Preparation and Molecular Recognition Ability of Mono[6-deoxy-6-(L-tyrosylamino)]-β-cyclodextrin. Formation and Guest Binding Properties of Distorted Cavity Formed Outside the Parent Cyclodextrin

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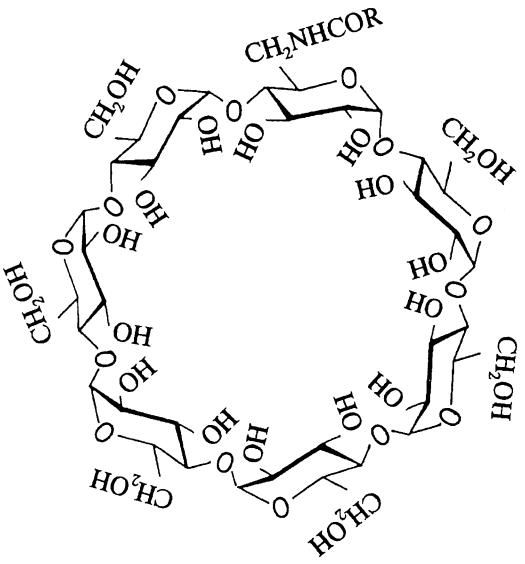
Mono[6-deoxy-6-(L-tyrosylamino)]-β-cyclodextrin (**2**) was prepared from *N*-(benzylcarbonyl)-L-tyrosine dicyclohexylamine salt (Z-Tyr DCHA salt) and mono(6-amino-6-deoxy)-β-cyclodextrin. **2** includes *N*-dansylphenylalanine enantioselectively (K_L/K_D=2.13). From an NMR study, it was suggested that the hydroxyphenyl group attached to **2** was located at the outside of the cavity and that **2** had an expanded and distorted cavity enclosed by the hydroxyphenyl moiety and the parent cyclodextrin wall.

Molecular recognition by modified cyclodextrins (CD) is currently of great interest in host-guest chemistry. Since CD has been known as a rigid host, efforts have been concentrated on obtaining more rigid and tighter cavities by chemical modification. Molecular recognition is a dynamic process based on distinct chemical interactions, not on passive fitting on "a lock and a key". Moreover, current reports have mentioned some evidence that CD can behave as a flexible host.[1] For this reason, the modification of CD should be carried out so as to achieve sufficient flexibility for conformational reorganization, induced-fit.[2] Reported evidence of flexibility was observed upon including the aromatic moiety. From this point of view we prepared some one-point aromatic amino acid-binding CDs.[3] These modified CDs have a phenyl group used as the cavity size control factor, or as a "self-guest molecule", and the flexible arm of sp^3 carbons between the parent CD cavity and the phenyl group. In our previous examination, the length of an arm as long as –C–C–CONH– was suitable. For example, 6-deoxy-6-[(*N*-formylphenylalanyl)amino]-β-CD (f-Phe-CD) formed an "intramolecular complex" including the f-Phe residue in the hydrophobic cavity and the intramolecular complex was released by adding the guest molecules. That phenomena is like induced-fit in an enzyme-substrate system. Moreover, although an enantioselective inclusion behavior was observed,[4,5] we never indicated a host with both large binding ability and high enantioselectivity for the guest molecules, since the distorted field where an enantiomer was recognized was formed by a partial interruption of the native CD cavity. In this report, a new host having a distorted and expanded cavity and displaying both high binding constants and molecular recognition is indicated. The cavity of the new host comprising a parent CD wall and a Tyr-residue is distorted, though the Tyr-residue is located outside of the cavity.

