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THE CRYSTAL STRUCTURE OF UNCOMPLEXED-HYDRATED CYCLOOCTAAMYLOSE

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SUMMARY

Cyclooctaamylose crystallizes from aqueous solution with space-group symmetry $P2_1$ and lattice parameters: $\underline{a}$ = 20.253(8), $\underline{b}$ = 10.494(5), $\underline{c}$ = 16.892(6)A and β = 105.32(1)°, Z = 2; the apparent formula per asymmetric unit is $C_{48}H_{80}O_{40}\cdot 17H_2O$. The macrocycle is in an open conformation but displays significant deviations from ideal eight fold molecular symmetry. Of the 19 water molecules thus far located, four of which have occupancy factors of one half, 12 may be characterized as being in the torus of the cycloamylose.

INTRODUCTION

The cycloamyloses, more commonly called cyclodextrins, are α-(1,4') linked cyclic oligomers of D-glucose that have been known for decades (1) to form "nonbonded" complexes in which guest molecules are included to varying extents in their hydrophobic torus. The lower homologs, α-cyclodextrin, α-CD (cyclohexaamylose), and β-cyclodextrin, β-CD (cycloheptaamylose) have been the focal point of considerable research interest, much of which arises from their suitability to serve as medium molecular weight enzyme models. Bender and Komiyama (2) have recently reviewed the literature relavent to this aspect of cyclodextrin chemistry. Topics addressed therein include the definition of the functional group(s) most likely responsible for the catalytic activity of the cyclodextrins toward hydrolysis reactions (under basic conditions), the elucidation of the forces responsible for the formation of the host-substrate complex and the complexing ability and catalytic properties of modified cyclodextrins.

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A thorough appreciation of the interactions between cyclodextrins and substrate molecules can only be achieved when the structural properties of the host system and its complexes are understood. Crystal structure analyses provide one method by which considerable insight into these properties can be gained. Meaningful interpretation of the conformational data from crystal structure determinations is best accomplished through a comparison of a series of related structures. We wish to extend the studies of Saenger and coworkers on uncomplexed-hydrated cyclodextrins (3,4) with a report of the initial results of the crystal structure determination for uncomplexed-hydrated γ-cyclodextrin, γ-CD (cyclooctaamylose). The accompanying report (5) presents a structure determination for γ-CD crystallized in the presence of organic substrate. These reports present the first single crystal X-ray structure determinations for this higher homolog of the cyclodextrin series.

METHODS

Crystals of uncomplexed-hydrated γ-CD were obtained by slowly cooling a saturated solution that had been heated until nearly all the previously crystallized γ-CD had redissolved. Large, colorless, transparent, prismatic cryatals were so obtained. A crystal measuring *ca.* 0.5x0.5x0.8mm^3 sealed in a thin walled glass capillary with a trace of mother liquor, was used for data collection with a Syntex P$\bar{1}$ autodiffractometer (monochromatized MoKα radiation, 0.71069A) equipped with a low temperature device (Syntex LT-1) that maintained the crystal at *ca.* 120K. Lattice parameters: $\underline{a}$ = 20.253(8), $\underline{b}$ = 10.949(5), $\underline{c}$ = 16.892(6)A and β = 105.32° resulted from least-squares refinement (6) with 46 automatically centered 2θ values in the angular range $31.5 < 2\theta < 42.6^{\circ}$; the space-group is $P2_1$ with z = 2. Intensities were measured in an ω-scan mode to a resolution of $2\theta < 60^{\circ}$. Of the 11052 unique reflections measured, 7143 reflections were classified as observed under the criterion $I > 3\sigma(I)$.

The initial structural model was determined by utilizing the 2.4-2.5A multiple vector in the Patterson map to determine the orientation of the local approximate 8-fold symmetry axis. Several models, less the primary hydroxyl groups, with ideal symmetry were generated to probe the 45° rotational ambiguity about the 8-fold axis. Translational ambiguity was resolved by application of the Karle translation function (7).

Development of the structural model to a conventional residual, R, of 0.20 was easily accomplished, though it was apparent early in the analysis that one sugar residue was not well ordered. Eight well ordered water oxygen atoms and six of eight primary hydroxyl oxygen atoms were located in difference Fourier maps at this stage. Further difference maps yielded probable positions for the missing hydroxyl oxygen atoms and 11 additional water oxygen atom sites. Least-squares refinement of the model confirmed the presence of disorder in the aforementioned sugar residue by producing thermal parameters for its atoms that are 5-10 times greater than the averages for the remaining residues. At this stage the R is 0.16 with anisotropic temperature factors. The thermal parameters are reasonable and representative for a structure in which there is generally nonresolvable disorder in part of the asymmetric unit. We are planning to continue our investigation of this structure in the hope that we can at least partially resolve the disorder. In the course of which additional water of hydration molecules may be located, but it is expected that they also will be disordered and/or of partial occupancy.

