Crystal and Molecular Structure of Maltose Monohydrate[1a]

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Abstract: The crystal structure of β-maltopyranose monohydrate (4-*O*-(α-D-glucopyranosyl)-β-D-glucopyranose monohydrate) has been determined. The unit cell is monoclinic with space group $P2_1$ and with dimensions a = 4.92 Å, b = 15.23 Å, c = 10.68 Å, and β = 97.53°. The structure was solved using Patterson search techniques and refined by difference Fourier and full-matrix least-squares procedures using isotropic temperature factors. The bond distances and angles are generally in the expected range. The conformations of the hydroxymethyl groups about the C(5)–C(6) and C(5′)–C(6′) bonds are gauche-trans and gauche-gauche, respectively. The conformational angles ϕ and ψ, for rotation about the bonds to the bridge oxygen, are 181 and 193°, respectively, and the bridge angle is 117°. The structure is held together by several sequences of hydrogen bonds which include an intramolecular hydrogen bond from O(2) to O(3′). This bond has been observed in all other previously determined structures containing the maltose linkage. Likewise, the conformation of maltose is quite similar to that observed in other structures containing the maltose residue. Examination of these structures provides strong evidence in favor of the generally accepted sixfold helical conformation of V-amylose and the recently proposed sixfold helical structure for B-amylose.

The glucose residue, both in its pure and derivatized states, forms the basis of a large class of naturally occurring polymers, of which cellulose (β-1,4 D-glucan) and starch (a mixture of amylose, a linear α-1,4 D-glucan, and amylopectin, a branched D-glucan containing both α-1,4 and α-1,6 linkages) are the most common. Cellulose structure studies were considerably aided by the determination of the crystal structure of cellobiose, its dimer residue.[2] For similar reasons, it is important to know the crystal structure of maltose which is the dimer residue of amylose and one of the principal dimer residues of a number of other linear polysaccharides, among them mycodextran, an alternating α-1,3 and α-1,4 linked D-glucan of fungal origin. Since amylose exists in a number of quite different but related crystalline forms,[3] it is apparent that there is a fair degree of flexibility in the 4-*O*-(α-D-glucosido)–glucopyranose linkage. The linkage has previously been studied crystallographically by Chu and Jeffrey[4a] in methyl-β-D-maltopyranose and by Hybl, Rundle, and Williams[4b] in the cyclohexaamylose–potassium acetate complex. A detailed knowledge of the maltose structure is a necessary complement to these two previous structures. Certain obvious differences and similarities were expected, and to the extent that these is confirmed the rationalization of future oligomeric and polymeric structures of the α-1,4-glucan type is facilitated. Carbohydrate chemists are particularly interested in the glycoside bond angle and its relation to conformation and intermolecular packing. In addition, only the crystallographic approach has been able to establish unequivocally the hydroxymethyl conformation of carbohydrates, a knowledge which is essential in understanding polysaccharide structures. Similarly, it can be hoped that eventually, when the crystal structures of a number of related oligomers have been determined, this information can be used for establishing potential energy functions which can then be used for predicting the structures of polysaccharides.

Crystal Data and Experimental Procedures

Maltose tends to separate from an aqueous solution either as a clear malleable film or as a microcrystalline precipitate of β-maltose monohydrate. After several attempts at growing crystals under a variety of conditions, small clusters of triangular crystal platelets were grown by slow diffusion of acetone into an aqueous maltose solution (concentration 0.10 g/ml). It was possible to remove and mount some of the larger flakes. Weissenberg diffraction patterns calibrated with powdered NaF gave the following unit cell data for β-maltose monohydrate (4-*O*-(α-D-glucopyranosyl)-β-D-glucopyranose monohydrate): molecular weight, 360.3; monoclinic, space group $P2_1$, from systematic extinctions $0k0$ absent with $k = 2n + 1$; $Z = 2$; a = 4.92 (σ = 0.03) Å, b = 15.23 (σ = 0.08) Å, c = 10.68 (σ = 0.04) Å; β = 97.53° (σ = 0.10); ρ_{obsd} = 1.538 g cm^{-3}, ρ_{calcd} = 1.532 g cm^{-3}. The unit cell is in good agreement with that first reported by French.[5]

Because of the curvature of the crystals acceptable intensity data could only be collected by rotation about the b axis. The data were collected on a single crystal of approximately 0.05 × 0.3 mm^2 in cross section and 1 mm in length, using a multiple film equiinclination Weissenberg camera with Cu Kα radiation. Corrections for absorption and extinction were neglected. In total, nine layer lines (zero–eight) were recorded. This includes approximately 55% of the unique data within the reflection sphere of the copper radiation. Intensities were measured using the positive print method as described by Buerger.[6] Of the 1075 reflections present in the measured portion of the recip-

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(2) R. A. Jacobson, J. A. Wunderlich, and W. N. Lipscomb, *Acta Crystallogr.*, **14**, 598 (1961).

(3) J. R. Katz and T. B. Van Itallie, *Z. Phys. Chem. Abt. A*, **150**, 90 (1930).

