The Microenvironmental Effect of Cyclodextrin on the Acid Dissociation of Some Azo Dyes in Aqueous Solutions

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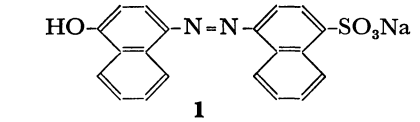
The effects of α- and β-cyclodextrins (CD) on the acid dissociations and tautomeric equilibria of sodium 4-(4′-hydroxy-1′-naphthylazo)-1-naphthalenesulfonate (**1**) and of the conjugate acid (**2**) of Methyl Orange were spectrophotometrically examined and were compared with the effect of a nonpolar solvent (THF). The apparent pK_a values for the dyes decreased with increasing CD concentration. The effect of CD is mainly explained in terms of the hydrophobic interactions between the azo dyes and CD, resulting in a shift in the tautomeric equilibria from less acidic azonium or hydrazone forms to more acidic azo forms of **1** and **2** in an aqueous solution.

The acid dissociation of an indicator dye is generally facilitated by the formation of an inclusion complex between the dye and cyclodextrin (CD) in an aqueous solution.[1-6] This phenomenon is interesting in connection with the microenvironmental effect of an enzyme on the acid dissociation of ionizing groups at the bonding or catalytic site.[7,8] The decrease in pK_a of a dye upon formation of a CD complex has been tentatively attributed to the space alkalinity of CD[1,2] which involves a number of electronegative oxygen atoms in a molecule. On the other hand, Broser *et al.*[4-6] have explained the change in pK_a in terms of a dipole-ion interaction between CD and a dye as follows. A dissociated dye may be more polarizable than the corresponding undissociated dye, so that a dipolar CD molecule associates more tightly to the former than the latter, resulting in enhancement of the acid dissociation of the dye. These arguments, however, completely neglect the hydrophobic property of the cavity of a CD molecule, a property later recognized to play an important role in the binding and catalytic processes of CD.[3,9] In a protein-[7,8] or micellar-[10] dye system, it has frequently been shown that the hydrophobic microenvironment of a binding site significantly affects the pK_a value of a dye. Recently, Connors and Lipari[11] and Miyaji *et al.*[12] reported that the dissociation of carboxylic and barbituric acids in aqueous solutions is retarded by the addition of CD. These observations cannot be explained in terms of the idea of Broser *et al.*, but are well explained in terms of the hydrophobic microenvironment of the CD cavity.

The present study was undertaken to elucidate the contribution of the hydrophobicity of the CD cavity to the acid dissociation of indicator dyes. For this purpose, the behavior of sodium 4-(4′-hydroxy-1′-naphthylazo)-1-naphthalenesulfonate (**1**) and of the conjugate acid (**2**) of Methyl Orange was examined in detail. Both dyes ate known to be good hydrophobic probes, which exhibit absorption spectra that are very sensitive to the microscopic environment around the dye molecules.[13-16] The association of CD with **2** has been examined in some detail by Broser *et al.*[4-6] and Yamada *et al.*[17]



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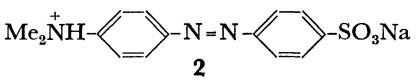

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Experimental

Materials. α-CD and β-CD were prepared by the method of Lane and Pirt.[18] These substances were separated and purified according to the directions given by Cramer and Henglein.[19] Methyl α-D-glucopyranoside and Methyl Orange of reagent grade were used without further purification. Dye **1** was prepared by coupling 4-diazonio-1-naphthalenesulfonate to 1-naphthol. The crude product was purified by repeated salting-out from an aqueous solution with sodium acetate, followed by recrystallization from water and then washing with hot ethanol. Tetrahydrofuran (THF) of reagent grade was used after distillation, bp 66—67 °C. The aqueous buffer solutions used in the present study were H_2SO_4–Na_2SO_4 (pH 1—2), sodium citrate–HCl (pH 2—4), citric acid–Na_2HPO_4 (pH 3—7), citric acid–NaOH (pH 4—6), Na_2HPO_4–KH_2PO_4 (pH 6—7), KH_2PO_4–$Na_2B_4O_7$ (pH 6—9), and $Na_2B_4O_7$–NaOH (pH 9—12). The ionic strengths (I_c) of buffer solutions were adjusted using Na_2SO_4 to 0.05 M for CD–**1** systems and 0.50 M for CD–**2** systems unless otherwise noted.

