X-ray Structural Studies on Two Forms of β-Cyclodextrin·barbital Complexes

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Abstract

The crystal structures of two forms of β-cyclodextrin·barbital complexes (Form I and Form II) were investigated by X-ray analysis. In Form I crystal, two β-cyclodextrin(β-CyD) molecules including two barbital molecules form a dimeric structure by the hydrogen bonds among their secondary hydroxyl groups. The unit cell volume of Form II is about twice as large as that of Form I and there exist two β-CyD dimers in Form II. Although the (Host)/(Guest) ratio is 1/1, only one barbital molecule can be found in Form II at the present stage. The similarities and differences between the two crystal structures are mainly discussed here.

1. Introduction

CyDs can be obtained by acting the cyclodextrin glucosyl transferases on starch and are the cyclic oligomers of α-1,4 linked D-glucopyranose residues. Well known α-, β-, and γ-CyD are constructed by six, seven, or eight D-glucopyranose units. β-CyD as well as the other CyDs has a conical cavity which can include various compounds and can change some properties such as the solubility and the stability of the included compounds. As β-CyD is also harmless and less expensive than the other CyDs, it has been used in the field of medicinal chemistry. As the barbituric acid derivatives(TABLE I) which have hypnotic and sedative properties are sparingly soluble in water, some work to improve the poor solubility by the aid of β-CyD had been done and it has been reported that the apparent solubility of them increased[1,2]. The stability constants and the thermodynamic parameters of the β-CyD inclusion complexes with barbital derivatives in aqueous medium have also been studied[3]. It is very interesting that these values are very different among the derivatives.

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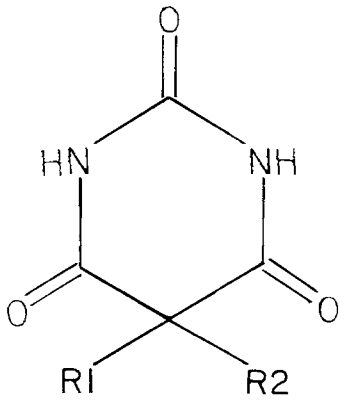
TABLE I. The barbituric acid derivatives.



R1	R2	
$-C_2H_5$	$-C_2H_5$	: Barbital
$-C_2H_5$		: Phenobarbital
$-C_2H_5$		: Cyclobarbital
$-C_2H_5$	$-CH_2CH_2CH(CH_3)_2$	: Amobarbital
$-CH_2CH=CH_2$	$-CH_2CH=CH_2$	: Allobarbital

It is our intention to do X-ray structure analyses of these inclusion complexes in order to elucidate how these derivatives having various substituents at C5 position are included in the β-CyD cavity and why some physicochemical constants and parameters are different among the derivatives. Firstly we chose barbital(5,5-diethylbarbituric acid) as a guest molecule and found two different crystals(Form I and II).

2. Experimental

2.1. Form I complex

The colorless plate crystals were obtained by gradually cooling 1.5 ml of the hot aqueous solutions containing about $2X10^{-4}$ mol of β-CyD and barbital, respectively. The specific gravity of the crystals was measured by the floatation method using carbon tetrachloride and o-dichlorobenzene. After a crystal was enclosed in the 0.5 mmϕ glass capillary with a little amount of mother liquid, the lattice constants and the intensity data were measured with nickel-filtered CuKα radiation using the Rigaku Rotor AFC-5FOS automated four-circle diffractometer (40kV, 200mA). A total of 8356 independent reflections with $F_o > 3\sigma F_o$ were collected by using ω-2θ scan mode up to 125° in 2θ. The crystal data are listed in TABLE II. As the cell dimensions and the location of the big peaks on the Patterson map were very similar to those of the β-CyD·aspirin complex[4], we regarded Form I as isomorphous with β-CyD·aspirin. Therefore as the β-CyD model we used the coordinates of dimeric β-CyDs from β-CyD·aspirin and then the guest and water molecules could be found by the successive Fourier syntheses. The refinement of the atomic coordinates are in progress by the full-matrix least-squares method[5] with restraints applied to the bond lengths and angles of β-CyD and barbital molecules and at the present stage, R factor is 25.4 % for the 4363 strong reflections using isotropic thermal parameters for all the non-hydrogen atoms.

