

Analysis of Hydrophobic Effect in the Complex Formation of Beta-Cyclodextrin-mono-(6-deoxy-6-sulfonate) with Quarternary Ammonium Ions

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Received July 24, 1986

β -Cyclodextrin substituted with a negatively charged sulfonate group at 6-position (β CDSO₃⁻) accelerated the alkaline hydrolysis of a positively charged meta-trimethylammonio-phenyl (Me₃N⁺-Ph) acetate by 3.5-fold when compared with β -cyclodextrin (β CD). To analyze the interactions in the complex formation, dissociation constants, K_d(am), were determined for complexes of β CD and β -CDSO₃⁻ with a variety of quarternary ammonium ions as an inhibitory effect of ammonium ions on the association of cyclodextrins with a dye, crystal violet. Quantitative analysis using substituent parameters of N-substituent groups revealed that ammonium ions were included from the most hydrophobic N-substituent side first into the cavity of β CDSO₃⁻ and that the positively charged N-atom remains out of the cavity. In the hydrolysis of positively charged acetate, however, meta-Me₃N⁺ group was included into the cavity to fit the acetyl group near the catalytic 2- or 3-hydroxy group of the host molecule and the electrostatic interaction between guest and host charged groups worked effectively to stabilize an inclusion complex.

KEY WORDS: β -cyclodextrin-mono-(6-deoxy-6-sulfonic acid)/ Trimethylammonio-phenyl acetate/ Quarternary ammonium ion/ QSAR of complex formation/

INTRODUCTION

Cyclodextrins have been studied as enzyme-models in the field of bioorganic chemistry because of their ability to form an inclusion complex with various types of organic compounds. In the inclusion reactions of β -cyclodextrin (β CD), hydrophobic and van der Waals interactions are mainly responsible for complex formation.¹⁾ Cyclodextrins have been chemically modified to improve their selectivities to a guest molecule and to increase catalytic functions.^{2,3)} Electrostatic interactions between charged groups play an important role in an enzymatic system such as acetylcholinesterase and trypsin.⁴⁾ Enzymes are macromolecular electrolytes, whereas cyclodextrins contain no charged group. Positively charged cyclodextrins, a β CD flexibly capped with a metal ion⁵⁾ and mono-(6-trimethylammonio-6-deoxy)- β CD,⁶⁾ have been synthesized to develop new host systems which include an electronically charged guest molecule. Complex formation with hydrophobic anions was significantly increased by charged cyclodextrins and their results were explained in terms of electrostatic and hydrophobic interactions between host and guest molecules.

In the present work, a negatively charged β CD-mono-(6-deoxy-6-sulfonic acid),

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β CDSO₃⁻, was prepared as a simple model of trypsin and acetylcholinesterase containing a negatively charged group in the vicinity of the active site. Electrostatic interaction between β CDSO₃⁻ and positively charged meta-trimethylammoniophenyl (Me₃N⁺-Ph) acetate was observed in the hydrolysis reaction of the acetate catalysed by β CDSO₃⁻. To investigate hydrophobic and electrostatic interactions in the complex formation of β CDSO₃⁻, we measured dissociation constants, Kd(am), of inclusion complexes of β CDSO₃⁻ with 15 different quarternary ammonium ions and analyzed the substituent effects of N-substituent groups on log 1/Kd(am) values.

EXPERIMENTAL

Materials β CD (Hayashibara Biochemical Laboratories Inc.) was recrystallized from water and dried over phosphorous pentoxide *in vacuo*. Quarternary ammonium salts used in this study were those reported in our previous papers^{7,8)} recrystallized before use from MeOH, MeOH/EtOAc, or MeOH/ether. meta- and para-Me₃N⁺-Ph acetates were prepared from corresponding dimethylaminophenol by esterification with acetyl chloride, followed by methylation of dimethylamino group with MeI.

