Supramolecular Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713649759

Molecular recognition by modified cyclodextrins with flexibility

Keiko Takahashi[a]; Kenjiro Hattori[a]
[a] Department of Industrial Chemistry, Faculty of Engineering, Tokyo Institute of Polytechnics, Atsugi, Kanagawa, Japan

To cite this Article Takahashi, Keiko and Hattori, Kenjiro(1993) 'Molecular recognition by modified cyclodextrins with flexibility', Supramolecular Chemistry, 2: 4, 305 — 308
To link to this Article: DOI: 10.1080/10610279308029823
URL: http://dx.doi.org/10.1080/10610279308029823

SUPRAMOLECULAR CHEMISTRY, Vol. 2, pp. 305–308
Reprints available directly from the publisher
Photocopying permitted by license only

Molecular recognition by modified cyclodextrins with flexibility

KEIKO TAKAHASHI* and KENJIRO HATTORI

Department of Industrial Chemistry, Faculty of Engineering, Tokyo Institute of Polytechnics, Atsugi Kanagawa 243-02, Japan

(Received July 30, 1992)

Several monosubstituted cyclodextrins having an aromatic moiety and a flexible arm were prepared. From NMR measurements, the structure of macrocyclic ring and the position of the phenyl moiety in the ring have been determined. The inclusion behaviour and molecular recognition ability of the hosts suggest that the optimal length of the arm, the size of hydrophobic moiety, and some weak interaction points, cause host molecules to be flexible and conformation reorganization, which makes it possible to engineer modified cyclodextrins for optimal function and selectivity.

INTRODUCTION

Molecular recognition by modified cyclodextrins (CDs) is currently of great interest in host–guest chemistry. Molecular recognition is a dynamic process based on distinct chemical interactions and not on the passive action of a lock and key. Moreover, recent reports have given evidence that CDs can behave as flexible hosts.[1] Modification of CDs should therefore be carried out to achieve sufficient flexibility for conformational reorganization or induced-fit. We have prepared some one-point aromatic amino acid-binding CDs (**3**,**4**).[2] These modified CDs have a phenyl group as the cavity-size control factor and a flexible arm composed of sp^3 carbons between the parent CD cavity and the phenyl group, which causes guest-induced hydrophobic wall movement.[3] We wish to report here the enantioselective inclusion behaviour of amino acid-binding CDs, and discuss the correlation between the structure of the CDs and enantioselectivity.

RESULTS AND DISCUSSION

Orientation of the phenyl residue against the macrocyclic ring

Since the phenyl moiety of the modified CDs indicated here interacted with the hydrophobic cavity like an 'intramolecular guest molecule', the orientation of the phenyl moiety should depend on the same factors as those for forming the host–guest complexes (van der Waals forces, electrostatic forces, hydrogen bonding) and depend on the length of the arm. NMR studies have given important information on the molecular geometry of CD inclusion complexes.[4] Our previous examination by ^{1}H-NMR has clarified the structural conformation of 6-*N*(*N'*-formyl-L-phenylalanyl)deoxyamino-β-cyclodextrin (**4**).

Direct evidence for the orientation of the phenyl residue of modified CDs with respect to the macrocyclic ring has been obtained by the ROESY method; a formyl-L-Phe residue was included into the CD cavity from the primary hydroxy group side of macrocyclic ring forming an 'intramolecular inclusion complex'. On the other hand, we have indicated that from the characteristic chemical shifts of the phenyl protons, the location of phenyl moiety, i.e. whether it is inside or outside of the cavity, could be easily determined.[5] Using this method, it was made clear that 6-*N*-phenylalanyldeoxyamino-β-cyclodextrin (**2**) and 6-*N*(*N'*-formyl-D- and 6-*N*(*N'*-formyl-L-phenylalanyl)deoxyamino-β-cyclodextrin (**3** and **4**) form intramolecular complexes and that the phenyl moiety of 6-*N*(*N'*-formyl-D- and 6-*N*(*N'*-formyl-L-phenylglycyl)deoxyamino-β-cyclodextrin (**5** and **6**) is outside the cavity. 6*N*-phenethylamino-β-CD, which has the same length of arm as **2**, **3** and **4**, also shows the characteristic shifts and forms an intramolecular complex. 6*N*-benzylamino-β-CD did not form any complexes.[5] From these results, the minimum length of arm between the nitrogen and the phenyl moiety for the phenyl moiety to be included into its own CD cavity is —C—C—C—. Since the decision is based on the large lowerfield shift of the *p*-proton, it cannot be applied for 6*N*-monotyrosinyldeoxyamino-β-cyclodextrin (**1**) which has a hydroxy moiety at the *p*-position. Measurement of the NOE of **1** using the ROESY method was attempted. The ε protons had no NOE crosspeak with the macrocyclic ring. The δ

