Immobilized β-Cyclodextrins. Preparation with Various Crosslinking Reagents and the Guest Binding Properties

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β-Cyclodextrin (β-CyD) was immobilized by crosslinking with 1,2-ethanediol diglycidyl ether, 1,4-buthanediol diglycidyl ether, 1,6-hexanediol diglycidyl ether, 1,3-butadiene diepoxide, 1,7-octadiene diepoxide, and epichlorohydrin. The molar ratio of the crosslinking residue to β-CyD (degree of crosslinking) for each macromolecular product was determined by the corresponding elemental analysis. The equilibrium constant K of complex formation of each crosslinking macromolecule for cresols, phenol, nitrophenols, and 2-naphthol was determined, respectively, by the use of electronic absorption spectroscopy in pH 4 and 10 (or 11) buffer solutions. The concept of a hydrophobic enhancement coefficient R was introduced in order to compare the effect of a hydrophobic crosslinking residue upon the complex formation of the immobilized CyD.

Cyclodextrin (CyD), a cyclic oligomer of the 6—8 glucose units, forms an inclusion complex with various kinds of guest compounds.[1] The complex formation, however, proceeds selectively, since it includes guest compounds in various ways of binding. From the viewpoint of selective complexation, CyD has been studied as an enzyme model, a selective catalyst for organic synthesis, a device for separation of mixtures, and so on.

Recently, the selective "host-guest" complex formation of β-CyD has been studied by many investigators with an interest in its utilization for the stationary phase of HPLC.[2a-d] Since CyDs are composed of natural D-glucose units and, therefore, are optically active, they are expected to be utilized as the stationary phase for the optical resolution of racemic compounds.[2b] Few investigations, however, have been made concerning the effect of the supporting groups of the stationary phase on the property of CyDs to include a guest compound. Furthermore, some kinds of these stationary phases which were investigated contain nitrogen atoms (amines and/or amides) to combine the CyD residue to the supporting gel. This existence of =NH groups is considered to result in complicated interactions between the guest molecules and the CyD residues. For instance, a hydrogen bonding of an =NH group should be very sensitive to pH and the structure of a guest molecule. It could therefore be difficult to analyze the mechanism of complex formation[2c] and, in consequence, to design an efficient stationary phase.

β-CyD forms inclusion complexes selectively with many kinds of aromatic compounds. They have been studied mainly as models of hydrolysis enzymes.[1,3,4] On the other hand, many investigators have already reported that β-CyD is very effective as a selective catalyst for bond formation.[5-7] Previously, we reported the selective synthesis of 4-hydroxybenzoic acid from phenol and carbon tetrachloride in β-CyD.[7] Moreover, we have succeeded in the immobilization of β-CyD by crosslinking with epichlorohydrin and its utilization as an immobilized catalyst for the selective synthesis of 4-hydroxybenzoic acid without any decrease in the catalytic activity.[8] An immobilized catalyst is expected to have the advantage to simplify the system by easy removal from a reaction system and repeated usage, in spite of the usual observation that the activity of the immobilized catalyst tends to decrease by immobilization.[9]

On the basis of these studies, we have prepared the immobilized β-CyD with various kinds of crosslinking reagents and have investigated the property of the inclusion phenomena.[10]

Here, we report the syntheses of various immobilized β-CyD macromolecules without nitrogen atoms, and their characteristic features to form inclusion complexes with phenol, nitrophenols, cresols, and 2-naphthol under various pH conditions. The effects of crosslinking residues on the formation of inclusion complexes are discussed from the viewpoint of hydrophobic interaction. A hydrophobic enhancement coefficient R is introduced, which indicates the contribution of the crosslinking residue to the hydrophobicity of the binding site in the immobilized CyD. The correlation between a crosslinking residue and a guest molecule is discussed by use of R.

Experimental

Materials. β-CyD from Nakarai Chemical Co. was purified by recrystallization from water. The crosslinking reagents, 1,2-ethanediol diglycidyl ether, 1,4-buthanediol diglycidyl ether, 1,6-hexanediol diglycidyl ether, 1,3-butadiene diepoxide, 1,7-octadiene diepoxide, and epichlorohydrin (Tokyo Kasei Kogyo Co.) were used without further purification. (See Fig. 1 for the structures.) Cresols, phenol, nitrophenols, 2-naphthol, and all other chemicals were

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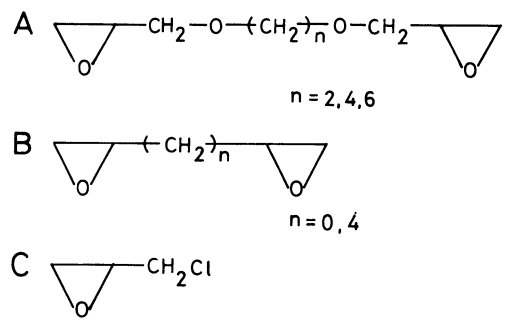


Fig. 1. Crosslinking reagents used for preparation of immobilized β-CyD.