Preparation of β CDSO₃⁻ β CD was tosylated by p-toluenesulfonyl chloride.⁶⁾ Mono-tosylated β CD was separated from other tosylates on hydrophobic adsorption chromatography,^{9,10)} then substituted by Na₂SO₃ to β CD-sulfonate.¹¹⁾ 6-Deoxy-6-sulfonation was confirmed by HCl hydrolysis of β CDSO₃⁻ to give a product chromatographically identical with authentic 6-deoxy-glucose-6-sulfonic acid which was synthesized from diacetone glucose.¹²⁾

Mono-6-tosyl- β -cyclodextrin A solution of p-toluenesulfonyl chloride (5 g) in dry pyridine was added to a solution of β CD (26.2 g) in dry pyridine (250 ml) cooled on ice. The mixture was stirred for 1 hr and kept at 5°C for 1 hr. Solvent was removed *in vacuo* at 40°C to dryness. Diethyl ether was added to the residue. The presipitate was collected and recrystallized twice from water to afford 13.5 g of crude product. HPLC analysis (LS410, 60% aq. MeOH, UV at 254 nm) of the product indicated a mixture of mono- and poly-tosyl β CD. This crude product (8.23 g) was dissolved in water and chromatographed on porous polystyrene gel (Amberlite XAD-2, ϕ 4×23 cm). Fractions eluted by 30% aq. EtOH gave a single product (4.19 g) on HPLC and TLC (silica gel, 8:1:1 AcOH/CHCl₃/H₂O and 100:30:27 EtOAc/MeOH/H₂O). Recrystallization from water gave colorless powder of β CDTs 3.91 g. The monosubstitution of β CD was confirmed by ¹H NMR spectra in DMSO-d₆; δ 3.0–3.8 (m, 28H), 4.8 (s, 7H), 5.7 (m, 14H), 7.43 (d, 2H), 7.73 (d, 2H). The ratio of peak areas due to the aromatic ring hydrogens (δ 7.43 and 7.73) and the anomeric hydrogens (δ 4.8) were 4:7.¹³⁾

β CD-mono-(6-sulfonic acid) ammonium salt A solution of β CDTs (2 g) in DMF (40 ml) was added to an aq. solution of Na₂SO₃ (3.2 g/120 ml). The mixture was stirred at 50°C until a spot of β CDTs disappeared on TLC (silica gel, 8:1:3 AcOH/CHCl₃/H₂O), and condensed *in vacuo* at 40°C to syrup. The syrup was solidified in EtOH: 5.3 g colorless solid. Chromatography on a DEAE-Sephadex A-25 column (ϕ 2.7×52 cm, linear gradient of NH₄HCO₃ from 0.02 M to 0.15 M) gave fractions free from

β CDTs and β CD on TLC. Water and NH_4HCO_3 were removed under reduced pressure to dryness. The residue was gel-filtrated on Sephadex G-15 ($\phi 2.7 \times 55$ cm) to afford 1.49 g of βCDSO_3^- ammonium salt as a colorless powder. Purity of the product was confirmed by silica gel TLC ($R_f=0.41$, 8:1:3 $\text{AcOH}/\text{CHCl}_3/\text{H}_2\text{O}$ and $R_f=0.43$, 3:1:2 $\text{BuOH}/\text{DMF}/\text{H}_2\text{O}$) and HPLC (IEX 260 SA SIL, 0.05 M NaCl, RI). ^1H NMR (D_2O) δ 4.9 (s, 7H), 3.5–4.0 (m, 28H). Anal. Calcd for $\text{C}_{42}\text{H}_{73}\text{O}_{37}\text{SN} \cdot 10\text{H}_2\text{O}$: C, 36.14; H, 6.71; S, 2.30. Found: C, 36.22; H, 7.04; S, 2.33. The 6-deoxy-6-sulfonylation of β CD was confirmed by the acid hydrolysis of βCDSO_3^- ammonium salt with 1 N HCl. After neutralization with aqueous ammonium bicarbonate, the products of hydrolysis were identified as D-glucose and 6-deoxy-D-glucose-6-sulfonate on TLC (Avicel, 2:1:1 $\text{BuOH}/\text{DMF}/\text{H}_2\text{O}$, detection with aniline hydrogen phthalate: $R_f=0.57$ for D-glucose; $R_f=0.40$ for 6-deoxy-D-glucose-6-sulfonate) and on HPLC (LS460K, elution with 0.025–0.05 M NaCl, detection with RI).