TABLE II. Crystal Data

	Form I	Form II
Chemical Formula*	$(\beta\text{-CyD})_2 \cdot (\text{Barbital})_2 \cdot 31H_2O$	$(\beta\text{-CyD})_4 \cdot (\text{Barbital})_4 \cdot 50H_2O$
Molecular Weight	3197.0	6177.7
Crystal System	Triclinic	Triclinic
Space Group	P1	P1
Z	1	1
Cell Dimensions;		
a (Å)	19.716(3)	34.341(5)
b (Å)	15.497(1)	15.529(2)
c (Å)	15.549(2)	15.568(2)
α (°)	103.63(1)	103.82(1)
β (°)	116.65(1)	100.58(2)
γ (°)	104.56(1)	106.67(1)
V ($Å^3$)	3762(1)	7434(1)
Dm (g/cm^3)	1.41(1)	1.38(2)
Dx (g/cm^3)	1.41	1.38

* β-CyD : $C_{42}H_{70}O_{35}$, Barbital : $C_8H_{12}N_2O_3$

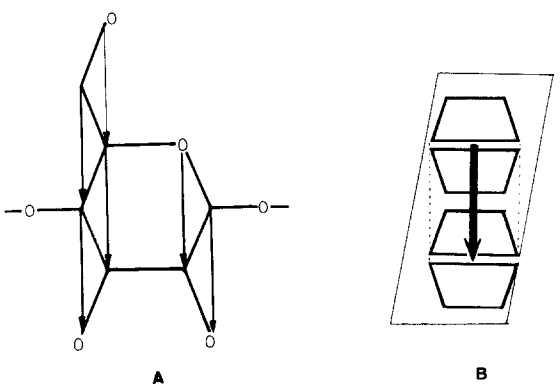
2.2. Form II complex

Form II was crystallized using similar conditions to that for Form I except for reducing the amount of water to 0.8 ml. Although both Form II and Form I crystals came out from the same batch, we could find the Form II crystals with higher certainty because the Form II crystals were much thinner than Form I crystals. The crystals were also enclosed in the glass capillary. The lattice constants and the intensity data were measured with graphite monochromatized CuKα radiation using the Rigaku AFC-5 automated four-circle diffractometer (40kV, 30mA). A total of 2827 independent reflections with $F_o > 3\sigma F_o$ were measured by using ω-2θ scan mode up to 70° in 2θ. The crystal data are also listed in TABLE II. We expected that four β-CyD molecules existed in an unit cell since the volume of the unit cell was about two times as large as those of the normal β-CyD dimers. Moreover as listed in TABLE III, there were two large peaks on the Patterson map in addition to the origin. A peak(b) 2.5 Å from the origin is characteristic of the interatomic vectors of the glucopyranose moiety and shows the orientation of CyD molecules. On the other hand, a peak(c) was found at 16.3 Å from the origin. Considering the existence of four β-CyD molecules in an unit cell, we judged that there existed two dimeric β-CyDs and peak(c) indicated inter-dimer vectors. Then a dimeric β-CyD model was constructed using the structure of the β-CyD·3,4-xylidine complex[6]. Based on a Patterson map, the orientations and the shifts were given to two dimer models, then the minimum R factor was searched by rotating the models around the molecular axes of dimeric β-CyDs. Thus we decided the position of the host molecules and then could find one guest molecule and fourteen water molecules by the successive Fourier method.

As the elemental analysis of Form II crystals indicated 1/1 (Host)-/(guest) ratio, we have to look for three more barbital and 36 more water molecules. However, at the present stage, we can not assign these molecules because the separation of the peaks on Fourier maps are not good owing to the use of low resolution reflection data and/or the existence of the notable disorder at these areas. The R factor is 21.9 % for all available reflections using isotropic thermal parameters.

