogen bonds (see Figs. 2, 3, 6, 7, and 10). This hydrogen-bond ring lies in the approximate "plane" of the six-membered chair itself with all of the hydroxyl-bond vectors pointing in the same direction, either clockwise or counterclockwise around the outer rim of the pyranoid ring. This geometry is probably not representative of aqueous solution and may be due to the absence of alternate hydrogen-bond partners. However, for one of these hydrogen bonds to change directions in vacuum by switching acceptor partner, there must be a concerted shift of the entire ring to avoid steric clashes, producing a large activation energy as all of the hydrogen bonds are broken roughly simultaneously. For this reason, during the minimization the algorithm works as if it has a memory of the starting hydrogen-bonding system and this pattern

will be retained into regions of glycosidic conformation space in which another pattern might be of lower energy, since the initial breaking of the bonds involves an increase in energy, regardless of the gain that might be achieved upon hydrogen bonding to another partner. This problem proved troublesome in regions of (ϕ, ψ) space where two hydrogen bonds involving the same atom are in competition or in regions corresponding to the end of one global hydrogen-bonding system and the beginning of another. The practical way to search for the lowest energy conformation of each grid point in such regions was to minimize from both directions and select the lowest energy solution. It should be remembered that this problem may be largely artifactual, in that it applies only to the molecule in vacuo with this potential energy function, and would not be present in aqueous solution.³⁹

There are also several possible combinations of orientations for the primary hydroxyl groups in the two sugar rings. In this paper, the *gauche-trans* (GT), *gauche-gauche* (GG), and *trans-gauche* (TG) terminology^{29,40} will be used to designate the conformation of the glucose exocyclic hydroxymethyl group, with the first term referring to the dihedral O5g-C5g-C6g-O6g and the second to the dihedral C4g-C5g-C6g-O6g. This terminology has been extended to the primary hydroxyl groups of the fructose residue, where the first term refers either to dihedrals O5f-C2f-C1f-O1f or O5f-C5f-C6f-O6f, and the last term refers either to dihedrals C3f-C2f-C1f-O1f or C4f-C5f-C6f-O6f. In the initial minimizations of the crystal structure, rotated to any (ϕ, ψ) grid point, the conformation of the primary hydroxyl group of the glucose residue was always found to be of the GG type, which corresponds to the conformation in the crystalline lattice of sucrose, since a substantial barrier must be surmounted before the system can reorient to a more favorable arrangement. However, in vacuum dynamics simulations in the absence of crystal neighbors,²⁵ transitions occurred from the initial form (GG) to the other two (GT and TG). Since the aim of an adiabatic map is to represent the lowest energy at each point of the grid, all of the initially identified minima were compared to the corresponding minimized solutions with GT and TG forms for this exocyclic group. In all cases, the TG orientation was preferred by about 3 kcal/mol compared to the GG orientation. This preference is due to the fact that the TG orientation of the glucose residue allows the formation of an intramolecular OH4g...OH6g

hydrogen bond. These alternate orientations were found not to produce significant shifts of ϕ and ψ values. Thus, in the final refinement of the adiabatic surface the minimizations were repeated with the TG geometry to produce the final "relaxed" map.

As described below, the two possible pyranoid intramolecular hydrogen-bond patterns, clockwise and counterclockwise, result in two different surfaces for both the A and B regions in (ϕ, ψ) space, while the loss of one hydrogen bond for the clockwise pattern results in only one possible low-energy orientation for the region C. Thus five partial maps were separately calculated from different starting sets of cartesian coordinates with the radial-like generation mentioned above. The final adiabatic map is the synthesis of these partial "relaxed" maps, with each point being the lowest potential energy value for each (ϕ, ψ) point of the grid regardless of from which partial map it comes.

RESULTS AND DISCUSSION

Partial "Relaxed" Maps

Starting Point

Table I lists values for selected internal coordinates of several conformations of sucrose calculated from these studies and compares them to the experimentally determined values for crystalline sucrose¹⁵ given in the first column. Values for relative potential energies, glycosidic dihedral and valence angles, and the exocyclic torsion angles of the hydroxymethyl and hydroxyl groups are included. The hydrogen-bond distances found in these conformations are also given. The puckering of the furanoid ring is specified by the Cremer-Pople pucker parameters ϕ_2 and q_2 for each conformation.³⁶ The pyranoid ring in the crystal structure is in the ⁴C₁ conformation, and since this conformation was retained in all of the other conformations studied, the pucker parameters for this ring are not listed in Table I.

From the crystallographic conformation, a minimization without any constraints on the glycosidic angles lowered the molecular energy by 21.97 kcal/mol. The resulting conformation (not reported in Table I) is located in the same region of conformation space with $\phi = -2.32$ and $\psi = -57.14$. The large decrease in energy is essentially due to the reorientation of the secondary hydroxyl groups. In the crystal structure,¹⁵ the atoms H'2g, H'3g, H'3f, and H'4f participate in intermolecular

Table I Summary of the Relative Potential Energies and Selected Internal Coordinates Calculated for the Five Local Minimum Energy Structures of Sucrose Identified for the Present Potential Energy Function, and the Corresponding Values for the Crystal Structure; Also Listed are the Cremer-Pople Pucker Parameters for the Fructose Ring and the Hydrogen-Oxygen Distances for the Interresidue Hydrogen Bonds Found in These Structures.

