Cyclodextrin-Containing Polymers. 2. Cooperative Effects in Catalysis and Binding

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ABSTRACT: Hydrolyses of *p*-nitrophenyl esters catalyzed by cyclodextrin-containing polymers were compared with those by their monomeric analogues. Polyacryloyl-β-cyclodextrin (poly-β-CD-A) showed a distinctly higher catalytic effect than the parent β-CD and exhibited a Michaelis–Menten type kinetics as β-CD does. Judging from Michaelis constant K_m, the polymer forms a less stable intermediate complex than β-CD with *p*-nitrophenyl acetate, whereas the polymer forms a more stable complex than β-CD with *p*-nitrophenyl *p*-nitrobenzoate, which has two benzene rings. Maximal rate constants k_2 of the polymer were larger than those of β-CD for both the substrates. It was also shown that the acceleration by polymers decreases as the distance between neighboring CD units increases. To confirm K_m values obtained from kinetics, the interactions of poly-β-CD-A with low molecular weight compounds of varying size were studied by spectrophotometry and solubility measurements. Poly-β-CD-A was found to be more efficient than β-CD in binding substrates which are too large to be accommodated in a single CD cavity but to be less efficient than β-CD in binding small substrates which can be completely included in a single cavity. The polymer showed 1:1 stoichiometry for a small substrate, but two CD residues of the polymer are required to bind a large substrate molecule. Both the kinetic and binding experiments suggest the existence of cooperative action between two neighboring β-CD groups on a polymer chain.

Cyclodextrins (CD) have drawn much attention because of their ability to form inclusion complexes in aqueous solution[1,2] and their selective catalytic activity,[3] and hence they have been studied as an enzyme model.[4] These studies have disclosed the substrate specificity of cyclodextrin both in the binding step and the catalytic step based on the size of their hydrophobic cavities.

Recently, a variety of esterolytic polymer catalysts[5] containing nucleophiles and binding moieties have been studied in order to realize enzymic efficiency and specificity. Although some cooperative action between binding and catalytic groups was observed, substrate specificity by steric control or multiple interactions has not been achieved in such systems.

When cyclodextrins are incorporated into a polymer chain as a definite structural unit and the cyclodextrin units are located in favorable positions, they can be expected to behave cooperatively in binding and in catalysis. Thus, we synthesized polymers containing cyclodextrins in order not only to obtain polymer catalysts equipped with substrate specificity but also to see whether the cyclodextrin moieties attached on the polymer chain behave independently or cooperatively in the binding and catalysis.[6]

In this paper, binding and catalytic properties of β-cyclodextrin incorporated into the polymer were investigated through the study of their effects on the ester hydrolysis and were compared with those of β-cyclodextrin. Furthermore, in order to clarify the substrate selectivity in the binding, the association equilibrium with low molecular weight compounds was studied.

Polymeric units used are shown in Figure 1.

Results and Discussion

Hydrolysis of *p*-Nitrophenyl Carboxylates. The pseudo-first-order rate constants (k') for the hydrolyses of a variety of *p*-nitrophenyl carboxylates catalyzed by poly-β-CD-A are compared with those by β-CD in Table I. Poly-β-CD-A showed distinctly higher catalytic effects than β-CD for all the substrates examined. The rate enhancements by the polymer, expressed as the $k'_{polymer}/k'_{\beta\text{-CD}}$ ratios, were 1.7, 1.6, and 2.1 for *p*-nitrophenyl acetate, propionate, and valerate, respectively, and 3 for *p*-nitrophenyl esters of three para-substituted benzoic acids. Thus the effect of the polymer depends on the acyl group of the esters.

Valeric acid is known to be bound within the hydrophobic cavity more strongly than acetic acid and propionic acid.[7] Benzoic acid is bound still more strongly. The rate enhancement by poly-β-CD-A may be ascribed at least in part to a hydrophobic interaction between the cyclodextrin cavity and the alkyl or phenyl groups of the acyl portion, in addition to the interaction between the cavity and the *p*-nitrophenyl group of the ester function. These two kinds of binding result in a cooperative action of neighboring cyclodextrin units to the substrate at the binding step and/or the catalytic step. These two steps were analyzed according to the Michaelis–Menten scheme.

$$CD_{unit} + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} CD\text{-}S \xrightarrow{k_2} CD' + P$$

The effect of the catalyst concentration on k' for *p*-nitrophenyl acetate (pNPA) and *p*-nitrophenyl *p*-nitrobenzoate (pNPpNB) is shown in Figure 2.

The rate constants for the reaction of the entirely complexed ester, k_2, and the Michaelis constants, K_m, were evaluated by the Lineweaver–Burk plots[8] of eq 1 as shown in Figure 3 and are shown in Table II

$$\frac{1}{k_{obsd} - k_{un}} = \frac{K_m}{(k_2 - k_{un})[CD]} + \frac{1}{(k_2 - k_{un})} \qquad (1)$$

where k_{obsd} and k_{un} denote the rate constant in the presence and absence of CD, respectively. K_m is Michaelis constant defined by $(k_2 + k_{-1})/k_1$.

