

Inclusion of Aromatic Compounds by a β -Cyclodextrin-Epichlorohydrin Polymer

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ABSTRACT: A condensation polymer (β -CD-E) of β -CD with epichlorohydrin was compared with β -CD and poly(β -CD acrylate) in regard to inclusion behavior in an aqueous solution. The stability of the inclusion complexes of β -CD-E with substrates of a single guest part is smaller than that of β -CD because of steric hindrance by substituents on β -CD. On the other hand, the stability of β -CD-E complexes for substrates with two guest parts is much larger than that of β -CD and is somewhat smaller than that of poly(β -CD acrylate). This is due to the cooperation in binding of the adjacent two CD units on a polymer chain.

KEY WORDS β -Cyclodextrin / Epichlorohydrin / β -Cyclodextrin Polymer / Inclusion / Fluorescence / Sodium 2-*p*-Toluidinylnaphthalene-6-sulfonate / Binding / Dansylamino Acid /

Cyclodextrins (CD) are known to form noncovalent inclusion complexes with various organic molecules in aqueous solution.^{1,2} In the preceding papers the authors reported the preparation of certain vinyl polymers containing cyclodextrins³ and their catalytic and inclusion behavior.⁴ The polymers containing β -CD were found to bind to large substrates having two guest parts more efficiently than β -CD but to bind to small substrates having one guest part less efficiently than β -CD. It was concluded that this polymer effect on the formation of complexes with large substrates was caused by the cooperation of two adjacent β -CD moieties on a polymer chain.⁵ This conclusion is supported by an inclusion study using the model dimers of β -CD.⁶

In this paper, the study of the polymer effect on the inclusion by β -CD was extended to a condensation polymer of β -CD, the preparation of which is already known⁷ and is much easier than that of vinyl polymers.³

RESULTS AND DISCUSSION

Characterization of β -CD-E

The molecular weight of the polymer was estimated to be 10^4 to 10^5 by GPC using Sephadex G-

15 and G-50 columns, the degree of polymerization corresponding to 10—100.

An ¹H NMR study did not identify the place of attachment of the glyceryl bridge or tail whether it is at the C-2, C-3 or C-6 position⁷ of the 1,4-glucoside unit. ¹³C NMR spectrum in Figure 1 presents some information on the place of substitution. The assignment of C-1 through C-6 is based on that of Colson *et al.*^{8a} Resonances a and b are indicative of the C-2 and C-3 substitution, respectively.^{3,8b} Resonance d may be an overlap of the C-3 and C-6 substitution. Resonance e is due to the terminal carbon of the glyceryl tail. Resonances f and g clearly shows the existence of the glycidyl group which may be responsible for gelation of the polymer after prolonged storage. Thus, we have no positive evidences for the C-6 substitution, but in view of the higher reactivity of the 6-OH group than that of the 2-OH or 3-OH group,⁹ the existence of the C-6 substitution is assumed as well as the C-2 and C-3 substitutions.

The polymer was found to be completely amorphous by examination of the X-ray powder pattern.

Solubilization

When an aqueous solution of β -CD (4.0×10^{-3} M) was shaken with an excess of the aromatic sub-