6-Tosyl-1,2-O-isopropylidene-D-glucofuranose To a solution of diacetone-D-glucose (Sigma, 20 g) in methanol (10 ml) was added 10 ml of 0.8% sulfuric acid. The solution was stirred for 3 hr at room temp, neutralized with IR45, and filtered. The solvent was removed *in vacuo* to dryness. Crystallization from methanol/ether provided isopropylidene (940 mg).

The isopropylidene, dried over phosphorus pentoxide at 60°C *in vacuo*, was dissolved into 4 ml of dry pyridine. To this solution, a solution of p-toluenesulfonyl chloride (188 mg) in 5 ml of CHCl_3 was added and stirred at 0°C for 3 hr, then at room temp until the isopropylidene disappeared. The solvent was evaporated to afford oily residue. Following standard workup with chloroform, the oily residue was purified by chromatography on silica gel (elution with CHCl_3), then crystallized from CHCl_3 /hexane to afford 1.0 g of tosylate. ^1H NMR (CDCl_3) δ 1.28–1.4 (s, 6H), 2.4 (s, 3H), 4.0–4.6 (m, 6H), 5.80(d, 1H, $J=4$ Hz, anomeric), 7.23(d, 2H), 7.70 (d, 2H).

6-Deoxy-D-glucose-6-sulfonic acid ammonium salt Aqueous solution of sodium sulfite (0.6 g/18 ml) was added to a solution of tosylate (1 g) in ethanol (10 ml) and refluxed for 24 hr. After cooling, the solution was passed through Amberlite IR 120 (H^+ type) ion exchange resin, and condensed to oily residue. The oily residue was dissolved in water, neutralized with ammonium bicarbonate, condensed *in vacuo*, and purified by ion exchange chromatography on DEAE-Sephadex A-25 (HCO_3^- type, elution with 0.02–0.07 M sodium bicarbonate). The yield was 190 mg. Anal. Calcd for $\text{C}_6\text{H}_{15}\text{O}_8\text{NS}$: C, 27.59; H, 5.79; S, 12.27. Found: C, 27.55; H, 5.93; S, 12.11.

Determination of the dissociation constants of β CD- and βCDSO_3^- -quarternary ammonium salt complexes Dissociation constants of inclusion complexes of cyclodextrin with quarternary ammonium salts were determined by a method of spectral competitive inhibition in which inclusion of quarternary ammonium salt was observed as the change in the spectra of cyclodextrin-dye complex due to a decrease in the concentration of cyclodextrin-dye complex. Crystal violet was used as a dye for the spectral competitive inhibition method.

Crystal violet (ca. 20 mg) was dissolved into water (100 ml) and diluted with

0.1 M phosphate buffer (pH 7.0) to 50-fold by volume. Quarternary ammonium salt was dissolved into this dye solution (ammonium-dye solution). Concentrations of quarternary ammonium salt were 10–40 mM in the ammonium-dye solution. Sample and reference cells were filled with 2.5 ml of the ammonium-dye solution and kept at $25 \pm 0.1^\circ\text{C}$ for 5 min in Shimadzu UV-360 spectrophotometer equipped with a magnetic stirring and thermoelemental cell compartment. A solution of cyclodextrin dissolved in the ammonium-dye solution (βCD , 40 mg/5 ml; βCDSO_3^- , 40 mg/2.5 ml) was successively added by a volume of 50–500 μl to a solution in the sample cell. The difference spectra were recorded at each addition of cyclodextrin.

Since inclusion complexes with p-propylphenyltrimethyl ammonium iodide and quinoline hydrochloride showed difference spectra in the absence of a dye, their dissociation constants were determined without crystal violet.

RESULTS AND DISCUSSION

Catalytic Effects of βCDSO_3^- on the Alkaline Hydrolysis of meta- and para- $\text{Me}_3\text{N}^+\text{-Ph}$ Acetates
Time course of alkaline hydrolysis of $\text{Me}_3\text{N}^+\text{-Ph}$ acetates was spectrophotometrically followed by the increase in the absorption of $\text{Me}_3\text{N}^+\text{-phenol}$ produced. Bimolecular rate constants, k_{un} , of uncatalyzed alkaline hydrolysis of meta- and para- $\text{Me}_3\text{N}^+\text{-Ph}$ acetates were 5.98 and $4.86 \times 10^{-3} \text{ s}^{-1}$, respectively, in 0.05 M carbonate buffer (pH 10.6) at 25°C . In the presence of cyclodextrins, apparent alkaline hydrolysis rate constant, k_{obs} , linearly increased with the concentration of cyclodextrins (0–15 mM). meta- $\text{Me}_3\text{N}^+\text{-Ph}$ acetate was hydrolyzed about 3.5 times faster by βCDSO_3^- than by βCD (Figure 1).