The maximal rate constant, k_2, for the pNPA-β-CD system was 1.15×10^{-3} s^{-1} and that for the pNPA-poly-β-CD-A system was 2.29×10^{-3} s^{-1}, so the rate enhancement factor $k_{2,polymer}/k_{2,\beta\text{-CD}}$ was 1.99. The rate enhancement factor for pNPpNB was 1.66. It seems that in the second step polymer effects are insensitive to the structure of the acyl portion. In contrast, the stability of the complex relative to β-CD complex depends on the structure of the acyl function. In the case of pNPA, the K_m of poly-β-CD-A was larger (1.1×10^{-2} M) than that of β-CD (0.83×10^{-2} M), whereas for pNPpNB the K_m of the polymer was smaller (12.7×10^{-2} M) than that of β-CD (22.8×10^{-2} M). As seen in K_m values of Table II, poly-β-CD-A formed a less stable complex than β-CD with pNPA while a more stable complex than β-CD with pNPpNB. Therefore, the apparent overall hydrolysis rate k_2/K_m for pNPpNB in the presence of poly-β-CD-A is three times larger than that of β-CD and for pNPA the polymer shows 1.5 times higher effect than β-CD.

In order to see the reliability of K_m values obtained from

Table I
Hydrolysis Rate Constants of *p*-Nitrophenyl Alkanoates and Benzoates with β-CD and Poly-β-CD-A[a]

p-Nitrophenyl esters	$k_{un} \times 10^{-4}$, s^{-1}	$k'_{\beta-CD}$[d] $\times 10^{-4}$, s^{-1}	$k'_{polymer}$[d] $\times 10^{-4}$, s^{-1}	$k'_{polymer}/k'_{\beta-CD}$
Acetate[b]	0.90	2.73	4.54	1.7
Propionate[b]	0.77	2.20	3.43	1.6
Valerate[b]	0.53	1.28	2.76	2.1
Benzoate[c]	0.08	0.10	0.31	3.1
p-Nitrobenzoate[c]	1.96	4.65	15.5	3.3
p-Chlorobenzoate[c]	0.18	0.58	1.81	3.1
p-Toluate[c]	0.04	0.11	0.33	3.0

[a] pH 8.7 Tris buffer, $I = 0.02$ at 25 °C, catalyst concentration 0.33×10^{-2} M. Substrate concentration 0.33×10^{-4} M. [b] In 0.5% acetonitrile. [c] In 20% acetonitrile. [d] $k' = k_{obsd} - k_{un}$.

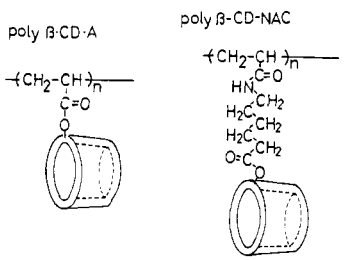


Figure 1. Cyclodextrin-containing polymers: polyacryloyl-β-cyclodextrin (poly-β-CD-A) and poly-*N*-acrylyl-6-aminocaproyl-β-cyclodextrin (poly-β-CD-NAC).

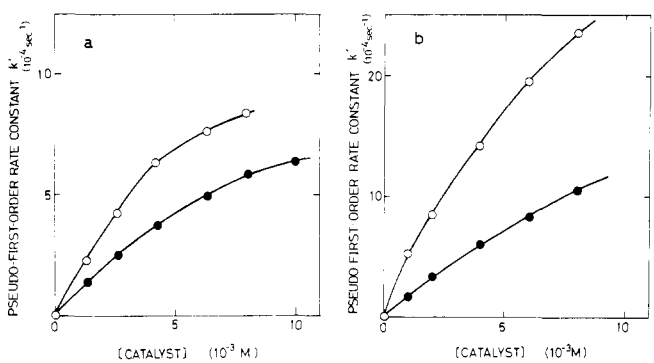


Figure 2. The pseudo-first-order rate constants for release of nitrophenol from *p*-nitrophenyl esters vs. catalyst concentration; pH 8.7 Tris buffer, $I = 0.02$ at 25 °C: (a) *p*-nitrophenyl acetate in 0.5% (v/v) acetonitrile; (b) *p*-nitrophenyl *p*-nitrobenzoate in 20% (v/v) acetonitrile; (●) β-CD; (○) poly-β-CD-A.

kinetic study, the dissociation constants K_d of the complexes were determined by the solubility method,[9] assuming 1:1 stoichiometry. The K_d value for pNPA is in fair agreement with the K_m for both the β-CD complex and the polymer complex (Table III). The K_m values for the pNPpNB complex cannot be directly compared with the K_d values because the K_m values were obtained in acetonitrile–water (20:80 v/v) solution due to the limited solubility of the substrate, whereas the K_d values were obtained in aqueous solution. The K_d for the polymer, however, is much smaller than that for β-CD. This trend is in accord with that in K_m.

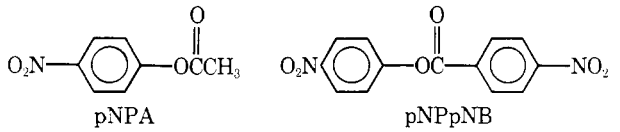
Judging from these independent estimations of the binding capacities of β-CD and poly-β-CD-A the following conclusion may be deduced. The polymer binds the pNPpNB molecule, which has two benzene rings, more strongly than β-CD, whereas it binds the small pNPA molecule, which can be completely included in a single cyclodextrin cavity, less strongly than β-CD. Cooperative action between β-CD moieties on a polymer chain should be taken into consideration for the binding of a large molecule by the polymer.