Apparatus. Absorption spectra were recorded using a Hitachi Model 124 spectrophotometer. The cells (1.0 cm) were maintained at 25±0.1 °C by means of a jacket through which water was circulated from a constant-temperature bath. The pH of each aqueous solution was measured by means of an Orion Model 801 A digital pH/mV meter.

Spectrophotometric Determination of an Apparent Dissociation Constant for a CD-Dye Inclusion Complex. The absorption spectra of dyes **1** and **2**, at a given concentration (*ca.* 0.03 mM for **1** and *ca.* 0.02 mM for **2**) and at a given pH, were recorded for various CD concentrations (0.0—2.0 mM). Each CD–dye system gave an isosbestic point, indicating the formation of a 1 : 1 complex of CD and the dye. The changes in absorbance with CD concentration were measured at 482 and 528 nm for β-CD–**1** systems in acidic and basic solutions, respectively, and at 507 and 530 nm for CD–**2** systems in strongly and weakly acidic solutions, respectively. According to Hildebrand and Benesi,[20] the change in absorbance (ΔA) is related to the total concentration (c_0) of CD by the following equation:

$$\frac{c_0 s_0}{\Delta A} = \frac{K_d}{\Delta\varepsilon} + \frac{c_0}{\Delta\varepsilon}, \quad (1)$$

where s_0 is the total dye concentration, and K_d and $\Delta\varepsilon$, the apparent dissociation constant of a CD–dye inclusion complex and the difference in the molar absorption coefficient between the free and complexed dyes, respectively. The plot of $c_0 s_0/\Delta A$ *vs.* c_0 gave a straight line for each CD–dye system, and the values of K_d and $\Delta\varepsilon$ were determined from the slope and intercept of the line.

Spectrophotometric Determination of the Apparent Acid Dissocia-

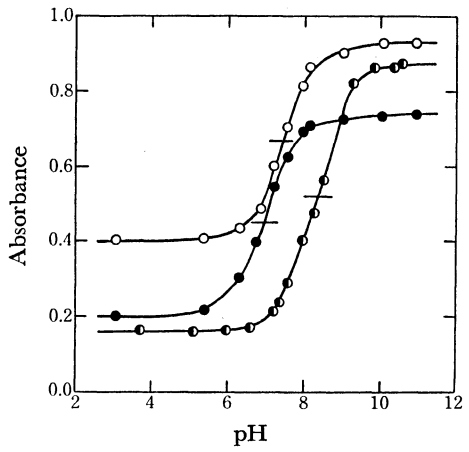


Fig. 1. Plots of absorbances of **1** (3×10^{-5} M) *vs.* pH's at 25 °C.
○: No additive and $\lambda=528$ nm, ●: [β-CD] = 2.02 mM and $\lambda=528$ nm, ◐: [THF] = 3.72 M and $\lambda=535$ nm.

tion Constant ($K_{a,app}$) for an Indicator Dye. Seven to ten buffer solutions were prepared with given concentrations of a dye and an additive, such as CD or THF, and with different pH values. The absorbance at an appropriate wavelength for each solution was plotted against the pH of the solution. Figure 1 illustrates examples of such plots for **1**. The $pK_{a,app}$ values were determined by graphical evaluations of the pH at which $A=(A_a+A_b)/2$, where A_a and A_b are the absorbances under sufficiently acidic and basic conditions, respectively.

