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Azo Dye–α-Cyclodextrin Adduct Formation[1]

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Association constants for the complexation of a series of azo dyes with α-cyclodextrin have been determined by means of changes in uv–visible spectra in aqueous solution at pH 7.0, an ionic strength of 0.15 *M* and at 25 and 35°C. Association constants for some of the systems were also determined in water–dimethyl sulfoxide mixtures. All dyes were azobenzenes with a hydroxy group at the 4′ position of one ring and a carboxy group at the 4 position of the other ring. The results were interpreted as demonstrating that the carboxy end of the dye entered the cavity of the α-cyclodextrin. An electrostatic model failed to account for all of the trends in the data. A view of the binding process involving a hydration shell in the region of the primary hydroxy groups of the cyclodextrin is presented.

INTRODUCTION

The ability of the cyclodextrins (cycloamyloses, cyclomaltohexaoses) to form inclusion complexes with a variety of molecules and ions is well known (*1*). That the solvent plays a key role in the complexation process is also well known. Complexation occurs for the most part in aqueous solution, although it can occur in other solvents (*2*). What still remains something of a mystery is the nature of the driving force leading to the formation of the inclusion complexes. Certainly, factors such as conformational changes, hydrophobic bonding, and van der Waals interactions have been postulated and rationalized as being important in the binding process. These proposals, however, while undoubtedly serving well as points of departure for further study, do not lend themselves well to easy application or testing.

The problem is complex. The bonding is nonspecific in that it does not involve the usual strong covalent or electrostatic forces encountered in other complexation reactions. The process, as has been well established, is principally driven by the solvent and most of the variations in stabilities are due to the differences in the sum over a large number of relatively weak interactions. These complexation reactions, however, being uniquely solvent dependent, are all the more interesting for that fact.

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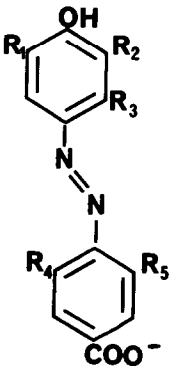

I $R_1, \ldots, R_5 = H$
II $R_1, R_2 = Cl$; $R_3, \ldots, R_5 = H$
III $R_1, R_2 = CH_3$; $R_3, \ldots, R_5 = H$
IV $R_1, R_2, R_4, R_5 = H$; $R_3 = CH_3$
V $R_1, R_3 = CH_3$; $R_2, R_4, R_5 = H$
VI $R_1, R_2, R_3 = CH_3$; $R_4, R_5 = H$
VII $R_1 = NO_2$; $R_3 = CH_3$; $R_2, R_4, R_5 = H$
VIII $R_1, R_2, R_4, R_5 = H$; $R_3 = C_2H_5$
IX $R_1, R_2, R_4, R_5 = H$; $R_3 = C(CH_3)_3$
X $R_1, R_2, R_4, R_5 = CH_3$; $R_3 = H$

SCHEME 1

In the work presented here, a complete solution to the problem is not proposed. These studies demonstrate that the examination of a series of equilibria involving guest species which differ systematically in structural characteristics can give some insight into the details of the binding process. In particular, the association constants for a series of well-characterized dyes (**I–X**) (Scheme 1) with α-cyclodextrin have been measured carefully, accounting for aggregation of the free dye and for multiple dye–α-cyclodextrin equilibria. In nine cases, enthalpies and entropies of binding were determined, and in four cases variations of the association constant with solvent composition (water–dimethyl sulfoxide) were determined. An analysis of the data suggests that a simple Born type of electrostatic interpretation of the interaction of a charged group with the cyclodextrin is inadequate to explain all of the results. The data also suggest that interaction of the guest with a structured hydration shell in the region of the primary hydroxyl groups of the sugar may be important. The work clearly shows that, although the range of association constant values is not great, small changes in the constant can be readily interpreted.

RESULTS

Dyes such as the ones studied here are known to aggregate in aqueous solution, even at disconcertingly low concentrations (*3*). Since such aggregation would affect any calculation of association constants for the dye–cyclodextrin system, a study of the dyes alone was conducted. The uv–visible spectra of the dyes were measured over a wide range of concentrations, and the molar absorptivities were found to be a strong function of concentration at higher concentrations. At lower dye concentrations dimerization was assumed, and dimerization constants were calculated for each dye, these being listed in Table 1.

Based on these constants, inclusion complex formation was studied in a dye concentration range where only the monomeric form of the dye was present. That the single species present at the lowest concentrations was the monomer is supported by the fact that the cyclodextrin–dye binding data were readily interpret-

TABLE 1

DIMERIZATION CONSTANTS OF DYES STUDIED IN WATER[a]

Dye	$K \times 10^{-1}$	Dye	$K \times 10^{-1}$
I	84	VI	1300
II	280	VII	404
III	120	VIII	330
IV	85	IX	2.5
V	420	X	350

[a] pH 7, ionic strength 0.15; units of K are M^{-1}.

able under that assumption but not under the assumption that, for example, the dimer was present as the sole form.