* To whom correspondence should be addressed.

protons of the Tyr residue had only two NOE crosspeaks with protons on the macrocyclic ring. These results indicate that the hydroxyphenyl group of the Tyr residue was outside the cavity of macrocyclic ring. But the δ proton is close to the primary hydroxy rim of the CD and **1** has a long enough arm. So the hydroxy group seems to disturb inclusion of the phenyl moiety into its own cavity. It is possible that the hydroxy group is near to the primary hydroxy group at C6 and hydrogen bonding occurs. This may cause the difference between **1** and **5** or **6**. The phenyl moiety on **5** and **6** is like a 'pendant', and that on **1** is 'capped' with an amide bond (covalent bonding) and hydrogen bonding. In other words, **1** forms an 'outside intramolecular complex'. Interestingly, these intramolecular complexes are released easily by adding guest molecules, like the induced-fit in an enzyme–substrate systems, which indicates that the modified CDs discussed here can behave as flexible hosts.

Structure of the macrocyclic rings

Evidence for the structure of the macrocyclic ring was also obtained from NMR studies. As all seven glucopyranose units of the parent β-CD are magnetically equivalent due to the presence of the molecular C_7 symmetry axis in solution, it is known that a single set of NMR resonances is observed as if there were only one glucopyranosyl residue. But for the modified CDs that have the symmetry-breaking constituent, the C_7 symmetry of the macrocyclic ring is disturned. The C1 resonances of 6-deoxyamino-β-cyclodextrin (ACD) are divided into only two sets of signals, one corresponding to a glucopyranose unit with an amino moiety, and the others corresponding to six unmodified glucopyranose units. This result means that the six unmodified glucopyranose units are magnetically equivalent. On the contrary, for monosubstituted CDs with a phenyl moiety, a clear distinction of the anomeric C1 proton resonances suggests that all glucopyranose units in the macrocyclic ring are magnetically non-equivalent and that the cavities are not symmetrical. But a single set of C1 protons was only observed in the case of the monosubstituted CDs with the *Z*-residue as the phenyl moiety, except for 6*N*(*N'*-*Z*-serinyl-γ-lactyl)deoxyamino-β-cyclodextrin (**7**). The ^{13}C-NMR measurements provide more direct evidence on the conformational structure of the macrocyclic rings. It has been reported that ^{13}C chemical shifts for the anomeric and aglycone carbon atoms (C1 and C4) in oligosaccharides can be directly correlated with one of the torsion angles (ψ) that is involved in the determination of the conformation of the glycoside linkage.[7,8] From the correlation between the anomeric glycosilation shift and the dihedral angle (ψ), a change in the observed ^{13}C chemical shift of *ca.* 2 ppm corresponds to an average change in ψ of *ca.* 10°. The representative data from the ^{13}C-NMR spectra are given in Table 1. As is shown in Table 1, only one C6 position was substituted with an aromatic amino acid. However, the resonances of the C1 and C4 carbons were observed as seven sets of signals. The $\Delta\delta$ for C4 was larger than that for C1 and independent of variations of substituents. Even for ACD, two sets of C4 carbon resonances were observed in spite of a single resonance for the C1 carbons. For this reason, the lowest chemical shift of C4 could be identified as the substituted glucose unit of the macrocyclic ring, and the total $\Delta\delta$ for C4 should not reflect the macrocyclic structure. The $\Delta\delta$ for C4,