Experimental

Materials. 6-Deoxy-6-[(*N*-formylphenylalanyl)amino]-β-cyclodextrin (f-Phe-CD) and 6-deoxy-6-[(*N*-formylphenylglycyl)amino]-β-cyclodextrin (f-pheGly-CD) were prepared according to a method which we reported previously.[2] Mono [6-deoxy-6-L-tyrosylamino)]-β-cyclodextrin (**2**:Fig. 1) was prepared according to the method for f-Phe-CD.[2] Mono (6-amino-6-deoxy)-β-cyclodextrin (ACD), 1.2 equivalents of Z-tyrosine dicyclohexylamine salt and *p*-toluenesulfonic acid were treated with dicyclohexylcarbodiimide in DMF at 5°C for 10 min. Crude 6-deoxy-6-[*N*-(benzoxycarbonyl)-L-tyrosylamino]-β-cyclodextrin (**1**) was yielded (80%) by the usual treatment. **1** was dissolved in an aqueous solution and treated with Pd/C under bubbling H_2 at 60°C for 1 h. After removing Pd/C, the reaction mixture was evaporated to dryness. The precipitate was recrystallized with water to give **2** (22% yield); (Found: C, 43.13; H, 6.93; N, 2.08%.



1 R=$HOC_6H_4CH_2CH(NHZ)$
2 R=$HOC_6H_4CH_2CHNH_2$
f-PheCD R=$C_6H_5CH_2CH(NHCHO)$
f-pheGlyCD R=$C_6H_5CH(NHCHO)$

Fig. 1. Structure of **2**.

Table 1. Association Constants[a)]

Host	Guest (K/mol^{-1} dm^3)				
	ANS	TNS	DNS-L-Phe	DNS-D-Phe	K_L/K_D
L-TyrCD[b)]	365±6	360±1	629±10	295±3	2.13
f-D-PheCD[c)]	78±11	167±27	83±28	160±36	0.52
f-L-PheCD[c)]	68±19	207±29	231±45	139±24	1.66
f-D-pheGlyCD[c)]	137±17	—[d)]	368±55	505±70	0.72
f-L-pheGlyCD[c)]	80±19	1026±47	262±56	381±52	0.69
β-CD[c)]	78±8	—[d)]	153±14	197±20	0.77

a) determined with fluorescence intensity at 540 nm excited at 350 nm; conditions: pH 7.0 solutions (1/15 mol dm^{-3} phosphate buffer), 25°C, [guest]=5.0×10^{-5} mol dm^{-3}, [cyclodextrin]=0.0—8×10^{-3} mol dm^{-3}. b) This work. c) Previous work.[3,4)] d) 2:1 host–guest complex.

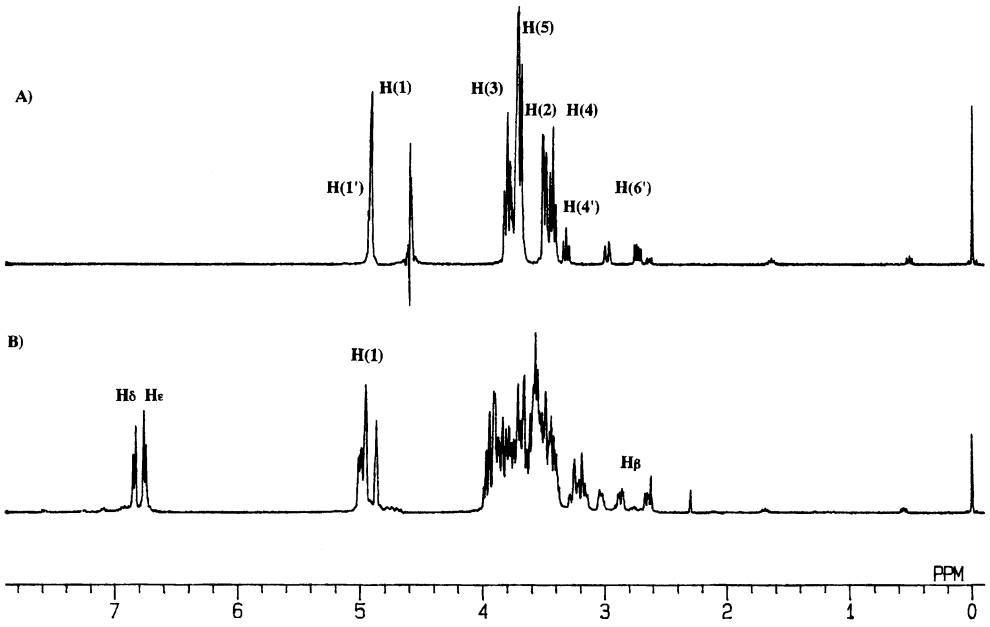


Fig. 2. 400 MHz ^{1}H NMR spectra of ACD (A) and **2** (B) in a 0.01 M solution in D_2O. (1M=1 mol dm^{-3})

Calcd for $C_{51}H_{80}N_2O_{36}\cdot 7H_2O$: C, 43.03; H, 6.67; N, 1.97%).