(4) (a) S. S. C. Chu and G. A. Jeffrey, *Acta Crystallogr.*, **23**, 1038 (1967); (b) A. Hybl, R. E. Rundle, and D. E. Williams, *J. Amer. Chem. Soc.*, **87**, 2779 (1965).

(5) D. French, *Acta Crystallogr.*, 7, 136 (1954).

(6) M. J. Buerger, "Crystal Structure Analysis," Wiley, New York, N. Y., 1960, Chapter 6.

rocal space, 781 had intensities above the background level.

Determination of Crystal Structure

The crystal structure of maltose monohydrate was solved by means of a systematic study of its sharpened Patterson function (the $E^2 - 1$ function[7]). Normally, a Patterson map of a structure of this complexity (over 500 independent Patterson peaks) is difficult, if not impossible, to interpret. There are certain characteristics of the pyranose ring, however, which may be expected to stand out in the Patterson map. To demonstrate this, Figure 1 shows a schematic view of β-D-glucose viewed from the direction perpendicular to the plane of the ring. The triangles shown by dashed lines should all be approximately equilateral with sides of 2.4 ± 0.2 Å and be lying in or near two parallel planes. Thus, the plane through the origin of Patterson space which corresponds to the above two planes will contain six peaks on the radius of a circle of approximately 2.4 Å, separated by approximately 60°. These peaks will be five to six times as strong as a single peak. In addition, vectors from the atoms above or below the center of each triangle should be readily distinguishable. The vector set of an α-D-glucose unit is similar but somewhat weaker because of one less coplanar triangle, *i.e.*, that involving O(1).

With this knowledge, visual examination of the sharpened Patterson revealed a likely orientation for one of the glucose residues which was roughly parallel to the 1, 0, 0 plane. The orientation of the second ring was not immediately obvious but was determined with the help of the Buerger minimum function[8] and a programmed vector search method.

Since the Harker section was not readily interpretable, the position of the molecule relative to the twofold axis had to be found by other means. The *bc* projection was used for this purpose, and the molecule was assigned an initial position relative to the *c* axis by considering packing and the minimum function. The position of the molecule relative to the *c* axis was then varied systematically and the position yielding the minimum *R* value (0.40) was selected. Several cycles of difference Fourier synthesis were then used to obtain the projected positions of the missing atoms, which led to an *R* value of 0.24 in projection. The position of the twofold screw axis in the *a* direction was determined in the same fashion. Further Fourier refinement followed by a full-matrix least-squares analysis[9] of the fractional coordinates, isotropic temperature factors, and layer line scaling constants yielded an *R* value of 0.117. A Fourier difference map obtained at this point showed peaks at the positions where the hydrogens bonded to ring carbons were expected, as well as several peaks in the positions consistent with hydroxyl hydrogens involved in hydrogen bonding. Inclusion of the hydrogens bonded to carbon atoms in their expected positions gave a final *R* value of 0.103. Further difference syntheses did not indicate the presence of additional hydrogens and refinement was terminated. Since the data were not of sufficiently high quality owing to curvature of the crystals, to warrant refinement of the thermal anisotropic parameters, this step of refinement was not attempted. The final atomic coordinates are listed in Table I. [The structure factors may be obtained by writing to one of the authors (A. S.).]

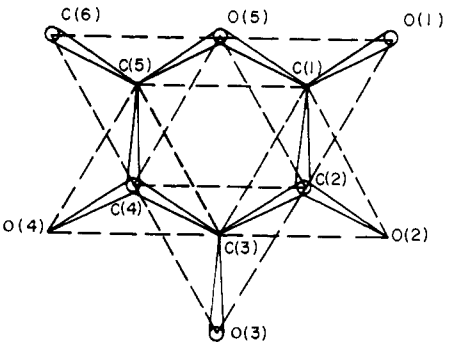

Figure 1. Schematic diagram of β-D-glucose viewed perpendicular to the plane of the ring (the hydrogens and O(6) are not included). The dashed lines depict the triangles used in the Patterson search.

Table I. Fractional Coordinates and Thermal Parameters of Maltose Monohydrate[a]