Thermodynamic Considerations on Hydrolysis of pNPA. Thermodynamic parameters were obtained for the binding processes in the catalytic hydrolyses of pNPA with poly-β-CD-A and with β-CD (Table IV). The binding process of poly-β-CD-A as well as β-CD[10,11] was associated with negative entropy and negative enthalpy changes. This may indicate that there is no substantial difference in the binding mechanism when β-CD units are in the monomeric[4,10] or polymeric form. However, appreciable differences were found in the absolute values of the enthalpy and entropy of binding for the polymer relative to those of β-CD. Bender *et al.*[4] reported that the entropy change for β-CD complex formation would arise from the release of highly structured water initially surrounding the nonpolar substrate and from the loss of translational and rotational degrees of freedom upon association of the two molecules. Thus the less reduction of entropy for the polymer relative to that for β-CD may be ascribed to the following reasons. The loss of freedom on complex formation for the polymer is smaller than that for β-CD and a smaller amount of structured water was released in the case of the polymer in comparison with β-CD. This is consistent with the concept of shallower or looser binding of poly-β-CD-A than β-CD. A major contribution to the negative enthalpy term for the β-CD complex is considered to be the release of water molecules from the CD cavity into solution.[12] The enthalpy change for the polymer may be less negative than with β-CD because the amount of excluded water from polymer is less than from β-CD. This also reflects the looser binding[13] for the polymer. This concept of looser binding is also consistent with the fact that the spectrum of *p*-nitrophenolate is less perturbed in the presence of excess poly-β-CD-A than in the presence of excess β-CD.

Activation parameters of the second step for pNPA hydrolysis are shown in Table V. In this step poly-β-CD-A was found to have a considerably smaller enthalpy of activation and a slightly smaller entropy of activation than β-CD. As a result poly-β-CD-A produces a higher overall catalytic rate than β-CD. These results might indicate that a different mechanism is operating in the catalysis by the polymer as compared to that by the monomer. The high local concentration of catalytic OH groups of pendent β-CD on the polymer chain may lead to multifunctional catalysis. The smaller ΔS^* for the polymer may be partly due to the difference of the initial state, i.e., the complex state.

Mode of Binding. A scale molecular model of cyclodextrin indicates that pNPpNB is too large to be completely included in a single β-CD cavity which can accommodate only one aromatic ring. Either the *p*-nitrophenyl group or the *p*-nitrobenzoyl group can enter the β-CD cavity. The binding, in which the *p*-nitrophenyl group is included into the CD cavity, may be productive as has been revealed for various substituted phenyl esters.[14,15] However, when the benzoyl group is included in the cavity, the binding may be nonproductive as shown by Lach et al.[16] and VanEtten et al.[17] on ethyl *p*-ami-

Table II
Kinetic Parameters for Catalyzed Hydrolysis of *p*-Nitrophenyl Esters[a]



pNPA pNPpNB

Substrate		$k_2 \times 10^3$, s^{-1}	$K_m \times 10^2$, M	$k_2/K_m \times 10^2$
pNPA[b]	β-CD	1.1_5	0.83	14
pNPA[b]	Poly-β-CD-A	2.2_9	1.1	21
pNPpNB[c]	β-CD	3.8_4	$22._8$	1.6_8
pNPpNB[c]	Poly-β-CD-A	6.2_5	$12._7$	4.9_2

[a] 25 °C, pH 8.7, I = 0.02. [b] 0.5% acetonitrile. [c] 20% acetonitrile.

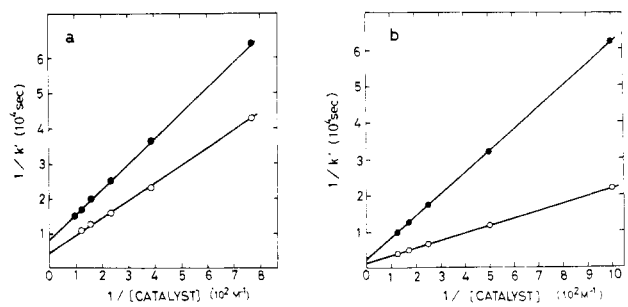


Figure 3. Lineweaver–Burk plots of Figure 2.

Table III
Michelis Constants and Dissociation Constants for β-CD and Poly-β-CD-A Complexes

Substrate	Catalyst	K_m[a] × 10^2, M	K_d[b] × 10^2, M
pNPA	β-CD	0.83	1.18
	Poly-β-CD-A	1.1	1.98
pNPpNB	β-CD	22.8[c]	0.17
	Poly-β-CD-A	12.7[c]	0.061

[a] Kinetic method at 25 °C. [b] Solubility method at 30 °C in pH 4.1 acetate buffer. [c] 20% acetonitrile.