The slopes in Figure 1 were $5.69 \times 10^{-3} \text{ s}^{-1} \text{ mM}^{-1}$ for βCDSO_3^- and $1.60 \times 10^{-3} \text{ s}^{-1} \text{ mM}^{-1}$ for βCD . para- $\text{Me}_3\text{N}^+\text{-Ph}$ acetate, however, was not effectively accelerated by cyclodextrins; $0.24 \times 10^{-3} \text{ s}^{-1} \text{ mM}^{-1}$ for βCDSO_3^- and $0.34 \times 10^{-3} \text{ s}^{-1} \text{ mM}^{-1}$ for βCD . Since the acetyl group of phenyl acetates have to locate near the catalytic 2- or 3-hydroxy group of the host molecule,¹⁴⁾ the $\text{Me}_3\text{N}^+\text{-Ph}$ group of acetate is included first into the cyclodextrin cavity. meta-Derivative easily takes a more favorable posi-

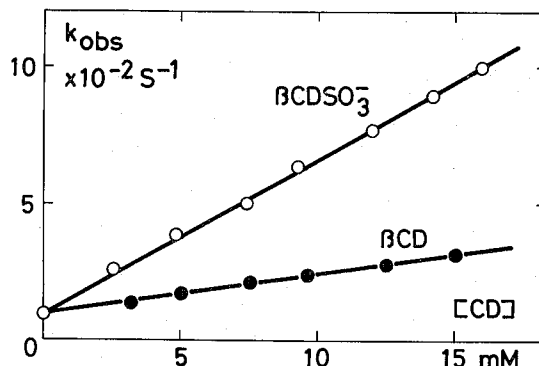


Fig. 1. Dependence of alkaline hydrolysis rate constant, k_{obs} , of meta-trimethylammonio-phenyl acetate on the concentration of βCD and βCDSO_3^- in 0.05 M carbonate buffer (pH 10.6) at 25°C .

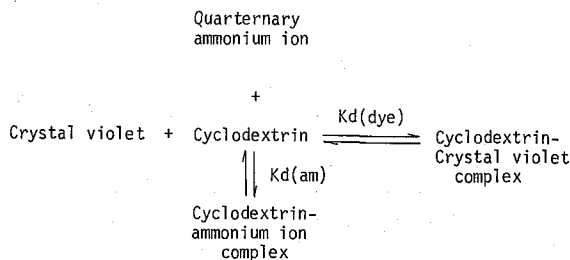
tion in the cavity for the hydrolysis than para-derivatives. At this relative position of the guest in the cavity, meta-Me₃N⁺ group is very close to 6-sulfonate group of the host. Then, electrostatic interaction between positively charged ammonium group and negatively charged sulfonate is responsible for 3.5 times greater acceleration of meta-Me₃N⁺Ph acetate hydrolysis catalyzed by β CDSO₃⁻ than hydrolysis by β CD.

Difference Spectra of a Cyclodextrin-Crystal Violet Complex Absorption spectra of crystal violet ($\lambda_{\text{max}}=589$ nm) showed a red shift by complexing with cyclodextrins. In the difference spectra of crystal violet with successive additions of β CD and β CDSO₃⁻ appeared two peaks at 581 and 616 nm for β CD, 581 and 615 nm for β CDSO₃⁻ (Figure 2).