TABLE III. The large peaks in addition to the origin on Patterson maps.

	U	V	W	L* (Å)
peak(a)	9.0/60	4.8/50	6.1/50	2.4
peak(b)	7.5/100	1.4/50	0.6/50	2.5
peak(c)	49.0/100	6.1/50	1.2/50	16.3



The peak(a) and peak(b) indicate the interatomic vectors of glucopyranose moiety illustrated in **A** for Form I and Form II, respectively. The peak(c) indicates the inter-dimer vectors illustrated in **B** for Form II.
* : Length from the origin to the peak.

2.3. Computations

All the crystallographic computations were carried out on an NEAC ACOS 850S computer at The Crystallographic Research Center, Institute for Protein Research, Osaka University.

3. Results and Discussion

3.1. Form I complex

The numbering scheme of the β-CyD and barbital molecules is shown in Fig. 1, and the inclusion status of Form I is shown in Fig. 2. Two β-CyDs form a dimeric structure by the intermolecular hydrogen bonds among their secondary hydroxyl groups. Such a structure has been frequently seen when β-CyDs are used for the host molecules. This fact is explained by the existence of the tight inter-molecular hydrogen bonds between two β-CyDs[6]. While one guest molecule could be assigned in the round shaped hydrophobic sphere, another one was included shallowly thrusting its one ethyl group from the primary hydroxyl side of β-CyD.

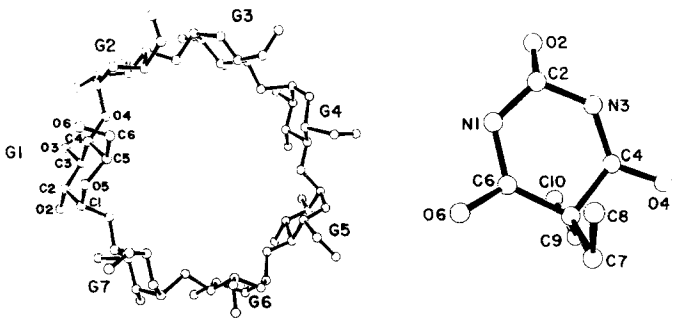


Fig. 1. The numbering schemes of each β-CyD and barbital molecules. G1,G2...G7 means the number of the glucosyl residues.

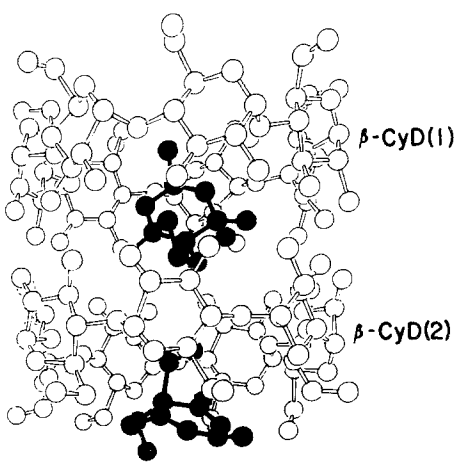


Fig. 2. The ORTEP drawing of the inclusion state of Form I. Barbital molecules are in the full lines and circles.