Table IV
Thermodynamic Parameters for Substrate Binding

	ΔH, kcal/mol	$T\Delta S$, kcal/mol	ΔG (298 K), kcal/mol
β-CD	−6.4	−3.6	−2.8
Poly-β-CD-A	−5.4	−2.7	−2.7

Table V
Activation Parameters for the Second Process

	$\Delta H^{\ddagger}$, kcal/mol	$\Delta S^{\ddagger}$, eu	$\Delta G^{\ddagger}$ (298 K), kcal/mol
β-CD	13.5	−26.8	21.5
Poly-β-CD-A	10.2	−36.6	21.1

nobenzoate and some alkyl benzoates. The unreactivity of the complexed ester was explained as due to its location in the cavity at some distance from the catalytic secondary hydroxyl groups.

In the poly-β-CD-A–pNPpNB system, molecular model studies show that three binding modes are possible as shown in Figure 4. Binding mode A may be productive and binding mode B may be nonproductive. Mode C may be productive. The stabilization of the polymer complex of large substrate relative to the β-CD complex can be reasonably explained by this binding mode.

The hydrolysis of pNPpNB was carried out in the presence of various polymers carrying β-CD units separated at varying distances from each other by a side chain or a main chain (Table VI). The polymers tested were poly-β-CD-A, poly-β-CD-NAC, and β-CD-A–acrylamide copolymer (1:6). Poly-β-CD-A, in which β-CD moieties are located most closely to each other, had an effect 3.3 times larger than β-CD. The β-CD-A–acrylamide copolymer, in which the β-CD units are not close to each other, produced a smaller effect than β-CD but almost the same effect as the monomer β-CD-A. Poly-β-CD-NAC was intermediate in its effectiveness. These results reveal a distinct trend; the acceleration effect of the polymer increased with a decrease in the distance between neighboring CD units. This observation can be explained by taking into account the cooperative action between neighboring β-CD units on the polymer chain.

It should be noted that the rate constants presented in Table VI are pseudo-first-order rate constants in the presence of 0.0033 M CD units concentration. They are not the maximal rate constants.

In conclusion the polymer complex with the larger substrate is stabilized relative to the complex of β-CD due to cooperative binding with neighboring β-CD moieties on the polymer chain and the reactivity of the polymer complex is increased due to the "intramolecular" catalysis, i.e., catalysis by the other β-CD units.

Interaction with Low Molecular Weight Compounds. The dissociation constants of complexes between poly-β-CD-A and low molecular weight compounds of varying size were determined by a spectrophotometric[14,18,19] or solubility method.[7,9,12] Table VII shows the dissociation constants of complexes of poly-β-CD-A and β-CD, assuming 1:1 stoichiometry. Poly-β-CD-A is less efficient than β-CD in binding small molecules such as *m*-chlorobenzoic acid and cinnamic acid. On the other hand the polymer is more efficient than β-CD in binding large substrates with two aromatic rings such as methyl red and orange I.[20] Furthermore the polymer complex of Congo red, which is larger than methyl red and orange I, corresponding in dimensions to the dimers of these dyes, is 15 times more stable than the β-CD complex[13,21] (Figure 5). These results suggest that there is a cooperative action of the β-CD units on the polymer chain in the binding of large substrates.

The dissociation constants of β-CD complexes are roughly independent of the size of the substrate. Congo red, which is the largest in this series, had a dissociation constant of about the same order as those of the smallest molecules, such as *m*-chlorobenzoic acid and cinnamic acid. This result suggests that only one aromatic ring can be included into the CD cavity, irrespective of the whole size of the substrate. By contrast, the dissociation constants of the polymer complexes depend on the size of the substrate. The K_d values changed 42-fold ranging from 0.38×10^{-3} M for Congo red to 16.3×10^{-3} M for cinnamic acid. This result may indicate that the aromatic rings of large substrates are included cooperatively by several CD cavities.

Stoichiometry of Binding. Most of cyclodextrin–substrate complexes in aqueous solution have been shown by Cramer to be of 1:1 stoichiometry from the presence of distinct isosbestic points in the spectrophotometric titrations.[1] Different stoichiometries have been suggested for a few cases.[1,14] In the case of methyl orange, for example, additional spectral perturbations were observed as the CD concentration is increased, indicating more complex modes of association. The substrate

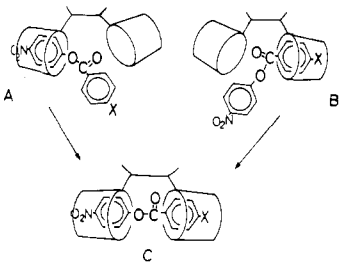


Figure 4. Binding mode of the poly-β-CD-A–*p*-nitrophenyl benzoate system.

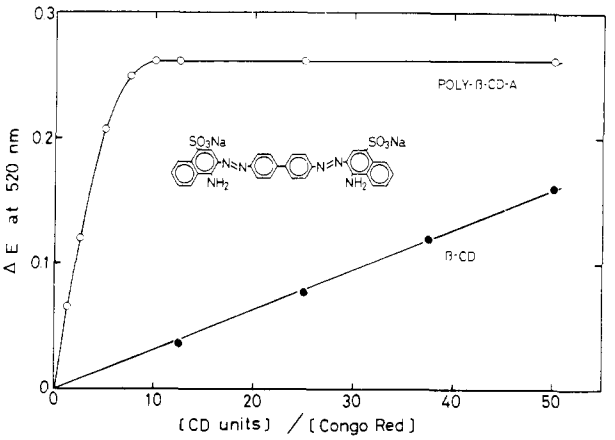


Figure 5. Spectral change of Congo red in the presence of β-CD and poly-β-CD-A; [Congo red] = 4×10^{-5} M at pH 7.54 Tris buffer, 25 °C.

apparently combines with two CD molecules. Another example is the complexes with long chain aliphatic carboxylic acids.[7] Solubility plots suggested that as many as four cyclodextrins interact with a single molecule of dodecanoic acid.