	Crystal	S1	S2	S3	S4	S5
Potential energy (kcal/mol)	30.5	0.0	1.0	2.8	2.5	2.7
$\phi^H = [H1g-C1g-O1-C2f]$	-8.03	0.21	-1.03	-16.61	-45.36	14.50
$\psi^O = [C1g-O1-C2f-O5f]$	-44.75	-59.55	-58.45	-167.72	168.24	54.30
$\psi^C = [C1g-O1-C2f-C1f]$	73.70	65.78	65.54	-47.12	-71.76	172.47
$\theta = [C1g-O1-C2f]$	114.30	116.57	117.15	118.52	121.15	117.44
$\chi_{2g} = [C1g-C2g-O2g-H'2g]$	93.27	-44.79	-165.02	-160.64	-51.70	-37.73
$\chi_{3g} = [C2g-C3g-O3g-H'3g]$	-91.44	-38.12	168.34	169.89	-40.66	-40.92
$\chi_{4g} = [C3g-C4g-O4g-H'4g]$	47.88	42.33	-159.02	-157.56	42.12	42.76
$\chi_{6g} = [O5g-C5g-C6g-O6g]$	-56.42	174.39	-179.78	177.09	170.31	170.22
$\omega_{6g} = [C5g-C6g-O6g-H'6g]$	88.77	35.89	-159.48	-169.10	42.10	40.82
$\chi_{1f} = [O5f-C2f-C1f-O1f]$	171.37	-176.47	-179.94	-157.14	-163.15	-172.60
$\chi_{1f} = [C2f-C1f-O1f-H'1f]$	-67.55	40.30	-74.92	21.12	-72.96	-44.95
$\chi_{3f} = [C2f-C3f-O3f-H'3f]$	67.01	42.23	-41.07	64.73	-58.89	-3.37
$\chi_{4f} = [C3f-C4f-O4f-H'4f]$	134.31	60.68	56.40	56.99	55.18	59.85
$\omega_{6f} = [O5f-C5f-C6f-O6f]$	-69.55	-62.65	-59.72	-60.16	-53.08	-55.98
$\chi_{6f} = [C5f-C6f-O6f-H'6f]$	59.46	50.64	38.84	38.38	44.77	75.06
q_2	0.353	0.362	0.389	0.399	0.443	0.249
ϕ_2	265.2	254.75	257.25	245.22	242.82	298.64
$d[O1-H'6f]$				1.983 Å	1.973 Å	
$d[O5g-H'1f]$					1.925 Å	
$d[O5g-H'6f]$	1.895 Å	1.983 Å	1.975 Å			
$d[H'2g-O5f]$						1.968 Å
$d[O2g-H'1f]$	1.851 Å		1.926 Å			
$d[H'2g-O1f]$		1.905 Å				
$d[O2g-H'3f]$				1.896 Å		
$d[O2g-H'6f]$						1.933 Å
$d[H'2g-O6f]$					1.963 Å	

hydrogen bonds in the crystal lattice. Thus, in this vacuum calculation, the dihedrals specifying the positions of these atoms deviated significantly from their crystallographic values in order to find alternate hydrogen-bond partners. Except for the dihedral χ_{2g} (deviation = 0.9°), the mean absolute shift of the other dihedrals is 74.2° . For the atom H'4g, which is not involved in any intermolecular linkage in the crystal, the dihedral χ_{4g} has not significantly changed (deviation = 0.3°). The primary hydroxymethyl groups of the glucose and fructose residues remained nearly in the same conformations as in the crystal: GG for the glucose group, and TG and GG for the C1 and C6 hydroxyl groups of the fructose, respectively. The two intramolecular hydrogen bonds present in the crystal structure ($O5g \cdots H'6f$ and $O2g \cdots H'1f$) are not significantly affected by this minimization.

In the preliminary search of the sucrose conformational energy on a regular 20° grid in (ϕ, ψ) space, the minimized energy of the structure constrained to remain at $(0, -60^\circ)$, the closest grid point to the crystallographic conformation, was calculated and found to be significantly (> 8 kcal/mole) lower than the energy of the crystallographic structure minimized without constraints. This lower energy resulted because the constrained minimization produced a complete reorientation of the lateral hydroxyl groups of the glucose ring, especially for the dihedrals χ_{2g} (93.3° to -43.3°) and χ_{1f} (-67.5° to 41.7°). Since the general conformations of the hydroxymethyl groups were not modified, the major difference between the two minimized conformations was the orientation of the hydrogen bond involving the oxygens O2g and O1f and in the orientations of the other glucose hy-

droxyl hydrogen atoms. In the first minimized structure, as in the crystallographic case, the hydrogen bond $O2g \cdots H'1f$ was observed, but in the second minimized structure, the inverse system $H'2g \cdots O1f$ was present. This result illustrates the importance of the hydrogen-bond orientations in vacuum calculations and the great complexity of the conformational energy map for this molecule. This preliminary reconnaissance of the conformational energy surface delineated three main low-energy regions (designated A, B, and C as described above), as well as demonstrating the need for more exhaustive searches from a variety of minimization starting points.

Region A

Since as a result of the multiple minimum problem, a minor perturbation of the molecular system such as imposing the grid constraint was sufficient to produce an important change in the conformation of this molecule and a significant decrease in the energy, it was necessary to characterize all possible major conformational types in this region. Thus several additional minimizations were carried out with different starting conformations corresponding to alternate possible orientations of the hydroxyl groups, both with and without the grid constraints.