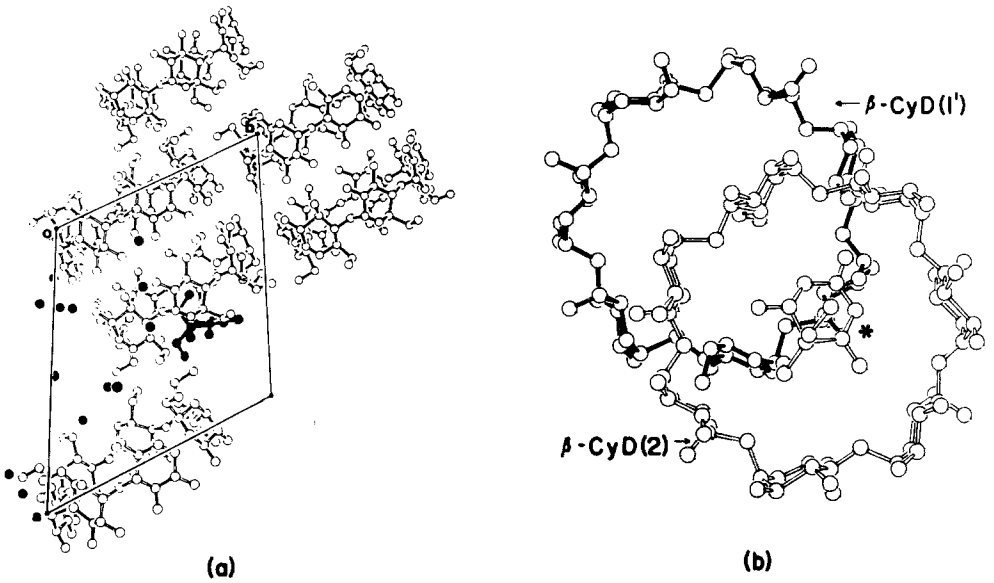


Fig. 3. The packing status of Form I complex; (a) The cage type packing system with the brickwork pattern. Only one barbital molecule which is in full lines and circles is drawn in the packing mode. Water molecules are also drawn in full circles. (b) The stacking of β-CyD(2), barbital and shifted β-CyD(1') molecules. Barbital molecule denoted by the asterisk is included in β-CyD(2).

Such an inclusion state was also observed in the structure of β-CyD·phenobarbital complex[7] which was determined as one of our serial structural studies of β-CyD·guest complexes, but in this case it was not the ethyl group but the more bulky phenyl group of phenobarbital which was included. These facts indicate that the β-CyD cavity has the selectivity to include the moiety which fits better into its own space when there are many guest molecules with different hydrophobic groups.

The packing of each molecule in the crystal is shown in Fig. 3. The β-CyD dimers are packed in the manner of the brickwork pattern which is one of the cage type packing structures and has been frequently seen in the β-CyD inclusion complex structure of the triclinic space group P1. It is obvious that the barbital molecule included shallowly in the β-CyD(2) can not escape easily because of the existence of the shifted β-CyD(1') as depicted in Fig. 3-(b). As the maximum molecular diameter of barbital molecule is larger than the minimum cavity diameter of β-CyD, a barbital molecule buried in the dimeric β-CyD also can not escape from the crystalline state. So barbital molecules are bound very tightly by β-CyDs. However, when the crystals are dissolved in aqueous medium and the matrix structure of β-CyD is broken, the barbital molecules included only at their ethyl groups are easily released.

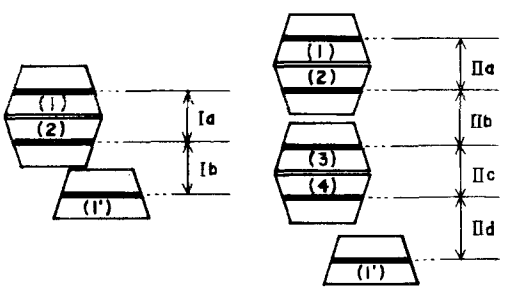
On the contrary, phenobarbital, which is stabilized by van der Waals forces between the hydrophobic β-CyD cavity and the phenyl ring in the β-CyD·phenobarbital crystal, is much more difficult to release and accounts for the eleven times larger stability constant for β-CyD·phenobarbital than β-CyD·barbital complex[3].

As shown in TABLE IV, the planes through seven glucosyl O4 atoms of β-CyD are layered one another with a 7 Å interval. There exist many water molecules in the space among these packed dimeric β-CyD units. Although in these spheres there are still many peaks at a low level on the difference Fourier map, these could be assigned as disordered water molecules. But what is important to remark here is the locations of ordered water molecules. At the present stage, we could find 19 water molecules, in which 13 waters occupied the same positions as those in β-CyD·aspirin complex used as the starting model. This fact implies that these waters are necessary to form the cage type matrix structure. Whereas disordered waters locating like clusters of grapes[6] except those relating to the host or guest molecules through hydrogen bonding are regarded as spacers to fill the vacant sphere.