β CD- and β CDSO₃⁻-crystal violet complexes were almost equal in the degree of red shift. Change in the absorption at minimum (581 nm) and maximum (615 or 616 nm) peaks followed the stoichiometry of the 1:1 inclusion complex formation between crystal violet and cyclodextrin. The value of dissociation constant, K_d(dye), of a dye-cyclodextrin complex determined at 581 nm was slightly different from that determined at 616 nm for β CD (or 615 nm for β CDSO₃⁻). In this study, difference spectra were measured as the difference between the absorptions at 581 nm and at 616 or 615 nm ($\Delta\text{Abs}=\Delta\text{Abs}_{616}-\Delta\text{Abs}_{581}$ for β CD and $\Delta\text{Abs}=\Delta\text{Abs}_{615}-\Delta\text{Abs}_{581}$ for β CDSO₃⁻). K_d(dye) values were determined as 0.198 ± 0.01 mM (number of data points=12) and 0.400 ± 0.01 mM (number of data points=10) for β CD and β CDSO₃⁻, respectively. Crystal violet formed a complex more stable with β CD than with β CDSO₃⁻.

Complex Formation of Cyclodextrin with Quarternary Ammonium Ions In the presence of quarternary ammonium ions, concentration of cyclodextrin-crystal violet complex decreases by the amount of cyclodextrin-ammonium ion complex formation. First, concentration of cyclodextrin-ammonium ion complex was estimated as the best fitting value in Scheme 1 by using ΔAbs , total concentrations of cyclodextrin and ammonium ion, and K_d(dye) value. K_d(am) in Scheme 1 is the dissociation constant of cyclodextrin-ammonium ion complex. Then, the value of K_d(am) was determined by the method of weight regression analysis from the estimated concentrations of cyclodextrin and its ammonium ion complex. Table 1 summarizes the values of K_d(am) for 15 quarternary ammonium ions determined from difference spectra.

K_d(am) values for unsubstituted and p-OH-phenyltrimethyl ammonium, quinuclidine, and N-methylquinuclidine were less for the complex with β CDSO₃⁻ than β CD,