Region A has two different families of conformations of low energy, essentially characterized by the orientations of the O-H groups, having either the $H'2g \cdots O1f$ or $O2g \cdots H'1f$ hydrogen bond as part of the intramolecular hydrogen-bond rings described above. Specifically, the conformation of lower energy has the $O2g-H'2g$ bond pointed toward the fructose residue (i.e., in the $H'2g \cdots O1f$ hydrogen bond), and the other hydroxyls of the glucose residue are all approximately in the equatorial plane and have the same orientation as the $O2g-H'2g$ bond vector. This hydroxyl arrangement and $O2g \cdots O1f$ hydrogen-bond orientation is opposite to that of the crystal structure. The other conformation, similar to that in the crystal structure for the $O2g-H'2g$ orientation and 1.0 kcal/mol higher in calculated energy, has the $O1f-H'1f$ bond pointed toward the pyranose rings ($O2g \cdots H'1f$ hydrogen bond) and all the hydroxyls of the glucose residue also point in the same general direction around the circumference of the ring. This orientation is the inverse of the preceding case and corresponds to a better orientation of the lateral groups in the absence of those intermolecular hydrogen bonds to neighboring molecules found in the crystal form. When looking at the glucose ring

Figure 2. Molecular representation of the stable conformation S1 with interresidue hydrogen bonds indicated by dashed lines.

Figure 3. Molecular representation of the stable conformation S2 with interresidue hydrogen bonds indicated by dashed lines.

from the direction perpendicular to the mean plane of the ring such that the carbon-identifying numbers are increasing in the clockwise direction, these two hydrogen-bond arrangements correspond to the hydroxyl groups of the glucose all approximately pointing in either the counterclockwise or clockwise directions (see Figs. 2 and 3). These two different hydrogen-bond patterns will be referred to as S1 and S2, respectively, in what follows and the minimized structures with these two different patterns will be referred to as the S1 and S2 conformations. These two patterns both refer to the "A" region of glycosidic conformational space. It should be noted that the S1 conformation could not be obtained directly from the crystallographic form by simple minimization due to the multiple minimum problem.

Figure 4. The S1 "partial" relaxed map for sucrose. The star refers to the S1 global minimum and the solid lines represent the iso-energy contours spaced at 2 kcal/mol intervals above the S1 minimum (2, 4, 6, and 8 kcal/mol). The dashed lines ($\perp \perp \perp$) surround the different interresidue hydrogen-bond domains (with a cut-off criterion of 2.05 Å for the $O \cdots H$ distance) with the tic marks on the dashes pointing toward the region where the given hydrogen bond is allowed.

Figures 2 and 3 illustrate these hydrogen-bonding systems and the orientation of all of the individual hydroxyl groups. The depicted conformations correspond to the TG form for the hydroxy-methyl group of the glucose residue. This change in glucose exocyclic orientation for both hydrogen-bonding patterns corresponds to a decrease in energy of approximately 2.5 kcal/mol. Except for this difference, the structural features described for the crystal geometry (with the exception of ϕ and ψ), are also present in the S1 and S2

conformations. Columns 2 and 3 of Table I summarize the main features of these two local minimum energy conformations. As can be noted from Table I, the final energies are similar and the difference (1.0 kcal/mol) is too small to exclude the S2 form.

Throughout the low-energy region A, these two different patterns of hydroxyl dispositions were found at each point on the (ϕ, ψ) grid. While separated by significant barriers, the potential energy differences between these two forms was not large. Furthermore, it is quite possible that the free

Figure 5. The S2 partial relaxed map for sucrose. The star refers to the S2 local minimum and the solid lines represent the iso-energy contours spaced at 2 kcal/mol intervals above the global S1 minimum (2, 4, 6, and 8 kcal/mol). The dashed lines ($\perp \perp \perp$) surround the different interresidue hydrogen-bond domains (with a cut-off criterion of 2.05 Å for the $O \cdots H$ distance) with the tic marks on the dashes pointing toward the region where the given hydrogen bond is allowed.

energy barrier between these two forms would be smaller in solution, or even that neither form actually represents a minimum free energy geometry in solution. Two complete and separate maps of this subregion A were generated, corresponding to these two hydrogen-bonding patterns within the limits ($-100^\circ < \phi < 100^\circ$) and ($120^\circ < \psi < 20^\circ$), as shown by the Figs. 4 and 5. These two separate energy maps of region A, generated from the S1 and S2 minimum energy geometries, will also be referred to as the S1 and S2 "partial" maps. In both cases, the orientations of the hydroxyl groups at each point of the grid were checked to ensure the continuity of the conformations. In the preparation of these maps, the energy barrier between these two forms prevented the spontaneous interconversion of the two cyclic hydrogen-bonding patterns in the minimization, although sufficiently energetic fluctuations could presumably lead to transitions between these two maps in dynamics simulations.

In the S1 ("counterclockwise") map (Fig. 4), the relative iso-energy contours are spaced at 2 kcal/mol intervals above the lowest energy on the 20° grid at $(0^\circ, -60^\circ)$. This geometry was indeed found to be the global minimum energy structure from all the minimizations with a 20° grid constraint, and served as a reference for the relative iso-energy levels (2, 4, 6, and 8 kcal/mol) in further relaxed maps. Thus, for the S2 map (Fig. 5), the slightly higher energy around $(\phi = 0, \psi = -60^\circ)$ is illustrated by the smaller surface inside of the 2 kcal/mol iso-contour, compared to that of the S1 map. The comparison of these iso-energy levels between the S1 and S2 maps shows very similar features, especially near the location of the minima. According to these energy maps, the S1-like conformations have lower energies around the global minimum, but to the left of this area, the S2-like forms seem to be at least as stable as those of the S1 surface.