RESULTS AND DISCUSSION

As indicated above, the refinement of the structural model of this uncomplexed-hydrated γ-CD is considerably more difficult than one routinely encounters for smaller asymmetric units. Nonetheless the general features of the model are physically and chemically reasonable and therefore most likely representative of the structure in the crystal. In view of the rather high R value, we will confine our discussion to the more general properties of the structure such as packing interactions and molecular conformation. Should our efforts to further characterize the disorder be successful, we will more closely examine bonding geometry in a future report.

The crystal packing consists of a herringbone arrangement of γ-CD molecules stacked parallel to the b-axis, Figures 1 and 2. Several features of the packing merit comment, particularly those associated with hydrophilic and hydrophobic interactions.

There is a hydrophilic channel consisting of water molecules and hydroxyl groups of symmetry related γ-CD molecules running through the tori of equivalent macrocycles related by translation in the b direction. As can be seen from the left side of Figure

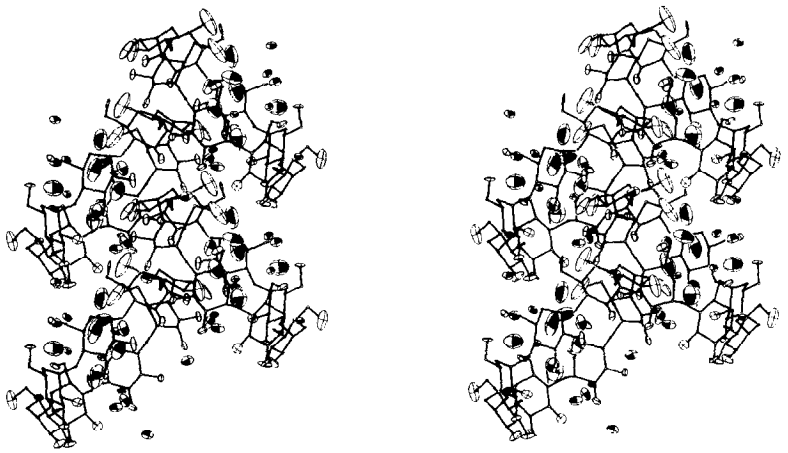

Figure 1. A stereoscopic projection (8) of the crystal packing illustrating two symmetry related hydrophilic channels separated by a hydrophobic barrier consisting of symmetry related sugar residues. A 2_1 symmetry axis (at 0,0,0) runs vertically through the center of the projection.

1, this channel does not run parallel to the *pseudo* molecular symmetry axis as does the hydrophobic channel in complexed α- and β-CD's (9,10). The angle between the b-axis (the channel axis) and the normal to the O(4) atom mean plane is 46.5°. The unit cell contains two such hydrophilic channels related by a crystallographic 2_1 axis.

The symmetry related hydrophilic channels are separated by a hydrophobic wall consisting of a stack of symmetry related sugar

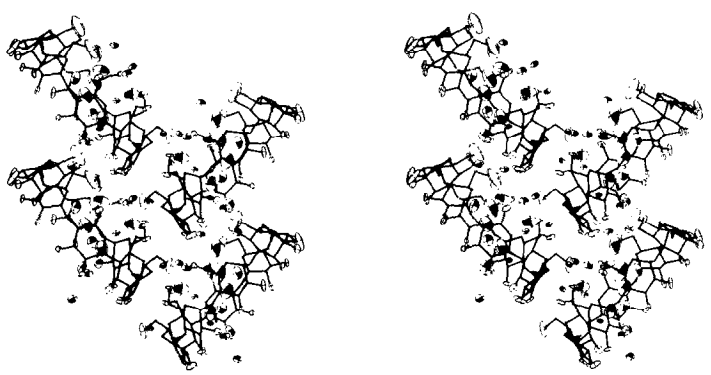

Figure 2. The crystal packing in the region of a second 2_1 axis (at ½,0,0). The diagram illustrates a mixed hydrophilic and hydrophobic region that separates the same hydrophilic channels presented in Figure 1.

residues so arranged that the hydrophobic "interior" of one residue interacts with the hydrophobic "exterior" of its symmetry related neighbor. The 2_1 axis at 0,0,0 runs through this stack and can be appropriately characterized as the stack axis. It is noteworthy that the greatest disorder in the macrocycle (as illustrated by the thermal ellipsoids in the figures) is in the residues in this stack. As can be seen from the central portion of Figure 1, said residues are not so disposed as to allow maximum hydrophobic interaction. The angle between the respective C(2)C(3)C(5)O(5) mean planes is 64.5°. The hydrophobic character of the intrastack region if nicely illustrated by the lack of water in that portion of the γ-CD torus lying between these residues.