The equilibrium dialysis method[22] was used to determine the stoichiometry and to measure the dissociation constants accurately, because by this method direct observation on the concentrations of complex and free substrate is possible. However, this technique can be used only with the polymer and not with cyclodextrin, which is too small. Equilibrium dialysis was carried out in the presence of 0.01 M poly-β-CD-A and various amounts of substrates. The plots of the results with orange I and benzoic acid shown in Figure 6 are based on the following equation:

$$[CD_0][S]/[CD\text{–}S] = K_d + n[S] \quad (2)$$

where CD_0 is the total CD, S is the free substrate, CD–S is the complex, n is the number of β-CD moieties bound to a substrate, and K_d is the dissociation constant. In a plot of $[CD_0][S]/[CD\text{–}S]$ vs. [S], the intercept is K_d and the slope is n.

The plot for benzoic acid followed the calculated line for 1:1 stoichiometry, whereas the plot for orange I coincided almost exactly with the theoretical line for a 2:1 stoichiometry. These results indicate that with orange I, which has two aromatic rings and is capable of forming a 2:1 inclusion complex, two cyclodextrin units on the polymer chain participate in the binding of the substrate.

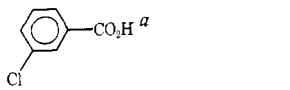
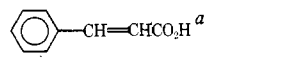
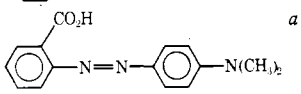
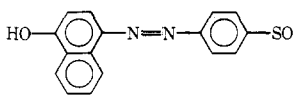
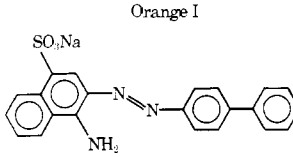
In the case of β-CD, the formation of a ternary complex with a large substrate requires a second β-CD molecule and this may be possible only in the presence of a considerable excess of β-CD.[23] Although rotational entropy is lost on formation of the poly-β-CD-A complex, the larger loss in translational entropy on forming the β-CD complex is eliminated when the association involves two CD residues carried by the same polymer chain.

Table VI
Hydrolysis of *p*-Nitrophenyl *p*-Nitrobenzoate with β-Cyclodextrin Derivatives[a]

Catalyst	$k' \times 10^4$, s^{-1}	$k'/k'_{\beta\text{-CD}}$
β-CD	4.65	
β-CD-A	3.45	0.74
Poly-β-CD-A	15.5	3.3
Poly-β-CD-NAC	8.6	1.8
β-CD-A-arcylamide copolymer (1:6)	3.8	0.8

[a] pH 8.7 Tris buffer, $I \approx 0.02$, 25 °C, in 20% acetonitrile, catalysts 0.33×10^{-2} M, ester 0.33×10^{-4} M.

Table VII
Dissociation Constants of β-CD and Poly-β-CD-A Complexes at 25 °C

Substrate	$K_d \times 10^3$, M: β-CD	Poly-β-CD-A
 [a]	3.7_8	5.5_6
 [a]	7.7_3	$16._3$
 Methyl red [a]	4.1_2	2.1_6
 Orange I [b]	2.4_7	1.5_2
 Congo red [b]	5.5	0.38

[a] Solubility method, pH 4.1 acetate buffer. [b] Spectrophotometric method.

The K_d value for the orange I–polymer complex was estimated as 0.8×10^{-3} M by equilibrium dialysis and this value is in fair agreement with the value of 1.5×10^{-3} M estimated by the spectrophotometric method. The K_d value for the benzoic acid–polymer complex was estimated as 5×10^{-3} M by equilibrium dialysis and that for *m*-chlorobenzoic acid was estimated as 5.56×10^{-3} M by the solubility study.

On increasing the substrate concentration at a fixed cyclodextrin (unit) concentration in equilibrium dialysis, more substrate is transferred to the polymer solution through the cellulose membrane. If the equilibrium constant is large enough, a point will be reached where nearly all the available cyclodextrin units are coordinated to the substrate. The maximum ratio of substrate to cyclodextrin attained may provide information on the stoichiometry of the complexes.[24,25]

To test this, saturation experiments were carried out with poly-β-CD-A. The results are shown in Figure 7, where the ratio of complexed substrate to total available CD units is plotted vs. $[S_0]/[CD]$. The ratio of the amounts of orange I and CD units approached a maximum of about 0.5. The maximum ratio with benzoic acid was substantially higher, approaching 0.75. This strongly suggests that with poly-β-CD-A, two CD

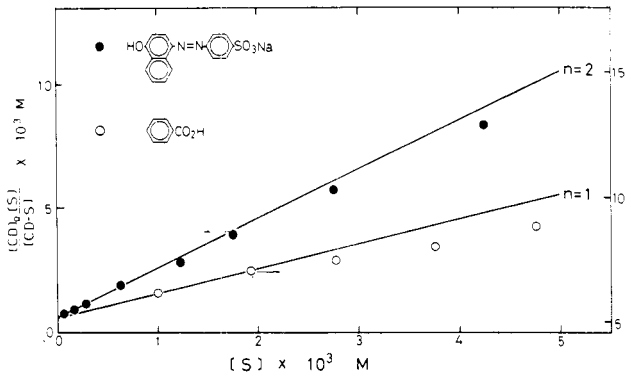


Figure 6. Binding of benzoic acid and orange I to poly-β-CD-A by equilibrium dialysis at 25 °C.