In Figs. 4 and 5, indications of the regions of (ϕ, ψ) space consistent with the existence of inter-residue hydrogen bonds are superimposed on the iso-energy contours in the two maps. In these maps, the dashed lines surround the zones where the hydrogen bonds exist with the tic marks on the dashes pointing toward the region where the hydrogen bond is allowed. These approximate lines correspond to a cut-off criterion of 2.05 Å, selected simply for the sake of clarity in these figures; a larger cutoff would expand the regions and produce greater overlaps. The resemblance between the two hydrogen-bonding systems is noticeable. On the S1

map, there are three hydrogen bonds: $O5g \cdots H'6f$, $H'2g \cdots O1f$, and $O6g \cdots H'6f$. The presence of two of them in the global minimum area explains the stability of S1 form, and the last hydrogen bond contributes to the stability of the leftmost protuberance of the low-energy region. In the S2 map, there are also three possible linkages ($O5g \cdots H'6f$, $O2g \cdots H'1f$, and $H'6g \cdots O6f$) approximately located in the same areas as in S1 map. However, these hydrogen bonds adopt the inverse orientation, except for the first one, which has no such possibility (see Fig. 3). As in the S1 map, the local minimum zone has two hydrogen bonds and the leftmost extension is stabilized by the third one.

Region B

In the initial exploration of (ϕ, ψ) space, a second region B of low-energy conformations was found near $(-20^\circ, 180^\circ)$. As was the case for region A, there exist two types of conformations for sucrose in this region, again with the glucose hydroxyls running either clockwise or counterclockwise, as defined above. Columns 4 and 5 of Table I summarize the main characteristics of these two minima, named S3 and S4, respectively. Like the low-energy structures in region A, these conformations have the primary hydroxyl of the glucose residue in the TG form. The S3 ($\phi = -16.6^\circ, \psi = -167.7^\circ$) and S4 ($\phi = -45.4^\circ, \psi = 168.2^\circ$) conformations have to be considered as local minima with potential energies higher than the lowest conformer, S1, by approximately 2.5 kcal/mol, but with no significant energy difference between the two.

As shown in Fig. 6, the general counterclockwise orientation of the hydroxyls of the glucose residue

Figure 6. Molecular representation of the stable conformation S4 with interresidue hydrogen bonds indicated by dashed lines.

Figure 7. Molecular representation of the stable conformation S3 with interresidue hydrogen bonds indicated by dashed lines.

for the S4 conformation is the same as in the S1 form with the O2g – H'2g bond pointed toward the fructose residue. Using this criterion, the S4 conformation can be considered as the continuation of the S1 surface into the B region. This result is illustrated by the corresponding dihedral values listed in Table I (mean absolute deviation of χ_{ig} and ω_{ig} between S1 and S4 forms = 4.0°). In the same way, as shown by Fig. 7, the S3 form can be considered as the continuation of the S2 geometry into this region (mean absolute deviation of χ_{ig} and ω_{ig} between S2 and S3 forms = 4.0°). The exocyclic torsion angles for the fructose residue show the inverse behavior. In their hydrogen-bond systems, the S3 and S4 forms have the same O1...H'6f linkage, but the S4 conformation has one additional hydrogen bond: O5g...H'1f. In addition, in the S3 form, the conformation is stabilized by an interring O2g...H'3f hydrogen bond, while in the S4 form, the O6f – H'6f group participates in a stabilizing hydrogen bond (H'2g...O6f).

For region B, minimized geometries for both the S3 and S4 (clockwise and counterclockwise) hydrogen-bond patterns were used as the starting points for two separate contour maps of this subregion with the radial-like generation of the 20° grid used previously. Since these conformations were calculated from local minima more than 2 kcal/mol higher in energy than the global minimum, these maps only depict three relative iso-energy contours, 4, 6, and 8 kcal/mol above the global minimum, the S1 conformer. In spite of the different positions of the local minima, a superposition of these two maps would lead to a significant overlap of the two inner contours, as in the S1 and S2 cases.

Figure 8. The S3 partial relaxed map for sucrose. The star refers to the S3 local minimum and the solid lines represent the iso-energy contours spaced at 2 kcal/mol intervals above the global S1 minimum (4, 6, and 8 kcal/mol). The dashed lines ($\perp \perp \perp$) surround the different interresidue hydrogen-bond domains (with a cut-off criterion of 2.05 Å for the O...H distance) with the tic marks on the dashes pointing toward the region where the given hydrogen bond is allowed.

For both maps, the regions of the space consistent with the formation of all possible hydrogen bonds were plotted superimposed upon the energy contours as before (also with a cut-off definition of 2.05 Å). For the S3 map (Fig. 8), the O1...H'6f hydrogen bond occurs throughout most of the low-energy area and the O2g...H'3f hydrogen bond is also possible for most of the area around and to the right of the minimum energy region. In addition, a third hydrogen bond (O5g...H'1f) also exists in the left and lower part of the map. For the lower-energy S4 map, in which the glucose hydroxyls run counterclockwise, there are four possible hydrogen-bond linkages: O1...H'6f, O5g...H'1f, H'2g...O3f, and H'2g...O6f, as shown in Fig. 9. The intersection of the O1...H'6f and H'2g...O6f regions on the map with this cut-off definition is a thin band within which the local minimum ($-45^\circ, 168^\circ$) is located, corresponding to both bonds being present but significantly stretched. This S4 local minimum also has another hydrogen bond (O5g...H'1f), as noted in Table I.