Scheme 1

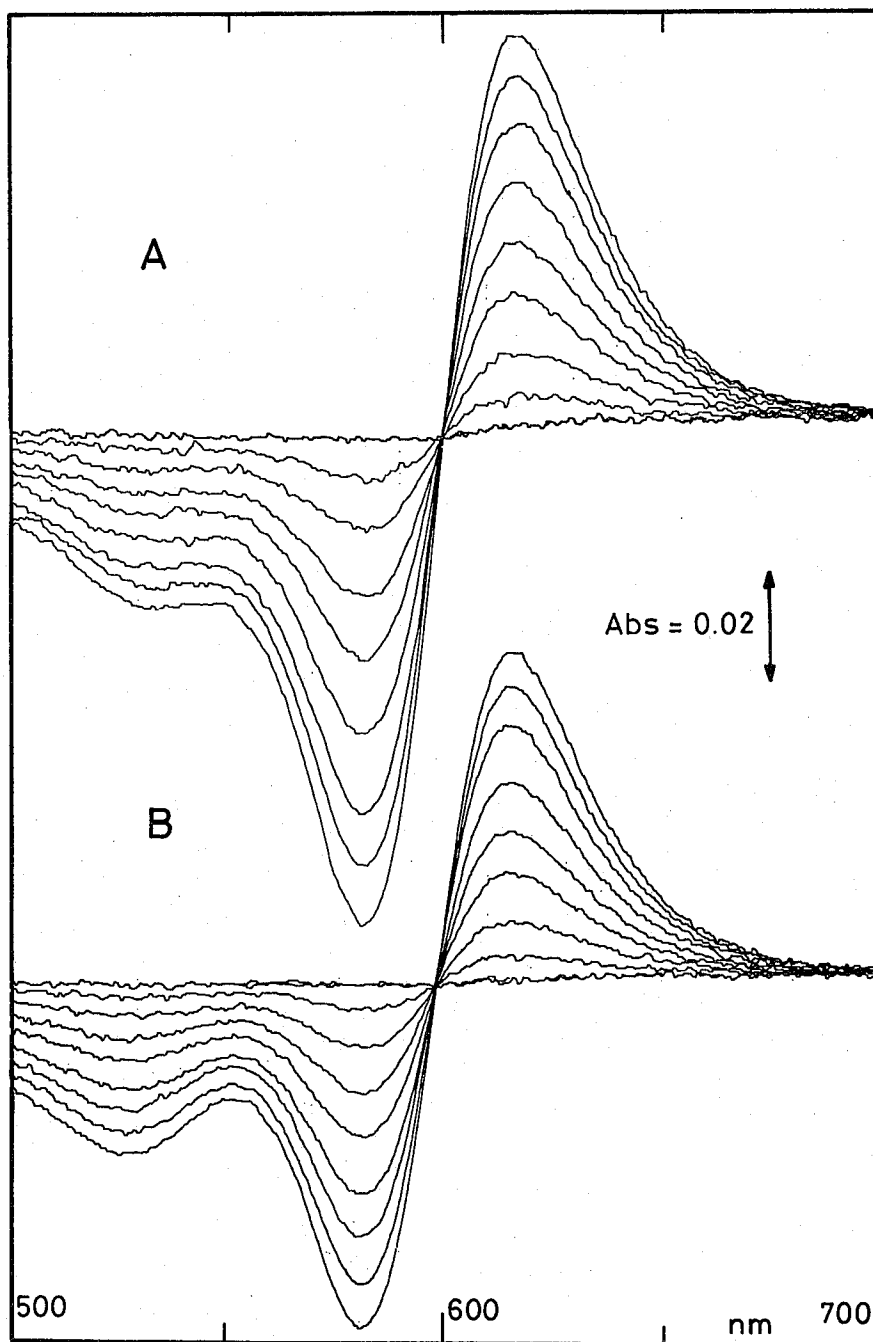


Fig. 2. Difference spectra due to the crystal violet-cyclodextrin inclusion complex formation. a) Difference spectra of the β CD-crystal violet system in the presence of 12.35 mM N-methylquinuclidine iodide. Concentrations of β CD were 0.014–0.506 mM. b) Difference spectra of the β CDSO₃[−]-crystal violet system in the presence of 11.08 mM para-chlorophenyltrimethyl ammonium iodide. Concentrations of β CDSO₃[−] were 0.025–0.881 mM.

Table 1. Dissociation constants $K_d(\text{am})$ of βCD and βCDSO_3^- complexes with quarternary ammonium ions determined by spectral competitive inhibition method.

Ammonium ions	$K_d(\text{am})$ with βCD mM	$K_d(\text{am})$ with βCDSO_3^- mM
$\text{PhN}^+(\text{Me})_3$		
p-H	58.1	50.7
p-F	62.6	112
p-Cl	27.1	63.7
p- CF_3	11.4	20.5
p-Me	38.4	28.9
p-Pr	1.37	1.24
p-OMe	31.2	33.7
p-OPr	5.03	5.58
p-OH	31.2	29.5
Me_4N^+	199	373
EtMe_3N^+	31.2	39.6
$\text{isoPrMe}_3\text{N}^+$	30.1	35.0
Quinuclidine HCl	58.4	16.6
N-Me quinuclidine	41.4	24.8
Quinoline HCl	12.4	18.5

as expected from electrostatic interaction between a positively charged guest and a negatively charged host. Other quarternary ammonium ions listed in Table 1, however, formed more stable complex with βCD rather than with negatively charged βCDSO_3^- . To find a general difference in the inclusion mechanism between two cyclodextrins, logarithmic values of $1/K_d(\text{am})$ of βCDSO_3^- complexes were plotted against those of βCD complexes with the corresponding ammonium ions (Figure 3). Almost linear relationship of the plot suggests that critical inclusion forces are the same between the two cyclodextrins.

Several intermolecular interactions are simultaneously responsible for complex formation of cyclodextrins. Experiments and theoretical considerations have shown that hydrophobic interaction and van der Waals forces probably dominate in the complex formation.^{1,3,14,15} Since diameter of βCD is greater than that of α -cyclodextrin, van der Waals interaction is less important in the inclusion reactions of βCD and its derivatives. We tried to estimate the contribution of hydrophobic interaction in the inclusion reaction of ammonium ions by βCD and βCDSO_3^- in Table 1.