Results and Discussion

Change in Absorption Spectrum of a Dye upon the Addition of CD. The absorption spectra of dyes **1** and **2** in a sufficiently acidic solution were markedly changed by the addition of CD, except for the case of the α-CD–**1** system for which the addition of α-CD gave only a slight change in the spectrum of **1**. The molecular cavity of α-CD may be too small to include the naphthyl groups of **1**. Methyl α-D-glucopyranoside had virtually no effect on the spectra of **1** and **2**. The spectral change in the β-CD–**1** system is illustrated in Fig. 2.

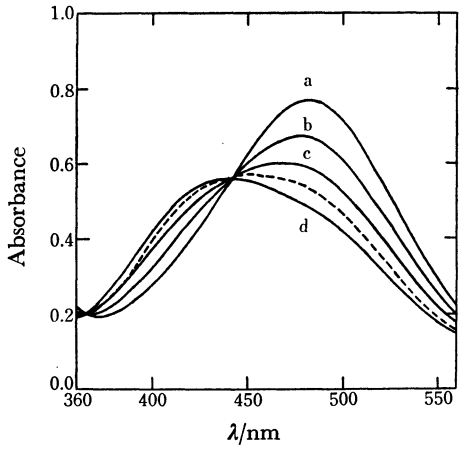
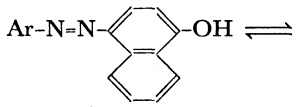


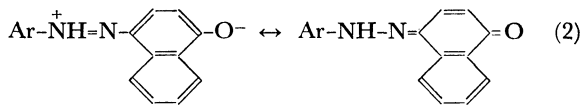
Fig. 2. Absorption spectra of **1** (3.97×10^{-5} M) at pH 4.19 and 25 °C.
Solid line: [β-CD] = (a) 0.00 mM, (b) 0.20 mM, (c) 0.51 mM, (d) 2.03 mM.
Dashed line: [THF] = 3.77 M.

The absorbance around 480 nm markedly decreased and that around 420 nm increased with increasing β-CD concentrations. Isosbestic points were observed at 365 and 441 nm, indicating that β-CD forms a 1 : 1 complex with **1**. The spectral change in **2** upon the addition of α- or β-CD was also very large as reported by Broser,[5] who also showed that both α- and β-CD form 1 : 1 complexes with **2** at low CD concentrations.

When a nonpolar solvent, THF, was added to a sufficiently acidic solution of **1** or **2**, a significant spectral change was observed, which was quite similar to that caused by the addition of CD. An absorption spectrum of **1** in the presence of THF is shown by the dashed line in Fig. 2. It is known that 4-(arylazo)-1-naphthols[14,16,21] and the conjugate acids of 4-aminoazobenzene derivatives[5,13,21] are in tautomeric equilibria.

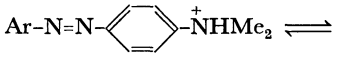


1a

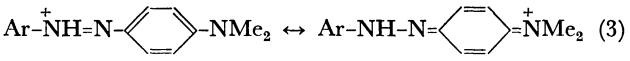


(2)

1b



2a



(3)

2b

The azonium or hydrazone forms (**1b** and **2b**) are predominant species in aqueous solutions, whereas the azo forms (**1a** and **2a**) are predominant in apolar solutions or microenvironments. Thus, the above spectral changes indicate that both **1** and **2** are bound to the hydrophobic regions of CD molecules to give mainly **1a**– and **2a**–CD complexes, respectively.

