Journal of Inclusion Phenomena and Molecular Recognition in Chemistry **17:** 277–290, 1994.

Inclusion of (Aminostyryl)-1-Methylpyridinium Dyes by β-Cyclodextrin and Its Use for Fluorescent-Probe Studies on Association of Cationic and Neutral Molecules with β-Cyclodextrin

JOON WOO PARK* and KYUNG HWA PARK
Department of Chemistry, Ewha Womans University, Seoul 120–750, Korea.

(Received: 2 November 1993; in final form: 8 March 1994)

Abstract. 2-(or 4-)-[4-(Dimethylamino)styryl]-1-methylpyridinium iodides (2-ASP and 4-ASP) form 1 : 1 inclusion complexes with β-cyclodextrin (β-CD). The association constant is about 1000 M^{-1} for both dyes in aqueous solution at 25°C. The complexation results in a 7.6- and 4.9-fold increase in the fluorescence intensities of 2-ASP and 4-ASP, respectively. The formation of inclusion complexes of n-alkylamines ($n = 4-10$), n-alkylammonium ions ($n = 4-10$), n-alkyl alcohols ($n = 4-9$), and n-alkyltrimethylammonium bromides (C_nTAB: n = 12, 14, 16) with β-CD was studied by a fluorescent-probe method using 2-ASP as a probe. The association constants K were obtained from nonlinear least-square regression analysis of the fluorescence titration data. The logarithm of the 1 : 1 association constants varies approximately linearly with the number of carbon atoms (n_c) of the alkyl chain up to $n_c = 7$ and then the dependence of log K on n_c is less pronounced for longer alkyl chains: for C_nTAB, 1 : 2 complexation was also observed. Combining the present result with a previous work [15], we found that the increasing order of stability of β-CD complexes of amphiphilic molecules with the same hydrocarbon tail but different head groups is ammonium < sulfonate < alcohol ≅ amine < sulfate. The association constants obtained from this method are compared with those given by other investigators and the discrepancies are discussed.

Key words: β-Cyclodextrin, inclusion complexes, fluorescence, aminostyryl dyes, association constants, fluorescent-probe.

1. Introduction

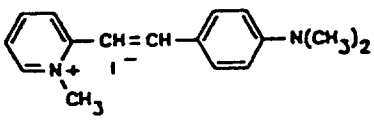
Cyclodextrins (CD) are cyclic oligosaccharides of truncated cone shape and form inclusion complexes with a variety of hydrophobic and amphiphilic species in aqueous solutions. Because of this characteristic, they are widely used as biomimetic systems and as novel media for various types of reactions [1]. A number of research groups have reported the results of studies on the binding of alkylamines [2–6], alkylammonium ions [3–10], and alkyl alcohols [11]. Also, formation of ternary complexes of aromatic hydrocarbons with aliphatic alcohol – β-CD [12–14] and amine – β-CD [2] complexes has been reported. In those binding studies, conductometric methods have been widely used for the ionic guests and the binding constants were calculated under the assumption of 1 : 1 complexation [3–5, 7, 8].

* Author for correspondence.

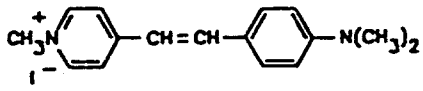
Electrochemical methods using surfactant selective electrodes [6, 10] and measurement of the speed of sound [9] have also been utilized. The association constants of alcohols [11] and amines [2] with CD were determined by absorption spectrophotometry using an inhibitory effect of the guest molecules on the binding of an azo dye to CD. However, the reported association constants differ widely among investigators, especially for guest molecules having long alkyl chains, depending primarily on the method used to determine the association constants.

In a previous report, we showed that a fluorescent-probe method using anilinonaphthalene sulfonates (ANS) is a reliable and convenient technique for studies of association between anionic surfactants and β-CD [15]. For fluorescent-probe studies of cationic guests, a cationic probe which behaves as a competitive inhibitor for the association is required.

In this article, we report that iodide salts of 2-(or 4-)-[4-(dimethylamino)styryl]-1-methylpyridinium (**1**, 2-ASP; **2**, 4-ASP), which have been used as potential sensitive fluorescent probes for biological systems [16] are suitable for the purpose. The association constants of various alkylamines, alkylammonium ions, and alkyl alcohols with β-CD were obtained and the results were correlated with the chain lengths of the hydrocarbon tails and the nature of head groups of the guest molecules. The validity of this method together with a comparison with those used by other investigators are also discussed.