Measurement. The NMR spectra were measured on a JEOL EX400WB spectrometer in D_2O using DSS as an internal reference (0.00 ppm). The association constants (K) between the host and various guest molecules were estimated by drawing Benesi-Hildebrand plots. The K values were obtained from fluorescence spectra which originated from the guest molecules. The detailed conditions are indicated in the footnote of Table 1.

Results and Discussion

Molecular Recognition Ability of CD Derivatives Binding with an Amino Acid Side Chain. Table 1 gives the association constants of **2** for some naphthyl derivatives; for a comparison, those of the other CDs are also indicated. Large association constants were displayed by **2**. The K values of **2** for 8-anilino-1-naphtalenesulfonic acid (ANS) and dansyl (DNS)-L-Phe were more than 4-times larger than that of the parent β-CD. The large K values for **2** suggest that although **2** has a bigger cavity than the parent β-CD, **2** indicated enantioselectivity for dansylphenylalanine (K_L/K_D=2.13). The selectivity was larger than that of either f-L or D-Phe-CD, which has a distorted cavity and can behave as a flexible host with phenyl group movement induced by the inclusion of a guest molecule.

Where Is the Tyrosine Moiety? Judging from the result of the association constants, the cavity of **2** should be enclosed. A determination of the position of the Tyr-residue in the CD cavity was necessary. NMR studies have provided important information concerning the molecular geometry of CD inclusion complexes.[6)] Our previous examination by ^{1}H NMR measurements also clarified the structural conformation of f-Phe-CD and f-pheGyl-CD. Direct evidence concerning the orientation of the phenyl residue of a modified CD with respect to the macrocyclic ring was accomplished using the ROESY method; the ε and/or ζ protons of the f-L-Phe-residue had NOE crosspeaks with the H3 protons of the A, B, C, G, and F glucopyranose units

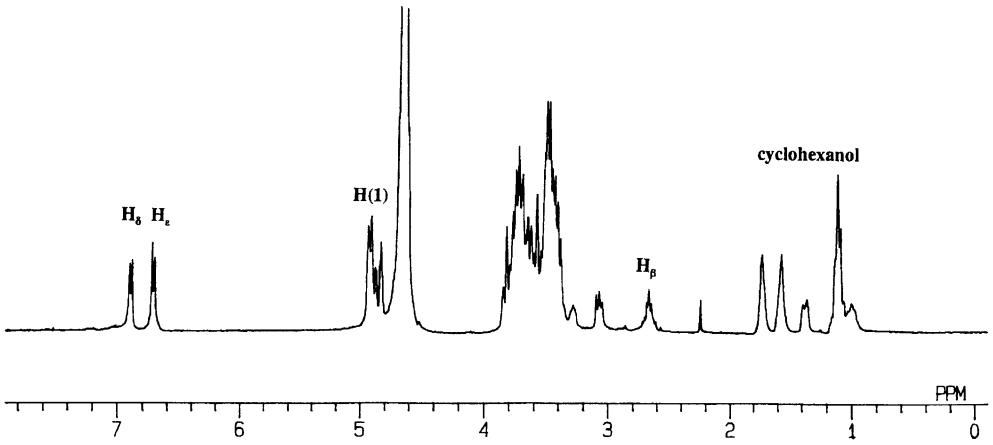


Fig. 3. 400 MHz ^{1}H NMR spectra of **2** in a 0.02 M solution in D_2O in the presence of cyclohexanol.