Effect of Ionic Strength on the K_d Values for CD–Dye Complexes. In order to determine which dipole-ion interactions and hydrophobic interactions contribute

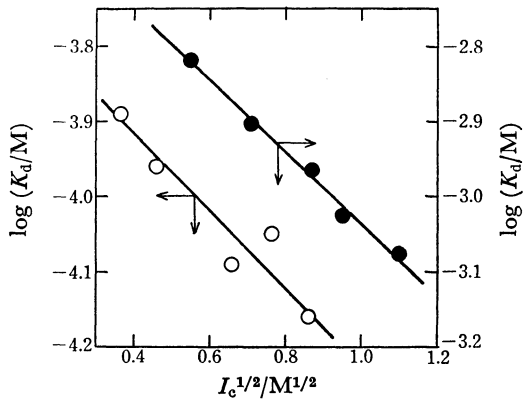
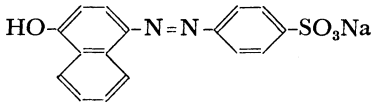


Fig. 3. Plots of $\log K_d$ *vs.* $I_c^{1/2}$ for α-CD–**2** systems at 25 °C. The I_c's were adjusted by the addition of Na_2SO_4.
○: pH = 6.7±0.1 (phosphate buffer),
●: 0.10 M H_2SO_4.

mainly to the association of the CD with the dyes, the effect of ionic strength (I_c) on the K_d values were examined for the CD–dye complexes. Electrostatic interactions, in general, weaken with increasing I_c.[7] If the dipole-ion interactions are dominant, as has been suggested by Broser,[5] the K_d value should increase with increasing I_c. On the other hand, hydrophobic interactions are generally enhanced by an increase in I_c (salting-out effect).[22] Figure 3 shows the plot of $\log K_d$ *vs.* $I_c^{1/2}$ for complexes of α-CD with **2** and its conjugate base. All $\log K_d$ values decrease with increasing I_c.

Dye **1** is too insoluble in an aqueous solution for I_c above 0.05 M to permit a precise examination of the effect of I_c on the K_d value for a β-CD–**1** system. However, Mochida *et al.* previously showed[23] that the K_d value for the complex of β-CD with Orange I (**3**), an analog of **1**, decreases with increasing I_c.



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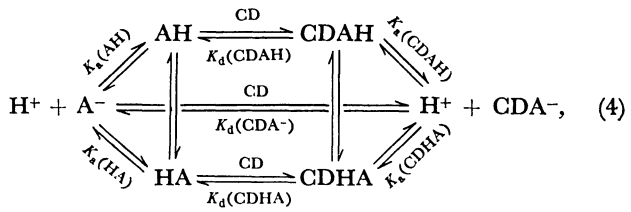
These observations strongly suggest that hydrophobic, rather than electrostatic, interactions play a dominant role in the associations of CD with dyes.

Effect of CD on the $pK_{a,app}$ *Values of Azo Dyes.* Table 1 gives the $pK_{a,app}$ values for dyes **1**—**3** in the absence and in the presence of α-CD, β-CD, and/or THF. The observed $pK_{a,app}$ values decrease with increasing CD concentration for all CD–dye systems. The addition of methyl α-D-glucopyranoside up to 100 mM had virtually no effect on the $pK_{a,app}$ values of the azo dyes.

In order to explain the dependence of the $pK_{a,app}$ values on the CD concentrations, a reaction system is postulated which involves inclusion, acid-dissociation, and tautomeric equilibrium processes, thus



where AH and HA represent the azo form and the azonium or hydrazone form of the azo dye, respectively, K_d and K_a, the dissociation constant of the inclusion complex and the acid-dissociation constant, respectively, for each species given in parenthesis. From reaction

Table 1. Observed and calculated values of $pK_{a,app}$ for CD–azo dye systems at 25 °C

Dye	Additive	Concentration / mM	$pK_{a,app}$ (obsd)	$pK_{a,app}$ (calcd)
1[a]	none	0.000	7.43	(7.43)
	α-CD	10.4	7.50	—
	β-CD	0.591	7.10	7.10
		2.02	7.02	7.01
	THF	3720	8.44	—
2[b]	none	0.000	3.37	(3.37)
	α-CD	0.104	3.07	3.07
		0.520	2.72	2.68
		1.00	2.58	2.53
	β-CD	0.402	3.16	3.12
		1.00	2.96	2.95
	THF	3720	2.88	—
3[c]	none	0.000	8.20	(8.20)
	β-CD	0.500	8.00	8.01
		1.00	7.89	7.89
		2.50	7.72	7.74
		5.00	7.62	7.65
		10.0	7.60	7.59

a) I_c=0.050 M; b) I_c=0.50 M; c) I_c=0.025 M.