1 (2-ASP)



2 (4-ASP)

2. Experimental

2.1. Materials

Guests used in this study are n-alkylamines $C_nH_{2n+1}NH_2$ ($n = 4 - 10$) and their protonated ammonium ions, n-alkyl alcohols $C_nH_{2n+1}OH$ ($n = 4 - 9$), and n-alkyltrimethylammonium bromides C_nTAB ($n = 12, 14, 16$). The n-alkylamines of $n = 5, 6, 7$ were obtained from Tokyo Kasei. C_{14}TAB was purchased from Fluka. Other guests were from Aldrich. Except for C_{12}TAB and C_{16}TAB, which were recrystallized from ethanol twice, guests from commercial sources were used as received. Iodide salts of 4-[4-(dimethylamino)styryl]-1-methylpyridinium (4-ASP) and 2-[4-(dimethylamino)styryl]-1-methylpyridinium (2-ASP) were obtained from Molecular Probes and used without further purification. β-CD was purchased

from Aldrich, and the concentration of β-CD solution was calculated from optical rotation data taken with a Jasco DIP-140 polarimeter at 25°C using $[\alpha]_D^{25} = 162.5°$ [1a]. Solutions were prepared with glass-distilled water and ionic strength was maintained constant at 0.10 M with NaCl. Alkylammonium ion solutions were prepared by dissolving alkylamines in 0.1 N HCl: the pK_a of conjugated acids of alkylamines is about 10.6 [17]. Others were made in phosphate buffer at pH 7.0.

2.2. Fluorescence spectra

These were recorded with a Hitachi F-3010 spectrofluorimeter at 25°C. The excitation wavelengths were set at the absorption maxima of the probes, 440 nm for 4-ASP and 435 nm for 2-ASP. The excitation and emission slit width was 5 nm.

2.3. Binding constants

Binding constants of ASPs with β-CD were determined from fluorescence titration data of ASPs with β-CD. The concentrations of ASPs were fixed at 4.0×10^{-6} M and the concentration range of β-CD was $(0.20 - 2.0) \times 10^{-3}$ M. The binding constants of guests with β-CD were determined from the dependence of the fluorescence intensities of 4.0×10^{-6} M 2-ASP – 1.0×10^{-3} M β-CD solutions on the concentration of guests. (For details, see following section).

3. Results and Discussion

3.1. Complexation of the ASPs with β-CD

The fluorescence intensity of the ASPs in aqueous solutions was greatly enhanced upon the addition of β-CD with a concomitant spectral blue shift (Figure 1). This is analogous to the observations made with the fluorescent dyes on reducing the polarity of the medium [18], and indicates the transfer of ASP molecules from the aqueous medium to the apolar β-CD cavity by inclusion complexation. The excited-state lifetime of (aminostyryl)pyridinium dyes is of the order of 10^{-10} s [18, 19], which is much shorter than the mean residence time of a molecule in the β-CD cavity, about 10^{-4} s [20]. Thus, we are safe in assuming that the complexation equilibria between ASPs and β-CD do not change appreciably during the excited lifetime of the dyes and the enhancement in the fluorescence intensity of ASP solutions upon addition of β-CD reflects the ground-state association of the dyes with β-CD [15].

If the ASPs form 1 : 1 complexes with β-CD (Equation 1), the change in fluorescence intensity (ΔI_F) caused by the addition of β-CD is related to the concentration of uncomplexed β-CD by Equation 2 [15].

$$\text{ASP} + \beta\text{-CD} = \text{ASP-}\beta\text{-CD}; \quad K_p = \frac{[\text{ASP-}\beta\text{-CD}]}{[\text{ASP}]\,[\beta\text{-CD}]} \tag{1}$$

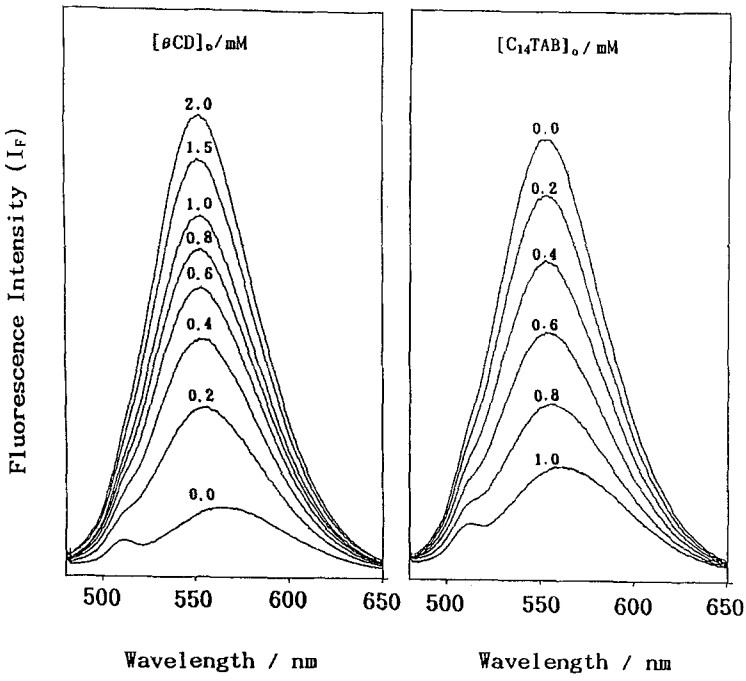


Fig. 1. Fluorescence spectra of 4.0×10^{-6} M 2-ASP solutions at 25°C in water: Left, at various concentrations of β-CD in the absence of guest; Right, at various concentrations of C_{14}TAB in the presence of 1.0 mM β-CD. The numbers in the spectra correspond to the concentrations of β-CD (left) and C_{14}TAB (right) in mM. The fluorescence intensity scale of the right spectrum is expanded 1.23 times compared to that of the left spectrum.