Table 1 Chemical shifts of the monosubstituted cyclodextrins[a]

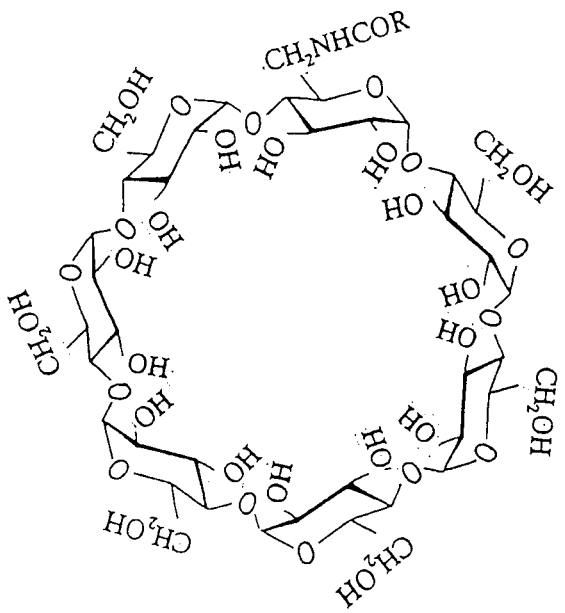
	δ (ppm)							
	L-TyrCD (D_2O)	*L-PheCD* (D_2O)	*formyl-L-PheCD* (D_2O)	*formyl-L-PheGlyCD* (D_2O)	*ACD* (D_2O)	*β-CD* (D_2O)	*L-TyrCD* ($NaOD/D_2O$)	*L-TyrCD* (*DMSO-d*$_6$)
C1 ($\Delta\delta$)[b]	101.44 (1.51)	102.69 (0.91)	102.80 (0.99)	102.58 (0.89)	102.87 (0.19)	103.71	102.60 (0.47)	103.22 (0.55)
	102.20	103.20	103.02	103.03	103.06		102.91	103.49
	102.25	103.60	103.62	103.24			103.07	103.77
	102.47		103.79	103.42				
	102.82			103.47				
	102.85							
	102.95							
C4 ($\Delta\delta$)[b]	81.24 (3.97)	81.97 (3.89)	82.04 (4.01)	81.71 (4.01	82.04 (1.96)	82.74	81.58 (3.26)	82.65 (3.46)
	81.33 (2.17)[c]	82.22 (1.39)[c]	82.26 (1.19)[c]	82.06 (1.19)[c]	82.26 (0.22)[c]		81.71 (0.68)[c]	83.12 (0.54)[c]
	81.66	82.44	82.47	82.33	84.00		81.91	83.49
	82.19	82.50	82.72	82.39			82.26	85.31[d]
	82.72	82.86	83.23	83.01			84.86[d]	
	83.41	83.36	86.05[d]	83.06				
	85.21[d]	85.86[d]		85.72[d]				

[a] Measured with an EX 400WB (JEOL) spectrometer at 27°C. [b] The variation of δ. [c] The variation of δ except the δ of C4 in substituted glucose units. [d] The δ of C4 in substituted glucose units.

Table 2 Association constants, K (M^{-1})[a]

Host	*Guest*				K_L/K_D
	ANS	*TNS*	*DNS-L-Phe*	*DNS-D-Phe*	
L-TyrCD (**1**)	365 ± 6	360 ± 1	629 ± 10	295 ± 3	2.13
L-PheCD (**2**)	68 ± 19	207 ± 29	175 ± 13	202 ± 45	0.87
formyl-D-PheCD (**3**)	78 ± 11	167 ± 27	83 ± 28	160 ± 36	0.52
formyl-L-PheCD (**4**)	68 ± 19	207 ± 29	231 ± 45	139 ± 24	1.66
formyl-D-PheGlyCD (**5**)	137 ± 17	–[b]	368 ± 55	505 ± 70	0.72
formyl-L-PheGlyCD	80 ± 18	1026 ± 47	262 ± 56	381 ± 52	0.69
β-CD	78 ± 8	–[b]	153 ± 14	197 ± 20	0.77

[a] Determined with fluorescence intensity at 540 nm, excited around 350 nm, pH 7.0 solutions (1/15 M phosphate buffer), 25°C with 5.0×10^{-5} M of guest molecule. [ANS] = 2×10^{-5} M, [cyclodextrin] = 1.0×10^{-3} M, [guest = $0\text{–}10^{-2}$ M. [b] 2:1 host–guest complex.



