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Binding and Catalytic Properties of Charged β-Cyclodextrins

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The effects of the signs and structures of charged groups in modified β-cyclodextrins on their binding and catalytic properties were examined in aqueous solutions by using ammonium salt of mono(6-sulfonato-6-deoxy)-β-cyclodextrin and hydrogencarbonate salts of mono(6-trimethylammonio-6-deoxy)- and mono[6-(1-pyridinio)-6-deoxy]-β-cyclodextrins as charged hosts. An ionic guest was bound more strongly to an oppositely charged host than to an identically charged host. Binding constants for charged host-guest systems were affected by a change in the ionic strength and by the addition of dimethyl sulfoxide to an aqueous solution. The catalytic effects of the charged hosts on the alkaline hydrolyses of *o*-, *m*-, and *p*-acetoxybenzoic acids were also different from those of the parent β-cyclodextrin. These results could be explained in terms of electrostatic, steric, and hydrophobic interactions between the hosts and guests.

Several intermolecular interactions have been proposed as being responsible for the formation of cyclodextrin (CD) inclusion complexes in aqueous solutions.[1-3] They are hydrophobic interactions, van der Waals interactions, hydrogen bonding, interactions due to the relief of high energy water from the CD cavity upon substrate inclusion, and interactions due to the relief of conformational strain in a CD-water complex upon substrate inclusion. These binding forces also play an important role in the binding processes of enzymes. This is one of reasons why CD has been widely studied as an enzyme model. However, an enzyme is a kind of polyelectrolyte. Electrostatic interactions between charged groups of an enzyme and an ionic substrate often contribute to the specificity of enzyme action.[4] On the other hand, CD is a neutral molecule and no electrostatic interaction can take part in its binding and catalytic processes. In order to enhance the substrate-specificity of CD, it is desirable to build up a molecular system in which a variety of intermolecular interactions, including electrostatic interactions, act simultaneously and cooperatively. Thus far, only a few studies have been reported which dealt with the binding and/or catalytic properties of charged CD's. Tabushi et al.[5] prepared a 1 : 1 complex of Zn(II) with β-CD functionalized by diethylenetriamine and showed that the positively charged host binds a negatively charged substrate, 2-oxo-1-adamantanecarboxylate, 330-times stronger than β-CD. Boger and Knowles[6] prepared positively charged triammonio-per-*O*-methyl-α-CD, which binds benzyl phosphate ca. 1000-times stronger than the corresponding monoammonium host. Matsui and Okimoto[7] examined the binding and catalytic properties of a positively charged CD, mono(6-trimethylammonio-6-deoxy)-β-CD [β-CDtma(+)]. The charged host exhibited abilities to bind an anionic dye more strongly than the parent host and to catalyze the alkaline hydrolyses of negatively charged *o*-, *m*-, and *p*-acetoxybenzoates in a manner significantly different from that of the parent β-CD.

The present investigation was undertaken to examine the effects of the sign and structure of charged groups in modified β-CD's on their binding and catalytic properties. For this purpose, we used the hydrogencarbonate salts of β-CDtma(+) and mono[6-(1-pyridinio)-6-deoxy]-β-CD [β-CDpy(+)] as positively charged CD's and the ammonium salt of mono(6-sulfonato-6-deoxy)-β-CD [β-CDsulf(−)] as a negatively charged CD. Electrostatic interactions are generally affected by such environmental factors as the ionic strength and polarity of the solution. The present study also dealt with the effects of these factors on the action of the charged hosts.

Experimental

Materials. The β-CD of a reagent grade was recrystallized from water and dried overnight in vacuo at 110 °C. Reagent-grade pyridine was dried over calcium hydride and distilled in the presence of fresh calcium hydride just before use. Mono[6-*O*-(*p*-tolylsulfonyl)]-β-CD (β-CDots) was prepared by a reaction of β-CD with *p*-toluenesulfonyl chloride in dry pyridine.[7] The β-CDtma(+) was prepared by a reaction of β-CDots with trimethylamine in *N*,*N*-dimethylformamide and then purified as described previously.[7] Reagent-grade dimethyl sulfoxide (DMSO) was distilled in the presence of calcium hydride in vacuo. Crystal Violet (CV), phenolphthalein (PP), ammonium 8-anilinonaphthalene-1-sulfonate (ANS), Methyl Orange (MO), *p*-nitrophenol (pNP), cyclohexanol (CHX), and *o*-acetoxybenzoic acid (*o*-ABA) were commercially available. The *m*- and *p*-acetoxybenzoic acids (*m*- and *p*-ABA) were prepared by reactions of the corresponding hydroxybenzoic acids with acetic anhydride in dry pyridine and were then recrystallized from chloroform.