Atom	*x*	*y*	*z*	*B*
C(1)	0.530 (2)	0.300 (2)	0.176 (1)	3.58 (0.22)
C(2)	0.557 (2)	0.323 (2)	0.042 (1)	3.64 (0.23)
C(3)	0.290 (2)	0.299 (2)	−0.044 (1)	2.96 (0.20)
C(4)	0.244 (2)	0.201 (2)	−0.028 (1)	3.62 (0.24)
C(5)	0.222 (2)	0.180 (2)	0.111 (1)	3.73 (0.24)
C(6)	0.212 (3)	0.080 (2)	0.134 (1)	4.92 (0.29)
O(1)	0.308 (2)	0.349 (1)	0.216 (1)	3.60 (0.16)
O(2)	0.621 (2)	0.414 (1)	0.030 (1)	4.46 (0.19)
O(3)	0.334 (2)	0.312 (1)	0.827 (1)	4.24 (0.18)
O(4)	−0.012 (2)	0.174 (1)	0.903 (1)	4.34 (0.18)
O(5)	0.475 (2)	0.211 (1)	0.186 (1)	3.78 (0.16)
O(6)	0.202 (2)	0.061 (1)	0.267 (1)	4.79 (0.20)
C(1′)	0.185 (3)	0.523 (2)	0.505 (1)	4.35 (0.26)
C(2′)	0.277 (2)	0.565 (2)	0.389 (1)	3.79 (0.24)
C(3′)	0.231 (3)	0.499 (2)	0.279 (1)	4.18 (0.28)
C(4′)	0.376 (2)	0.411 (2)	0.318 (1)	3.96 (0.25)
C(5′)	0.265 (2)	0.376 (1)	0.438 (1)	3.48 (0.23)
C(6′)	0.383 (3)	0.287 (2)	0.482 (1)	4.47 (0.29)
O(1′)	0.260 (2)	0.581 (1)	0.608 (1)	4.95 (0.21)
O(2′)	0.119 (2)	0.641 (1)	0.358 (1)	4.87 (0.21)
O(3′)	0.349 (2)	0.531 (1)	0.172 (1)	5.03 (0.21)
O(5′)	0.325 (2)	0.441 (1)	0.535 (1)	3.98 (0.17)
O(6′)	0.676 (2)	0.291 (1)	0.512 (1)	4.48 (0.20)
O(W)	−0.187 (2)	0.377[b]	−0.263 (1)	4.68 (0.20)
H[C(1)]	0.731	0.311	0.231	5.0
H[C(2)]	0.737	0.291	0.011	5.0
H[C(3)]	0.106	0.335	0.979	5.0
H[C(4)]	0.418	0.169	0.940	5.0
H[C(5)]	0.046	0.214	0.145	5.0
H[C(6)-1]	0.024	0.056	0.097	5.0
H[C(6)-2]	0.388	0.047	0.121	5.0
H[C(1′)]	0.968	0.511	0.502	5.0
H[C(2′)]	0.483	0.583	0.407	5.0
H[C(3′)]	0.012	0.491	0.256	5.0
H[C(4′)]	0.571	0.418	0.331	5.0
H[C(5′)]	0.428	0.366	0.419	5.0
H[C(6′)-1]	0.279	0.267	0.563	5.0
H[C(6′)-2]	0.337	0.279	0.372	5.0

[a] The estimated standard deviations are given in parentheses (×10³, except for *B*). Coordinates of hydrogens are chosen to agree with previously examined structures. [b] Invariant.

(7) H. Hauptman and J. Karle, "The Solution of the Phase Problem. I. The Centrosymmetric Crystal," ACA Monograph No. 3, Edwards Bros., Ann Arbor, Mich., 1953.

(8) M. J. Buerger, "Vector Space," Wiley, New York, N. Y., 1959, Chapter 10.

(9) W. R. Busing, K. O. Martin, and H. A. Levy, "Fortran Crystallographic Least-Squares Program," ORNL-TM-305, Oak Ridge National Laboratory, Oak Ridge, Tenn., 1962.

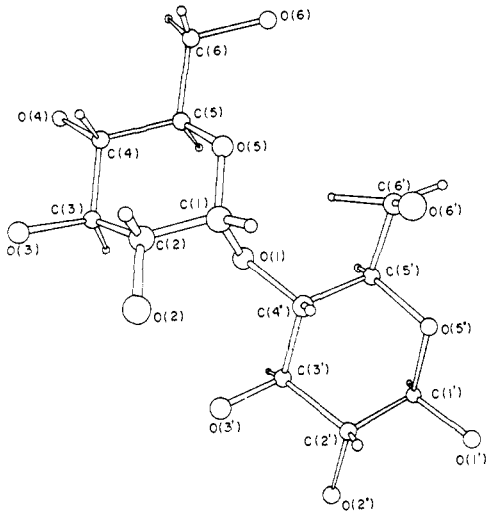

Figure 2. Structure of β-maltose viewed along the a axis of the unit cell.

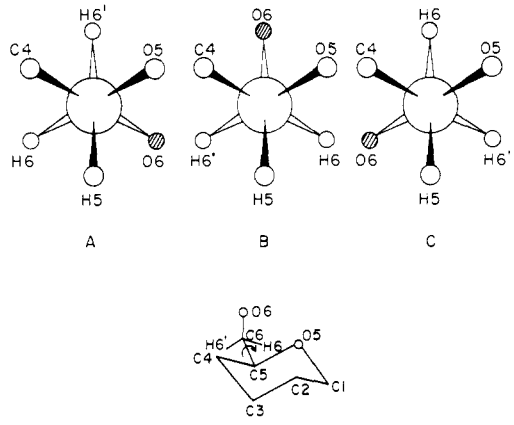

Figure 3. Preferred conformations of the C(6) hydroxymethyl group in carbohydrates.[10] The view is down the C(5)–C(6) bond. (Refer to the diagram of the ring for numbering of atoms.) In conformation A O(6) is gauche with respect to O(5) and trans with respect to C(4), in B it is gauche to both O(5) and C(4), and in C it is trans to O(5) and gauche to C(4).