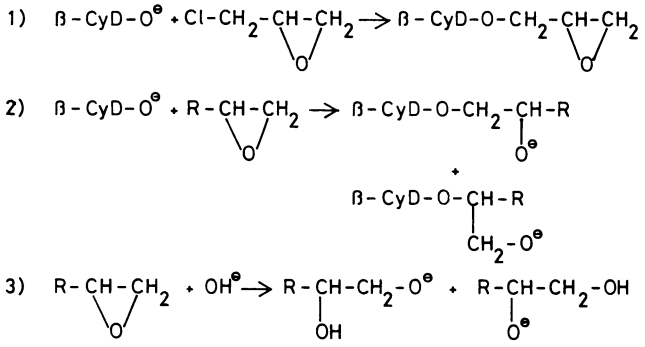
purified by the usual methods, respectively.

Preparation of Immobilized β-CyD. Immobilized β-CyDs were prepared according to the modified method we reported previously.[10] Fifty grams (44 mmol) of β-CyD were completely dissolved in 80 ml of a 50 wt% aqueous sodium hydroxide solution, to which 440 mmol of a crosslinking reagent was added dropwise. The mixtures were kept at 50 °C which being vigorously stirred with a mechanical stirrer. As the crosslinking reaction proceeded, the viscosity of the solution increased. After several hours the solid gel appeared. Then, 150 ml of acetone was added. The resulting solids were repeatedly washed with acetone and water, and were dried in vacuo at 60 °C for 24 h.

Evaluation of the Equilibrium Constants for Complex Formation of the Immobilized β-CyDs with Guest Molecules. The equilibrium constants (K) of complex formation of the immobilized β-CyD were measured for cresols, phenol, nitrophenols, and 2-naphthol. Each K value was measured at pH 4 for all guest molecules, at pH 10 for nitrophenols and 2-naphthol, and at pH 11 for phenol and cresols. The experiments were performed by incubating 200 mg of the immobilized macromolecules and the guest compounds in 10 cm^3 of buffer solutions under pH 4, 10, or 11 at 25 °C. The charged concentrations of the guest compounds ($[G]_0$) and the ionic strength were 1.0×10^{-3} and 1.0 mol dm^{-3}, respectively. After 40 h the concentrations of the solutes in the liquid phase ($[G]_{free}$) were determined by electronic absorption spectroscopy. Completions of the equilibria were confirmed by the consistency of the concentration measured after a 60 h incubation with that after 40 h. During the long-term incubation, especially in a pH 4 buffer solution, a very slow fragmentation of one part of the immobilized macromolecule was observed, probably due to the hydrolysis of the macromolecule, resulting in a dispersion of microparticles. In order to compensate the scattering by dispersion, a blank test was performed by incubation of the immobilized macromolecules in a pH 4 buffer solution for 40 h without guest compounds. The scattering absorption caused by the microparticles was measured and subtracted from the corresponding absorption in the presence of the guest compounds.

The equilibrium constants (K) for the complex formation were calculated by

$$K = \frac{[\text{Complex}]}{[\beta\text{-CyD}]_{free}\,[G]_{free}}$$



Scheme 1.

$$= \frac{[G]_0 - [G]_{free}}{([\beta\text{-CyD}]_0 - [G]_0 + [G]_{free})[G]_{free}}, \quad (1)$$

where $[G]_0$, $[G]_{free}$, and $[\beta\text{-CyD}]_0$ refer to the concentrations of the guest molecule in an initial solution, the guest molecule in the filtrate after the complex formation, and the CyD residue in the macromolecule matrix, respectively.

Equation 1 was defined on the basis of 1:1 complex formation between CyD and a guest molecule.[1] The numerator of Eq. 1 ($[G]_0-[G]_{free}$) indicates the concentration of the complex.

Evaluation of the Equilibrium Constant for Complex Formation of Free β-CyD with Guest Molecules. The equilibrium constants of the complex formation of free β-CyD with the guest molecules were evaluated according to the method reported by VanEtten et al. by using electronic absorption spectroscopy.[3b]

Results

Preparation and Characterization of Immobilized β-CyDs. Immobilization of β-CyD was carried out by a substitution or ring-opening reaction between the hydroxyl groups of β-CyD and the chlorine atoms of epichlorohydrin or the epoxide rings of the crosslinking reagents in an alkaline solution at 50 °C according to the reaction shown in Scheme 1. As shown in Scheme 1, the first step of the crosslinking reaction is a nucleophilic attack by the $-O^-$ group of β-CyD to the crosslinking reagent.[11] The spontaneous ring-opening reaction by free hydroxide OH^- proceeds at the same time (the 3rd reaction in Scheme 1), followed by the propagation reaction by the nucleophilic $-O^-$ group newly produced.

The immobilized β-CyD was obtained as a white to pale-yellow solid in sponge or beads of 1—3 mm in diameter. They are insoluble in water, methanol, ethanol, acetone, and chloroform.

The IR spectra of free β-CyD, epichlorohydrin-immobilized CyD, and 1,6-hexanediol diglycidyl ether-immobilized CyD were measured by a KBr pellet method, giving the following results: 1) The IR spectra of the immobilized CyDs maintain the specific absorptions due to free CyD. 2) The new absorption

derived from a $-(CH_2)_n-$ residue (2860—2875 cm^{-1}) appears for the immobilized CyDs. 3) The specific absorption derived from an epoxy ring (ca. 1250 cm^{-1}) is not observed for the immobilized CyDs. 4) The specific absorption derived from a $-CH_2Cl$ group (735—720 cm^{-1}) does not appear for the epichlorohydrin-immobilized CyD. 5) The absorption derived from a C–O–C stretching vibration (1070—1150 cm^{-1}) for free CyD is sharp, while that for the immobilized CyD is broad because of the crosslinkage. A new absorption derived from the C–O–C stretching vibration of the crosslinking residue (1110 cm^{-1}) appears for the 1,6-hexanediol diglycidyl ether-immobilized CyD.