of CD, and the δ proton of that had with the H3 proton of the B unit, with the H5 protons of the A, B, C, and G units and with the H6 of the A and E or F units. Upon considering these experimental facts, we concluded that the f-L-Phe-residue was included in the CD cavity from the primary hydroxyl group side of the macrocyclic ring forming the "intramolecular inclusion complex". We also indicated that from the characteristic chemical shifts of the phenyl protons, the location of the phenyl group, whether inside or outside of the cavity, can be easily determined.[5] Phe-CD without a formyl substituent also displayed the characteristic shifts, and formed an intramolecular complex, whereas f-pheGly-CD, which has a shorter arm length than that of Phe-CD, did not form intramolecular complex. The decision based on the large lower-field shift of the *para* proton cannot be applied to **2** with the hydroxyl group on the *p*-position. The author tried to measure NOE of **2** using the ROESY method. The 400 MHz ^{1}H NMR spectrum of **2**, and that of ACD for a comparison, are shown in Figs. 2B and 2A, respectively. In the ROESY spectrum, although the crosspeaks between the anomeric C1 protons and the aglyconic C4 protons were clearly observed, the protons of the Tyr-residue have the NOE crosspeak with neither protons on the macrocyclic ring nor with the Hα and Hβ protons of the Tyr-residue. These results indicate that the hydroxyphenyl group of the Tyr-residue is outside the cavity of the macrocyclic ring. Compound **2** has a sufficient arm length to include the Tyr-residue in its hydrophobic cavity. The hydroxyl group appeared to disturb the phenyl moiety inclusion. It is possible that the hydroxyl group is near to the primary hydroxy group on the C6 position, and that hydrogen bonding occurs. This may be the difference between **2** and f-pheGly-CD. The phenyl group on f-pheGly-CD is like a "pendant", and that on **2** may be "capped" with amide bond(covalent bonding) and hydrogen bonding. In other words, **2** forms an "outside intramolecular complex". The ^{1}H NMR resonances of the Tyr-residue changed with excess cyclohexanol (Fig. 3). The chemical shifts of Tyr-residue were almost the same as that in a dilute alkaline solution in which hydroxy group of Tyr is dissociated (Fig. 4). These results suggest that the outside intramolecular complex of **2** was released by the inclusion of cyclohexanol molecule like induced-fit in the enzyme-substrate system.

Structure of the Macrocyclic Rings. Evidence concerning the structure of the macrocyclic ring was also obtained by NMR studies. Since all seven glucopyranose units of the parent β-CD are magnetically equivalent due to the presence of the C_7 symmetry axis on

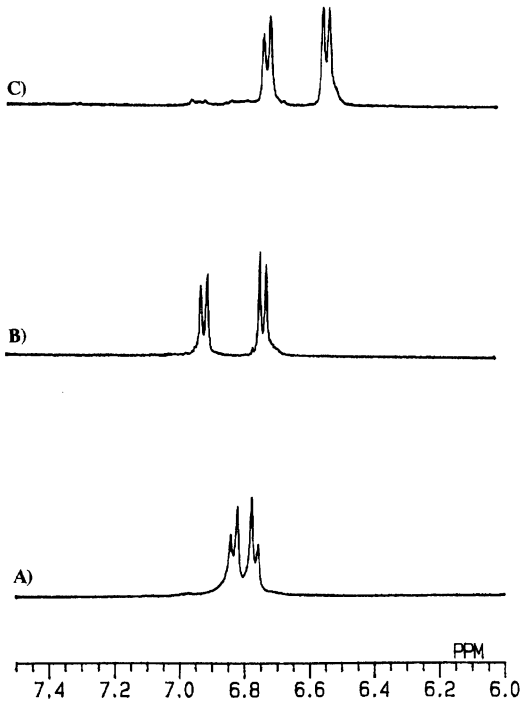


Fig. 4. Phenyl ring proton region of the 400 MHz ^{1}H NMR spectra of **2** in the absence (A) and presence (B) of cyclohexanol in D_2O and in NaOD/D_2O (C).