Thin-layer chromatography (TLC) was performed on Kieselgel 60F_{254} (Merck) with a mixed solvent of acetic acid–chloroform–water [80 : 10 : 20 (v/v)]. A saturated solution of cerium(IV) sulfate in concentrated sulfuric acid was used as a spray reagent to detect the parent and modified β-CD's. The charged β-CD's were separated from the reaction mixtures by liquid chromatography with a 4.5×43 cm CM-cellulose (Serva) column, a 5×49 cm DEAE-sephadex (Pharmacia) column, and/or a 4×73 cm Sephadex G-15 (Pharmacia) column.

Apparatus. Absorption, fluorescence, and NMR spectra were recorded using a Hitachi Model 220 spectrophotometer, a Hitachi Model 650-10S fluorescence spectrophotometer, and JEOL Model JNM-MH-100 and Nicolet Model NT-300

Table 1. Binding Constants (K_a/mmol^{-1}dm^3) for 1:1 Inclusion Complexes of Parent and Charged β-CDs with Organic Guests at 25 °C

Host	CV(+)[a]	PP(−)[b]	pNP(−)[c]	ANS(−)[d]	MO(±)[e]	CHX(0)[f]	DMSO(0)[g]
β-CD	4.0	29	0.77	0.091	0.30	0.71	0.0018
β-CDsulf(−)	2.6	3.4	0.053	0.037	0.18	0.37	0.0016
β-CDtma(+)	0.43	25	1.37	0.17	0.44	0.34	0.0009
β-CDpy(+)	—	50	1.52	0.26	—	0.43	0.0009

a) Crystal Violet at pH 5.0. b) Phenolphthalein at pH 10.5. c) *p*-Nitrophenolate at pH 10.5. d) 8-Anilinonaphthalene-1-sulfonate at pH 6.8. e) Methyl Orange in 0.1 mol dm^{-3} H_2SO_4. f) Cyclohexanol at pH 10.5. g) Dimethyl sulfoxide at pH 10.5.

NMR spectrometers respectively. The optical rotation was mesured using a Union Giken Model PM-101 polarimeter.

Preparation of β-CDpy(+). A solution of β-CDots (6.00 g, 4.65 mmol) in dry pyridine (120 cm^3) was heated at 100 °C for 12 h. The reaction mixture was evaporated in vacuo at 40 °C to dryness. The residue was dissolved in water, and acetone was added to the solution to give a white precipitate. After being collected, the precipitate was dissolved in water (10 cm^3) and then chromatographed on a CM-cellulose column with 0.05 mol dm^{-3} ammonium hydrogencarbonate as an eluent. Each fraction (10 cm^3) was assayed by UV at 270 nm, polarimetry at 589 nm, and/or TLC. Fractions which gave only one spot with R_f 0.25 (fractions from No. 76 to No. 160) were combined and evaporated to dryness at 40 °C in vacuo. The residue was dissolved in water, and the solution was decolorized by activated charcoal and evaporated to dryness to afford 1.06 g (0.84 mmol; yield, 18% based on β-CDots) of the hydrogencarbonate salt of β-CDpy(+) as a white powder. The ^{1}H NMR spectrum of β-CDpy(+) in D_2O gave peaks due to pyridinium protons at δ=8.07 (t, meta-H), 8.58 (t, para-H), and 8.82 (d, ortho-H) ppm with a ratio in peak area of 2:1:2. The mono-substitution of the CD hydroxyl groups by the 1-pyridinio group was confirmed by a comparison of the peak area of the CD protons with that of the pyridinium protons.