Description of the Structure

A diagram of one molecule of β-maltose monohydrate showing the numbering system is shown in Figure 2 and the packing of molecules is shown in Figure 4. Both glucose residues are in the expected C1 chair conformation with no apparent deviations from the normal unstrained conformation. According to the nomenclature of Sundaralingam,[10] the orientation of the C(6)–O(6) bond is gauche-trans [dihedral angles defined by O(5)–C(5)–C(6)–O(6) and C(4)–C(5)–C(6)–O(6) are 61 and 178°, respectively (the dihedral angle for the bond C(5)–C(6) is defined as the angle that the projection of the bond O(5)–C(5) makes with respect to the projection of the bond C(6)–O(6))] and the orientation of the C(6′)–O(6′) bond is gauche-gauche (dihedral angles defined by O(5′)–C(5′)–C(6′)–O(6′) and C(4′)–C(5′)–C(6′)–O(6′) are 61 and 60°, respectively). These orientations which represent two of the three most probable conformations for the hydroxymethyl group (see Figure 3 for explanation)

(10) M. Sundaralingam, *Biopolymers*, 6, 189 (1968).

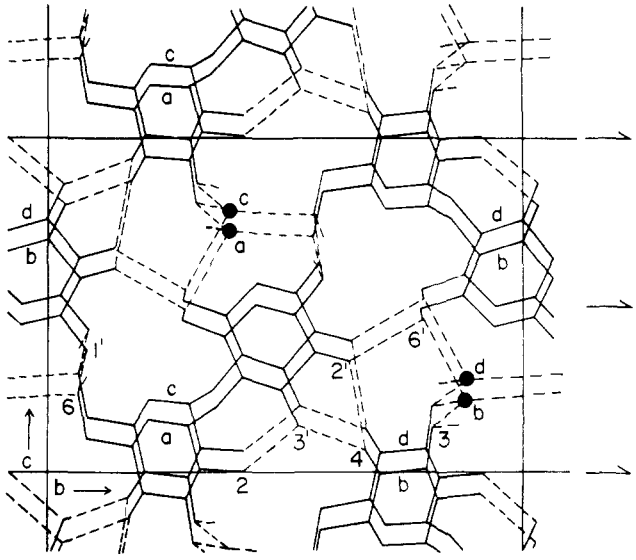

Figure 4. Packing of maltose molecules in the crystal structure, viewed along the a axis of the unit cell. Two unit cells in the a direction are superimposed slightly out of register to permit better visualization of hydrogen bonds shown in dashed lines. The water molecules are indicated by black dots. (Refer to Table III for explanation of the symmetry code.)

coincide with those found in the respective glucoses[11–13] and in methyl β-maltopyranoside.[4a] It is noteworthy that maltose possesses both the gauche-trans and gauche-gauche conformations of the hydroxymethyl group, while the cellobiose structure[2] has the same gauche-trans conformation in both residues. Recent conformational energy calculations[14] indicate that the gauche-trans form is the favored one, but because the energy barrier between conformations is not high, it appears that interaction with the surroundings could be the determining factor in the selection. To date no glucose structure has been reported with the trans-gauche conformation which leads to an unfavorable "peri" interaction, or eclipsing, between O(6) and O(4).

The covalent bond distances and angles are listed in Table II. No unusual deviations from the bond lengths and angles typically found in carbohydrates were observed.[4,12,15–17]

Hydrogen Bonding

The crystal structure of maltose is characterized by an extensive system of hydrogen bonds. Three sequences are distinguishable, and they are shown in Figure 4. One of the sequences is roughly parallel to the bc plane, while the other two sequences are perpendicular to it. Since the positions of the hydroxyl hydrogens were not determined, the direction of the sequences is generally undefined. A list of hydrogen bond distances and angles about the hydrogen bonded oxygens is given in Table III. The mean oxygen–oxygen distance is 2.79 Å. All hydroxyl oxygens

(11) T. R. R. McDonald and C. A. Beevers, *Acta Crystallogr.*, **5**, 654 (1952).
(12) G. M. Brown and H. A. Levy, *Science*, **147**, 1038 (1965).
(13) W. G. Ferrier, *Acta Crystallogr.*, **16**, 1023 (1963).
(14) P. R. Sundararajan, Ph.D. Thesis, University of Madras, India, 1969.
(15) S. H. Kim and G. A. Jeffrey, *Acta Crystallogr.*, **22**, 537 (1967).
(16) G. M. Brown and H. A. Levy, *Science*, **141**, 921 (1963).
(17) H. M. Berman and S. H. Kim, *Acta Crystallogr., Sect. B*, **24**, 897 (1968).