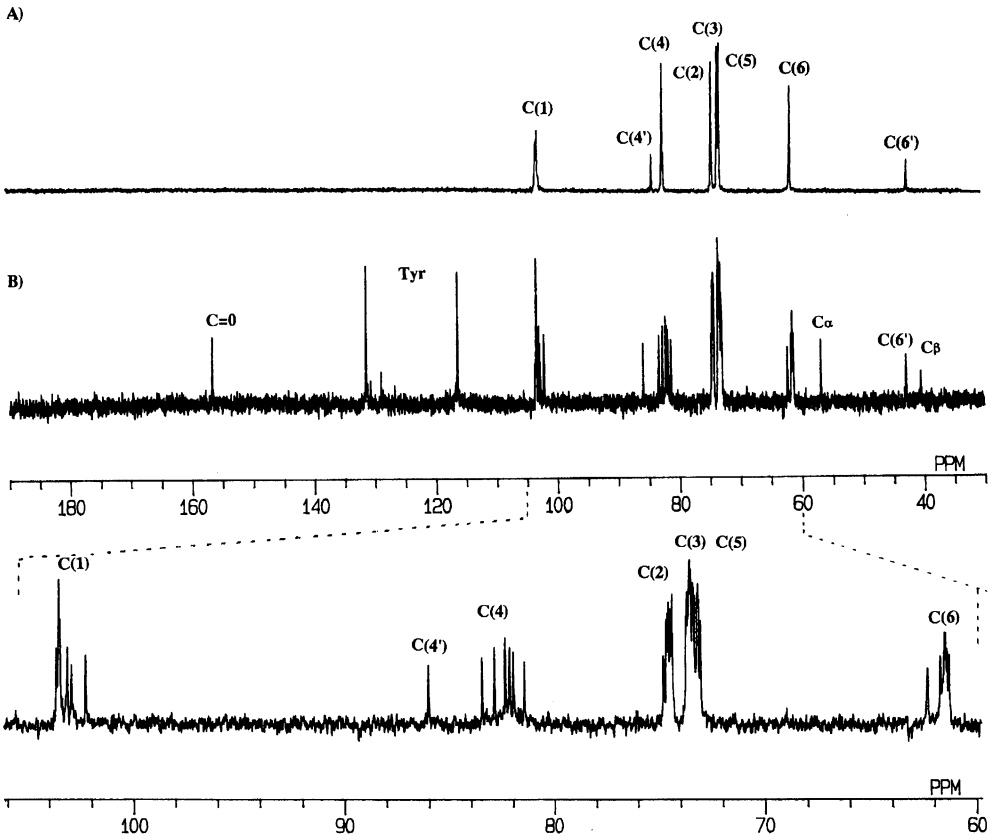


Fig. 5. 100 MHz ^{13}C NMR spectra of ACD (A) and 2 (B) in a 0.02 M solution in D_2O. The assignments are also given.

Table 2. ^{13}C Chemical Shifts of the Monosubstituted Cyclodextrin[a)]

Assignment	$\Delta\delta$/ppm						
	L-TyrCD (D_2O)	L-TyrCD (NaOD/D_2O)	L-TyrCD (DMSO-d_6)	f-L-PheCD (D_2O)	f-L-pheGlyCD (D_2O)	ACD (D_2O)	β-CD (D_2O)
C1 ($\Delta\Delta\delta$)[b)]	101.44 (1.51)	102.60 (0.47)	103.22 (0.55)	102.80 (0.99)	102.58 (0.89)	102.87 (0.19)	103.71
	102.20	102.91	103.49	103.02	103.03	103.06	
	102.25	103.07	103.77	103.62	103.24		
	102.47			103.79	103.42		
	102.82				103.47		
	102.85						
	102.95						
C4 ($\Delta\Delta\delta$)[b)]	81.24 (3.97)	81.58 (3.26)	82.95 (3.46)	82.04 (4.01)	81.71 (4.01)	82.04 (1.96)	82.74
	81.33 (2.17)[c)]	81.71 (0.68)[c)]	83.12 (0.54)[c)]	82.26 (1.19)[c)]	82.06 (1.19)[c)]	82.26 (0.22)[c)]	
	81.66	81.91	83.49	82.47	82.33	84.00[d)]	
	82.19	82.26	85.31[d)]	82.72	82.39		
	82.72	84.86[d)]		83.23	83.01		
	83.41			86.05[d)]	83.06		
	85.21[d)]				85.72[d)]		

a) Measured with EX 400WB(JEOL) spectrometer at 27°C. b) The variation of $\Delta\delta$. c) The variation of $\Delta\delta$ except the $\Delta\delta$ of C4 carbon in substituted glucose unit. d) The $\Delta\delta$ of C4 carbon in substituted glucose unit.

