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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 cyclodextrins[3] 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 known[7] 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 ^{1}H 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 position[7] of the 1,4-glucoside unit. ^{13}C 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 gellation 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-

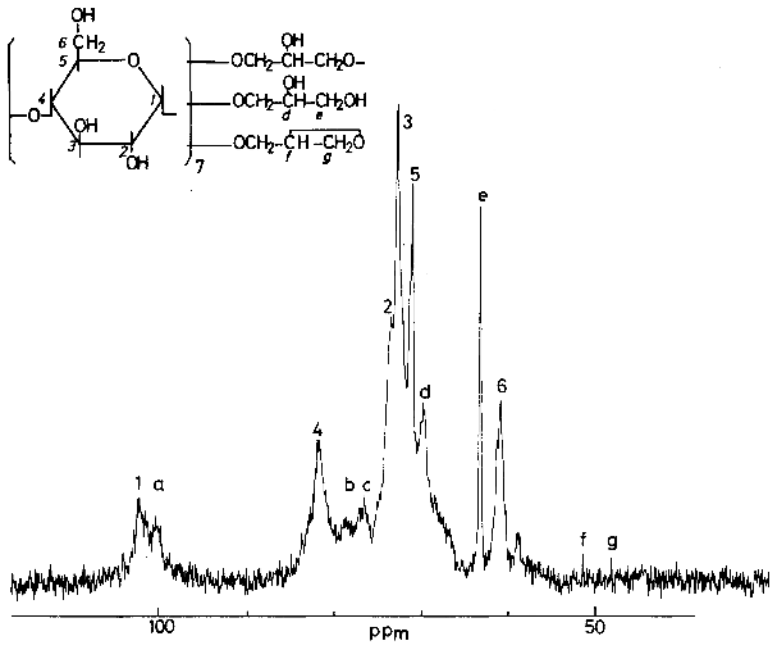

Figure 1. ^{13}C NMR spectrum and the proposed structure of β-CD-E: TMS external reference, 15 wt% in D_2O, 16384 accumulations.

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 ($\times 10^{-3}$ M^{-1})	K_a, β-CD-E ($\times 10^{-3}$ M^{-1})	S/S_0[b]
4-Dimethylaminoazobenzene	0.35	7.0	40.0
m-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
p-Xylene	〃	0.3	2.2

[a] [β-CD] = 4.0×10^{-3} M, [β-CD-E] = 5.6×10^{-3} unit M.
[b] S_0, solubility in H_2O; S, solubility in aqueous β-CD-E solution (5.6×10^{-3} 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^{F}_{max} (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. [CD] = 1.0×10^{-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 inclusion[5] (*vide infra*).

Fluorescence Enhancement

Fluorescence enhancement by inclusion of hydrophobic microenvironmental probes, sodium 1-anilinonaphthalene-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 existense 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

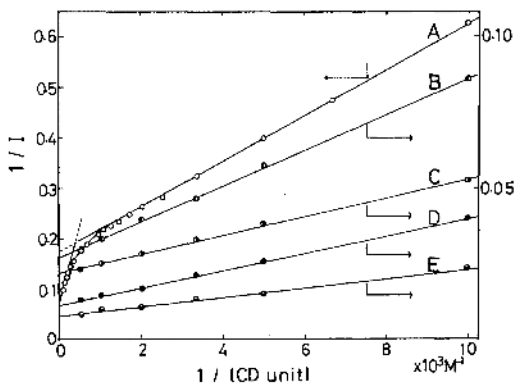

Figure 2. Double reciprocal plots for titration of TNS by β-CD (A), bis(β-CD) glutarate (B), bis(β-CD) succinate (C), β-CD-E (D), and poly(β-CD-A) (E): 0.1 M phosphate buffer (pH 5.9), 25°C; *I*, fluorescence intensity.

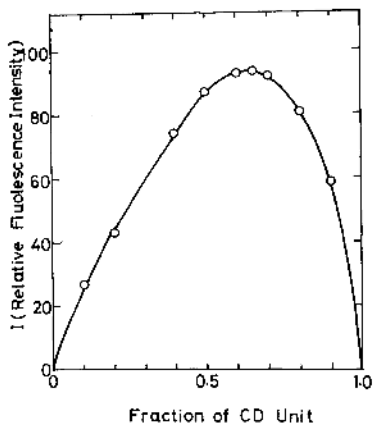

Figure 3. Continuous variation plot of β-CD-E–TNS system: [TNS] + [β-CD unit] = 1.00×10^{-4} M, 25°C.

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

	CD : TNS	K_d/M	λ_m^F / nm	Relative fluorescence intensity
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
$(\beta\text{-CD})_2$G	2 : 1	1.2×10^{-4}	447	8
$(\beta\text{-CD})_2$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 cases with poly(β-CD-A) and model dimers, bis-β-CD succinate $(\beta\text{-CD})_2$S and glutarate $(\beta\text{-CD})_2$G (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 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 cooparative binding for substrates with two guest parts.

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
$-CH_2C_6H_5$	5.6	20.0

[a] $[\beta\text{-CD}] = 1.0 \times 10^{-3}$ M; [DNS-amino acid] $= 1.0 \times 10^{-4}$ M.
[b] DNS–NHCH(R)COOH.

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 2*N* 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]

^{13}C 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 described[4] using Klotz's method.[10]

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