TABLE I. Binding constants (K) of ASPs with β-CD and the enhancement of fluorescence intensities of ASPs upon binding with β-CD at 25°C in water.[a]

ASP	K/M^{-1}	I_{CD}/I_W
2-ASP	1,070 ± 110	7.6 ± 0.4[b]
4-ASP	1,000 ± 100	4.9 ± 0.7[c]

[a] Error ranges are standard deviations of 4 measurements.
[b] Measured at 552 nm.
[c] Measured at 580 nm.

$$\Delta I_F/[\beta\text{-CD}] = \Delta I_\infty K_p - \Delta I_F K_p \quad (2)$$

ΔI_∞ is the maximum change in fluorescence intensity when all of the ASP molecules form the complex. Since the total concentration of β-CD, $[\beta\text{-CD}]_0$, is much higher than the total concentration of ASP, $[\beta\text{-CD}]$ can be replaced by $[\beta\text{-CD}]_0$.

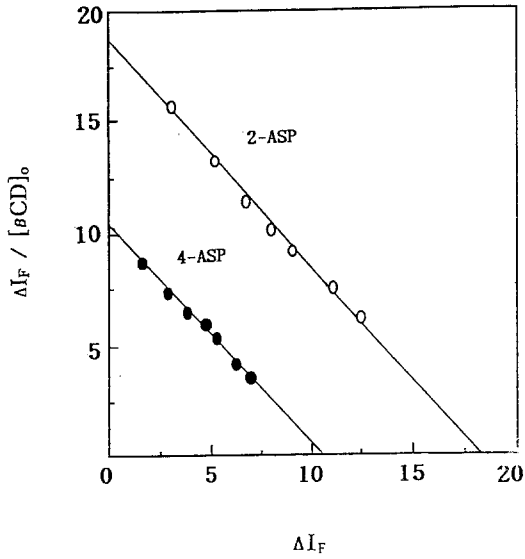


Fig. 2. Plots of fluorescence titration data of ASPs with β-CD according to Equation 2: (○) 2-ASP; (●) 4-ASP.

Figure 2 shows the plots of experimental data according to Equation 2. Good linearity (coefficient of correlation $r \geq 0.997$) confirms 1 : 1 complexation under our experimental condition of $[\beta\text{-CD}]_0 \leq 2$ mM. The binding constants and ΔI_∞ were calculated from the plots. The results are summarized in Table I.

The binding constants of ASPs with β-CD do not depend appreciably on the position of the dimethylaminostyryl moiety on the *N*-methylpyridinium ring. This can be taken as evidence that the dimethylaminostyryl moiety of the dyes is preferentially included in the β-CD cavity. We also determined the binding constant of 4-(styryl)-1-methylpyridinium iodide (stilbazole methiodide) with β-CD from the dependence of the rate of luminescence quenching of $Ru(bpy)_3^{2+}$ by the styrene derivative on the concentration of β-CD. The binding constant was found to be 280 and 210 M^{-1} for the *trans* and *cis* isomers, respectively. These values are significantly less than that for the ASPs, suggesting that the dimethylamino group of the dimethylaminostyryl moiety interacts with β-CD to give enhanced stability of the complexes. The enhancement of fluorescence intensity upon binding to β-CD is about 1.5 times greater for 2-ASP than that for 4-ASP: 2-ASP is a more sensitive probe than 4-ASP, thus, 2-ASP was used as a competitive fluorescent probe for binding studies of other non-fluorescent guests with β-CD [21].

3.2. BINDING CONSTANTS OF NON-FLUORESCENT GUESTS WITH β-CD

Guests such as alkylamines, alkylammonium ions, and alkyl alcohols do not bear a chromophore. Thus, neither absorption nor luminescent methods can be applied directly for the studies of binding of the guests with β-CD. However, indirect competitive binding using solvatochromic [11] or medium sensitive fluorescent dyes [15] can be used. In this study we used 2-ASP as a competitive inhibitor, i.e. a probe.