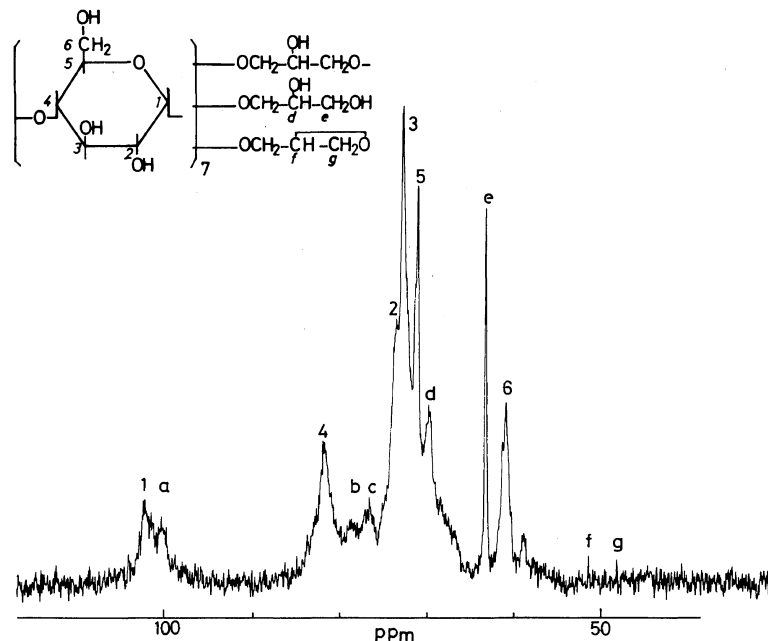


Figure 1. ^{13}C NMR spectrum and the proposed structure of β -CD-E: TMS external reference, 15 wt% in D_2O , 16384 accumulations.

Table I. Association constants (K_a) and solubilities of β -CD and β -CD-E complexes at 30°C^a

Substrate	K_a , β -CD ($\times 10^{-3} \text{ M}^{-1}$)	K_a , β -CD-E ($\times 10^{-3} \text{ M}^{-1}$)	S/S_0^b
4-Dimethylaminoazobenzene	0.35	7.0	40.0
<i>m</i> -Chlorobenzoic acid	2.2	0.5	2.1
Dibenzyl	Crystallized	1.3	6.9
Diphenylmethane	"	0.8	4.6
1,1-Diphenylethylene	"	2.0	9.3
<i>p</i> -Xylene	"	0.3	2.2

^a $[\beta\text{-CD}] = 4.0 \times 10^{-3} \text{ M}$, $[\beta\text{-CD-E}] = 5.6 \times 10^{-3} \text{ unit M}$.

^b S_0 , solubility in H_2O ; S , solubility in aqueous β -CD-E solution ($5.6 \times 10^{-3} \text{ unit M}$).

strates listed in Table I, crystallization of inclusion complexes occurred in many cases (Table I, second column). But when an aqueous solution of β -CD-E was used instead, no precipitation took place and solubilization of substrates was always realized. This is one notable feature of β -CD polymers in solubilization.

The last column in Table I shows solubilization expressed in relative solubility in aqueous β -CD to that in water. It is seen that solubilization is comparatively small with the substrates of one benzene

ring such as *m*-chlorobenzoic acid and *p*-xylene and relatively large with the substrates of two benzene rings, the largest being with 4-dimethylaminoazobenzene. Association constant K_a in Table I also indicates this trend. When β -CD and β -CD-E are compared in K_a values, β -CD-E forms a less stable complex with *m*-chlorobenzoic acid, whereas β -CD-E forms a more stable complex with 4-dimethylaminoazobenzene. This is reasonable since β -CD-E is less favorable for the inclusion of substrates with a single guest part owing to steric interference of the

Table II. Effects of β -CD, poly(β -CD-A), and β -CD-E on the fluorescence of dyes^a

Dye	I/I_0^b			λ_{\max}^F (nm)			
	β -CD	Poly- (β -CD-A)	β -CD-E	None	β -CD	Poly- (β -CD-A)	β -CD-E
ANS ^c	10.4	70	61	515	495	475	475
TNS ^d	25.3	571	316	500	460	437	440
DNS-Phe ^c	150	600	450	548	500	500	500

^a pH 5.9 phosphate buffer. $[\text{CD}] = 1.0 \times 10^{-3}$ M.

^b Relative fluorescence intensity.

^c 1.0×10^{-5} M.

^d 1.6×10^{-5} M.

glyceryl substituent and because β -CD-E is favorable for the inclusion of substrates with two guest parts owing to the cooperation of two CD moieties for inclusion⁵ (*vide infra*).