Preparation of β-CDsulf(−). To an aqueous solution (300 cm^3) of sodium sulfite (3.11 g, 24.7 mmol) was added β-CDots (6.03 g, 4.7 mmol); the resulting mixture was heated at 100 °C for 20 h. The reaction mixture was evaporated in vacuo at 40 °C to dryness, and the residue was washed twice with hot ethanol. After being dissolved in water, the residue was chromatographed on a DEAE-sephadex column. Linear gradient elutions were applied with 1.2 dm^3 of 0.02 mol dm^{-3} NH_4HCO_3 and 1.2 dm^3 of 0.1 mol dm^{-3} NH_4HCO_3 and then with 0.3 dm^3 of 0.1 mol dm^{-3} NH_4HCO_3 and 0.3 dm^3 of 0.5 mol dm^{-3} NH_4HCO_3. Each fraction (15 cm^3) were assayed by polarimetry at 405 nm and TLC. Fractions which gave only one spot with R_f 0.10 were combined and evaporated to dryness at 40 °C. The desalting of the resulting residue was carried out with a Sephadex G-15 column to give 2.16 g (1.78 mmol; yield 38% based on β-CDots) of the ammonium salt of β-CDsulf(−). The mono-substitution of the CD hydroxyl groups by the sulfonato group was confirmed by the oxidative degradation of β-CDsulf(−) with hot nitric acid, followed by a turbidimetric determination of the resulting sulfate ion with barium chloride and gelatin.

Determination of Binding Constants for Inclusion Complexes. The charged or parent β-CD was added to an aqueous buffer solution containing 0.008 mmol dm^{-3} CV (pH 5.0, citric acid-Na_2HPO_4), 0.03 mmol dm^{-3} PP (pH 10.5, $NaHCO_3$-NaOH), 0.05 mmol dm^{-3} pNP (pH 10.5, $NaHCO_3$-NaOH), 0.01 mmol dm^{-3} ANS (pH 6.8, NaH_2PO_4-Na_2HPO_4), or 0.02 mmol dm^{-3} MO (0.1 mol dm^{-3} H_2SO_4). After thermal equilibrium at 25 °C had been reached, the UV or fluorescence spectra were recorded. Binding constants (K_a) for 1:1 inclusion complexes of the hosts with the guests were determined by a least-squares curve-fitting analysis of relationships between absorbances and host concentrations with a microcomputer. The K_a values for the DMSO and CHX systems were estimated by a least-squares curve-fitting analysis of the inhibitory effect of the guests on the complexation of the hosts with PP at pH 10.5 in a similar manner described previously.[8]

Kinetics. The rates of the alkaline hydrolyses of *o*-, *m*-, and *p*-ABAs were measured by following the appearance of the absorptions of the corresponding phenoxide anions in the same way as described previously.[7] The K_a values and catalytic rate constants (k_c) for ABA-host systems were also determined in the same way as described previously.[7]

Results and Discussion

Binding Properties of the Charged β-CD's. Table 1 shows the K_a values determined for 1:1 inclusion complexes of the parent and charged hosts with a variety of guests. A positively charged guest, CV(+), was bound more strongly to β-CDsulf(−) than to β-CDtma(+). On the contrary, negatively charged guests, PP(−), pNP(−), and ANS(−), were bound more strongly to β-CDtma(+) and β-CDpy(+) than to β-CDsulf(−). The K_a values for inclusion complexes with amphoteric [MO(±)] and neutral [CHX(0), DMSO(0)] guests were not so much affected by the charges of the hosts. These results indicate that electrostatic attractive interactions do act between an oppositely charged host and its guest, and repulsive interactions between those with charges identical in sign. However, the effect of electrostatic interactions on the binding constants was relatively small. The K_a value for an oppositely charged host-guest system was, at most, 3 times that for a corresponding β-CD system. It seems that the replacement of one of the OH groups of β-CD by a charged group results in a decrease in hydrophobic interactions between the host and a guest. In fact, the K_a value for a β-CD-CHX(0) system, where hydrophobic interactions play an important role in complexation,[8] was decreased by a factor of ca. 2 by the introduction of a charged group to the host.