3.2. Form II complex

As with the Form I complex, four β-CyDs in an unit cell form two dimeric structures by the hydrogen bond among the secondary hydroxyl groups. As described above, we could assign no more than one guest molecule. As shown in Fig. 4, the inclusion status is very similar to Form I, that is, the hydrophobic ethyl group is inserted in the β-CyD cavity from the primary hydroxyl group side leaving also the base skeletone of barbital i.e. pyrimidine-tri-one ring near the primary hydroxyl side of β-CyD.

TABLE IV. The distances between O4-planes.



	distances (Å)
Ia	6.8
Ib	7.0
IIa	7.1
IIb	8.9
IIc	7.0
IId	10.6

The number in the parentheses is β-CyD unit number.

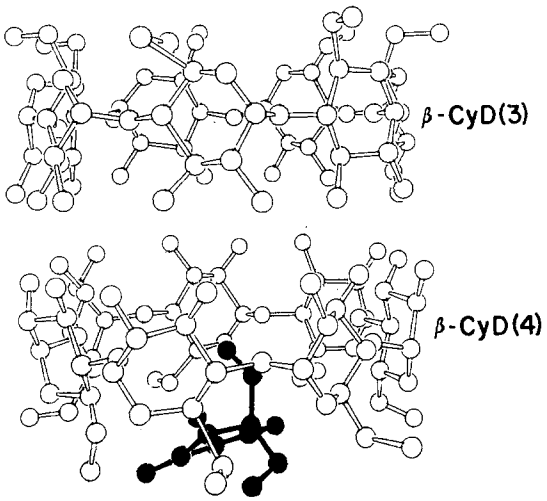


Fig. 4. The ORTEP drawing of the inclusion state of Form II. β-CyD(1) and β-CyD(2) are not drawn. Barbital molecule is in full lines and circles.

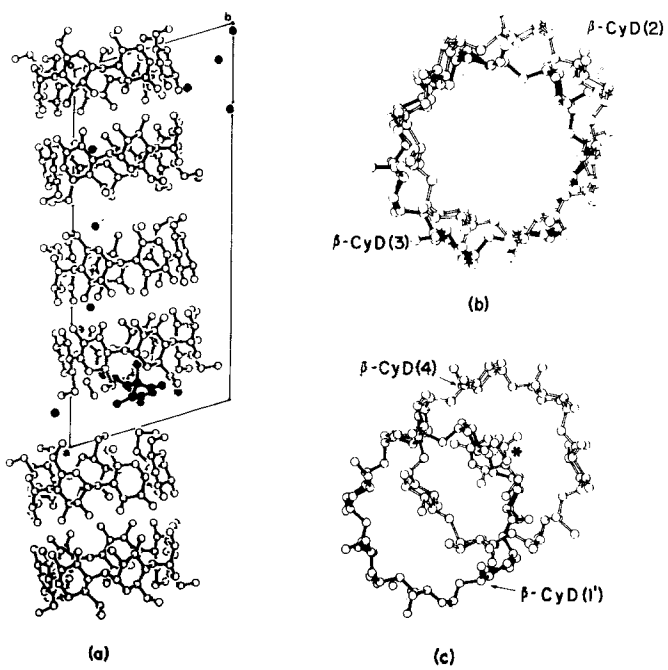


Fig. 5. The packing status of Form II complex; (a) Both channel and cage type packing systems of β-CyD molecules. Barbital and water molecules are drawn by full lines and circles. (b) The stacking of β-CyD(2) and β-CyD(3) molecules in the channel type packing area. (c) The stacking of β-CyD(4) and shifted β-CyD(1') molecules in the cage type packing area. Barbital molecule included in β-CyD(4) is denoted by an asterisk.