In the structure-reactivity analyses, partition coefficient determined in the 1-octanol/water system is used as a linear-free-energy related hydrophobic parameter (Hansch-Fujita analysis).¹⁷ Parameter π , estimated from the difference of partition coefficients of substituted derivative and its parent compound, is defined for the hydrophobicity parameter of a substituent group. Takayama et al.⁸ have measured partition coefficients of quarternary ammonium salts as those of ion-pair formation-partition equilibrium constants with picrate and separated them into the sum of hy-

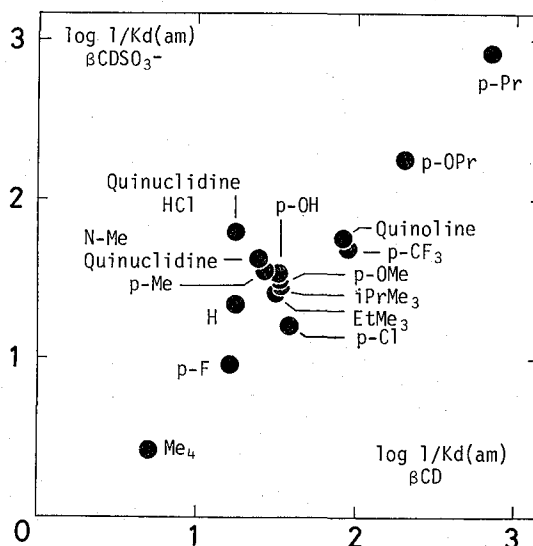


Fig. 3. Plot of $\log 1/K_d(\text{am})$, dissociation constants, of inclusion complex of quarternary ammonium ions by βCD against those of by βCDSO_3^- .

dropohbic, electronic, steric, and hydrogen-bonding effects of N-substituents. They suggested that the effects other than hydrophobic effect were derived from the interaction at the ion-pair formation between quarternary ammonium ion and picrate. Hydrophobicity of ammonium ions is mostly represented as the sum of hydrophobic effects of N-substituent groups (Table 2). Dissociation constants, $\log 1/K_d(\text{am})$ values, were first plotted against hydrophobic substituent parameter π values of the N-substituent groups attached to a trimethylammonio radical (Figure 4a).

Complex of quinuclidine HCl was not included in the plot because of the lack of π value. The plot in Figure 4a was biphasic. Complexes of ammonium ions substituted with hydrophobic groups such as p-Pr-, CF_3 -, Cl-, Me-, and PrO-phenyltrimethylammonium ions formed a stable complex with cyclodextrins and their $\log 1/K_d(\text{am})$ values increased linearly with their π values. Complexes with as less hydrophobic ammonium ions as p-OH-phenyltrimethy, tetramethy, ethyltrimethyammonium ions, however, deviated from the linear relation between the hydrophobic ammonium ions and their π values. Parametres such as electronic σ_1 and steric E_s° effects did not improve the biphasic correlation in Figure 4a.

Biphasic behavior of the plot reflects the inclusion direction of a guest ion in the cavity of a host cyclodextrin. Linear part of the plot proposes that ammonium ions are predominantly included into the cyclodextrin cavity from the substituted phenyl group side first, Me_3N^+ groups remain extended out of the cavity. For these ions, the substituted phenyl group is the most hydrophobic group among four N-substituent groups: π values of substituted phenyl groups are greater than the sum of π values for three methyl groups, $0.54 \times 3 = 1.62$. On the other hand, any of four N-alkyl groups of tetramethyl, ethyl- and isopropyltrimethyl ammonium ions are less hydrophobic and less bulky than a substituted phenyl group. Three N-alkyl substituents

Table 2. Substituent parameters used for the analysis of $\log 1/K_d(\text{am})$.¹⁾

Substituents	$\pi^{2)}$	$\pi^{3)}$	σ_1	E_s^c
Ph, Me, Me				
p-H	1.68	1.68	0.12	-2.31
p-F	1.82	1.82	0.13 ⁵⁾	-2.31
p-Cl	2.39	2.39	0.15	-2.31
p-CF ₃	2.56	2.56	0.18	-2.31
p-Me	2.24	2.24	0.10	-2.31
p-Pr	3.17	3.17	0.10	-2.31
p-OMe	1.88 ⁴⁾	1.88 ⁴⁾	0.10	-2.31
p-OPr	2.86 ⁴⁾	2.86 ⁴⁾	0.10	-2.31
p-OH	1.76 ⁴⁾	1.76 ⁴⁾	0.10	-2.31
Me, Me, Me	0.54	1.62	-0.03	0.00
Et, Me, Me	1.08	2.16	-0.03	-0.34
isoPr, Me, Me	1.49	2.57	-0.03	-1.09
CH(C ₂ H ₅) ₃	2.75	2.75	-0.03	0.00

1) Unless otherwise noted, values were from Ref. 8.