its molecule in solution, it has been known that a single set of NMR resonances is observed as if there were only one glucopyranosyl residue. For modified CDs that have a symmetry-breaking constituent, however the C_7 symmetry of the macrocyclic ring is perturbed. As is shown in Fig. 2A, the C1 resonances of ACD are divided into only two sets of signals, one corresponding to a glucopyranose unit with an amino moiety, and the other to six unmodified glucopyranose units. This result means that the six unmodified glucopyranose units are magnetically equivalent. On the contrary, in the case for **2** (Fig. 2B), good discrimination in the anomeric C1 proton resonances suggest that all of the glucopyranose units in the macrocyclic ring are magnetically

nonequivalent, and that the cavity is unsymmetrical. The same discrimination has been observed in f-Phe-CD. The ^{13}C NMR measurements provided more direct information concerning the conformational structure of the macrocyclic rings. It has been reported that the ^{13}C chemical shifts for the anomeric and aglycone carbon atoms (C1 and C4 carbon atoms) in oligosaccharides can be directly correlated with one of the torsion angles (ψ) which are involved in the determination of the conformation of the glycoside linkage.[7,8] Based on the correlation between the anomeric glycosylation shift and the dihedral angle (ψ), a change in the observed ^{13}C chemical shift of ca. 2 ppm corresponds to the average change in ψ of ca. 10°. The representative data of the ^{13}C NMR spectra are given together in Figs. 5A and 5B, and Table 2. As is shown in Table 2, although only one C6 position was substituted with an aromatic amino acid, the resonances of the C1 and C4 carbons were observed as seven sets of signals. The $\Delta\Delta\delta$ of C4 was bigger than that of C1 and independent of the variation of the substituent. Even for ACD, lowershifted C4 carbon resonances were observed (Fig. 5A). For this reason, the lowest chemical shift of C4 could be assigned as the substituted glucose unit of the macrocyclic ring;[5] the total $\Delta\Delta\delta$ of C4 should not reflect the maclocyclic structure. The $\Delta\Delta\delta$ of C4, except for the lowest shift, was comparable to that of C1. This result indicates that the cavities of all of the modified CDs with the aromatic group indicated here are distorted, especially that of **2**. It appears that the ring current effect from the substituted phenyl groups can induce such a nonequivalence of the C5 and C6 carbons, but cannot cause such an effect in the C1 carbons of each glucose unit, since they are far from the phenyl group wherever it is oriented. In the case of **2**, the C2 and C3 carbon shifts were also observed as 7 or less sets of resonances, in spite of outside intramolecular complex formation, suggesting that the cavity of **2** was formed with complicated distortion. In 1 mol dm^{-3} of a NaOH solution, a split of the C1 and C4 carbon was also observed, although the $\Delta\Delta\delta$ values decreased and the C2 and C3 carbon signals were observed as a single resonance. The ^{1}H NMR resonances of the macrocyclic moiety were also simplified. Since the hydroxyl groups at the C6, C2, and C3 position of glucose units are deprotonated, hydrogen bonding cannot occur under these conditions and repulsive forces between the alkoxide ions on the macrocyclic ring are effective. In a DMSO solution in which hydrogen bonding is strengthened, the same phenomena has been observed. These results indicate that hydrogen bonding between the glucopyranose units and the hydrophobic interaction between the tyrosine moiety and CD moiety should play important roles in the formation of the distorted cavity in **2**. The complicated distortion of the macrocyclic ring in **2** may be caused by hydrogen bonding between the hydroxyl group on the Tyr-residue and that on the C6 position of the CD ring.

In conclusion, **2** has an expanded and distorted cavity which may be induced by the hydroxyphenyl moiety and hydrogen bonding between the substituent and the hydroxyl group in the macrocyclic ring or the hydroxyl moiety in the macrocyclic ring with each other. This twisted cavity should permit the recognition of an enantiomer. In addition, It should be pointed out that measurement of the ^{13}C chemical shifts for the anomeric and aglycone carbon atoms are useful regarding the conformational structure of the macrocyclic rings in CD chemistry.

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