1 R=$HOC_6H_4CH_2CHNH_2$

2 R=$C_6H_5CH_2CHNH_2$

3,4 R=$C_6H_5CH_2CH(NHCHO)$

5,6 R=$C_6H_5CH(NHCHO)$

Figure 1 Various modified cyclodextrins.

except the lowest shift, is comparable with that of C1. It seems that the ring current effect from the substituted phenyl groups can induce such non-equivalencies of the C5 and C6 carbons, but cannot cause non-equivalencies of the C1 carbons of each glucose unit because they are distant from the phenyl group, whichever way it is oriented. This result indicates that the cavities of all the modified CDs with aromatic moieties discussed here, especially that of **1**, are distorted. In 1 M alkaline solution, the split of the C1 and C4 carbons was also observed, though the $\Delta\delta$ values decreased. The ^{1}H-NMR spectrum of the macrocyclic moiety was also simplified. Since the hydroxy groups at the C6, C2 and C3 positions of the glucose units are deprotonated, hydrogen bonding cannot be a factor under these conditions, but the repulsive force between alkoxide ions on the macrocyclic ring has had an effect. In DMSO solution, in which hydrogen bonding is strengthened, the same phenomenon was observed. These results indicate that hydrogen bonding between glucopyranose units and hydrophobic interactions between the phenyl residue and the CD moiety should play an important role in the formation of the distorted cavity. Schematic representations of the structures of various 6*N*-monosubstituted CDs are shown in Figure 2.

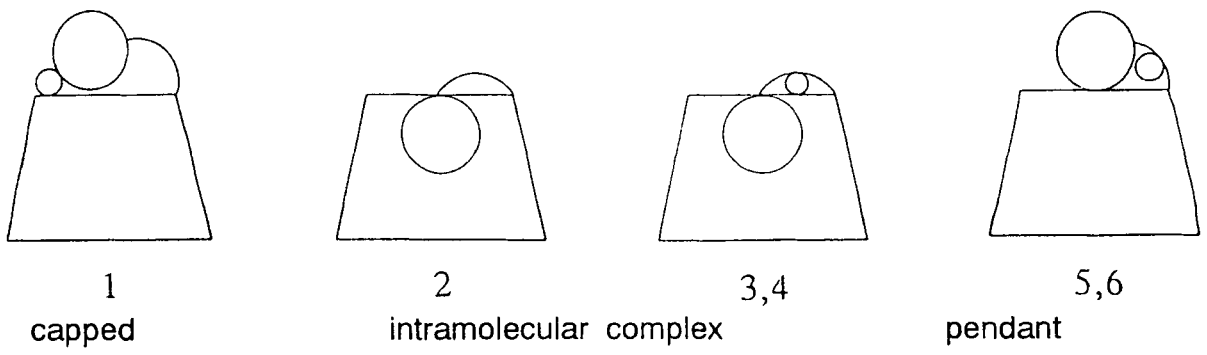


Figure 2 Schematic representation of most likely averaged conformation.