Among the guests examined, ANS(−) is known to be a fluorescent probe: The wavelength (λ_{max}) of the emis-

Table 2. The Wavelength (λ_{max}) and Relative Intensity (I_{rel}) of the Emission Maximum of Fluorescence for Host-ANS(−) Systems at 25 °C and pH 6.8[a]

Host	λ_{max}/nm[b]	I_{rel}[b]	f[c]
β-CD	495±1	10.5±0.4	0.48
β-CDsulf(−)	502±2	4.5±0.4	0.27
β-CDtma(+)	487±1	23.1±0.3	0.63
β-CDpy(+)	485±3	2.3±0.1	0.72

a) [Host]=10.0 mmol dm^{-3}, [ANS(−)]=0.010 mmol dm^{-3}. b) The λ_{max} and I_{rel} values for ANS(−) in the absence of a host were 519±1 nm and 1.0 respectively. c) The fraction of ANS(−) bound to each host.

sion maximum decreases and the relative intensity (I_{rel}) of the peak increases with decreasing polarity of microenvironment around the probe[9] or with decreasing internal rotation of the probe[10,11] accompanied by an increase in viscosity or by the association with biopolymers. Similar spectral changes have been observed for a β-CD-ANS(−) system, where the hydrophobic nature of the β-CD cavity is responsible for the spectral changes.[12] Table 2 shows the λ_{max} and I_{rel} values for the emission maxima of ANS(−) in the presence of 10 mmol dm^{-3} neutral and charged β-CD's. The λ_{max} values decreased in the order of β-CDsulf(−)>β-CD>β-CDtma(+)>β-CDpy(+). The I_{rel} values increased in the order of β-CDpy(+)<β-CDsulf(−)<β-CD<β-CDtma(+).[13] It is unlikey that the significant blue shifts observed for β-CDtma(+) and β-CDpy(+) systems are brought about by a decrease in microenvironmental polarity, since the cavity of the charged β-CD's may be less hydrophobic than that of neutral β-CD. It is more plausible that the internal rotaion of ANS(−) is restricted by the attractive electrostatic interactions between the charged β-CD's and the guest. Similar effects of electrostatic interactions were reported for complexes of ANS(−) with biological membranes and detergent micelles.[14] The I_{rel} value for a β-CDpy(+) system was unusually low, compared with that for a β-CDtma(+) system. The pyridinio moiety of β-CDpy(+) may interact with the singlet excited species of ANS(−) to accelerate the quenching of the excited species. This presumption was substantiated by the fact that the addition of *N*-ethylpyridinium chloride (Etpy) in place of β-CDpy(+) to an aqueous ANS(−) solution also caused a decrease in I_{rel} (I_{rel}=0.84 at [Etpy]=18 mmol dm^{-3}).

Effects of Ionic Strength and an Organic Solvent on Binding Constants. Thermodynamic stabilities (log K_a) for inclusion complexes of β-CD, β-CDsulf(−), and β-CDtma(+) with CV(+) and PP(−) increased in proportion to the square root of ionic strength (I_c) in a manner similar to that described preveously.[7] Ionic strength was adjusted by use of potassium sulfate, which does not interact with the cavity of β-CD.[15] Regression analysis of the relationships between them gave intercepts (A) and slopes (B) (Table 3). It is notable that the B values for identically charged host-guest systems [β-CDtma(+)-CV(+) and β-CDsulf(−)-PP(−)] are larger than those for oppositely charged host-guest systems. The B values for neutral β-CD systems were intermediate between those for the charged β-CD systems. Hydrophobic interactions are generally strengthened by an increase in I_c (salting-out effect). This may be one reason why the B values for the neutral β-CD systems are positive. Electrostatic interactions are lowered by an increase in I_c (Debye-Hückel theory). In identically charged host-guest systems where electrostatic repulsive interactions as well as hydrophobic interactions are acting between host and guest, an increase in I_c weakens the repulsive interactions and strengthens the hydrophobic interactions. Thus, the B values for these systems become larger than those for the β-CD systems. In oppositely charged host-guest systems, electrostatic attractive interactions are weakened by an increase in I_c, although hydrophobic interactions are strengthened. Then, the B values for these systems become smaller than those for the β-CD systems.