4, we can easily derive the following relations from the law of mass action:

$$pK_{a,app}(c_0=0) = \log\{K_a(AH)^{-1}+K_a(HA)^{-1}\}, \quad (5)$$

$$pK_{a,app} = pK_{a,app}(c_0=0) - \log\frac{1+c_0K_d(CDA^-)^{-1}}{1+c_0\{K_d(CDAH)+K_d(CDHA)\}^{-1}}, \quad (6)$$

and

$$pK_{a,app}(c_0=\infty) = pK_{a,app}(c_0=0) - \log\frac{K_d(CDAH)+K_d(CDHA)}{K_d(CDA^-)}, \quad (7)$$

where $pK_{a,app}$ (c_0=0) and $pK_{a,app}$ (c_0=∞) are the $pK_{a,app}$ values at null and infinite CD concentrations, respectively. Equations similar to Eq. 6 have been derived[11,12] for reaction systems which involve inclusion and acid-dissociation processes, but which did not involve tautomeric equilibrium processes. The values of $\{K_d(CDAH)+K_d(CDHA)\}$ and $K_d(CDA^-)$ in Eqs. 6 and 7 are equal to the K_d in Eq. 1 for each CD–dye system in sufficiently acidic and basic solutions, respectively, and can be experimentally determined (Table 2). The $pK_{a,app}$ values in the presence of CD were calculated on the basis of Eq. 6 using the data in Table 2 and the $pK_{a,app}$ (c_0=0) values determined experimentally, and are compared with the observed values (Table 1). They are in good agreement with

Table 2. The values of $\{K_d(CDAH)+K_d(CDHA)\}$, $K_d(CDA^-)$ and $\{pK_{a,app}(c_0=0)-pK_{a,app}(c_0=\infty)\}$ for CD–azo dye systems at 25 °C

System	$K_d(CDAH)+K_d(CDHA)$ / mM	$K_d(CDA^-)$ / mM	$pK_{a,app}(c_0=0)-pK_{a,app}(c_0=\infty)$
β-CD-**1**	0.390	0.134	0.46
α-CD-**2**	1.25	0.0882	1.15
β-CD-**2**	2.70	0.380	0.85
β-CD-**3**	3.00	0.588	0.71

each other to within the experimental error. This fact indicates that the chemical equilibria of Reaction 4 are valid.

The azonium and hydrazone forms (HA in Eq. 4) are the dominant species in aqueous solutions. This fact means that they are of weaker acidity than the corresponding azo form (AH).[21] However, the latter becomes dominant in the CD complexes. This shift in tautomeric equilibrium may contribute significantly to the decrease in the $pK_{a,app}$ of the dye. The change in microscopic dielectric constant around the dissociative groups of the dyes upon inclusion in the hydrophobic CD cavity may also affect the $pK_{a,app}$ values.[11,12] As the effect of THF on the $pK_{a,app}$ values shows (Table 1), the decrease in dielectric constant causes an increase in the $pK_{a,app}$ of **1**, but a decrease in that of **2**. Thus, the decrease in $pK_{a,app}$ with the formation of the inclusion complexes should be larger for the CD–**2** system than for the CD–**1** system. Indeed, this is the case, as the differences between the $pK_{a,app}(c_0=0)$ and $pK_{a,app}(c_0=\infty)$, calculated on the basis of Eq. 7, show (Table 2).

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