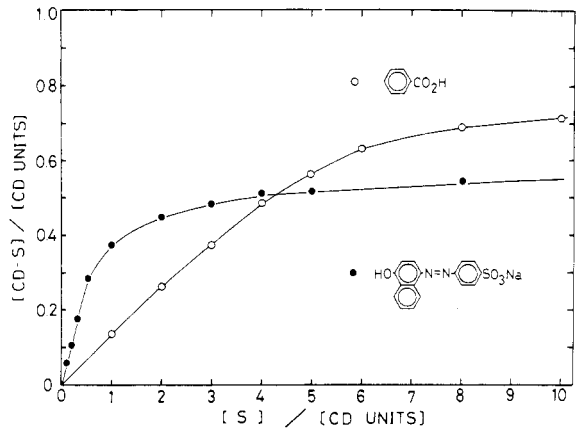


Figure 7. Maximum ratio of complexed substrate to β-CD units in poly-β-CD-A complexes from equilibrium dialysis at 25 °C.

units are complexed to orange I while a 1:1 complex is mainly formed with benzoic acid.

Recently an x-ray analysis showed that the crystalline complex of α-cyclodextrin and methyl orange was a 2:1 complex[26] whereas the α-cyclodextrin–para-substituted aniline complex was a 1:1 complex.[27]

Cooperation of two adjacent β-CD moieties is obviously important for complex formation with large substrates.

Experimental Section

Materials. β-CD was obtained from Hayashibara Biochemical Laboratory Inc. and was purified as described previously.[6] Poly-β-CD-A and poly-β-CD-NAC were prepared as described previously[6] and were purified by gel chromatography on Sephadex G-15 and dialysis against distilled water. The molecular weights of the polymers were estimated to be 10^4–10^5.

***p*-Nitrophenyl Carboxylates.** *p*-Nitrophenyl acetate (pNPA) obtained from Tokyo Kasei Ltd. was twice recrystallized from ethanol. Other esters were prepared by the method of Bodansky and du Vigneaud.[28] The carboxylic acid (0.05 mol) and *p*-nitrophenol (0.05 mol) were dissolved in dry ethyl acetate (100 ml). The mixture was cooled to 0 °C and dicyclohexylcarbodiimide (0.08 mol) was added. The mixture was kept at 0 °C for 1 h and then at room temperature overnight. Dicyclohexylurea was removed by filtration and the solvent was evaporated off. The resulting yellow solid was recrystallized from dry ethanol, acetone–ether, or acetone. When the esters were liquid, the ester products were finally purified by column chromatography on silica gel using carbon tetrachloride, and chloroform as eluents.

Kinetic Measurements. The release of *p*-nitrophenolate from esters was followed by measuring the absorbance at 400 nm. The reaction was initiated by addition of 17 μl of a stock solution of the ester in acetonitrile. The final concentration of *p*-nitrophenyl ester in each reaction mixture was 0.33×10^{-4} M. Plots of log $(A_\infty - A)$ vs. time for the reaction in the absence and in the presence of cyclodextrins (and polymer) gave straight lines. The pseudo-first-order rate constants were calculated from the plots. The rate of hydrolysis was measured to at least 30% completion of the reaction. The infinite absorbance value was obtained after hydrolysis was completed by addition of a drop of concentrated sodium hydroxide solution. The rate constants reported are averages of the values in two or three runs which agreed within 5%.

K_d. Spectrophotometric Method.[1] The dissociation constants of the CD (or CD unit)–substrate complexes were calculated from a relationship between the observed spectral changes and the added concentrations of CD or polymer. The change in absorbance of the aromatic chromophore in the presence of various amounts of CD or polymer was determined using a Hitachi Spectrophotometer Model 124 with a thermostatically controlled cell compartment. The substrate concentration was held constant at 10^{-4} to 10^{-5} M. The range of CD (unit) concentration (depending on the K_d value) varied from 5.0×10^{-4} to 10^{-2} M. If saturation of binding could not be attained due to limited solubility of β-CD, the dissociation constant of the complex was determined from a plot of the Benesi–Hildebrand equation:[29]

$$\frac{[S_0][CD_0]}{\Delta E} = \frac{K_d}{\Delta\epsilon} + \frac{[CD_0]}{\Delta\epsilon} \tag{3}$$

in which $[S_0]$ is the total concentration of substrate, $[CD_0]$ is the total concentration of CD (CD unit), K_d is the dissociation constant of the complex, $\Delta\epsilon$ is the difference between molar extinction coefficients of the free and complexed substrate, and ΔE is the change in the extinction of the solution on adding CD or polymer.