Region C

Unlike the other regions, only one typical minimum energy geometry was found in the C region, located at $\phi = 14.5^\circ$ and $\psi = 54.3^\circ$, with a potential energy 2.7 kcal/mol higher than the global minimum. The important features of this conformation, named S5, are reported in the last column of Table I. As shown in Fig. 10, the general orientation of the hydroxyls of the glucose residue is

Figure 9. The S4 partial relaxed map for sucrose. The star refers to the S4 local minimum and the solid lines represent the iso-energy contours spaced at 2 kcal/mol intervals above the global S1 minimum (4, 6, and 8 kcal/mol). The dashed lines ($\perp \perp \perp$) surround the different interresidue hydrogen-bond domains (with a cut-off criterion of 2.05 Å for the $O \cdots H$ distance) with the tic marks on the dashes pointing toward the region where the given hydrogen bond is allowed.

Figure 10. Molecular representation of the stable conformation S5 with interresidue hydrogen bonds indicated by dashed lines.

similar to that of the S1 and S4 forms; that is, counterclockwise. This is consistent with the mean absolute change of the χ_{ig} and ω_{ig} dihedrals between S1 and S5 forms (3.9°). In terms of the orientation of the hydroxyl-bond vectors of the fructose residue, this local minimum represents the continuation of the S2 and S4 forms (mean absolute deviation of χ_{if} and ω_{if} between S2 and S5 = 19.7°). As can be seen from Fig. 10, the $O2g - H'2g$ hydroxyl group contributes to the stabilization of the local minimum through two hydrogen bonds. This group is pointed toward the fructose residue and the $H'2g$ atom is the donor in an $H'2g \cdots O5f$ hydrogen bond, while the $O2g$ atom is

Figure 11. The S5 partial relaxed map for sucrose. The star refers to the S5 local minimum and the solid lines represent the iso-energy contours spaced at 2 kcal/mol intervals above the global S1 minimum (4, 6, and 8 kcal/mol). The dashed lines ($\perp \perp \perp$) surround the different interresidue hydrogen-bond domains (with a cut-off criterion of 2.05 Å for the $O \cdots H$ distance) with the tic marks on the dashes pointing toward the region where the given hydrogen bond is allowed.

the acceptor in the other $O2g \cdots H'6f$ linkage. The participation of the $O5f$ atom in this system precludes the existence of the alternate clockwise conformer, as in regions A and B, because the corresponding system would have only one hydrogen bond ($O2g \cdots H'6f$).

From this local minimum, a map was generated (Fig. 11) as for the preceding cases and it was found that the probable zone is significantly smaller than in the other cases, with the 8 kcal/mole contour contained between $(-40^\circ < \phi < 60^\circ)$ and $(0 < \psi < 100^\circ)$. The three relative iso-energy contours (4, 6, and 8 kcal/mol) enclose a compact area inside which only the two hydrogen bonds present in the local minimum energy structure are possible.

Adiabatic Map

Figure 12 depicts the synthesis of the five local maps. In this final map each point of the grid corresponds to the conformation of lowest energy found among all those examined, producing an approximation to the adiabatic map for conformational transitions in sucrose. We emphasize that this map retains the three separate minima A, B, and C found in the preliminary mapping. Thus the steric repulsions, in spite of the relaxation of the internal parameters, are basically preponderant outside these three regions and the subsequent refinements significantly affect only the allowed regions around the minima. Although the S2 mini-

Figure 12. Adiabatic energy map for sucrose. The solid lines represent the iso-energy contours at 2, 4, 6, and 8 kcal/mol above the global S1 minimum. The stars refer to the various minima calculated with the present potential energy function. The solid circles indicate the experimentally determined conformations for sucrose (Su), raffinose (Ra), 1-kestose (1K), 6-kestose (6K), melezitose (Me), planteose (Pl), and stachyose (St).

imum energy conformation is not explicitly a stationary point on this combined map, many S2-like conformations also contribute to the adiabatic map in the left part of region A. The region of the minimum itself and the right-hand portion of the

adiabatic map are mostly from the S1 map. Within the column ($-100^\circ < \phi < 100^\circ$), the 8 kcal/mol contour surrounds more than one-third of this total surface. Only the C region is not contiguous with the others within this contour.

Since the barrier between the A and C regions is greater than 8 kcal/mole, transitions to the C conformational region will be infrequent at moderate temperatures, but even if the C region is discounted, the remaining allowed conformational space encompasses an area far larger than that found in earlier molecular modeling works.^{17,32} With their "HSEA" calculations, Bock and Lemieux found only a very narrow allowed zone around the crystallographic point¹⁷ (variations of 10° and 20° , respectively, in ϕ and ψ). Ferretti et al.³² have obtained a different rigid map by including rotations of the hydroxymethyl groups of the fructofuranose ring. Their most probable region is slightly larger than that found by Bock and Lemieux, while their upper iso-energy lines cannot be interpreted easily because of high energy values. In the present work, the corresponding region A is much broader and two additional allowed regions were located. Furthermore, the energy differences between the global minimum and the higher ones are not sufficiently large as to completely exclude these conformations. These observations demonstrate that small changes in internal parameters within physically acceptable ranges can considerably modify the general features of conformational energy maps.

