THE CRYSTAL STRUCTURE OF A COMPLEX OF CYCLOHEPTAAMYLOSE WITH 2,5-DIIODOBENZOIC ACID

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SUMMARY

The molecular structure of the 1:1 complex of cycloheptaamylose with 2,5-diiodobenzoic acid has been determined by X-ray crystallography. The iodine atoms of the guest molecule are disordered and were not used in the structure determination. The cycloheptaamylose molecules form channels in the crystal by means of head to head and tail to tail association using the two-fold crystallographic axis.

INTRODUCTION

Cycloheptaamylose (CHPA) is a cyclic polymer of D-glucose containing seven glucose units with α-1:4 glycosidic linkages. Cyclohexaamylose, cycloheptaamylose and cyclooctaamylose all form inclusion complexes with a wide variety of substances (1-6). In a previous paper (7) we reported the interrelated space group symmetry for complexes of CHPA with small organic molecules.

The cycloamyloses had been shown to cause a remarkable stereoselective acceleration of the cleavage of phenyl esters in homogeneous aqueous solutions (8,9). The mechanism of rate acceleration was shown to involve the complexed ester molecule and an alkoxide ion derived from the secondary hydroxyl groups of the cycloamylose (8,9). The magnitude of rate acceleration was explained on the basis of the stereochemistry of amylose-guest complex (8,9). 2,5-Diiodobenzoic acid (a substrate analogue) was chosen as the guest molecule in this present crystal structure analysis to try to determine this stereochemistry of binding.

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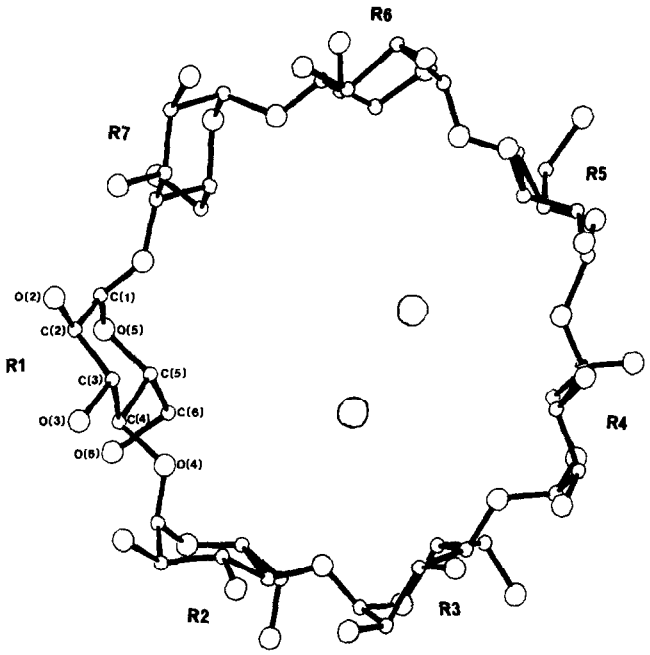

Fig. 1. Numbering scheme of the CHPA molecule. R1, R2, etc. refer to the residue number and I1 and I2 are the two major iodine sites of the guest molecule 2:5-diiodobenzoic acid.

METHODS

The CHPA-2,5-diiodobenzoic acid complex was prepared as previously described (7). The crystal form used here is identical to that found previously and the cell dimensions are the same to within 0.1 A.

Space group C2: $\underline{a}$ = 19.192(13) Å, $\underline{b}$ = 24.759(20) Å, $\underline{c}$ = 15.739(13) Å, β = 109.6(2)°.

The present full three-dimensional data set was collected on a Nonius automated 4-circle diffractometer at the University of Leeds (England) and consisted of 1885 unique reflections to 1.2 A resolution. Chemical analysis of this complex had indicated a 1:1 complex of CHPA to 2,5-diiodobenzoic acid. Attempts to locate the positions of the iodine atoms on a Patterson map were unsuccessful. In fact, the Patterson maps (2 A resolution) of all heavy-atom complexes mentioned in the preliminary paper (7) were uninterpretable in terms of full occupancy of the heavy-atom site. At this point the focus of our efforts moved from the heavy atoms of the guest molecule to the CHPA molecule itself. The orientation and position of the CHPA molecule with respect to the unit cell were determined using the rotation function (10) and translation search (11). The starting coordinate set for the search was obtained from computer modeling using a conformation similar to the cyclohexaamylose (12) and the coordinates of one of the glucose units from that paper. The resulting coordinates after the translation search were refined using the block-diagonal least-squares program in the X-ray 72 system. Individual atomic pa-