Molecular recognition ability of monoamino acid-binding CD derivatives

Table 2 gives association constants of compounds **1**–**6** and β-CD with some naphthyl derivative guests. **1**, **4** and **5** display large association constants. The *K* values of **1** for ANS AND DNS-L-Phe are more than 4 times larger than that of the parent β-CD. The large *K* values are due to the enclosed hydrophobic cavity with an outside intramolecular complex. Enantioselectivity for dansylphenylalanine was observed in **1**, **3** and **4**. The difference between the 'pendant' style and the 'capping' style seems to reflect molecular recognition ability. Interestingly, **2** cannot recognize enantiomers. The difference between **2** and **4** is only the formyl moiety, and the difference between **2** and **1** is only the hydroxy moiety. **2** forms intramolecular complexes. Then why can **2** not recognize enantiomers? It is possible for both the formyl and the hydroxy moieties to interact with the hydroxy moiety on rim of the CD cavity through hydrogen bonding. We have not yet any direct evidence from structural results that hydrogen bonding plays an important role in the formation of the geometry of the complex. Some hydrogen bonding points are necessary for the molecular recognition ability with this system. Some hydrogen bonding causes a distorted cavity and controls the flexibility of the host CDs. Indeed, seven sets of C1 proton resonances were observed in **7** which has five hydrogen bonding points. However, without the phenyl moiety, even with several hydrogen bonding points, the macrocyclic ring is not distorted.

These results suggest that the optimal length of the arm, the hydrophobic moiety, and some weak interaction paths, cause flexible host molecules and conformational reorganization (induced fit), which makes it possible to engineer modified CDs for optimal function and selectivity.

EXPERIMENTAL

Materials

Various monosubstituted CDs, 6*N*-monotyrosinyldeoxyamino-β-cyclodextrin (**1**), 6-*N*-phenylalanyldeoxyamino-β-cyclodextrin (**2**), 6-*N*(*N'*-formyl-D- and L-phenylalanyl)deoxyamino-β-cyclodextrin (**3** and **4**) and 6-*N*(*N'*-formyl-D- and L-phenylglycyl)-deoxyamino-β-cyclodextrin (**5** and **6**), 6*N*(*N'*-*Z*-serinyl-γ-lactyl)deoxyamino-β-cyclodextrin (**7**) and 6*N*-serinyllactyldeoxyamino-β-cyclodextrin (**8**) were prepared according to the method reported previously.[2] 6-Deoxyamino-β-cyclodextrin (ACD) and 1.2 mol of each *N*-protected amino acid were treated with dicyclohexylcarbodiimide in dimethylformamide (DMF). *N*-free compounds (**1**, **2**, **8**) were prepared from *Z*-protected compounds with Pd/C and H_2 bubbling treatment in aqueous solution at 60°C for 1 h.

Measurements

NMR spectra were taken using a JEOL EX4000WB spectrometer in D_2O using DSS as 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 originating from the guest molecules. The detailed conditions are indicated in the footnotes of Tables 1 and 2.

ACKNOWLEDGMENT

The work was supported by a Grant-in-Aid from Hayashi Memorial Foundation for Female Natural Scientists.

REFERENCES

1 Schneider, H.J.; Blatter, T.; Cuber, U.; Juneja, R.; Schiestel, T.; Shneider, U.; Theis, I.; Zimmermann, P.; in *Frontiers in Supermolecular Organic Chemistry and Photochemistry* (Schneider, H.J. and Dürr, H., eds.), VCH Weinheim, **1991**, p. 49.
2 Takahashi, K.; Ohtsuka, Y.; Nakada, S.; Hattori, K.; *J. Incl. Phenom. Molec. Recog. Chem.* **1991**, *10*, 63.
3 Takahashi, K.; Ohtsuka, Y.; Hattori, K.; *Chem. Lett.* **1990**, 2227.
4 (a) Inoue, Y.; Okuda, T.; Miyata, Y.; Chûjô, R.; *Carbohydr. Res.* **1984**, *125*, 65. (b) Lipkowitz, K.B.; Raghothama, S.; Yang, J.; *J. Am. Chem. Soc.* **1992**, *114*, 1554.
5 Moon, H.; Toda, F.; Wadamori, M.; Takahashi, K.; Hattori, K.; *Chem. Lett.* **1993**, submitted.
6 Takahashi, K.; *Bull. Chem. Soc. Jpn.* **1993**, *66*, 550.
7 Bock, K.; Brigbole, A.; Sigurskjold, B.W.; *J. Chem. Soc., Perkin Trans. II.* **1986**, 1711.
8 Backman, I.; Erbing, B.; Jansson, P.; Kenne, L.; *J. Chem. Soc., Perkin Trans. II* **1988**, 889.