In Table II we have reported the main conformational features of the seven known crystal structures of oligosaccharides containing the sucrose moiety [α -D-glucopyranosyl (1 $\rightarrow$ 2) β -D-fructofuranose]. Column 1 indicates the five-membered

Table II Summary of the Conformations of the Seven Known Crystal Structures of Oligosaccharides that Contain the Sucrose Moiety.^a

Name	Fructofuranoose Conformation	Cremer-Pople Pucker Parameters		Glcpl-2Ff Linkage Parameters			Reference
		ϕ_2	q_2	ϕ_H	ψ^0	θ	
Sucrose: Glcpl-2Ff	$^3T^4$	265.2	0.353	-8	-45	114.3	15
Raffinose: Galpl-6Glcpl-2Ff	4T_3	274.3	0.397	-36	11	122.1	42
Planteose: Glcpl-2Ff6-1Galp	$^3T^4$	265.7	0.387	-11	-27	118.9	43
Melezitose: Glcpl-2Ff3-1Glcpl	4T_3	275.7	0.388	-21	-31	119.3	44
Stachyose: Galpl-6Galpl-6Glcpl-2Ff	4T_3	272.0	0.410	-10	-48	117.7	45
1-Kestose: Glcpl-2Ff1-2Ff	$^4E - ^4T^3$	100.6	0.298	-32	-66	119.4	34
	3E	254.4	0.416				
	4T_3	272.9	0.422	-28	-55	121.3	
6-Kestose: Glcpl-2Ff1-6Ff	3E	252.3	0.348				32

^aSee text for an explanation of the conformational designations and pucker parameters.

ring conformation with the corresponding numbering of fructofuranose,³⁷ and columns 2 and 3 give the pucker parameters (ϕ_2 and q_2) calculated by the Cremer-Pople algorithm. In order to facilitate the description of the conformations for five-membered rings, on Fig. 13 we have drawn two corresponding conformational wheels, one referring to the accepted numbering of fructofuranose^{34,37} and the other referring to the numbering of ribose.³³ We have also reported in Fig. 13 the phase angle values calculated according to the Altona-Sundaralingam and Cremer-Pople algorithms. In all that follows, we have used the fructofuranose numbering and the Cremer-Pople parameters. All crystal structures mentioned in Table II (except

for 1-kestose) have pseudo-rotation phases ranging from 252 to 276, which corresponds to the conformational region ${}_3E \rightleftharpoons {}^4T_3$. These conformations describe one part of the northern hemisphere, which is also statistically the most populated region for the 4800 ribose conformations observed by Harvey and Prabhakaran.⁴¹ In the 1-kestose case,³⁴ the furanose ring adjacent to the (1 $\rightarrow$ 2)-linkage has a pseudo-rotation phase value equal to 100.6, which corresponds to an intermediate form between ${}_4E$ and ${}_4T$. This conformational point is located in the southern hemisphere, which is a less frequently observed region for ribose.⁴¹ These crystal structures clearly demonstrate the flexibility of the furanose ring.

Figure 13. Conformational wheels for five-membered rings. The inner circle refers to the β -D-fructofuranose numbering scheme (B) and the Cremer-Pople pseudo-rotation phase values. The outer circle refers to the 2-deoxy- β -D-ribose numbering scheme (A) and the Altona-Sundaralingam pseudo-rotation phase values.

The last three columns of Table II give the ϕ^H , ψ^O , and θ parameters for the (1 $\rightarrow$ 2)-linkage of the crystallographic structures. As can be seen from the adiabatic map in Fig. 12, the (ϕ, ψ) points of all of these crystal structures are included within the allowed region A. Six of them are within the 4 kcal/mol contour while only one conformation (raffinose) is located on the outer contour line (8 kcal/mol) of region A. Thus the adiabatic map of sucrose is in good agreement with other crystal forms with this (1 $\rightarrow$ 2)-linkage. Care must be exercised in interpreting this distribution, however, since the vacuum adiabatic energy map was not calculated for crystalline lattices of sucrose.

For sucrose, this enlargement of the probable low-energy area can be due to the cooperative effects of the variation of the glycosidic bond angle, the deformation of the two rings, and the reorientation of the OH bonds. It is difficult to evaluate the contribution of each factor even though some variations seem more important than others. For the glycosidic bond angle θ , the calculated conformations have larger values (116.6° to 121.1°) than the crystallographic form (114.3°), but these values are comparable to those of other crystal structures (Table II, column 6). This can be explained by the general tendency to move the atoms of the two rings in such a way as to diminish the most important van der Waals contacts, in the absence of lattice or neighboring constraints. Although the variations of the endocyclic dihedrals of the glucose residue are not large (this ring always remains very close to the 4C_1 stable form), the rearrangement of the atoms of this ring could contribute significantly to the stabilization of the minima, since a small change in their positions often results in a major shift in the farthest atoms of the lateral groups. For the fructose residue, this phenomenon is magnified by the relative flexibility of the five-membered ring. With this potential, the calculated minima adopt an envelope form for S1 and S2 ($\phi_2 = 254.8^\circ$ and 257.3° , compared to the value 252° for the nearest perfect envelope, ${}_3E$) and intermediate envelope-twist forms for S3 (${}_3T^2$), S4 (${}_3T^2$), and S5 (4T_5) (245.2° , 242.8° , 298.6°). All of these forms lie in the family of easily interconverting forms^{33,37} ${}_3T \rightleftharpoons {}_3E \rightleftharpoons {}_3T \rightleftharpoons {}^4E \rightleftharpoons {}^4T$, which also contains the crystallographic point [ϕ_2 of 262.5° (${}_3T^4$), compared to the pure twist value of 270° (4T)]. These minima belong to the northern hemisphere and no inversion to the opposite family of interconverting conformers (southern hemisphere) occurred during the minimizations. Nevertheless, this hindered ring flexibility with the

${}_3T$ – 4T range seems to have an important influence on the enlargement of the probable conformational area. Finally, the reorientation of the O-H bonds, described above, also significantly contributes to the stabilization of the conformations by the formation of several hydrogen bonds.