The addition of the cationic or neutral guests to a solution containing both 2-ASP and β-CD decreases the fluorescent intensity of 2-ASP. A typical dependence of the fluorescence spectrum on the concentration of guest is shown in Figure 1. The shape and position of a spectrum taken at a high concentration of β-CD in the presence of a guest were not different from that taken at a low concentration of β-CD in the absence of the guest, when the intensities of the two spectra are the same. When β-CD is absent from the solution, little effect of the guests on the fluorescence of ASP is observed under the experimental condition of [guest] < 10 mM or below the critical micelle concentrations (*cmc*) of C_nTAB: at higher concentrations of guests, the fluorescence intensity of ASP is enhanced and the emission maximum shifts to shorter wavelength, presumably due to a change in the polarity and viscosity of the medium or the binding of the dyes to micelles [18]. This indicates that the observed spectral change of 2-ASP upon addition of guests to 2-ASP/β-CD solutions arises from competition of 2-ASP and guest molecules for binding to the host β-CD: binding of a guest (G) to β-CD depletes the uncomplexed β-CD and thus results in dissociation of the 2-ASP/β-CD complex.

$$\text{ASP} + \beta\text{-CD} = \text{ASP-}\beta\text{-CD} \tag{2}$$

$$\text{G} \quad + \beta\text{-CD} = \beta\text{-CD-G} \tag{3}$$

The variation of fluorescence intensity of 2-ASP/β-CD solutions at 552 nm with the concentrations of various alkylamines are shown in Figure 3. Similar figures were also prepared for the binding of alkylammonium ions and alkyl alcohols, but are not shown. The concentrations of free β-CD in equilibrium at various concentration of the guests are calculated from the emission intensity and the parameters (Table I) of the 2-ASP-β-CD association. From these, the concentrations of β-CD complexed with a guest $[\beta\text{-CD}]_b$ were calculated at various guest concentrations by a relationship, $[\beta\text{-CD}]_b = [\beta\text{-CD}]_0 - [\beta\text{-CD}]$. Figure 4 shows a typical dependence of $[\beta\text{-CD}]_b$ on [guest]. The binding constants K of the guests with β-CD are determined from data given in Figure 4 (and corresponding figures drawn for binding of other guests). If a guest forms a 1 : 1 complex only, the calculation of the K_1 value is straightforward from Equation 4 [15].

$$K_1 = \frac{[\beta\text{-CD-G}]}{[\beta\text{-CD}]\,[\text{G}]} = \frac{[\beta\text{-CD}]_b}{([\beta\text{-CD}]_0\text{-}[\beta\text{-CD}]_b)([\text{G}]_0\text{-}[\beta\text{-CD}]_b)} \tag{4}$$

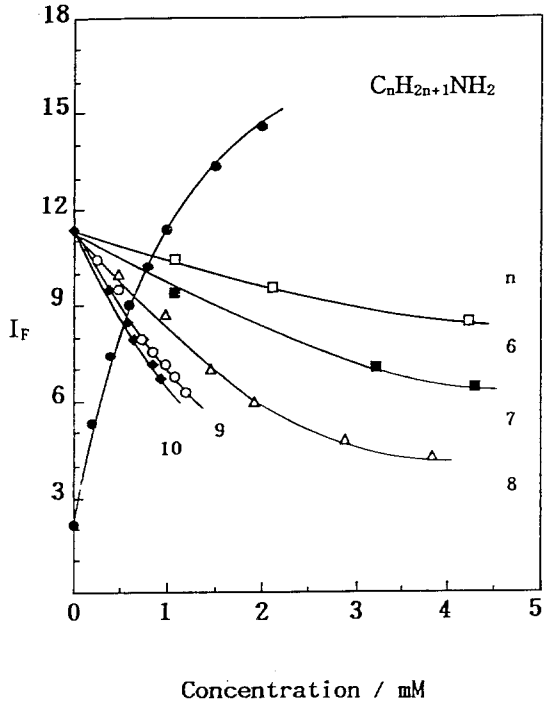


Fig. 3. Dependence of fluorescence intensity of 4.0×10^{-6} M 2-ASP solution at 552 nm on the concentration of β-CD (•), and on the concentration of alkylamines at a fixed concentration of β-CD, 1.0×10^{-3} M.

The consistency of the calculated K_1 values for a given guest–β-CD system, regardless of the concentrations of the guest, supports the validity of the assumption of 1 : 1 complexation. This was the case for alkylamines, alkyl alcohols, and alkylammonium ions where the alkyl chains contain ≤ 10 carbon atoms. The K_1 values are listed in Tables II and III.

