

Structure of the β -Cyclodextrin (β -CD) Inclusion Complex with *p*-Ethylaniline (PEA)

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Abstract. $(\beta\text{-CD}\cdot\text{PEA})_2\cdot 32\text{H}_2\text{O}$; $(\text{C}_{42}\text{H}_{70}\text{O}_{35})_2\cdot (\text{C}_8\text{H}_{11}\text{N})_2\cdot 32\text{H}_2\text{O}$, $M_r = 3088\cdot 8$, $P2_1$, $a = 15\cdot 300$ (1), $b = 32\cdot 311$ (2), $c = 15\cdot 584$ (2) Å, $\beta = 102\cdot 40$ (1)°, $V = 7524$ (1) Å³, $Z = 2$, $D_m = 1\cdot 367$ Mg m⁻³. The structure was solved by the single isomorphous replacement method with the aid of anomalous dispersion of iodine atoms, and refined by the full-matrix and block-diagonal least-squares method using 3420 reflections to $R = 0\cdot 25$. The two independent β -CD molecules associate tail-to-tail with intermolecular O(3)···O(3) hydrogen bonds to form a dimer. The β -CD dimer includes two guest PEA molecules in its cavity. These dimeric β -CD molecules were found to stack along the b axis to produce a new type of packing.

Introduction. Cyclodextrins have attracted considerable attention as model compounds for enzymatic reactions (Griffiths & Bender, 1973). It is very interesting that they can accelerate the cleavage of amides in a similar way to the hydrolytic enzyme, α -chymotrypsin (Komiya & Bender, 1977). In order to elucidate the acceleration mechanism, we have chosen here PEA as a substrate analogue or product, crystallized it with

β -CD, and determined the whole structure. A colourless triangular prismatic crystal was obtained by slow cooling of an equimolar β -CD and PEA aqueous solution. A crystal (0.40 × 0.30 × 0.30 mm) was sealed in a glass capillary with mother liquor and used for collection of X-ray diffraction data by a 2θ - ω scan mode. The isomorphism of the β -CD·PEA complex and the β -CD·*p*-iodoaniline complex [$P2_1$, $a = 15\cdot 292$ (3), $b = 32\cdot 490$ (5), $c = 15\cdot 531$ (4) Å and $\beta = 102\cdot 62$ (2)°] enabled us to calculate the three-dimensional difference Patterson map and to define the locations of three iodine atoms in an asymmetric unit. The three iodine sites were refined using 275 three-dimensional reflections at 3 Å resolution (Dickerson, Weinzierl & Palmer, 1968). The centric and Kraut R

Table 1. Refinement of the three iodine parameters

Refinement of parameters for the three iodine atoms at 3 Å resolution. Occupancy, R_k , and R_c indicate an occupancy of the atom, Kraut R and centric R factors respectively.

Site	x	y	z	Occu- pancy	B (Å ²)	R_k	R_c
I(1)	0.026 (7)	0.312 (0)	0.106 (7)	0.695	23.72	0.16	0.58
I(2)	0.904 (3)	0.664 (2)	0.885 (3)	1.000	0.10		
I(3)	0.006 (4)	0.549 (2)	0.048 (4)	0.740	0.10		

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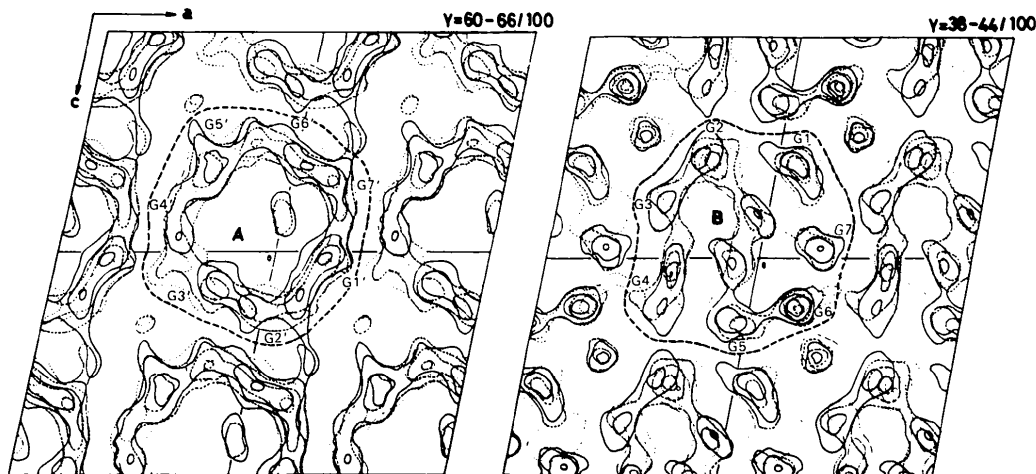