K_d. Solubility Studies. Solubility measurements were carried out by the method of Higuchi and Lach.[9] Amounts of substrates in excess of their normal solubilities were weighed into 20-ml vials. Quantities corresponding to 10^{-2} M β-CD (or unit) were measured in, and water was added to give a final volume of 10 ml in each vial. The vials were sealed and incubated in a constant temperature water bath at 30 °C for 24–28 h until the system reached equilibrium. Then 1 or 2 ml of the supernatant solution were removed and diluted with water, and their total substrate concentration was measured by ultraviolet spectrophotometry. Dissociation constants were determined assuming 1:1 stoichiometry as follows:

$$\text{cyclodextrin} + \text{substrate} \rightleftharpoons \text{complex} \tag{4}$$

$$K_d = \frac{[CD][S]}{[CD\text{–}S]} = \frac{[S]\{[CD_0] - ([S_0] - [S])\}}{[S_0] - [S]} \tag{5}$$

where S is the free substrate and CD_0 and S_0 are the total CD and the total substrate, respectively.

An acetate buffer of pH 4.1 was employed when the substrates were *p*-nitrophenyl esters to prevent hydrolysis of esters and suppress phenol ionization.

Equilibrium Dialysis.[22] Dialysis tubing (Visking Union Carbide Co.) was freed of soluble material by boiling it in distilled water for 1 h and then leaching it in frequent changes of water for 2 days. Five milliliters of solutions containing 10 mg of polymer and various amounts of substrate was placed inside the dialysis tubing and equilibrated at 30 °C for about 48 h with 6 ml of substrate solution with occasional shaking. Control samples containing only substrate were also dialyzed. After equilibration, the substrate concentration was determined by absorbance measurements.

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References and Notes

(1) F. Cramer, W. Saenger, and H. Ch-Spatz, *J. Am. Chem. Soc.*, **89**, 14 (1967).
(2) P. V. Demarco and A. L. Thakker, *Chem. Commun.*, 2 (1970).
(3) F. Cramer and H. Hettler, *Naturwissenschaften*, **54**, 625 (1967).
(4) (a) D. W. Griffiths and M. L. Bender, *Adv. Catal.* **23**, 209 (1973); (b) M. L. Bender, "Mechanisms of Homogeneous Catalysis from Protons to Proteins", Wiley-Interscience, New York, N.Y., 1971, p 373.
(5) (a) C. G. Overberger and R. C. Glowaky, *J. Am. Chem. Soc.*, **95**, 6014 (1973); (b) C. G. Overberger and T. W. Smith, *Macromolecules*, **8**, 407 (1975); (c) T. Kunitake, F. Shimada, and C. Aso, *J. Am. Chem. Soc.*, **91**, 2716 (1969); (d) C. G. Overberger and K. N. Sannes, *Agnew. Chem., Int. Ed., Engl.*, **13**, 99 (1974).
(6) (a) M. Furue, A. Harada, and S. Nozakura, *J. Polym. Sci., Polym. Lett. Ed.*, **13**, 357 (1975); (b) A. Harada, M. Furue, and S. Nozakura, *Macromolecules*, preceding paper in this issue.
(7) H. Schlenk and D. M. Sand, *J. Am. Chem. Soc.*, **83**, 2312 (1961).
(8) H. Lineweaver and D. Burk, *J. Am. Chem. Soc.*, **56**, 658 (1934).

(9) (a) T. Higuchi and J. L. Lach, *J. Pharm. Sci.*, **43**, 349 (1954); (b) J. Cohen and J. L. Lach, *ibid.*, **52**, 132 (1963); (c) J. L. Lach and T.-F. Chin, *ibid.*, **53**, 69 (1964).
(10) E. A. Lewis and L. D. Hansen, *J. Chem. Soc., Perkin Trans. 2*, 3401 (1973).
(11) T. Takeo and T. Kuge, *Stärke*, **24**, 331 (1972).
(12) J. A. Thoma and L. Stewart, "Starch; Chemistry and Technology", Vol. 1, R. L. Whistler and E. F. Paschall, Ed., Academic Press, New York, N.Y., 1965, p 209.
(13) T. Takeo and T. Kuge, *Stärke*, **24**, 281 (1972).
(14) R. L. VanEtten, J. F. Sebastian, G. A. Clowes, and M. L. Bender, *J. Am. Chem. Soc.*, **89**, 3242 (1967).
(15) H. J. Brass and M. L. Bender, *J. Am. Chem. Soc.*, **95**, 5391 (1973).
(16) J. L. Lach and T.-F. Chin, *J. Pharm. Sci.*, **53**, 924 (1964).
(17) R. L. VanEtten, G. A. Clowes, J. F. Sebastian, and M. L. Bender, *J. Am. Chem. Soc.*, **89**, 3253 (1967).
(18) W. Lautsch, W. Broser, W. Biedermann, and H. Gnichtel, *Angew. Chem.*, **66**, 123 (1954).
(19) J. L. Hoffman and R. M. Bock, *Biochemistry*, **9**, 3542 (1970).
(20) K. Mochida, A. Kagita, Y. Matsui, and Y. Date, *Bull. Chem. Soc. Jpn.*, **44**, 341 (1971).
(21) K. Sensse and F. Cramer, *Chem. Ber.*, **102**, 509 (1969).
(22) I. M. Klotz, F. Walker, and R. Pivan, *J. Am. Chem. Soc.*, **68**, 1486 (1946).
(23) A. Wishnia and S. J. Lappi, *J. Mol. Biol.*, **82**, 77 (1974).
(24) S. Kopolow, T. E. Hogen Esch, and J. Smid, *Macromolecules*, **6**, 133 (1973).
(25) S. Kopolow, Z. Machacek, U. Takaki, and J. Smid., *J. Macromol. Sci., Chem.*, **7**, 1015 (1973).
(26) K. Harata and H. Uedaira, *Nature (London)*, **253**, 190 (1975).
(27) K. Harata and H. Uedaira, 30th Annual Meeting Reprints of the Chemical Society of Japan, Tokyo, April, 1975, p 198.
(28) M. Bodansky and V. du Vigneaud, *J. Am. Chem. Soc.*, **81**, 5688 (1959).
(29) H. A. Benesi and J. H. Hildebrand, *J. Am. Chem. Soc.*, **71**, 2703 (1949).