CONCLUSIONS

It has long been known that internal flexibility must be considered in analyses of allowed conformational space for polymers, and the role of flexibility in the biological function of proteins and nucleic acids has been extensively investigated. For example, Olson and Sussman³⁸ have noted the importance of valence angles in determining the energy barriers to pseudo-rotation in furanose rings, and Brant and Dimpfl¹² observed the conformational energy dependence of maltose upon the glycosidic valence angle. Recent adiabatic energy maps for maltose have demonstrated that the internal relaxation of a molecule in response to environment can potentially have important physical consequences.

In the case of sucrose, when the monomer units were kept rigidly fixed at their crystallographic bond lengths and angles, but with the glycosidic and exocyclic torsions allowed to vary to avoid steric clashes, the allowed low-energy region in glycosidic (ϕ, ψ) space was restricted to the immediate region of the crystallographic conformation, and was so small that the 6 kcal/mole contour was entirely contained within one $20^\circ \times 20^\circ$ grid square of the present map, a result quite similar to that obtained previously by Bock and Lemieux using rigid mapping.¹⁷ As seen in Fig. 12, however, when molecular flexibility is taken into account, a broader range of conformations becomes possible at moderate temperatures. In the case of sucrose, the effects of this relaxation upon the predicted low-energy structure are not dramatic, but such a relaxed surface is essential to interpret the dynamics of a molecular system, since the relaxed energy surface should be an approximation to the actual surface over which most molecular trajectories move during the course of normal structural fluctuations.

Since the molecule is not rigid, it is difficult to discuss either the structure or dynamics of such a complex system containing a large number of internal coordinates in terms of only two internal coordinates, the glycosidic torsions ϕ and ψ . As a typical structural oscillation in (ϕ, ψ) space occurs,

the remainder of the molecule will dynamically relax to relieve the increasing internal strains as the system moves further away from the energy minimum. If the relaxation rate is fast on the time scale of the conformational excursion, then the adiabatic surface will be a very good approximation to the surface over which the molecule moves. This will generally be the case for bond stretching and bending motion. In some cases, particularly involving torsional changes, reaching the adiabatic surface at any point in (ϕ, ψ) space from an arbitrary starting point may require the activated crossing of a substantial energy barrier, as in the exchange of hydrogen-bond partners, which may not be fast on the trajectory time scale. In such cases, the trajectory may remain on a higher energy local (ϕ, ψ) surface corresponding to its current conformation of the internal coordinate in question, in much the same way that an optically excited molecule can remain on an excited surface above the ground state curve in spectroscopy. The degree to which the observed dynamics will follow the adiabatic surface under these conditions will depend upon the similarity of the adiabatic and local "excited" surfaces. In the present study, S2 is an example of such an excited surface, for the most part lying above the adiabatic surface; in this case the topology of the two surfaces is similar.

The observed broadening of the primary conformational minimum energy region for sucrose is consistent with the nmr results^{17,18} indicating a relatively rigid geometry, since while the region of allowed fluctuations has expanded, the well is still relatively steep and narrow, and no alternate low-energy geometries are nearby for conformational transitions to be significantly frequent, as is the case for maltose^{10,11} and cellobiose.⁴⁶ The primary minimum of the adiabatic map, termed the A region above, was found to contain the crystallographic conformation, with the global minimum energy conformer being quite close to the crystallographic values for ϕ and ψ , but with the opposite donor-acceptor orientation for the O2g...O1f hydrogen bond. Although internal relaxation softens the nonbonded repulsions that make the primary minimum steep and narrow, this effect is somewhat offset by the fact that the simultaneous existence of both the H'2g...O1f and O5g...H'6f intramolecular hydrogen bonds is only possible in a restricted region around this primary minimum (see Fig. 4). Due to the presumed overemphasis of the present potential energy function on intramolecular hydrogen bonding in vacuum, this contribution to the well steepness may be dimin-

ished in aqueous solution, where alternate solvent hydrogen-bond partners may compete successfully for the O5g...H'6f hydrogen bond. The experiments of Bock and Lemieux,¹⁷ confirmed by McCain and Markley,^{18,19} indicate that in aqueous solvent this intramolecular hydrogen bond is absent, while it is present in nonhydrogen-bonding solvents. It would be most interesting to use molecular dynamics simulations of sucrose in aqueous solution to calculate a potential of mean force for conformational transitions in solution, and to determine whether the hydrogen bond between the O2g and O1f hydroxyl groups persists in aqueous solution, as suggested by the nmr results.

The authors thank S. Perez, L. Madsen, A. D. French, S. Ha, and W. E. Reiher for helpful discussions. This work was supported in part by NIH grant number GM34970 and Hatch project NYC 143431, USDA, and by a travel grant to V.T. from INRA.