Table 3. The Values of Parameter A and B for the Equation; $\log(K_a/\text{mmol}^{-1}\,\text{dm}^3)=A+BI_c^{1/2}$

Guest	Host	A	B	n[a]	r[b]
CV(+, pH 5.0)	β-CDtma(+)	−0.62	0.54	6	0.972
	β-CD	0.46	0.29	6	0.996
	β-CDsulf(−)	0.26	0.27	6	0.946
PP(−, pH 10.5)	β-CDtma(+)	1.35	0.17	12	0.915
	β-CD	1.41	0.24	6	0.974
	β-CDsulf(−)	0.42	0.50	6	0.988

a) The number of data. b) Correlation coefficient.

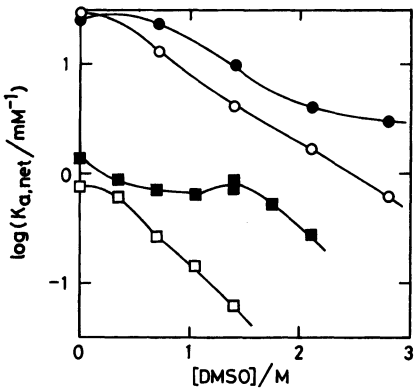

Fig. 1. Changes of log K_a with increasing concentration of DMSO (1 M=1 mol dm^{-3}).
○: β-CD-PP(−) system, ●: β-CDtma(+)-PP(−) system, □: β-CD-pNP(−) system, ■: β-CDtma(+)-pNP(−) system.

The addition of an organic solvent, DMSO, to the media also changed the log K_a values for β-CD and β-CDtma(+) complexes with PP(−) and pNP(−) (Fig. 1). DMSO is bound to the hosts (Table 1), so that it competitively inhibits the complexation of the hosts with the anionic dyes. In order to shed light on the

Table 4. Kinetic and Equilibrium Parameters for Host-ABA Systems in Alkaline Solutions at 25 °C

Host	Substrate	pH	[DMSO] %(v/v)	k_c/k_{un}	K_a mol^{-1}dm^3	$K_a k_c/k_{un}$ mol^{-1}dm^3
β-CD	*o*-ABA	12.0	0	7.7	83	640
	m-ABA	11.9	0	6.3	91	570
		11.8	20	7.4	21	160
		11.9	40	17	16	270
	p-ABA	10.9	0	1.2	710	860
β-CDsulf(−)	*o*-ABA	12.0	0	2.0	830	1700
	m-ABA	11.9	0	0.00	59	0
		11.8	20	0.00	37	0
		12.8	40	0.00	30	0
	p-ABA	10.9	0	0.30	220	67
β-CDtma(+)	*o*-ABA	12.0	0	2.2	430	960
	m-ABA	11.9	0	9.3	290	2700
		11.8	20	9.9	59	580
		11.9	40	43	9.4	410
	p-ABA	10.9	0	1.6	770	1200
β-CDpy(+)	*o*-ABA	12.0	0	0.00	120	0
	m-ABA	11.0	0	5.3	190	980
		11.8	20	3.6	91	330
		11.9	40	11	63	690
	p-ABA	10.9	0	0.32	530	170

solvent effect of DMSO, the net K_a values ($K_{a,net}$) for these systems were calculated from the apparent K_a values by use of Eq. 1, where K_i is the binding constants for the host-DMSO systems:

$$K_{a,net} = K_a(1 + K_i[\text{DMSO}]). \quad (1)$$

This equation can easily be derived on the basis of the law of mass action under conditions that the concentration of DMSO is much larger than that of a host. In neutral β-CD systems where hydrophobic interactions play an important role in complexation, the log $K_{a,net}$ values decreased greatly with increasing [DMSO]. On the other hand, the log $K_{a,net}$ values for the β-CDtma(+) systems were not so much lowered. An addition of DMSO to an aqueous solution results in a decrease in dielectric constant of the solution, which contributes to strengthening electrostatic attractive interactions between the oppositely charged host and guests. However, the attractive interactions were not so large as to overcome an accompanied decrease in hydrophobic interactions.