For C_nTAB with $n = 12$, 14 and 16, the calculated K_1 values depended strongly on the concentration of the guest surfactants. This suggests that complexes other than those with the presumed 1 : 1 stoichiometry also form in those systems. Figure 4 shows that $[\beta\text{-CD}]_b$ values are greater than the total concentration of surfactants $[G]_0$ when $[G]_0 < [\beta\text{-CD}]_0$. The results indicate that more than one β-CD molecule can bind to a single guest molecule forming 1 : 2 as well as 1 : 1 complexes. Another equilibrium (Equation 5) should be considered in addition to Equation 2.

$$\beta\text{-CD-G} + \beta\text{-CD} = (\beta\text{-CD})_2\text{-G}; \quad K_2 = \frac{[(\beta\text{-CD})_2\text{-G}]}{[\beta\text{-CD-G}]\,[G]} \tag{5}$$

When Equations 2 and 4 are combined and the mass balances of the guests and β-CD are used, the total concentration of guest is related to [β-CD] by Equation 6.

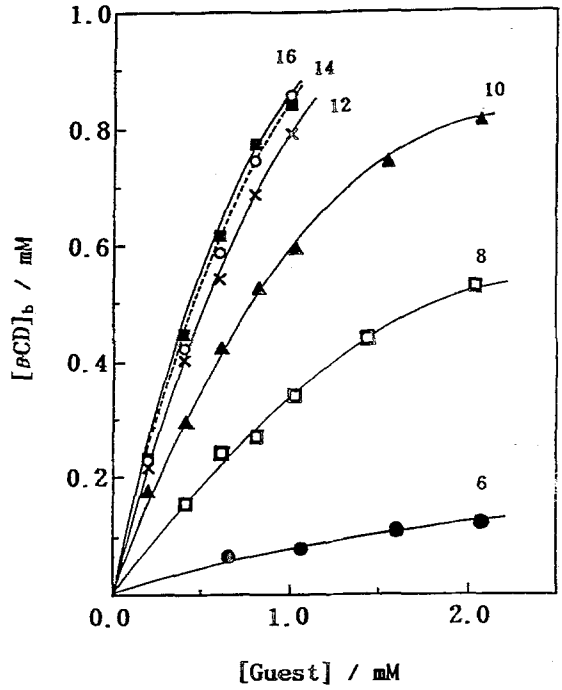


Fig. 4. Plots of alkylammonium (n = 6, 8, 10) and alkyltrimethylammonium (n = 12, 14, 16) ion-bound β-CD against the total concentrations of the guests. The initial concentration of β-CD was 1.0×10^{-3} M. The numbers of carbon atoms of the hydrocarbon tail of guests are shown.

$$[\mathrm{G}]_0 = \frac{1 + K_1[\beta\text{-CD}]_0 + K_1K_2[\beta\text{-CD}]_0^2}{K_1[\beta\text{-CD}]_0 + K_1K_2[\beta\text{-CD}]_0^2} ([\beta\text{-CD}]_0 - [\beta\text{-CD}]) \tag{6}$$

The binding constants K_1 and K_2 are calculated by nonlinear least-square regression analysis. The results are included in Table II. We also attempted to fit the data for other guests where the alkyl chain is nonyl or decyl to Equation 6. The K_2 values were found to be less than 10 M^{-1}, which is too small to have any physical meaning. Moreover, the fitting was not significantly better than that for a simple 1 : 1 binding.

3.3. Dependence of binding constants on the length of hydrocarbon tail and polar head groups

The interior of the cavity of cyclodextrins is lined with $-CH_2$ groups and ether oxygen atoms and provides a relative hydrophobic environment. Thus the interior favors interaction with the hydrocarbon chain of the amphiphilic molecules. The inclusion of polar head groups of the guest molecules in the cavity of β-CD is expected to be disfavored by the large desolvation energy [1a]. X-ray crystallo-

TABLE II. Binding constants of alkylammonium and alkyltrimethylammonium ions with β-CD at 25°C in water.

	K_1, M^{-1}		K_2, M^{-1}	
n	this work[a]	lit. (Method)[b]	this work[a]	lit. (Method)[b]
		$C_nH_{2n+1}NH_3^+$		
5	5 ± 1			
6	43 ± 3			
7	200 ± 15			
8	750 ± 55	710[c](E)		< 10[c](E)
9	2270 ± 490			
10	3770 ± 200	3550[c](E)		< 16[c](E)
		$C_nH_{2n+1}N(CH_3)_3^+$		
12	22100 ± 5500	394[d](S), 4900[e](C) 17800[c](E), 18100[f](E)	52 ± 32	25[c](E)
14	44000 ± 6500	9610–11700[g](C) 39800[c](E)	118 ± 12	56[c](E)
16	59800 ± 15000	2240[h](C), 22000[i](C) 72400[c](E)	390 ± 70	126[c](E)

[a] (Average $\pm$ standard deviation) of at least 5 measurements at different guest concentrations for $C_nH_{2n+1}NH_3^+$, and of 4 independent determinations for $C_nH_{2n+1}N(CH_3)_3^+$.

[b] E: Electrochemical; S: Measurement of the speed of sound; C: Conductometry.

[c] Reference 6.

[d] Reference 9.

[e] Reference 4.

[f] Reference 10.

[g] Reference 5.

[h] Reference 8.