Table II. Bond Distances and Bond Angles[a]

Bond distances			Bond angles			
Atoms		Distance	Atoms			Angle
i	j	D(ij), Å	i	j	k	(ijk), deg
C(1)–C(2)		1.50 (0.02)	C(2)–C(1)–O(1)			109.7 (1.4)
C(1)–O(1)		1.43 (0.02)	C(2)–C(1)–O(5)			110.2 (1.5)
C(1)–O(5)		1.39 (0.02)	O(1)–C(1)–O(5)			108.7 (1.3)
C(2)–C(3)		1.54 (0.01)	C(1)–C(2)–C(3)			109.7 (1.1)
C(2)–O(2)		1.42 (0.02)	C(1)–C(2)–O(2)			110.4 (1.5)
C(3)–C(4)		1.52 (0.03)	C(3)–C(2)–O(2)			111.5 (1.5)
C(3)–O(3)		1.44 (0.01)	C(2)–C(3)–C(4)			107.0 (1.4)
C(4)–C(5)		1.54 (0.02)	C(2)–C(3)–O(3)			108.3 (1.0)
C(4)–O(4)		1.44 (0.01)	C(4)–C(3)–O(3)			106.7 (1.5)
C(5)–C(6)		1.53 (0.03)	C(3)–C(4)–C(5)			110.2 (1.5)
C(5)–O(5)		1.47 (0.01)	C(3)–C(4)–O(4)			110.8 (1.5)
C(6)–O(6)		1.46 (0.02)	C(5)–C(4)–O(4)			105.6 (1.1)
C(1′)–C(2′)		1.51 (0.02)	C(4)–C(5)–C(6)			111.7 (1.5)
C(1′)–O(1′)		1.42 (0.02)	C(4)–C(5)–O(5)			107.1 (1.1)
C(1′)–O(5′)		1.44 (0.02)	C(6)–C(5)–O(5)			106.0 (1.4)
C(2′)–C(3′)		1.55 (0.02)	C(5)–C(6)–O(6)			111.0 (1.5)
C(2′)–O(2′)		1.41 (0.02)	C(1)–O(1)–C(4′)			117.2 (1.1)
C(3′)–C(4′)		1.55 (0.03)	C(1)–O(5)–C(5)			115.8 (1.5)
C(3′)–O(3′)		1.44 (0.02)	C(2′)–C(1′)–O(1′)			107.3 (1.7)
C(4′)–O(1)		1.44 (0.02)	C(2′)–C(1′)–O(5′)			111.1 (1.2)
C(4′)–C(5′)		1.55 (0.02)	O(1′)–C(1′)–O(5′)			107.3 (1.2)
C(5′)–C(6′)		1.52 (0.02)	C(1′)–C(2′)–C(3′)			108.8 (1.7)
C(5′)–O(5′)		1.44 (0.02)	C(1′)–C(2′)–O(2′)			109.1 (1.1)
C(6′)–O(6′)		1.43 (0.01)	C(3′)–C(2′)–O(2′)			109.2 (1.1)
			C(2′)–C(3′)–C(4′)			109.8 (1.1)
			C(2′)–C(3′)–O(3′)			110.6 (1.7)
			C(4′)–C(3′)–O(3′)			107.1 (1.3)
			O(1)–C(4′)–C(3′)			107.9 (1.2)
			O(1)–C(4′)–C(5′)			109.0 (1.7)
			C(3′)–C(4′)–C(5′)			108.7 (1.2)
			C(4′)–C(5′)–C(6′)			113.8 (1.3)
			C(4′)–C(5′)–O(5′)			107.6 (1.7)
			C(6′)–C(5′)–O(5′)			110.4 (1.2)
			C(5′)–C(6′)–O(6′)			111.0 (1.7)
			C(1′)–O(5′)–C(5′)			112.7 (1.2)

[a] Standard deviations are given in parentheses.