Polymerization of Vinylanthracene Monomers. 2. 2-Vinylanthracene and 2-Propenyl-2-anthracene

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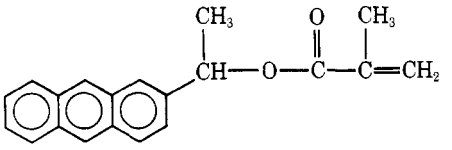
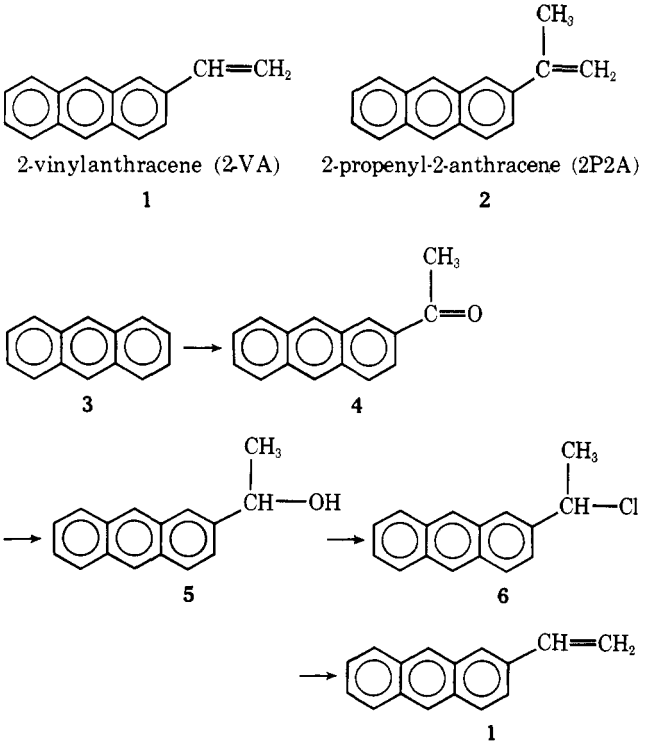
ABSTRACT: The synthesis of 2-vinylanthracene and 2-propenyl-2-anthracene and their polymerizations by free radical, anionic, cationic, and Ziegler techniques have been investigated. 2-Vinylanthracene and 2-propenyl-2-anthracene have been polymerized to high molecular weight polymers ($\bar{M}_n > 10^5$) by anionic addition type initiators. The polymerization reaction must be carried out with high purity monomer at low temperature (<−40 °C) to maximize molecular weight. Poly-2-vinylanthracene and poly-2-propenyl-2-anthracene are linear, soluble polymers with conventional vinyl structures. Both polymers undergo facile cross-linking and insolubilization in air/light. The cross-linking appears to result both from oxidation leading to free radicals and photodimerization of anthracene groups.

Until recently[1] *no high molecular weight polymers of* vinylanthracene were known. It was believed that the synthesis of such polymers by simple addition polymerization was not possible. This conclusion was based on several generally accepted facts: (1) anthracene is an efficient radical quencher and inhibits radical polymerization of vinyl monomers, including styrene, (2) anthracene inhibits or severely retards the ionic polymerization of vinyl monomers, (3) 9-vinylanthracene could not be polymerized to a high molecular weight polymer by any known technique.[2] It was concluded that the inability to prepare high molecular weight poly-9-vinylanthracene was



inherently associated with the anthracene ring structure and, therefore, no real attempts were made to investigate other anthracene monomers. Katz[3] and Hawkins[4] reported the syntheses of 1- and 2-vinylanthracenes but were only able to obtain oligomers by standard polymerization techniques. It has recently been shown[5] that under certain conditions the anthracene containing monomer, 1-(2-anthryl)ethylmethacrylate, can be polymerized to a conventional high molecular weight vinyl type polymer by free-radical methods. During investigations of this anthryl methacrylate polymerization it was established that monomer purity is extremely important in attaining high molecular weight products. In view of this finding it was decided to re-examine the polymerization of 2-vinylanthracene and its α-methyl analogue, 2-propenyl-2-anthracene;



Experimental Section

Synthesis of 2-Vinylanthracene. 2-Vinylanthracene (2-VA) (1) was synthesized from anthracene by the following reaction scheme which is a modification of the procedure of Etienne et al.[6]