Fig. 1. Two parts of the 'best Fourier map' along the b axis at 3 Å resolution. A and B indicate the two independent β -CD molecules in an asymmetric unit.

factors were 0.58 and 0.16 respectively. The coordinates of these sites are listed in Table 1.*

Phase determination was carried out using the single isomorphous replacement method with the aid of anomalous dispersion of the iodine atoms (Matthews, 1966), yielding a mean figure of merit $m = 0.77$ at 3 Å resolution. Two β -CD molecules in an asymmetric unit were traced from the best Fourier map as shown in Fig. 1. The R factor for the 154 determined atoms was 0.45 for 2220 selected strong reflections.

The following refinement was by the full-matrix least-squares method with constraints applied to the bond lengths and angles of the two β -CD moieties. In succeeding difference Fourier maps, we could assign two PEA molecules and many water molecules. The occupancies of the PEA molecules were tentatively assigned as 0.80 and 0.75, and some of the water molecules were found to be in disorder. Final refinement by a block-diagonal least-squares method with anisotropic temperature factors for all atoms reduced the R to 0.25 for 3420 reflections at 1.2 Å resolution. The atomic scattering factors used were taken from *International Tables for X-ray Crystallography* (1974). All the numerical computations were done on an ACOS 700 computer at The Crystallographic Research Center, Institute for Protein Research, Osaka University, using the programs UNICS (1979) and ORXFLS4 (Busing, Martin & Levy, 1978).

Discussion. The average bond lengths and angles in the two β -CD molecules are shown in Fig. 2. They are all normal values. All the glucose units are in a 4C_1 chair conformation and intramolecular O(2)···O(3) hydrogen bonds are formed between neighbouring glucose units. Seven glucoside oxygen atoms [O(4)] of each β -CD molecule form a plane with a maximum atomic deviation of -0.22 and 0.23 Å respectively. These two planes are almost parallel (dihedral angle, 2.67°). The molecular structure of β -CD is well represented by the dihedral angles between the above-mentioned O(4) plane and the plane formed by the four atoms C(2), C(3), C(5) and O(5) of each glucose unit; the angles range from 77 to 90° . They are generally larger than those of the β -CD-ethanol complex (69 to 86°) (Tokuoka, Abe, Fujiwara, Tomita & Saenger, 1980). Therefore, the β -CD cavity of this complex opens more narrowly at the secondary hydroxyl side than does that of the ethanol complex. The two independent β -CD molecules associate tail-to-tail with

* Lists of phase angles and figure of merit in the phase determination, structure factor table, atomic parameters, observed bond lengths and angles, dihedral angles in β -CD, hydrogen-bond distances and the nearest-region distances have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 35878 (44 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

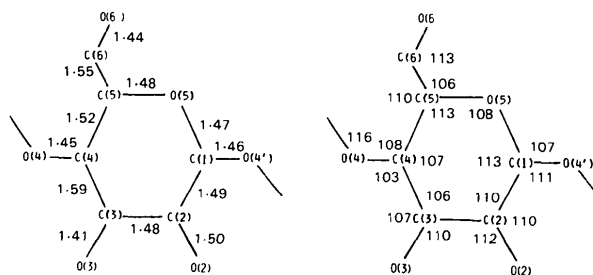


Fig. 2. The average bond distances ($\bar{\sigma}_{\max} = 0.24$ Å) and angles ($\bar{\alpha}_{\max} = 13^\circ$) of 14 glucose units in two β -CD molecules. Average standard deviations $\bar{\sigma}$ were obtained according to $\bar{\sigma} = [\sum(X_j - \bar{X})^2 / (m - 1)]^{1/2}$ with X_j = each individual observation, \bar{X} = mean of the m observations, $m = 14$.