Fluorescence Enhancement

Fluorescence enhancement by inclusion of hydrophobic microenvironmental probes, sodium 1-anilinoanthracene-8-sulfonate (ANS), potassium 2-*p*-toluidinylnaphthalene-6-sulfonate (TNS), and 5-dimethylaminonaphthalenesulfonylphenylalanine (DNS-Phe) was compared among β -CD, β -CD acrylate polymer (poly(β -CD-A))⁵, and β -CD-E. As shown in Table II, β -CD-E exhibits a much larger fluorescence enhancement than β -CD toward all of the probes and a slightly smaller enhancement than poly(β -CD-A). The blue shift of emission maximum with β -CD-E is also similar to that with poly(β -CD-A). These facts indicate that CD moieties in β -CD-E cooperate in binding the large substrate which has both the phenyl and naphthalene rings as in the case of poly(β -CD-A).⁵

Characteristics of the interaction of TNS with β -CD-E was studied by the fluorescence titration method^{5,10} and is depicted in Figure 2 along with the data with other β -CD derivatives.^{5,6} In these experiments the concentration of TNS was kept constant and the fluorescence intensity was measured at various concentrations of β -CD units. A straight line for β -CD-E shows the existence of a homogeneous binding mode throughout the concentration range, whereas a curved line for β -CD shows the existence of two binding modes, *i.e.*, 2:1 for β -CD:TNS at high concentrations of CD and 1:1 at low concentrations.⁵

The stoichiometry of the binding between β -CD

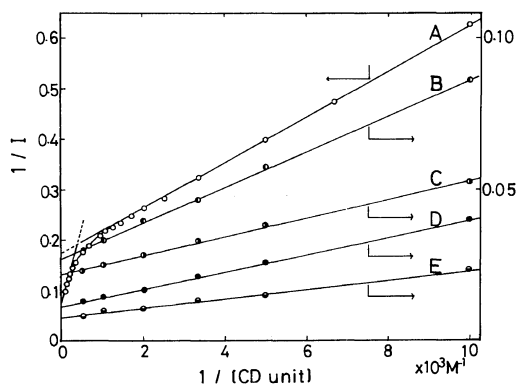


Figure 2. Double reciprocal plots for titration of TNS by β -CD (A), bis(β -CD) glutarate (B), bis(β -CD) succinate (C), β -CD-E (D), and poly(β -CD-A) (E): 0.1 M phosphate buffer (pH 5.9), 25°C; I , fluorescence intensity.

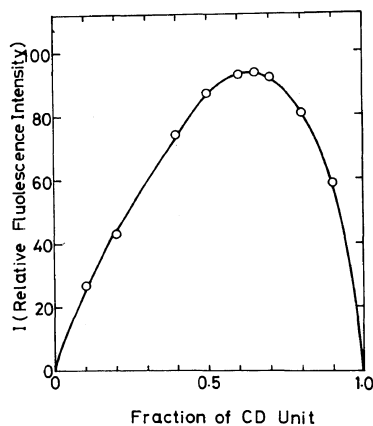


Figure 3. Continuous variation plot of β -CD-E-TNS system: $[\text{TNS}] + [\beta\text{-CD unit}] = 1.00 \times 10^{-4}$ M, 25°C.

Table III. Dissociation constants and fluorescence properties of complexes of TNS with β -CD derivatives

	CD : TNS	K_d/M	λ_m^F	Relative fluorescence intensity
			nm	
β -CD	1 : 1	2.5×10^{-4}	462	1
	2 : 1	5×10^{-2}	447	3
β -CD-E	2 : 1	3.0×10^{-3}	440	12
$(\beta\text{-CD})_2\text{G}$	2 : 1	1.2×10^{-4}	447	8
$(\beta\text{-CD})_2\text{S}$	2 : 1	0.6×10^{-4}	447	9
Poly(β -CD-A)	2 : 1	1.0×10^{-4}	438	20

and TNS was studied by the continuous variation method, watching the fluorescence intensity at various ratios of the two reactants. As shown in Figure 3 the curve for β -CD-E has a maximum at a β -CD unit molar fraction of 0.66, which corresponds to a 2 : 1 (β -CD : TNS) stoichiometry. Thus, β -CD-E was found to bind to TNS at a 2 : 1 stoichiometry throughout the entire concentration range. This was also the case with poly(β -CD-A) and model dimers, bis- β -CD succinate (β -CD)₂S and glutarate (β -CD)₂G (Fig. 2^{5,6}).