Table III. Hydrogen Bond Distances and Angles about Hydrogen-Bonded Oxygens

Atoms[a]		D(ij),	Atoms[a]			∠(ijk),
i	j	Å	i	j	k	deg
$O(2)_a$	$O(3')_a$	2.79	$C(2)_a$	$O(2)_a$	$O(3')_a$	116.5
$O(3')_a$	$O(4)_b$	2.77	$C(3')_a$	$O(3')_a$	$O(2)_a$	118.9
$O(4)_b$	$O(2')_a$	2.82	$C(3')_a$	$O(3')_a$	$O(4)_b$	102.3
$O(2')_a$	$O(6')_b$	2.79	$C(4)_b$	$O(4)_b$	$O(3')_a$	145.4
$O(6')_b$	$O(W)_b$	2.74	$C(4)_b$	$O(4)_b$	$O(2')_a$	126.8
$O(W)_b$	$O(6)_a$	2.80	$C(2')_a$	$O(2')_a$	$O(4)_b$	114.0
$O(6)_a$	$O(1')_b$	2.81	$C(2')_a$	$O(2')_a$	$O(6')_b$	113.7
$O(1')_b$	$O(6)_c$	2.79	$C(6')_b$	$O(6')_b$	$O(2')_a$	111.1
$O(3)_b$	$O(W)_b$	2.83	$C(6')_b$	$O(6')_b$	$O(W)_b$	109.9
$O(W)_b$	$O(3)_d$	2.78	$O(6')_b$	$O(W)_b$	$O(6)_a$	117.3
			$C(6)_a$	$O(6)_a$	$O(W)_b$	100.8
			$C(6)_a$	$O(6)_a$	$O(1')_b$	106.8
			$C(6)_c$	$O(6)_c$	$O(1')_b$	125.2
			$C(1')_b$	$O(1')_b$	$O(6)_a$	115.5
			$C(1')_b$	$O(1')_b$	$O(6)_c$	98.8
			$O(6)_a$	$O(1')_b$	$O(6)_c$	122.2
			$O(W)_b$	$O(6)_a$	$O(1')_b$	97.9
			$O(W)_d$	$O(6)_c$	$O(1')_b$	95.5
			$C(3)_b$	$O(3)_b$	$O(W)_b$	127.5
			$C(3)_d$	$O(3)_d$	$O(W)_b$	97.7
			$O(3)_b$	$O(W)_b$	$O(6')_b$	90.7
			$O(3)_d$	$O(W)_b$	$O(6')_b$	104.1
			$O(3)_b$	$O(W)_b$	$O(6)_a$	109.3
			$O(3)_b$	$O(W)_b$	$O(3)_d$	121.8
			$O(3)_d$	$O(W)_b$	$O(6)_a$	112.3

Symmetry Codes

	x′	y′	z′
a	x	y	z
b	$-x$	$0.5 + y$	$-z$
c	$1.0 + x$	y	z
d	$1.0 - x$	$0.5 + y$	$-z$

[a] The subscript letters refer to symmetry-related residues given at the bottom of the table.

with the exception of O(2) and O(6) are involved in two hydrogen bonds, and the water molecule is involved in four approximately tetrahedral hydrogen bonds. The direction of the sequence containing O(2) (the sequence in the *bc* plane) is determined if it is assumed that O(2) acts as a hydrogen donor. This is reasonable in view of the angle $C(2)_a$–$O(2)_a$→$O(3')_a$ of 116.5°. The sequence then continues with $O(3')_a$–H→$O(4)_b$, since the angle for this hydrogen bond, $C(3')_a$–$O(3')_a$→$O(4)_b$, is 102.3° which allows a more stable hydrogen bond than the reverse, *i.e.*, $C(4)_b$–$O(4)_b$→$O(3')_a$ for which the angle is 145.4°. The full sequence is accordingly

$$O(2)_a \rightarrow O(3')_a \rightarrow O(4)_b \rightarrow O(2')_a \rightarrow O(6')_b \rightarrow O(W)_b \rightarrow O(6)_a$$

(The subscripts refer to the various symmetry-related molecules as specified in Table III and Figure 4.) The sequence ends with $O(6)_a$, which is also involved in another hydrogen-bond sequence zigzagging parallel to the *a* axis: $O(6)_a \leftrightarrow O(1')_b \leftrightarrow O(6)_c \leftrightarrow O(1')_d$. The direction of bonding in this sequence cannot be determined.

Similarly, the direction of the third sequence, which also zigzags parallel to the *a* axis and involves the atoms $O(3)_b \leftrightarrow O(W)_b \leftrightarrow O(3)_d \leftrightarrow O(W)_d$, cannot be further defined.

All available hydrogens are utilized in this arrangement. The acceptor capacities of the ring and bridge oxygens as well as of O(2) are not utilized. In the case of the bridge oxygen, and to some degree O(5), steric effects may prevent hydrogen bonding. The explanation for the lack of bonding to O(2) and O(5′) is less clear but may be simply that it is impossible to produce a regular structure utilizing these oxygens as well as the other hydrogen-bonding groups.

It is interesting to note that the $O(2)_a \rightarrow O(3')_a$ intramolecular hydrogen bond has now been observed in all known crystal structures which contain the maltose linkage, in spite of vastly different intermolecular hydrogen bonds in these structures. This hydrogen bond thus appears to be one of the principal forces controlling the conformations which are based on α-1,4 linked glucoses.