The immobilized macromolecules prepared are listed in Table 1 with the results of an elemental analysis, the degree of crosslinking, and reaction conditions. The degree of crosslinking m, a molar ratio of a crosslinking residue to a β-CyD residue, is determined by calculation according to Eq. 2, by using the results of the elemental analysis of carbon in the case of the immobilized macromolecule crosslinked by diol diglydidyl ethers:

$$C(\%) = \frac{12.011[42 + (6 + n)m] \times 100}{(\text{MW of a crosslinking reagent})m + (\text{MW of } \beta\text{-CyD})}, \quad (2)$$

where m and n are the degree of crosslinking and the number of the methylene moiety of the crosslinking reagent shown in Fig. 1, respectively. The numerator of Eq. 2 intends the summation in atomic weight of the whole carbon atoms of the immobilized β-CyD per β-CyD residue. The degree of crosslinking m for the epichlorohydrin-immobilized β-CyD is calculated by a similar method.

The crosslinking reagents are not so much soluble in an aqueous solution that the reaction time required for the crosslinkage increases with the number of the hydrophobic methylene units in a reagent molecule.

The degree of crosslinking in the present system was determined to be 3—6, as shown in Table 1. Even by using the same crosslinking reagent and under the similar reaction conditions, the resulting immobilized macromolecules exhibited different values in the degree of crosslinking, depending on the lot (cf. I-3a, -3b, and -3c).

Evaluation of the Equilibrium Constants for Complex Formation of the Immobilized β-CyDs with Guest Molecules. The equilibrium of the complex formation between a β-CyD residue of the immobilized macromolecule and a guest molecule is completed by incubating the aqueous mixtures for 40 h. Since the equilibrium constant K refers the ratio of the concentration of the inclusion complex to the mathe-

Table 1. Immobilization of β-CyD by Various Crosslinking Reagents[a]

Immobilized β-CyD	Crosslinking reagent[b]	Reaction time/h	Elemental analysis		m[c]
			C/%	H/%	
I-1	A (n=2)	2.2	48.80	7.28	4.5
I-2	A (n=4)	2.2	51.20	7.77	4.6
I-3a	A (n=6)[d]	6.4	53.12	8.19	4.5
I-3b	A (n=6)[d]	7.5	53.47	8.44	5.0
I-3c	A (n=6)[d]	5.8	54.25	8.70	5.8
II-1	B (n=0)	0.4	47.82	6.93	5.6
II-2	B (n=4)	4.1	51.68	7.57	3.6
III	C	0.8	47.35	6.88	3.9

a) The molar ratio of the charged crosslinking reagent to β-CyD is 10 except I-3b. Detail reaction conditions are described in Experimental of the text. b) The structure of the crosslinking reagents are illustrated in Fig. 1. c) The degree of crosslinking calculated by the results of the elemental analysis. d) The stirring and adding rates are different because of the difficulty in completely the same operation in a heterogeneous system.

Table 2. The Equilibrium Constant (K) of the Complex Formation of the Free and Immobilized β-CyD with Cresols and Phenol at 25 °C

Immobilized β-CyD[a]	Crosslinking reagent[b]	m[c]	K/mol^{-1} dm^3							
			Cresol						Phenol	
			ortho		meta		para			
			pH 4	pH 11	pH 4	pH 11	pH 4	pH 11	pH 4	pH 11
I-1	A (n=2)	4.5	89	21	28	14	108	23	58	6
I-2	A (n=4)	4.6	165	24	23	17	193	37	117	11
I-3a	A (n=6)	4.5	167	56	167	21	165	29	68	9
III	C	3.9	300	40	70	42	704	103	331	23
β-CyD	—	—	82	81	635	176	461	135	101	17

a) See Table 1 for abbreviation. b) See Fig. 1 for the chemical structures. c) m: Degree of crosslinking.

matical product of the concentration of a free β-CyD residue and that of a free guest molecule, the practical K value can be evaluated by Eq 1. The equilibrium constants for the 1:1 complex formation of the β-CyD residues in the immobilized macromolecules with *o*-, *m*-, and *p*-cresols, and phenol (Table 2), with *o*-, *m*-, and *p*-nitrophenols (Table 3), and with 2-naphthol (Table 4) were obtained at 25 °C and are shown in tables, respectively. The 1:1 complex formation is confirmed by the independence of the K value from the charged amount of the immobilized CyDs in the range of 30—200 mg. The reproducibility of K was checked by using the immobilized beads of I-3a at various diameters. The equilibrium constants for *m*-nitrophenol at pH 4, for example, were 264, 268, and 254 mol^{-1} dm^3 for the small (1—3 mm in size, 2 mm in an average diameter), medium (2—7 mm in size, 4 mm in an average diameter), and large (4—7 mm in size, 6 mm in an average diameter) macromolecular beads, respectively. The result of these series of K values indicates that the reproducibility for the K measurement is fairly excellent and, in addition, the macromolecules employed here swell sufficiently for the measurement of the equilibrium constants in a limited time.