Effect of the Charged Hosts on the Alkaline Hydrolyses of ABAs. Kinetic parameters determined for the alkaline hydrolyses of ABAs in the presence of the hosts are shown in Table 4, where k_{un} and k_c are the first-order rate constants for free and complexed substrates, respectively. The substrates bear carboxylate anions in alkaline solutions. For *m*-ABA systems where the most remarkable effects of the charged hosts were observed among the substrates examined, kinetic parameters were also determined in the presence of DMSO.

The results were too complicated to be explained simply in terms of electrostatic interactions between hosts and guests in such a manner as described previously.[7] However, a few remarkable features are found among them. The positively charged β-CDtma(+) and the neutral β-CD accelerated, more or less, the hydrolyses of all the substrates examined. The degree of acceleration (k_c/k_{un}) was larger for β-CDtma(+)-*m*- and *p*-ABS systems than for the corresponding β-CD systems, and the reverse was true for *o*-ABA systems. Another cationic host, β-CDpy(+), decelerated the hydrolyses of *o*- and *p*-ABAs, though it accelerated the hydrolysis of *m*-ABA to a lesser extent than β-CDtma(+) or β-CD. The negatively charged β-CDsulf(−) decelerated the hydrolyses of *m*- and *p*-ABAs and accelerated that of *o*-ABA to a lesser extent than β-CD.

It is known that the degree of acceleration is closely related to the distance between the catalytic and reaction sites of the host-guest complex (proximity effect).[1,16] The catalytic site of each host is an alkoxide ion derived by the acid dissociation of a secondary OH group of the host.[1] The reaction site is an ester carbonyl carbon. Thus, the fact that the k_c/k_{un} value for a β-CDtma(+)-*m*- or *p*-ABA system is larger than that for the corresponding β-CD system implies that the reaction site is located more closely to the catalytic site in the former than in the latter. Electrostatic attractive interactions between the host and guest may be responsible, at least in part, for such a change in the geometry of an inclusion complex. Steric interactions between the charged groups of the host and guest may

also contribute to a change in the geometry. It has been reported[17] that the replacement of the primary OH group(s) of β-CD by some bulky group(s) results in significant changes in the rates of the alkaline hydrolyses of phenyl acetates. The trimethylammonio group of β-CDtma(+) is so bulky that it may be advantageous for the acceleration of the hydrolyses.

When the reaction site of a guest is deeply included within the cavity of a host, the site is protected from the nucleophilic attack of either the alkoxide ion of the host or the hydroxide ion in a bulk solution. Then, the hydrolysis of the guest is retarded by complexation. Such non-productive binding is probably brought about in β-CDsulf(−)-*m*- or *p*-ABA and β-CDpy(+)-*o*- or *p*-ABA systems. We could speculate for the case of a β-CDsulf(−) system that *m*- or *p*-ABA is inserted into the cavity of β-CDsulf(−) from the side of the acetyl group to minimize the electrostatic repulsive interactions between the sulfonate anion of the host and the carboxylate anion of the guest. However, it is difficult to reasonably explain at the present stage of investigation why β-CDpy(+) retards the hydrolyses of *o*- and *p*-ABA on one hand and β-CDtma(+) accelerates the reactions on the other hand. Further information on the geometries of the inclusion complexes is necessary in order to solve these problems.

The addition of DMSO to an aqueous solution also caused significant changes in the values of k_c/k_{un} and K_a. Generally, the k_c/k_{un} value increased and the K_a value decreased with increasing DMSO concentration. Similar DMSO effects have been reported by Trainor and Breslow.[18] The addition of DMSO may enhance the dehydration of the alkoxide ion of the hosts to facilitate its nucleophilic attack to the guests. On the other hand, DMSO is competitively bound to the hosts, as was previously described (Table 1), to weaken the binding of hosts to guests. A decrease in the polarity of a solvent with the addition of DMSO also contributes to the weakening of the hydrophobic interactions between the hosts and guests. Thus, the value of a net catalytic parameter, $K_a k_c/k_{un}$, did not always increase with increasing DMSO concentration.

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