The packing around a second 2_1 axis, that at ½,0,0, is illustrated in Figure 2. The interactions it gives rise to alternate between hydrophobic and hydrophilic. The appropriate O(6) hydroxyl oxygen atoms are turned into the hydrophilic regions while the H atoms of the C(6) methylene group are directed toward the hydrophobic region.

The molecular conformation is illustrated in two views in Figures 3 and 4. The molecule displays an open conformation that

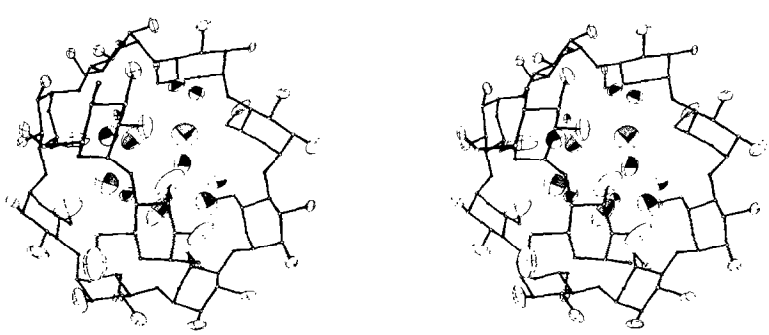

Figure 3. The conformation of the γ-cyclodextrin molecule. A fragment of a symmetry related molecule and the water molecules "included" in the torus are illustrated.

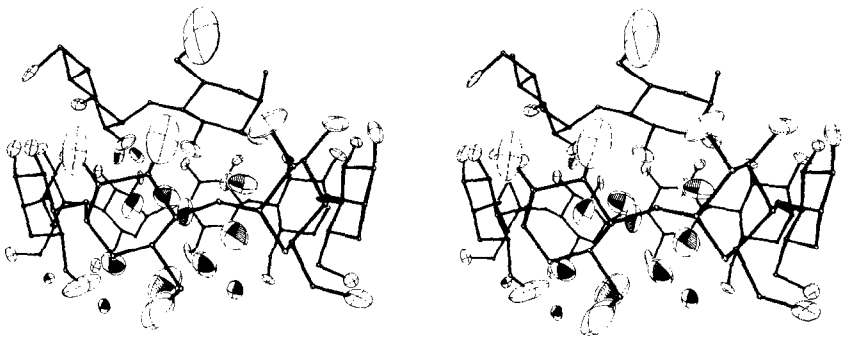

Figure 4. The contents of Figure 3 rotated through 90° about the horizontal axis to further illustrate the conformation of the macrocycle and the distribution of the "included" water.

obviously deviates significantly from 8-fold symmetry. A roughly quantitative appraisal of the deviations from ideal symmetry is provided by the data in Table I. The angle between the normals of the mean planes fit to atoms C(2)C(3)O(5)C(5) of adjacent sugar residues (ideally all equal) and between these normals and the mean plane fit to the eight O(4) atoms are tabulated. Ideally, the latter set of

Table I. Interplanar Angles Characterizing the γ-Cyclodextrin Molecular Conformation

Plane	ESD[a]	O(4),n	n,n+1
O(4)[b]	0.13A		
n=1[c]	0.08	18.6	50.4
n=2	0.01	1.9	44.6
n=3	0.02	17.9	46.4
n=4	0.04	6.5	44.6
n=5	0.02	12.9	41.1
n=6	0.04	10.6	45.5
n=7	0.03	8.3	45.3
n=8	0.02	20.1	45.2

[a]The tabulated ESD's do not include coordinate errors.

[b]The least-squares mean plane fit to the eight O(4) atoms.

[c]A arbitrary index sequentially assigned to glucose residues to identify least-squares planes fit to atoms C2), C(3), O(5), C(5).

angles would be all equal and would determine the pitch of the cone that describes the symmetrical CD torus. The conformation of uncomplexed-hydrated γ-CD much more closely resembles that found for the β-CD analog (4) than it does that of the α-CD analog (3). From these three examples, it appears appropriate to conclude that if strain energy plays a significant role in the mechanism of complex formation (9). its contribution is important only in the case of α-CD.

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