As shown in Tables 2—4, the equilibrium constant K depends on the crosslinking reagents. This suggests that the interaction between the crosslinking residue and the guest molecule exists in the complex formation of the immobilized β-CyD with the guest molecule. The effect of the degree of crosslinking on the complex formation constant K was examined by

Table 3. The Equilibrium Constant (K) of the Complex Formation of the Free and Immobilized β-CyD with Nitrophenols at 25 °C

Immobilized β-CyD[a]	Crosslinking reagent[b]	m[c]	K/mol^{-1} dm^3					
			ortho		meta		para	
			pH 4	pH 10	pH 4	pH 10	pH 4	pH 10
I-1	A (n=2)	4.5	432	12	115	35	142	128
I-2	A (n=4)	4.6	294	12	87	40	112	149
I-3a	A (n=6)	4.5	897	10	268	27	262	50
III	C	3.9	215	33	151	114	161	646
β-CyD	—	—	338	826	204	238	218	164

a) See Table 1 for abbreviation. b) See Fig. 1 for the chemical structures. c) m: Degree of crosslinking.

Table 4. The Equilibrium Constant (K) of the Complex Formation of the Free and Immobilized β-CyD with 2-Naphthol at 25 °C

Immobilized β-CyD[a]	Crosslinking reagent[b]	m[c]	K/mol^{-1} dm^3	
			2-Naphthol	
			pH 4	pH 10
I-1	A (n=2)	4.5	339	269
I-2	A (n=4)	4.6	324	100
I-3a	A (n=6)	4.5	1460	883
III	C	3.9	159	211
β-CyD	—	—	721	459

a) See Table 1 for abbreviation. b) See Fig. 1 for the chemical structures. c) m: Degree of crosslonking.

Table 5. The Equilibrium Constant (K) of the Complex Formation of the Three Kinds of Immobilized β-CyD (I-3) Prepared by Crosslinking with 1,6-Hexanediol Diglycidyl Ether with Three Isomers of Cresol as the Guest Molecules at 25 °C

Immobilized β-CyD[a]	m[b]	K/mol^{-1} dm^3					
		ortho		meta		para	
		pH 4	pH 11	pH 4	pH 11	pH 4	pH 11
I-3a	4.5	167	56	167	21	165	29
I-3b	5.0	169	40	149	32	150	40
I-3c	5.8	385	58	252	17	289	29

a) See Table 1 for abbreviation. b) m: Degree of crosslinking.

using the immobilized β-CyDs which are crosslinked by 1,6-hexanediol diglycidyl ether and cresols as a guest molecule. The results are summarized in Table 5.

Discussion

Preparation of Immobilized β-CyDs. Immobilization of β-CyD was successfully performed by using various crosslinking reagents. These macromolecules involve no nitrogen atoms (amines and/or amides) in the linkage. The results of the IR spectra of the immobilized CyDs are in good agreement with the immobilizing reactions shown in Scheme 1. The specific absorptions of the CyD residue in the IR spectra indicate that the structure of CyD is maintained in the immobilized CyD macromolecule. The absence of the absorption bands due to an epoxy ring and a chloromethyl group indicates that the immobilizing reaction proceeds the nucleophilic attack by the hydroxyl group of β-CyD to the epoxy ring and the chloromethyl group. The insolubility of the immobilized CyD in water suggests that the macromolecule has a tangly network composed of crosslinking residues and CyD residues. Since the CyD residues have many hydroxyl groups, in fact the strong IR absorptions due to the hydroxyl groups are observed, the crosslinked macromolecules are able to swell in water.

The number of the crosslinking residues which can bind to one CyD residue (m) is 3—6, as shown in Table 1. Harada et al. reported that the substitution of the secondary hydroxyl groups by the crosslinking residues was observed in the ^{13}C NMR spectrum of the epichlorohydrin-immobilized CyD.[11] In general, however, the reactivity of the primary hydroxyl group of CyD is thought to be much higher than that of the secondary hydroxyl group of CyD.[12] In the immobilizing reactions examined here, some parts of the crosslinking residue probably bind to the secondary hydroxyl group of CyD (as the case reported before[11]), besides the primary one, because of the present severe reaction conditions with extraordinarily high concentration of sodium hydroxide. The immobilized β-CyDs I-3a, 3b, and 3c shown in Table 1 were prepared by the same crosslinking reagent (1,6-hexanediol diglycidyl ether). The charged molar ratio of the crosslinking reagent to β-CyD is the same for I-3a and I-3c, and other reaction conditions are similar to each other. The resulting immobilized β-CyDs, however, exhibit different values of the degree of crosslinking. This is probably due to the heterogeneity of the reaction system. For instance the addition rate of the crosslinking reagents and the stirring rate of the mixtures may be effective for the constitution of the macromolecules in the heterogeneous system. In case of the macromolecule I-3b, the charged molar ratio of the crosslinking reagent was 7.5 times to CyD, and less than those for I-3a and I-3c. The degree of crosslinking, however, exhibits 5.0 which is higher than that of I-3a. This reverse phenomena would be explained by the longer reaction time for I-3b. The difference in their inclusion properties will be discussed later.