[i] Reference 7.

graphic studies of some α-CD complexes confirm this [22]. Therefore, it can be reasonably assumed that the hydrocarbon tail of the guest molecules are included in β-CD.

The variations of the binding constants with the carbon numbers (n) of the hydrocarbon tails of the guest molecules are shown in Figure 5. Log K_1 varies linearly with n for $n \leq 7$. The slopes were 0.80, 0.57 and 0.47 for alkylammonium ions, alkylamines, and alkyl alcohols, respectively. The increment of log K_1 per methylene group becomes progressively less when n_c exceeds 7. This behavior agrees well with previous reports for n-alkyl sulfonates [4, 15]. Based on the structure of β-CD and geometrical consideration of inserting a hydrocarbon chain into a β-CD cavity, we showed that the maximum number of carbon atoms of an

TABLE III. Binding constants of alkylamines and alkyl alcohols with β-CD at 25°C in water.

n	K_1, M^{-1} this work[a]	K_1, M^{-1} lit. value[b]	K_1, M^{-1} this work[a]	K_1, M^{-1} lit. value[b]
	$C_nH_{2n+1}NH_2$		$C_nH_{2n+1}OH$	
4	26 ± 2	10 ± 5[c]	15[e] ± 2	17[d]
5	71 ± 3		57 ± 3	63[d]
6	240 ± 20		210 ± 14	220[d]
7	650 ± 25		790 ± 70	700[d]
8	1690 ± 100		1630 ± 84	1480[d]
9	3680 ± 420		3700 ± 400	
10	5670 ± 400			

[a] (Average ± standard deviation) of at least 5 measurements at different guest concentrations.

[b] By absorption spectrophotometry using an azo dye as probe.

[c] Reference 2.

[d] Reference 11.

[e] K_1 values for (R)- and (S)-forms of 2-butanol were found to be 13 ± 2 M^{-1}.

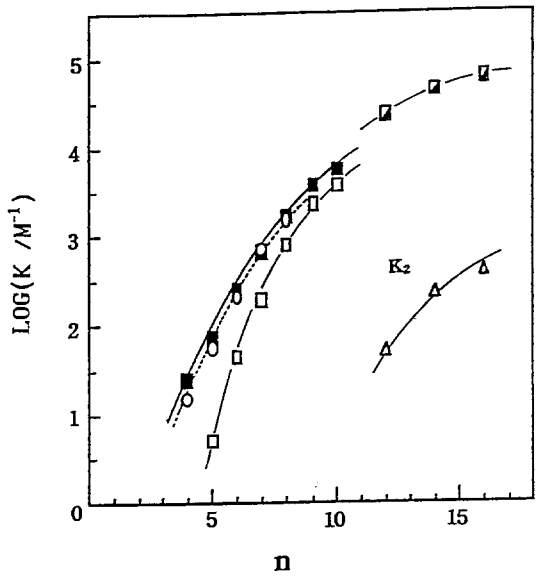


Fig. 5. Dependence of the binding constants of alkylamines (■), alkyl alcohols (○), alkylammonium ions (□), and alkyltrimethylammonium ions (◪, △) with β-CD on the chain length of the hydrocarbon of the guests, (△), K_2 for C_nTAB_2; others, K_1 for the respective types of guests.

alkyl chain that can be accommodated inside the cavity of β-CD is 8 [15]. It is generally believed that one CH_2 group adjacent to the polar head groups makes little or no contribution to the hydrophobic effect [23]. Considering only these factors, one may expect to observe saturation phenomena at $n \cong 8$ in the log K_1 *vs.* n plot. However, Figure 5 does not show such an effect. The interaction of the protruding hydrocarbon tail with the externally hydrated surface of β-CD, which might be energetically favored over the interaction with bulk water, was given as a plausible explanation for this [4, 15].

Figure 5 shows that the head group influences the binding of guest molecules to β-CD. With the results of previous work for alkyl sulfonates and sulfates [15], we found that the increasing order of stability of the complexes of β-CD with guest molecules having the same hydrocarbon tail, but different polar head groups, is $-NH_3^+ < -SO_3^- < -OH \cong -NH_2 < -OSO_3^-$. The great stability of alkyl sulfate complexes can be attributed to the presence of the ether oxygen atom, which behaves as a methylene group in hydrophobic interaction [15]. Davis *et al.* [24] demonstrated that $-NH_2$ and $-OH$ groups contribute about $+21$ kJ/mole, whereas the ether oxygen atom contributes $+15$ kJ/mole, to the free energy change in transferring amphiphilic molecules from water to hydrocarbon. This explains successfully the order between alcohols or amines and sulfates. The order among $-NH_3^+$, $-SO_3^-$, and $-OH$ (or $-NH_2$) seems to reflect the degree of solvation near the head groups. The $-NH_3^+$ group has the same amount of formal charge as $-SO_3^-$ but is solvated more than $-SO_3^-$ due to its smaller size, giving a greater unfavorable contribution to inclusion by β-CD: the greater stability of the dodecyltrimethylammonium (C_{12}TAB)/β-CD complex compared with the dodecylammonium/β-CD complex seems to support this argument [6]. The $-NH_2$ and $-OH$ groups are uncharged and less hydrated than the ionic groups, giving a less disfavorable contribution to the inclusion.