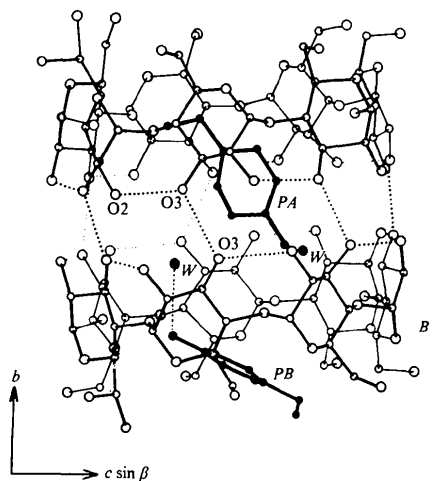


Fig. 3. A projection of the crystal structure along the a axis. PA, PB and W indicate two *p*-ethylaniline molecules and a water molecule respectively.

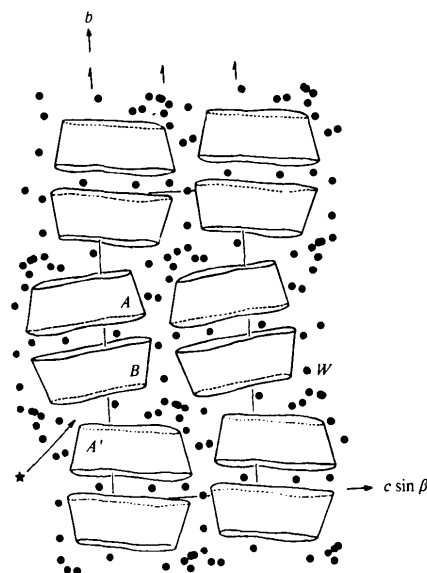


Fig. 4. A schematic representation of the molecular packing along the a axis. A and B indicate the independent β -CD molecules; solid circles represent water molecules. The asterisk indicates the position where two β -CD molecules come into closest contact.

intermolecular O(3)···O(3) hydrogen bonds to form a dimer as shown in Fig. 3. This dimer structure is similar to that found for the other dimeric β -CD complexes (Hamilton, Sabesan, Steinrauf & Geddes, 1976; Stezowski, Jogun, Eckle & Bartels, 1978; Harding, MacLennan & Paton, 1978) but, as shown in Fig. 4, the dimers stack along the *b* axis to produce a new type of packing, 'bent structure'. The dimers are separated by the normal van der Waals distances and come into closest contact at the position marked by an asterisk in Figs. 4 and 5, forming a hydrophobic region.

Two PEA molecules (*PA* and *PB* in Fig. 3) are found in a β -CD dimer. One PEA (*PA*) is included com-

pletely in the β -CD dimer and forms an NH···O hydrogen bond to a water molecule in the cavity. The other PEA (*PB*) is situated in the above-mentioned hydrophobic region and forms a hydrogen bond similar to that of the *PA* molecule. Both PEA's are in normal van der Waals contact with β -CD molecules. It is very interesting that the amino groups of the guests are located near the nucleophilic secondary hydroxyl groups of the host molecules.

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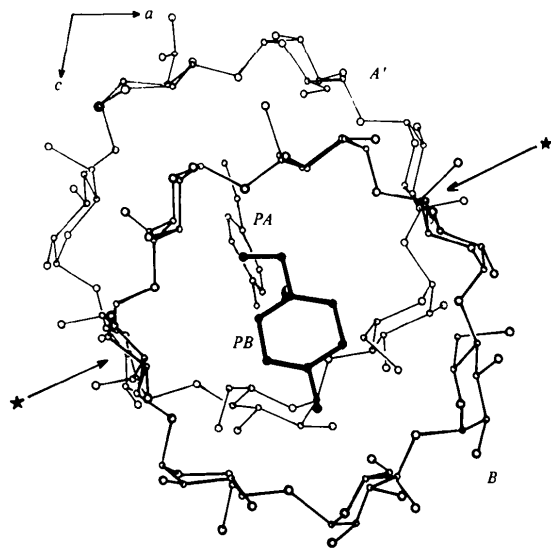


Fig. 5. A projection of the crystal structure along the *b* axis. *A*' is β -CD molecule *A* related by the twofold screw symmetry. A dimeric β -CD (*B*) is in closest contact with another dimer (*A*') at the position denoted by an asterisk.