Extension to Polysaccharides

One of the primary motivations for studying the structure of maltose was to gain insight into the conformation of polymers containing the maltose linkage. The structures of mycodextran and amylose are of special interest in this connection. The structure of mycodextran has been studied in this laboratory and will be discussed in a separate communication. Amylose has been studied in a number of forms and by a number of workers. Rao, *et al.*,[18] reviewed the structure of V-amylose and concluded that a sixfold helix was the most probable one, in agreement with the

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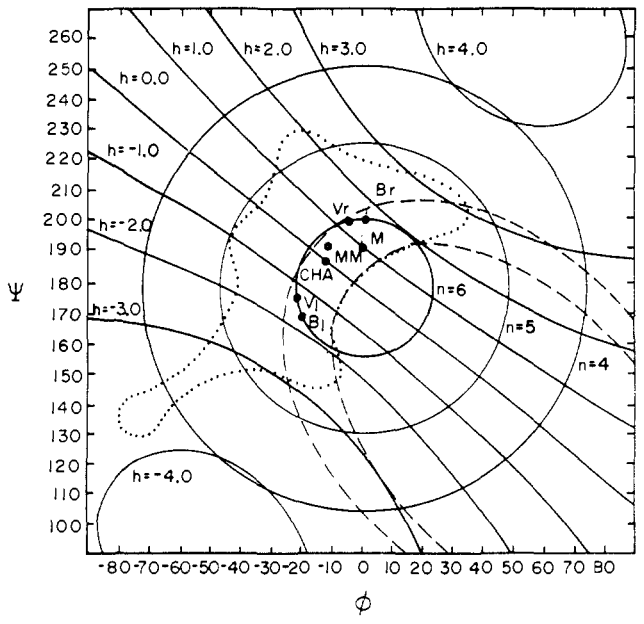

Figure 5. Allowed conformational region (dotted line) for maltose with a bridge angle of 117°. Dashed lines bound the area of acceptable O(2)→O(3′) hydrogen bond lengths (2.6–3.0 Å). The n and h contours are, respectively, the number of residues per turn of helix and the axial rise per residue in ångström units (positive h indicates a right-handed helix): M, β-maltose (this work); MM, β-methylmaltose;[4a] CHA, cyclohexaamylose;[4b] V_l and V_r, left- and right-handed V-amylose;[4b,18,19] B_l and B_r, left- and right-handed sixfold B-amylose.[23]

conclusions of earlier workers.[4b,19] In their most recent study, Sundararajan and Rao[20] favored a right-handed helix for this structure. The naturally occurring B-amylose has been investigated by Kreger,[21] who proposed a helical structure with three residues per turn; by Spark[22a] and Rao and Sundararajan,[22b] who proposed structures with four residues per turn; and most recently, by Blackwell, Sarko, and Marchessault,[23] who proposed a structure with six residues per turn.

Figure 5 shows a limited conformational energy contour map of the type commonly used in studying polymer conformations.[18,23] The axes represent the two conformational angles, C(4′)–O(1)–C(1)–O(4) (rotation about the C(1)–O(1) bond) and C(1)–O(1)–C(4′)–O(1′) (rotation about the C(4′)–O(1) bond), to be referred to as ϕ and ψ, respectively. (The conformational angle ϕ, for rotation about the C(1)–O(1) bond, is defined as the angle that the projection of the bond C(4′)–O(1) makes with respect to the projection of the vector O(4)→C(1). Similarly, the conformational angle ψ, for rotation about the C(4′)→O(1) bond, is defined as the angle that the projection of the bond C(1)–O(1) makes with respect to the projection of the vector O(1′)→C(4′). The (ϕ,ψ) = (0,0) starting position corresponds to O(4), C(1), O(1), C(4′), and O(1′) lying in the same plane, and the rotations are considered positive when, viewing along C(1)–O(1) and C(4′)–O(1) toward O(1), the rotations are performed counterclockwise.[18] Contour lines of integral n and h (the number of residues per turn of helix and the axial rise per residue in ångströms, respectively) are shown. The dotted line in the figure bounds the area in which there are no critically unfavorable contact distances. All areas outside this region have one or more contacts shorter than the limits proposed by Ramachandran.[24] The dashed lines in the figure bound O(2)···O(3′) distances from 2.6 to 3.0 Å, and the overlap of this area with the allowed area represents conformations for which this hydrogen bond is allowed.

The dihedral angles ϕ and ψ for both crystalline β-maltose (ϕ = 181, ψ = 193°) and crystalline methyl-β-maltose[4a] (ϕ = 169, ψ = 193°) are noted in Figure 5. Both of these conformations lie inside but near the n = 6 contour (as does cyclohexaamylose,[4b] included for comparison). This observation clearly supports the accepted sixfold V-amylose and the proposed sixfold B-amylose.[23] In addition, both methyl-β-maltose and β-maltose lie in the region of positive h corresponding to right-handed helicity. In fact, β-maltose is quite close to both the right-handed V- and B-amyloses, being about 10° away in ψ and within 5° of each in the ϕ direction. The argument for right-hand chirality is somewhat weakened by the fact that in right-handed B- and V-amyloses the O(2)→O(3′) hydrogen bond would be slightly longer than in both left-handed helices. Slight changes in the starting coordinates of the glucopyranose residue could, of course, bring this hydrogen bond to a more favorable value.