Dissociation constants and fluorescence properties of complexes of TNS with certain β -CD derivatives are summarized in Table III. The stability of the 2 : 1 complex with β -CD-E is larger than that with β -CD and smaller than those with poly(β -CD-A) and model dimers. In the cases of poly(β -CD-A) and the dimers, the substitution is exclusively on one of the secondary hydroxyl groups. This substituent makes the inclusion complex less stable than that of β -CD since the substituent hinders the entrance of guests into the cavity of β -CD.^{4,5} A substrate like TNS has two guest parts and can be bound cooperatively by two CD units on the polymer or dimers, yielding eventually more stable complexes than with β -CD. In the case of β -CD-E, more than two substitutions as an average have taken place on β -CD on either the primary or secondary hydroxyl groups. These substituents make the steric hindrance more severe than poly(β -CD-A) or the dimers, so that the complexes with β -CD-E become less stable.

It may be noteworthy to point out a possible application of β -CD-E in binding. Fluorescence enhancement of dansylamino acid (DNS-amino acid) by β -CD is used for the quantitative determination of amino acids.¹¹ As shown in Table

Table IV. Effects of β -CD and β -CD-E on the fluorescence of DNS-amino acids^a

R ^b	$I_{\beta\text{-CD}}/I_0$	$I_{\beta\text{-CD-E}}/I_0$
-H	1.5	2.8
-CH ₃	1.5	2.8
-CH(CH ₃) ₂	2.8	5.9
-CH ₂ C ₆ H ₅	5.6	20.0

^a [β -CD] = 1.0×10^{-3} M; [DNS-amino acid] = 1.0×10^{-4} M.

^b DNS-NHCH(R)COOH.

IV, β -CD-E shows a fluorescence enhancement of all dansylamino acids tested that is higher than β -CD. This enhancement was largest in the case of dansylphenylalanine, again manifesting the importance of the cooperative binding for substrates with two guest parts.

EXPERIMENTAL

Preparation of Soluble β -CD-Epichlorohydrin Polymers(β -CD-E)

The condensation reaction was carried out using a modified method of Wiedenhof *et al.*⁷ Into a mixture of a solution of β -CD (12.0 g, 0.0105 mol) in 150 ml of water and 40 ml of 20% NaOH solution, 10.0 g (0.127 mol) of epichlorohydrin was added dropwise at 60°C in 45 minutes. After the reaction mixture was kept at 65°C for 24 hours, it was neutralized with 2N HCl, dialyzed with distilled water for several days, and freeze-dried. Larger amounts of epichlorohydrin resulted in gellation during the reaction, yielding only insoluble polymers. The Beilstein test of the polymer was

negative.

Materials

β -CD was a product of Hayashibara Biochemical Laboratory Inc. and was purified as described previously.³

Measurement

Solubility was measured according to the previously described method, dissociation constants being calculated assuming 1:1 stoichiometry.⁴

¹³C NMR spectra were recorded on a JEOL FX90Q spectrometer operated at 22.50 MHz in the pulse Fourier transform mode.

Fluorescence was measured in a 0.1 M phosphate buffer (pH 5.9) using a Union Giken FS-401 spectrofluorometer as described previously.⁵ Determination of dissociation constants K_d of inclusion complexes by fluorimetric titration was carried out as previously described⁴ using Klotz's method.¹⁰

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