REFERENCES

1. Rao, V. S. R., Sundararajan, P. R., Ramakrishnan, C. & Ramachandran, G. N. (1967) in *Conformation in Biopolymers*, Vol. 2, Ramachandran, G. N., Ed., Academic Press, London, pp. 721-737.
2. Brant, D. A. (1972) *Ann. Rev. Biophys. Bioeng.* **1**, 369-408.
3. Rees, D. A., Morris, E. R., Thom, D. & Madden, J. K. (1982) in *The Polysaccharides*, Vol. 1, Aspinall, G. O., Ed., Academic Press, New York, pp. 195-290.
4. French, A. D. & Murphy, V. G. (1973) *Carbohydr. Res.* **27**, 391-406.
5. French, A. D. & Murphy, V. G. (1977) *Polymer* **18**, 489-494.
6. Joshi, N. V. & Rao, V. S. R. (1979) *Biopolymers* **18**, 2993-3004.
7. Brady, J. W. (1986) *J. Am. Chem. Soc.* **108**, 8153-8160.
8. Brady, J. W. (1987) *Carbohydr. Res.* **165**, 306-312.
9. Gelin, B. R. & Karplus, M. (1975) *J. Am. Chem. Soc.* **97**, 6996-7006.
10. Ha, S. N., Madsen, L. & Brady, J. W. (1988) *Biopolymers*, **27**, 1927-1952.
11. Tran, V., Buleon, A., Imbert, A. & Perez, S. (1989) *Biopolymers*, **28**, 679-690.
12. Brant, D. A. & Dimpfl, W. L. (1970) *Macromolecules* **3**, 655-664.
13. Prabhakaran, M. & Harvey, S. C. (1987) *Biopolymers* **26**, 1087-1096.
14. Homans, S. W., Pastore, A., Dwek, R. A. & Rademacher, T. W. (1987) *Biochemistry* **26**, 6649-6655.
15. Brown, G. M. & Levy, H. A. (1973) *Acta Crystal B* **29**, 790-797.

16. Hanson, J. C., Sieker, L. C. & Jensen, L. H. (1973) *Acta Crystal* **B29**, 797–808.
17. Bock, K. & Lemieux, R. U. (1982) *Carbohydr. Res.* **100**, 63–74.
18. McCain, D. C. & Markley, J. L. (1986) *Carbohydr. Res.* **152**, 73–80.
19. McCain, D. C. & Markley, J. L. (1986) *J. Am. Chem. Soc.* **108**, 4259–4264.
20. Christofides, J. C. & Davies, D. B. (1985) *J. Chem. Soc. Chem. Commun.* 1533–1534.
21. Davies, D. B. & Christofides, J. C. (1987) *Carbohydr. Res.* **163**, 269–274.
22. Mathlouthi, M. & Luu, D. V. (1980) *Carbohydr. Res.* **81**, 203–212.
23. Mathlouthi, M., Luu, C., Meffroy-Biget, A. M. & Luu, D. V. (1980) *Carbohydr. Res.* **81**, 213–233.
24. Mathlouthi, M. (1981) *Carbohydr. Res.* **91**, 113–123.
25. Tran, V. & Brady, J. W. (1990) *Biopolymers* **29**, 977–997.
26. Brooks, B. R., Bruccoleri, R. E., Olafson, B. D., States, D. J., Swaminathan, S. & Karplus, M. (1983) *J. Comput. Chem.* **4**, 187–217.
27. Ha, S. N., Giammona, A., Field, M. & Brady, J. W. (1988) *Carbohydr. Res.* **180**, 207–221.
28. Reiher, W. E. (1985) Ph.D. thesis, Harvard University, Cambridge.
29. Goebel, C. V., Dimpfl, W. L. & Brant, D. A. (1970) *Macromolecules* **3**, 644–554.
30. Rees, D. A. & Smith, P. J. C. (1975) *J. Chem. Soc. Perkin II*, 836–840.
31. Perez, S., Roux, M., Revol, J. F. & Marchessault, R. H. (1979) *J. Mol. Biol.* **129**, 113–133.
32. Ferretti, V., Bertolasi, V. & Gilli, G. (1984) *Acta Crystal* **C40**, 531–535.
33. Stoddart, J. F. (1971) *Stereochemistry of Carbohydrates*, Wiley, New York.
34. Jeffrey, G. A. & Park, Y. J. (1972) *Acta Cryst.* **B28**, 257–267.
35. Altona, C. & Sundaralingam, M. (1972) *J. Am. Chem. Soc.* **94**, 8205–8212.
36. Cremer, D. & Pople, J. A. (1975) *J. Am. Chem. Soc.* **97**, 1354–1358.
37. French, A. D. (1988) *J. Plant Physiol.*, in press.
38. Olson, W. K. & Sussman, J. I. (1982) *J. Am. Chem. Soc.* **104**, 270–278.
39. Brady, J. W. (1989) *J. Am. Chem. Soc.*, **111**, 5155–5165.
40. Marchessault, R. H. & Perez, S. (1979) *Biopolymers* **18**, 2369–2374.
41. Harvey, S. C. & Prabhakaran, M. (1986) *J. Am. Chem. Soc.* **108**, 6128–6136.
42. Berman, H. M. (1970) *Acta Crystal* **B26**, 290–299.
43. Rohrer, D. C. (1972) *Acta Crystal* **B28**, 425–433.
44. Avenel, D., Neuman, A. & Gillier-Pauraud, M. (1976) *Acta Crystal* **B32**, 2598–2605.
45. Gilardi, R. & Flippen-Anderson, J. L. (1987) *Acta Crystal* **C43**, 806–808.
46. French, A. D. (1988) *Biopolymers*, in press.

Received October 3, 1988

Accepted March 22, 1989