Complex Formation of the Immobilized β-CyD. From the data in Tables 2, 3, and 4, one can conclude the general tendency for the complex formation. 1) The different crosslinking residues result in a different equilibrium constant K. This indicates that the crosslinking residues affect the complex formation. 2) The long crosslinking residue which has long hydrophobic moieties (A n=6) results in a large equilibrium constant, especially at pH 4. This indicates that the hydrophobic residue plays an important role in complex formation. 3) The equilibrium constant K at pH 4 is generally larger than that at pH 10 or 11. In a pH 4 buffer solution, the guest molecules are electrically neutral, while in pH 10 or 11 they are anionic, since the pK_a values of nitrophenols, cresols, and phenol are 8.0—9.3, 10.0—10.3, and 9.8, respectively. The hydroxyl group of CyD cannot dissociate into ionic species under these conditions since the pK_a of CyD is more than 12.[1] Therefore, the present result of the larger K value at pH 4 than at pH 10 or 11 indicates that the immobilized CyDs include the neutral guest molecule strongly in its hydrophobic cavity and do the anionic molecule weakly. In other words, the hydrophobic interaction is very important for inclusion complex formation.

In order to clarify the importance of the hydrophobic interaction between the crosslinking residues and guest molecules, the relation between the degree of crosslinking and the equilibrium constant K was investigated. Three types of immobilized CyDs exhibiting different degrees of crosslinking were synthesized by using 1,6-hexanediol diglycidyl ether as a crosslinking reagent. The equilibrium constants were measured for these three kinds of immobilized CyDs against cresols as guest molecules, since cresol is considered to be a proper guest molecule in order to examine the hydrophobic interaction between the guest molecule and the new binding site composed of the CyD cavity and the crosslinking residue.

In general, the hydrophobic modifying residue of CyD can affect the inclusion complex formation of the modified CyD with a guest molecule in various ways. From a micro point of view, two kinds of effects can be reasonably considered: 1) The hydrophobic modifying residue is competitively included in the cavity of CyD[13,14] so as to inhibit the inclusion of a guest molecule, resulting in the decrease of the equilibrium constant K. 2) The hydrophobic modifying residue forms a cap and/or a floor of the CyD cavity[15] so as to promote the inclusion complex formation by the

cavity, resulting in the increase of K. From a macro point of view, the other two kinds of effects are also considered: 3) The modifying residue can reduce the flexibility of the CyD residue and the movement of the guest molecule near the CyD cavity by the steric hindrance so as to hinder the inclusion complex formation,[1] resulting in the decrease of K. 4) The hydrophobic environment composed of the modifying residue can interact with a hydrophobic guest molecule so as to increase the local concentration of the guest molecule or the frequency factor of the complex formation,[16] resulting in the increase of K.

As shown in Table 5, the K value of I-3c (m=5.8) at pH 4 is much larger than those of I-3a and I-3b (m=4.5 and 5.0, respectively). From the general considerations described above, this result can be explained by the larger contribution of 2) and/or 4) than 1) and/or 3). In other words, the hydrophobic crosslinking residues in the macromolecules do enhance the complex formation with the hydrophobic guest molecules in the micro and/or macro ways.

In a preliminary communication,[10a] we reported that the K values of the 1,2-ethanediol diglycidyl ether-immobilized CyDs with the different degree of crosslinking (m=3.1 and 5.9) for p-nitrophenol are not so different from each other (K=130 and 119 mol^{-1} dm^3, respectively). This is considered to be a rather special case, since p-nitrophenol is known to interact with the CyD cavity[1] in a special way, for instance, the dipole-dipole interaction[1,17] and the crosslinking residue of 1,2-ethanediol diglycidyl ether is rather hydrophilic.

Evaluation of the Hydrophobic Coefficients of the Immobilized β-CyD. As described above, the CyD residue and the crosslinking residue in the immobilized CyD coopetate to form an inclusion complex. The crosslinking residue can affect the complex formation not only by the so-called "general polymer effect" but also by altering the quality of the binding site. To distinguish these two factors, further analysis of the equilibrium constant K was attempted by comparing the complex formation property of free CyD with that of the immobilized CyD.

A direct comparison of the K values of free and immobilized CyD for the same guest molecule under the same pH condition reflects not only the effect of the crosslinking residue to alter the complex formation ability of the CyD residue but also the other contribution of the crosslinking or "general polymer effect," for example, the effect on the diffusion of the guest molecule to access to the binding site of the CyD residue, the swelling property of the immobilized CyD, the degree of freedom of the CyD residue, and so on. These varieties of "general polymer effects" by the crosslinking residue conceal the pure property of the binding site in the immobilized CyD. To compensate this disturbance, a concept of the hydrophobic enhancement coefficient R is introduced.

The hydrophobicities of the free CyD and the immobilized CyDs can be estimated by the ratio of the corresponding K value at pH 4 to that at pH 10 (or 11), since the guest molecule is neutral or hydrophobic at pH 4 and anionic or hydrophilic at pH 10 (or 11). The influence of the crosslinking residue to the complex formation property of the CyD residue can be evaluated by the increase of the hydrophobicity by crosslinking.