Fig. 5 shows the packing in the crystal. Two β-CyD dimers stack like a tube in an unit cell(Fig. 5-(a) and (b)) forming a channel type packing system. At the present stage, there is only one direct hydrogen bond among the primary hydroxyl groups of β-CyDs, so the interactions here are weaker than those of the secondary hydroxyl side which play an important role to form a dimer. However, comparing the distances between O4-planes(TABLE III), the distance of IIb is shorter than that of IId but is longer than that of Ib. So there must be some important interactions in this area. On the contrary, at the unit cell edge along the a-axis, the next unit β-CyD(1') is arranged so as to close the barbital molecule in β-CyD(4) as well as in Form I complex(Fig. 5-(a) and (c)). As the distances between the barbital and β-CyD(1') are longer than that of the Form I complex, the host-guest interactions may be weak in this crystal. If we regard two dimers in the channel type packing as a β-CyD tetramer, this tetramer packs also in the way of the brickwork pattern, that is the cage type mode. This is the first time that both channel and cage type packing systems appear in a crystal at the same time. But we can not explain why Form II crystals with such a packing system results in the smaller amount of crystalline water.

4. Conclusion

Comparing Form I with the Form II complex, we can pick out the following points;

Similarities:

1) Both crystals are colorless and plates.
2) The (Host)/(Guest) ratio is 1/1.
3) Two β-CyDs form a dimer by hydrogen bonds between the secondary hydroxyl sides.
4) Both crystals are formed on the basis of the cage type packing mode with the brickwork pattern so as to fix the guest molecules at the primary hydroxyl side.
5) One barbital molecule is shallowly included inserting an ethyl group into the β-CyD cavity.

Differences:

1) The specific gravity of Form I crystals is larger than that of Form II.
2) The unit cell volume of Form II is about two times as large as that of Form I, whereas the volume per one dimeric β-CyD of Form I is a little larger than that of Form II.
3) The packing along the molecular axis of β-CyD of Form I is closer than that of Form II.
4) Although the distance between two O4-planes of each β-CyD in Form I is about 7 Å, the intervals of those in Form II range from 7 to 11 Å.
5) Form II constructs a β-CyD tetramer-like structure, which form the channel type packing.

The important points to be considered about the formation of the two different crystals of β-CyD·barbital complex are in Differences 1) and 3). As the (Host)/(Guest) ratio is equal in both form, Form II, having a smaller crystal volume per one β-CyD dimer, is considered to have a lighter density, but not actually. We can not make a sweeping statement about this fact because the positions of the whole water molecules are not clear, but if the environment of water molecules around the β-CyD dimer for both complexes is the same, we may say that the β-CyD dimer units pack more closely along the molecular axis in Form I. By detailed observation of the water molecules existing in the loose packing area of Form II, we will explain the problems more clearly. J.STEZOWSKI et al. have obtained the stable and metastable crystals of the β-CyD·n-propanol complex[8]. M.YAMAZAKI et al. also have obtained the stable and metastable crystals of the β-CyD·water complex[9]. In the latter case, both crystals had the cage type packing by monomeric β-CyD and their cell dimensions are almost the same. The main difference is the number of water molecules. On the contrary in the former complex, β-CyD forms a typical dimeric structure and the cell dimensions are considerably different. And that in the stable crystal dimeric β-CyD packs in the cage type system with a brickwork pattern, however, dimeric β-CyD showed a channel type packing system in the metastable crystal. In view of these facts, our crystals are similar to the β-CyD·n-propanol crystals. But as we could not observe the transition from the metastable to the stable crystals in aqueous solution as seen in the β-CyD·n-propanol crystals, we can not conclude which form is in the stable state at the present stage. Considering the more loose packing status of Form II than that of Form I, we can expect that Form I complex may be in a more stable crystalline state.

Now we proceed with the refinement of Form I structure and collect the high resolution reflection data of Form II crystals using a Rotor-diffractometer. In the near future we hope to solve the above problems more clearly.

5. Acknowledgements

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6. References

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