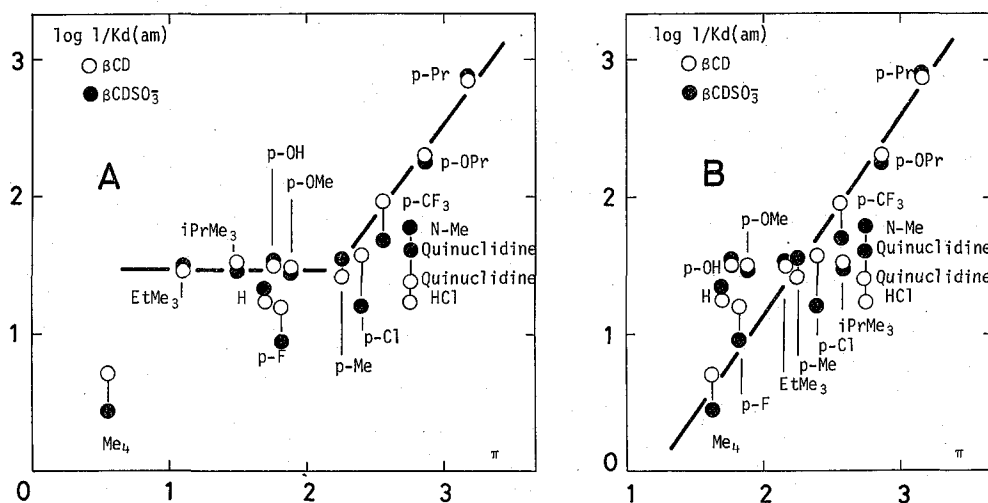
2) π values of the most hydrophobic group among N-substituents.3) π values of substituted phenyl group or the sum of three N-substituent groups.4) π values were corrected for the electronic effect of trimethylammonio group on the hydrogen bonding of oxygen atom with solvent (Ref 17). p-OMe and p-OPr were corrected by the equation $\pi(\text{OR}) + 0.27 \times 0.80$. p-OH by the equation $\pi(\text{OH}) + 0.94 \times 0.80$.5) M. Charton, *Prog. Phys. Org. Chem.*, **13**, 119 (1981).

Fig. 4. Plot of $\log 1/K_d(\text{am})$ values against hydrophobic parameter π values. a) Plot against π values of N-substituent groups attached to a trimethylammonio radical. Complexes by βCD ($\circ$) and by βCDSO_3^- ($\bullet$). b) Plot against π values of substituted phenyldimethyl and alkylidimethyl groups. Complexes by βCD ($\circ$) and by βCDSO_3^- ($\bullet$).

of these ammonium ions—trimethyl, ethyldimethyl, and isopropyldimethyl groups—are able to be included into the cyclodextrin cavity. When $\log 1/K(\text{am})$ values were plotted against the sum of π values for three N-substituent groups, the deviation from the linearity was effectively improved (Figure 4b). Quinuclidine HCl and N-methyl quinuclidine were included from a bicyclic alkyl group as expected from their π values.

Although most ammonium ions studied formed a more stable complex with unmodified βCD than with βCDSO_3^- , inside of the cavity βCDSO_3^- was as hydrophobic as that of βCD when estimated from the degree of red-shift of crystal violet complex. The analysis of $\log 1/K_d(\text{am})$ values of quarternary ammonium ion-cyclodextrin complexes by a hydrophobic substituent parameter π showed that predominant inclusion force of βCDSO_3^- was hydrophobic interaction, the dependency on which was same to that of βCD , and that ammonium ions were included from the most hydrophobic substituent sides first into the cyclodextrin cavity. The positively charged nitrogen atom remained out of the cavity, so that the distance between nitrogen atom of the guest and 6- SO_3^- group of the host was large. This is the reason why electrostatic charge attraction between the negatively charged cyclodextrin 6- SO_3^- group and the positively charged nitrogen atom of ammonium ions did not so effectively strengthen the stability of inclusion complexes as hydrophobic interaction.

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