On the basis of the above consideration, the hydrophobic enhancement coefficient R is defined by

$$R = \frac{K_{imm}(\text{pH 4})}{K_{imm}(\text{pH 10 of 11})} \Big/ \frac{K_{free}(\text{pH 4})}{K_{free}(\text{pH 10 or 11})}, \quad (3)$$

where K_{imm} and K_{free} are the equilibrium constants of complex formation of the immobilized β-CyDs and the free β-CyD, respectively. The ratio K_{imm}(pH 4)/K_{imm}(pH 10 or 11) indicates the ability of the immobilized CyD to discriminate a neutral guest molecule from an anionic one, reflecting the property of the hydrophobic field composed of the crosslinking residues and the CyD residue. (This hydrophobic field binds the hydrophobic part of guest molecule and repulses the anionic part of guest molecule.) In the similar way, the ratio K_{free}(pH 4)/K_{free}(pH 10 or 11) indicates the ability of free CyD to discriminate a neutral from an anionic. (This original property will be altered by the crosslinking.) In short, the ratios K_{imm}(pH 4)/K_{imm}(pH 10 or 11) and K_{free}(pH 4)/K_{free}(pH 10 or 11) reflect the hydrophobicity of the immobilized and free CyD, respectively. Thus, the ratio of these two ratios is the hydrophobic enhancement coefficient by crosslinking (R), which indicates how the crosslinking affects on

Table 6. The Hydrophobic Enhancement Coefficient (R) for Cresols and Phenol

Immobilized β-CyD[a]	Crosslinking reagent[b]	R[c]			
		Cresol			Phenol
		ortho	meta	para	
I-1	A (n=2)	4.2	0.6	1.4	1.7
I-2	A (n=4)	6.9	0.4	1.5	1.9
I-3a	A (n=6)	3.0	2.2	1.7	1.3
III	C	7.5	0.5	2.0	2.4

a) See Table 1 for abbreviation. b) See Fig. 1 for the chemical structures. c) The coefficient R is calculated by the Eq. 3 in the text.

the property of the CyD binding site.

Table 6 lists the enhancement coefficients R which are calculated by using the K values listed in Table 3 according to the Eq. 3. It is surprising that the actual R values change to a great extent depending on the kind of the guest molecule but do not change with the kind of the crosslinking residues. In fact, they are 3.0—7.5 for *o*-cresol, 0.4—0.6 for *m*-cresol (except I-3a), 1.4—2.0 for *p*-cresol, and 1.3—2.4 for phenol. The coefficients Rs for *o*-cresol are largest among these, which means that the immobilization enhances the hydrophobicity of the CyD binding site so that the ability of CyD to discriminate *o*-cresol from *o*-cresolate is strengthened by the immobilization. For *p*-cresol and phenol, the Rs are larger than 1 but smaller than those for *o*-cresol, which means that the above ability is strengthened a little. For *m*-cresol, in contrast with other three guests, the Rs are smaller than 1 (except for I-3a), meaning that the ability of CyD to discriminate *m*-cresol from *m*-cresolate is weakened by the crosslinking. This large dependence of the R values on the guest molecule and the slight dependence of the crosslinking residue suggest that the quality of the effect of the crosslinking residue on the binding site alters with the structure of a guest molecule. In other words, the interaction between the crosslinking residue and the guest molecule may vary with the steric structure of the included guest molecule.

This might be explained in, for example, the following way: the crosslinking residue can form a cap and/or a floor of the CyD residue in the immobilized CyD.[11,15] The main interaction between the immobilized CyDs and cresols is a hydrophobic interaction. The most hydrophobic part of a cresol molecule is the methyl group and, then, the benzene ring.[18] Under the condition of pH 4, the CyD cavity includes these hydrophobic parts of a guest molecule, and the hydroxyl groups of the host and the guest molecules may interact with each other through hydrogen bonding. At pH 11 the hydrophobic interaction works in a similar way as that described above, even though the difference between the strengths of the interactions at pH 4 and at pH 11 is unknown. However, repulsion may arise between the anionic oxygen of cresolate and the hydrophobic field composed of the CyD residue and the crosslinking residue. The former hydrophobic interaction can promote a deep inclusion of cresolates into the CyD cavity at the position of the methyl group and the benzene ring. The latter repulsion, however, can make the anionic oxygen of the cresolates far from the CyD cavity or the hydrophobic crosslinking residue.

If these two effects, a hydrophobic interaction and a repulsion, cooperatively work to make the actual R value different from each other, depending on the isomers of cresolate, the structures of the inclusion complexes of the isomers are possibly illustrated as shown in Fig. 2. In the case of *o*-cresolate ($R \gg 1$), the methyl group of *o*-cresolate inhibits a deep inclusion of the benzene ring because of a steric hindrance, so that the anioic oxygen may be compelled to locate near the hydrophobic crosslinking residue which forms a cap or a floor for the CyD cavity. This situation can induce a strong repulsion between the anionic oxygen and the crosslinking residue, which results in the large R values for *o*-cresol. For *p*-cresolate, and phenolate ($R>1$), the inclusion may be deeper than that of *o*-cresolate so that the repulsion may exist but being weaker than that for *o*-cresolate. This results in the larger R values for *p*-cresol and phenol than 1, but smaller than those for *o*-cresol. On the contrary, the inclusion of *m*-cresolate ($R<1$) may be achieved by the structure shown in Fig. 2(b), where the methyl group of *m*-cresolate is deeply included in the CyD cavity. However, the anionic oxygen may be able to avoid the hydrophobic crosslinking residue. In this structure, the interaction between the benzene ring of the guest molecule and the hydrophobic crosslinking residue of the immobilized CyD may become favorable, resulting in the strengthening of the inclusion of the guest molecule by capping the CyD cavity with the crosslinking residue. These interactions, as a whole, result in the small R values for *m*-cresol. In the case of macromolecule I-3a (crosslinked by 1,6-hexanediol diglycidyl ether), the R for *m*-cresol is 2.2, which is exceptionally large. This peculiarity is probably derived from a strong hydrophobic field composed of a long hydrophobic residue of this macromolecule, resulting in the strong repulsion between the anionic oxygen of *m*-cresolate and the binding site of the immobilized CyD. The hydrophobic field may be so large that even for *m*-cresol R is larger than 1.