It must be noted that the map in Figure 5 is calculated for a C(1)–O(1)–C(4′) angle of 117°. Maps for different bridge angles vary somewhat, but within the few degrees of variation commonly observed in unstrained bond angles, the map does not change significantly and none of the observations made here are altered. A map similar to the one shown in Figure 5 but calculated for a bridge angle of 112° would extend the pitch range, and an n = 7 contour would appear at the very center. A sevenfold helix has been reported for a complex of amylose and *tert*-butyl alcohol.[25]

One does not expect to learn much about *intermolecular* bonding in the parent polysaccharide from the crystal structure of its dimer; nevertheless, there is strong reason to believe that the packing in the c axis direction (*cf.* Figure 4) has significance with respect to the crystal structure of the polymer. The period along this direction for the maltose hydrate structure is 10.68 Å, which almost coincides with the fiber repeat of 10.4–10.6 Å always reported for B-amylose fiber.[23] When one compares the projections of, for example, the molecules marked with the letter c in Figure 4 with the cylindrical projection of the proposed structure for B-amylose (Figure 3 in Blackwell, Sarko, and Marchessault[23]), the similarity is obvious. The packing of the maltose molecules almost duplicates the arrangement of glucose residues in two contiguous turns of the B-amylose structure, in that one maltose molecule may represent two glucose residues in one

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turn of the B-amylose helix, and the equivalent maltose molecule in the adjacent unit cell (in the *c* direction) may represent the residues in the next turn of the helix. The similarity is complete down to the interturn hydrogen bonding through the water molecule, with the only exception that in maltose this bonding is O(6)–O(W)–O(3), while in B-amylose it is O(6)–O(W)–O(2). This difference is minor in contrast to the support which the present result lends to the idea that in the B-amylose structure the hydrogen bonding between successive turns of the helix is *via* water of hydration.

Ion-Electrode Study of Alkali Metal Adenosine Triphosphate Complexes

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Abstract: Association constants of the ion pairs $KATP^{3-}$ and $NaATP^{3-}$ have been measured by direct potentiometry using K^+-sensitive electrodes of the valinomycin and glass membrane type. Formation constants taken at 25° are substantially greater than those previously reported from indirect measurements.

The adenine nucleotides, in particular adenosine 5′-triphosphate (adenosine 5′-(tetrahydrogen triphosphate)), take part in many important biochemical reactions. The hydrolysis of adenosine 5′-triphosphate is the chief source of energy for the active transport of K^+ and Na^+ ions in nerve, muscle,[1] and blood cells.[2] It is now generally recognized that adenosine 5′-triphosphate and its analogs exist in various metal-complexed forms in biological fluids and it is important for obvious reasons to know the stabilities of these complexes.[3] The alkali metals form complexes of weak but measurable stability. Melchior[4] measured the difference in the pK_a value for the reaction

$$HATP^{3-} \rightleftharpoons ATP^{4-} + H^+$$

obtained by replacing $(CH_3)_4N^+$ with Na^+ or K^+, and obtained a value of 9.8 ± 0.2 for the stability constant of the complex $MATP^{3-}$, where M is the alkali metal. In this study the ionic strength was maintained around 0.2 *M* and the assumption was made that the tetraalkylammonium ions did not associate with the ligand. By a similar technique of potentiometric titrations, using the same assumptions, Smith and Alberty[5] obtained values of 11.5 ± 1.0 and 14.3 ± 0.4 for the $KATP^{3-}$ and $NaATP^{3-}$ species, respectively, at an ionic strength of 0.2 maintained with tetra-*n*-propylammonium bromide. The difference in stabilities between the sodium and potassium complexes was considered significant by the authors. From the apparent stability constants of $CaATP^{2-}$ and $MgATP^{2-}$ complexes in the presence of sodium chloride, O'Sullivan and Perrin[6] estimated the stability constant of $NaATP^{3-}$ species to be 15 ± 2 M^{-1}. The value for the $KATP^{3-}$ complex was estimated to be 14 ± 2 M^{-1} by a similar method.

Although there is apparent agreement among the results of these investigations, they have all been, in essence, indirect methods. Furthermore, the assumption that the tetraalkylammonium ions do not associate with a polyvalent anion like ATP^{4-} is open to question. Since the stability constants have been determined at constant ionic strength, they are applicable only under the conditions specified, and the interactions of the ions of interest with the solvent or medium ions cannot be distinguished. A more direct determination of the thermodynamic stability constants of alkali metal complexes of adenosine triphosphate therefore seems appropriate and desirable.

In recent years ion-selective electrodes have been successfully used as concentration and activity probes in several thermodynamic and kinetic studies.[7-10] The introduction of a new valinomycin[11,12] electrode of the liquid exchanger type with a very high selectivity for potassium over sodium of 5000:1 promises to be an excellent tool for investigating biological phenomena involving changes in potassium activity in the presence of a larger concentration of sodium ions. In the work reported here the thermodynamic stability constant for the $KATP^{3-}$ complex has been measured using a valinomycin electrode and a Corning monovalent cationic glass electrode. The stability constant for the $NaATP^{3-}$ complex measured with the latter electrode is also reported.

Experimental Section

Reagents. The Σ-grade disodium salt of adenosine 5′-triphosphate ($Na_2H_2ATP \cdot 3.5H_2O$) manufactured by Sigma Chemicals

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