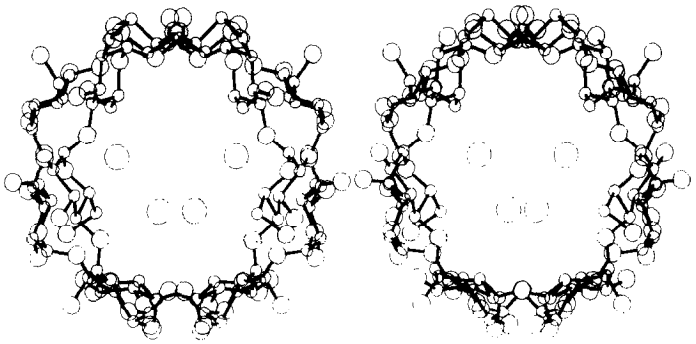

Fig. 2. View of the CHPA molecules along its channel axis which is also the crystallographic c axis. The biggest circles in the cavity are the major iodine sites of the guest molecules.

rameters and isotropic temperature factors were refined. A subsequent difference Fourier map showed the iodine positions almost continuously disordered around the seven-fold axis. Further refinement with 10 iodine partial sites and six well behaved water molecules brought the conventional R-index to 0.19 with 1885 reflections in the 1.2 A resolution sphere.

RESULTS AND DISCUSSION

The crystallographic refinement is complicated by the disordering of the guest molecule. We are at present working on a satisfactory method of treating this disorder. In view of this a discussion of the intramolecular bond distances and angles will be deferred until refinement is complete.

Each glucose unit is in C1 chair conformation and the glucose units are α-1:4 linked. The seven-fold symmetry of the CHPA molecule appears to be well maintained. This is reflected in the seven glycosidic oxygens being very nearly planar, the largest deviation from the least squares plane formed with these seven oxygens is 0.07 Å. In the case of the crystal structure of the cyclohexaamylose-iodoaniline complex this largest deviation was found to be 0.13 Å (5).

The hole through the CHPA molecule forms a continuous channel by the head-to-head and tail-to-tail stacking of the CHPA molecules. The closest stacking distance is through the secondary hydroxyl groups which form several intermolecular hydrogen bonds and also form an intensive regular network of

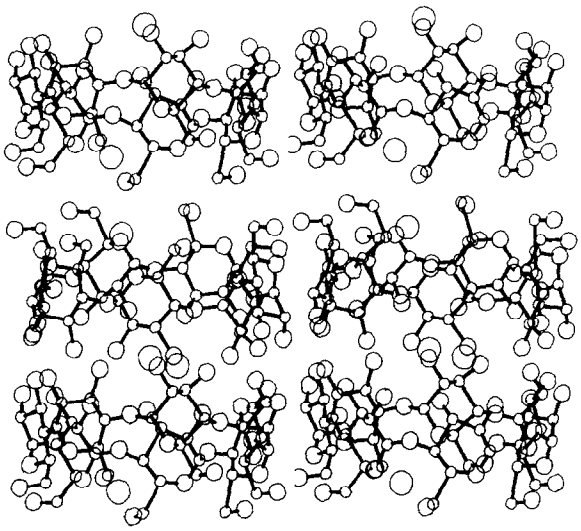

Fig. 3. View of the CHPA molecules perpendicular to the channel axis.

intramolecular hydrogen bonds. The guest molecule is also very close to the guest of the neighbor in this direction. The primary hydroxyl groups on the other end of the molecule are separated from the nearest neighbor by a longer distance. There is a continuous channel of well-defined water molecules through the structure parallel to the other channel through the torus of the CHPA. Each CHPA molecule is tilted at an angle of 10° to the channel direction; this tilt was found to be 7° in cyclohexaamylose (6).

Difference Fourier synthesis based solely on the atoms of the CHPA showed extensive electron density only within the CHPA cavity. At each end of the cavity there is a torus of heavy electron density parallel to each other and separated by about 6.9 Å, and normal to the seven-fold axis of the CHPA. Only a single such torus was observed in the difference Fourier synthesis of the m-iodo benzoic acid derivative. This establishes the carboxylic acid to be at the primary hydroxyl end of the cavity and the benzene ring is very near the middle.

It is curious that the close contacts to the next amylose molecule in the

direction of the secondary hydroxyl groups is maintained even at the expense of overcrowding of the guest. The close intermolecular contacts in this direction are probably of sufficient importance to justify calling the structure a dimer consisting of 2:2 CHPA-disordered guest. It is therefore of great interest to note the recent finding of a 2:1 complex between CHPA and the fluorescent probe 6-p-toluidinylnaphthalene-2-sulfonate (13).

At present in addition to completing this refinement we are examining the 2 Å resolution maps of the other isomorphous complexes, m-iodobenzoic acid, m-bromobenzoic acid, m-toluic acid, m-iodophenol, 2-bromo, 5-t-butyl phenol, and ethyl p-aminobenzoate (benzocain) (7). It is hoped that one or more of these may show less disorder and enable the position of the guest molecule to be determined with more precision.

We also have a full three-dimensional data set on a complex of CHPA with p-mercuri acetate aniline which crystallized in space group P1. The mercury atoms are again disordered. This structure is of interest as a model of the reversible binding of mercurials in biological systems.

ACKNOWLEDGEMENT

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