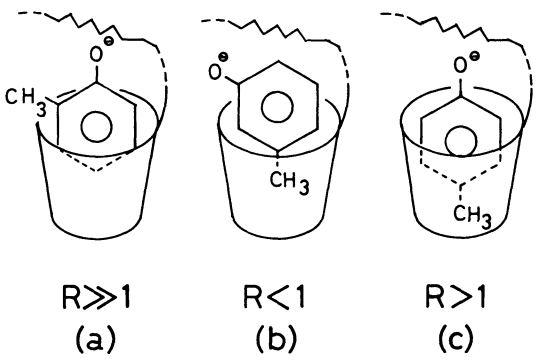


Fig. 2. Proposed structures of the complexes of the immobilized β-CyD, with *o*-, *m*-, and *p*-cresol.

The hydrophobic enhancement coefficients Rs for nitrophenols and 2-naphthol are listed in Table 7, respectively. Here again, the R value changes with the guest molecule and not so much with the crosslinking reagent. In the case of nitrophenols, the R values for the ortho-isomer is quite large in the order of the

Table 7. The Hydrophobic Enhancement Coefficient (R) for Nitrophenols and 2-Naphthol

Immobilized β-CyD[a]	Crosslinking reagent[b]	R[c]			
		Nitrophenol			2-Naphthol
		ortho	meta	para	
I-1	A (n=2)	90	3.7	0.9	0.8
I-2	A (n=4)	61	2.4	0.6	2.1
I-3a	A (n=6)	225	11.0	4.0	1.1
III	C	16	1.4	0.2	0.5

a) See Table 1 for abbreviation. b) See Fig. 1 for the chemical structures. c) The coefficient R is calculated by the Eq. 3 in the text.

magnitude of R, ortho≫meta>1>para, except for the case of the macromolecule I-3a (crosslinked by 1,6-hexanediol diglycidyl ether) for *p*-nitrophenol. It is known that a nitro group is deeply included in a CyD cavity and, in consequence, plays an important part for the inclusion complex formation.[1,17] According to the results of two-dimensional NMR studies using a nuclear Overhauser effect (NOESY), the nitro groups of *m*- and *p*-nitrophenol are suggested to be included in the CyD cavity from the secondary hydroxyl side (the wide side) of the CyD cavity; in contrast with these two guests, the nitro group of *o*-nitrophenol will be included in the cavity from the primary hydroxyl side (the narrow side) of the CyD cavity.[19] This suggests that in the immobilized CyD *o*-nitrophenol may locate near the narrow side of a CyD residue which is connected by the crosslinking residues to form a crowded and strong hydrophobic field; in consequence there may be a strong repulsion resulting in the very large R values.

For *m*- and *p*-nitrophenol, the order of the R values is reverse to that for *m*- and *p*-cresol. Though the reason is unknown there may be a special participation of the nitro group, since the interaction of the nitro group with CyD cavity can not be explained only by hydrophobic interaction and hydrogen bonding.[17]

For 2-naphthol the R is rather near unity. This might be due to the fact that the main interaction between the binding site and 2-naphthol is a hydrophobic interaction.

Conclusion

1. Many kinds of immobilized β-CyDs can be successfully synthesized as solid products by crosslinking β-CyD with six kinds of crosslinking reagents. The immobilized β-CyD prepared in the present investigations involves no nitrogen atoms (amines and/or amides) in the linkage. The degree of crosslinking was 3.6—5.8 on the basis of the elemental analysis.

2. The equilibrium constants Ks of the immobilized β-CyDs were determined for cresols, phenol, nitrophenols, and 2-naphthol. The independence of K from the amount of immobilized CyD indicates 1:1 complex formation between the CyD residue and the guest molecule.

3. The K values measured at pH 4 (a neutral guest) are generally larger than those measured at pH 10 or 11 (an anionic guest). The K values reflect the property of the crosslinking residue, indicating the participation of the crosslinking residue to the inclusion complex formation. Macromolecules containing the long crosslinking residues (I-3a, 3b, and 3c) exhibit large K values, which indicate the existence of a hydrophobic interaction between the crosslinking residue and the guest molecule. A high degree of crosslinking results in the large K values, indicating that the crosslinking residue promotes complex formation but does not inhibit it.