3.4. Comparison of methods for determination of binding constants

As can be seen from Tables II and III, and in the data of a previous work [15], the association constants of amphiphilic molecules with β-CD determined by the present method agree well with the corresponding values from electrochemical [6], conductometric [3, 4], and azo dye-probe absorption spectrophotometric [2, 11] measurements for guest molecules where the alkyl chains contain ≤ 8 carbon atoms and thus form 1 : 1 complexes only. However, large discrepancies between this method and others are noticed for guest molecules with longer alkyl chains.

The largest discrepancy is shown in the results from conductometric measurement which has been the primary tool for studies of the processes. This method relies on the difference in the equivalent electric conductance (λ) between the associated and unassociated amphiphilic ions, which is about 20 % [3–5, 7, 8]. The values of association constants were evaluated from conductometric titration data by a nonlinear least squares method on the assumption of 1 : 1 complexation [3–5,

7, 8], even when the data indicate the presence of 1 : 2 guest–β-CD complexes [5]. Since the λ value is inversely proportional to a hydrodynamic radius of an ion, the difference in λ values between 1 : 1 and 1 : 2 guest–β-CD complexes would be much less than 20%. This might make it difficult to differentiate 1 : 1 and 1 : 2 complexation and the apparent K_1 values reflect both 1 : 1 and 1 : 2 complexation. This seems to be the reason why the apparent K_1 value increases and the conductance difference ($\Delta\lambda = \lambda_{\text{unassociated}} - \lambda_{\text{associated}}$) decreases as the initial concentration of amphiphiles is lower [5], which cannot be explained from a thermodynamic point of view and the definition of ionic conductance. The large discrepancy in the reported K_1 values for the C_{16}TAB – β-CD system determined by the same conductometric method (see Table II) can be attributed to the difference in the initial conditions. As the concentration of a guest is lowered, the formation of the 1 : 2 complex becomes less significant and the value of K_1 calculated on the assumption of a 1 : 1 complex is the lower limit and approaches the value defined in Equation 3. The trend of apparent K_1 *vs.* [guest] reported by Palepu and Reinsborough [5] seems to support the validity of the association constants determined by the present method. Applicabilities of the present method for non-ionic guests as well as ionic guests and in buffered solutions are another merit, compared to the conductometric method.

As far as we know, there is only one report in which the speed of sound is used to study inclusion complexation with cyclodextrins [9]. Junquera *et al.* [9] fitted the speed of sound *vs.* [C_{12}TAB] titration data obtained in the presence of β-CD to an equation which is essentially the same as that used for the conductometric method [3, 4]. They also assumed the formation of only a 1 : 1 complex, though data indicated the presence of a 1 : 2 complex.

The electrochemical method [6, 10] uses guest-sensitive membrane electrodes and monitors the concentration of the unassociated (free) guests directly. In this aspect, the method is similar to the present method which monitors the concentration of the unassociated host (β-CD) by the fluorescence intensity of probe molecules. The values of association constants determined by the present method are in excellent agreement with those from the electrochemical method. However, the types of electrode depend on the nature of the guests and are not commercially available.

The present fluorometric method is analogous to the reported absorption spectrophotometric method which uses azo dyes as competitive inhibitors [2, 11]. However, the fluorometric method has some advantages over the absorption method. The concentration range for fluorescence measurements is usually 2–3 orders of magnitude less than that for absorption spectrophotometry. Also the fluorescence spectrum is generally much more sensitive to the solvent medium, i.e. upon inclusion complexation with β-CD, than the absorption spectrum. Because of these effects, the probe concentration is much lower in the fluorometric method than in the absorption method making knowledge of the exact concentration of the probe

practically unnecessary. The availability of various medium sensitive fluorescent dyes is another advantage.

In conclusion, (aminostyryl)-1-methylpyridinium dyes, ASPs, form inclusion complexes with β-CD. The styryl moieties are the preferred binding sites. The competitive binding of 2-ASP and alkylamines, alkyl alcohols or alkylammonium ions with β-CD enables us to determine the binding constants of the amphiphiles with β-CD by a fluorescence-probe method. Combining the present result with a previous work [15], we found that the increasing order of stability of β-CD complexes with amphiphilic molecules with the same hydrocarbon tail but different head groups is ammonium < sulfonate < alcohol ≅ amine < sulfate. The fluorescence-probe method has several advantages over other methods used for studies on inclusion complexation of cyclodextrins, and can be applied to many other systems and conditions.