4. A hydrophobic enhancement coefficient R has been introduced. The R values indicate how the crosslinking residue effects on the property of the binding site for complex formation. The R values calculated from the K constants are independent of the crosslinking reagents, but dependent on the structure of the guest molecule, suggesting the expected potential for high guest selectivity of the immobilized CyD.

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References

1) M. L. Bender and M. Komiyama. "Cyclodextrin Chemistry," Springer-Verlag, Berlin (1978).

2) a) D. W. Armstrong, W. DeMond, and B. P. Czech, *Anal. Chem.*, **57**, 481 (1985), and the references cited therein; b) A. Harada, M. Furue, and S. Nozakura, *J. Polym. Sci., Polym. Chem. Ed.*, **16**, 189 (1978); c) M. Tanaka, Y. Mizobuchi, T. Sonoda, and T. Shono, *Anal. Lett.*, **14(A4)**, 281 (1981); d) K. Takahashi, S. Nakada, M. Mikami, and K. Hattori, *Nippon Kagaku Kaishi*, **1986**, 1032.

3) a) R. L. VanEtten, G. A. Clowes, J. F. Sebastian, and M. L. Bender, *J. Am. Chem. Soc.*, **89**, 3253 (1967); b) R. L. VanEtten, J. F. Sebastian, G. A. Clowes, and M. L. Bender, *ibid.*, **89**, 3242 (1967).

4) a) T. Ikeda, R. Kojin, C-J. Yoon, H. Ikeda, M. Iijima, K. Hattori, and F. Toda, *J. Incl. Phenom.*, **2**, 669 (1984); b) C-J. Yoon, H. Ikeda, R. Kojin, T. Ikeda, and F. Toda, *J. Chem. Soc., Chem. Commun.*, **1986**, 1080.

5) R. Breslow and P. Campbell, *J. Am. Chem. Soc.*, **91**, 3085 (1969).

6) a) I. Tabushi, K. Fujita, and H. Kawakubo, *J. Am. Chem. Soc.*, **99**, 6456 (1977); b) I. Tabushi, K. Yamamura, K. Fujita, and H. Kawakubo, *ibid.*, **101**, 1019 (1979).

7) M. Komiyama and H. Hirai, *J. Am. Chem. Soc.*, **106**, 174 (1984).

8) a) M. Komiyama, I. Sugiura, and H. Hirai, *J. Incl. Phenom.*, **2**, 823 (1984); b) M. Komiyama, I. Sugiura, and H. Hirai, *Polym. J.*, **17**, 1225 (1985); c) M. Komiyama, I. Sugiura, and H. Hirai, *J. Mol. Catal.*, **36**, 271 (1986).

9) a) C. U. Pittman, Jr., L. R. Smith, and R. M. Hanes, *J. Am. Chem. Soc.*, **97**, 1742 (1975); b) T. Mukaiyama and H. Iwakiri, *Chem. Lett.*, **1985**, 1363; c) H. Hirai and N. Toshima Ed. "Kobunshi Sakutai Shokubai, (Macromolecular Complex Catalysts)," Japan Scientific Societies Press, Tokyo (1982).

10) a) M. Komiyama and H. Hirai, *Polym. J.*, **19**, 773 (1987); b) M. Komiyama, I. Sugiura, and H. Hirai, *J. Coord. Chem.*, **18**, 181 (1988); c) I. Sugiura, K. Kawamura, N. Toshima, and K. Komiyama, *Polymer Preprints, Japan* **37**, No. 10, 3423 (1988).

11) A. Harada, M. Furue, and S. Nozakura, *Polym. J.*, **13**, 777 (1981).

12) For example, S. Umezawa and K. Tatsuta, *Bull. Chem. Soc. Jpn.*, **41**, 464 (1968).

13) H. Schlenk and D. M. Sand, *J. Am. Chem. Soc.*, **83**, 2312 (1961).

14) A. Ueno, F. Moriwaki, T. Osa, F. Hamada, and K. Murai, *J. Am. Chem. Soc.*, **110**, 4323 (1988).

15) a) J. Emert and R. Breslow, *J. Am. Chem. Soc.*, **97**, 670 (1975); b) I. Tabushi, K. Shimokawa, N. Shimizu, H. Shirakata, and K. Fujita, *J. Am. Chem. Soc.*, **98**, 7855 (1976).

16) a) I. Sakurada, Y. Sakaguchi, T. Ono, and T. Ueda, *Makromol. Chem.*, **91**, 243 (1966); b) S. Yoshikawa and O-K. Kim, *Bull. Chem. Soc. Jpn.*, **39**, 1515 (1966); c) C. G. Overberger, R. Corett, J. C. Salamone, and S. Yaroslavsky, *Macromolecules*, **1**, 331 (1968); d) T. Kunitake, F. Shimada, and C. Aso, *J. Am. Chem. Soc.*, **91**, 2716 (1969).

17) M. Kitagawa, H. Hoshi, M. Sakurai, Y. Inoue, and R. Chujo, *Carbohydr. Res.*, **163**, C1 (1987).

18) I. Sanemasa and Y. Akamine, *Bull. Chem. Soc. Jpn.*, **60**, 2059 (1987).

19) K. Kawamura, N. Toshima, and H. Hirai, 57th National Meeting of the Chemical Society of Japan, Sendai, September 1988, Abstr. I, No. 1G215.