Acknowledgements

This work was supported by the Basic Science Research Institute Program of the Ministry of Education of the Republic of Korea (BSRI-92-335) and a Grant from the Korea Science and Engineering Foundation (91-0300-03).

Notes and References

1. For reviews, see: (a) M.L. Bender and K. Komiyama: *Cyclodextrin Chemistry*, Springer Verlag, New York, 1977; (b) J.H. Fendler: *Membrane Mimetic Chemistry*, Wiley-Interscience, New York, 1982; (c) *Inclusion Compounds*, Edited by J.L. Atwood, J.E.D. Davies, and D.D. MacNicol, Academic Press, 1984, Vols. 2 and 3; (d) K. Kalyanasundaram: *Photochemistry in Microheterogeneous Systems*, Academic Press, New York, 1987, pp. 300–317; (e) V. Balzani and F. Scandola: *Supramolecular Photochemistry*, Ellis Horwood, New York, 1991, pp. 288–318.
2. K. Kano, I. Takenoshida, and T. Ogawa: *J. Phys. Chem.* **86**, 1833 (1982).
3. I. Satake, T. Ikenoue, T. Takeshita, K. Hayakawa, and T. Maeda: *Bull. Chem. Soc. Jpn.* **58**, 2746 (1985).
4. I. Satake, S. Yoshida, K. Hayakawa, T. Maeda, and Y. Kusumoto: *Bull. Chem. Soc. Jpn.* **59**, 3991 (1986).
5. R. Palepu and V.C. Reinsborough: *Can. J. Chem.* **66**, 325 (1988).
6. D. Jezequel, A. Mayaffre, and P. Letellier: *Can. J. Chem.* **69**, 1865 (1991).
7. R. Palepu, J. Richardson, and V.C. Reinsborough: *Langmuir* **5**, 218 (1989).
8. T. Okubo, H. Kitano, and N. Ise: *J. Phys. Chem.* **80**, 2661 (1976).
9. E. Junquera, E. Aicart, and G. Tardajos: *J. Phys. Chem.* **96**, 4533 (1992).
10. W.M.Z. Wan Yunus, J. Tayer, D.M. Bloor, D.G. Hall, and E. Wyn-Jones: *J. Phys. Chem.* **96**, 8979 (1992).
11. Y. Matsui and K. Mochida: *Bull. Chem. Soc. Jpn.* **52**, 2808 (1979).
12. S. Hamai: *J. Phys. Chem.* **94**, 2595 (1990).
13. A. Muñoz de la Peña, T.T. Ndou, J.B. Zung, K.L. Green, D.H. Live, and I.M. Warner: *J. Am. Chem. Soc.* **113**, 1572 (1991).
14. S. Hamai, T. Ikeda, A. Nakamura, H. Ikeda, A. Ueno, and F. Toda: *J. Am. Chem. Soc.* **114**, 6012 (1992).
15. J.W. Park and H.J. Song: *J. Phys. Chem.* **93**, 6454 (1989).
16. (a) L.M. Loew and L.L. Simpson: *Biophys. J.* **34**, 353 (1981); (b) L.M. Loew, L.B. Cohen, B.M. Saltzberg, A.L. Obaid, and F. Bezanilla: *Biophys. J.* **47**, 71 (1985); (c) J. Rafael and D.G. Nicholls: *FEBS Lett.* **170**, 181 (1984).

17. *Handbook of Chemistry and Physics*, R.C. Weast Ed., 1989, CRC Press, 70th Ed., D161–162.
18. (a) H. Ephardt and P. Fromherz: *J. Phys. Chem.* **95**, 6792 (1991); (b) H. Ephardt and P. Fromherz: *J. Phys. Chem.* **97**, 4540 (1993).
19. C.J. Tredwell and C.M. Keary: *Chem. Phys.* **43**, 307 (1979).
20. N.J. Turro, T. Okubo, and C.-J. Chung: *J. Am. Chem. Soc.* **104**, 1789 (1982).
21. Addition of guests to 4-ASP-β-CD solutions *increased* the fluorescent intensity when the concentration of the guests is lower than that of β-CD. This is indicative of the formation of ternary complexes. Because of this complication, we could not determine the binding constants of the guests with β-CD using 4-ASP as a probe. This is another reason why we used 2-ASP as a probe in the present study.
22. (a) K. Harata: *Bull. Chem. Soc. Jpn.* **49**, 1493 (1976); (b) K. Harata: *Bull. Chem. Soc. Jpn.* **49**, 2066 (1976).
23. C. Tanford: *Hydrophobic Effect: Formation of Micelles and Biological Membranes*, 2nd Ed., John Wiley & Sons, New York, 1980, p. 14.
24. S.S. Davis, T. Higuchi, and J.H. Rytting: *Adv. in Parmaceutical Sci.* **4**, 73 (1974).