Association constants for the dye–cyclodextrin equilibria were determined spectrophotometrically and are listed in Table 2. The association constant is defined as being the equilibrium constant for the reaction

$$CD + X \rightleftharpoons CD \cdot X$$

where CD is the cyclodextrin molecule, X the guest molecule, and CD · X the complex. Also listed in some cases are constants for the binding of a second α-cyclodextrin molecule to the existing complex, and enthalpies and entropies of binding. In those cases where a second α-cyclodextrin molecule bound to the dye, care was taken to verify that each constant was determined in a concentration range where only one of the equilibria was important. Both constants and measured values for the molar absorptivities were then used to reproduce the entire absorption–concentration dependency; and, in that region where both equilibria were important, the calculated absorbance agreed with the measured absorbance within the experimental error. An error analysis indicates that the constants are known to about 20%.

DISCUSSION

While a change in the interaction with the solvent is the driving force for the binding of any guest into the cavity of the cyclodextrin, steric factors are also critical. If a potential guest molecule is too large, it does not bind. Molecular models and calculations using crystallographic data (*4*) indicate that the cavity in α-cyclodextrin is just large enough to accommodate (and allow to pass through) a benzene ring. Experimental work with azo dyes also shows that monosubstituted benzene rings can also pass through the cavity but with greater difficulty largely reflected in rate constants for binding rather than in equilibrium constants (*5*, *6*). Thus dye **I** binds to cyclodextrin, but dye **X** does not because of the methyl groups on both rings. Inspection of space filling models indicates that dyes **II**–**IX** can enter the cyclodextrin cavity only if the carboxylate end enters first. Given that mode of entry, a methyl group in the 2′ position (the primed ring being the phenolic ring) will prevent deep penetration of the dye into the cavity and prevent

TABLE 2

ASSOCIATION CONSTANTS AND THERMAL PARAMETERS FOR THE BINDING OF DYES TO α-CYCLODEXTRIN

Dye	Solvent	$K_{25} \times 10^{-2}$	$K_{35} \times 10^{-2}$	$\Delta H°$	$\Delta S°$
I	H_2O	134[a]	64.5	−13.4	−25.9
II	H_2O	730[b]	444	−9.12	−8.32
III	H_2O	223[c]	127	−10.2	−14.3
	5% DMSO[d]	30.9			
	10% DMSO	26.7			
	20% DMSO	6.67			
	30% DMSO	4.01			
IV	H_2O	15.9	11.7	−5.69	−4.42
	5% DMSO	11.7			
	10% DMSO	9.43			
	20% DMSO	4.15			
	30% DMSO	2.72			
V	H_2O	12.4	9.90	−4.22	0.00
VI	H_2O	12.6	9.90	−4.45	−0.74
VII	H_2O	8.00	5.85	−5.66	−5.70
VIII	H_2O	4.69	4.07	−2.62	3.44
	5% DMSO	3.55			
	10% DMSO	3.13			
	20% DMSO	2.12			
	30% DMSO	1.57			
IX	H_2O	1.65	1.56	−1.14	6.33
	5% DMSO	1.65			
	10% DMSO	1.58			
	20% DMSO	1.50			
	30% DMSO	1.52			
X	No binding observed				

Note. Association constants are given in units of M^{-1}. $\Delta H°$ is in kcal/mole and $\Delta S°$ is in cal/deg mol. All constants refer to pH 7.0 and ionic strength 0.15.

[a] Constant for the binding of a second cyclodextrin, 150 M^{-1}.

[b] Constant for the binding of a second cyclodextrin, 300 M^{-1}.

[c] Constant for the binding of a second cyclodextrin, 660 M^{-1}.

[d] Percent by volume.

groups in the 3′ and 5′ positions from coming into contact with the sugar. Thus it is expected that groups in these positions will not have any effect on the stability of the complex except perhaps through electronic effects transmitted through the ring system. This is born out in dyes **IV–VI**, which have comparable association constants. Dye **VII** also has a similar steric requirement, but the nitro group seems to have a further influence on the binding constant. Dyes **III**, **V**, **VIII**, and **IX** differ in the size of the substituent at the 2′ position; their stability constants

reflect this size variation, becoming smaller as the size of the substituent becomes larger.

This view of steric effects is supported by the fact that dyes **I–III** bind with a second α-cyclodextrin molecule. This is due to the absence of a restrictive group at the 2′ position.

An interpretation of the detailed variation in the association constants remains. One view is simply as follows. The interior of the α-cyclodextrin cavity is a region of low dielectric constant compared with that of the bulk solvent. Placing a charged group within the cavity amounts to moving it from a region of high to low dielectric constant, an unfavorable process. Using the Born equation, the ΔG° for transferring a singly charged ion from water to dioxane is approximately 50 kcal/mol. Space filling models show that dye **X** would exist with the carboxylate group well inside the cavity. This particular substrate fails to bind. Dyes **I–III** can exist bound to the cyclodextrin with the carboxylate having passed through and completely protruding from the cavity, the solvation of this group then being essentially the same in the complex as in the free dye. Dyes **IV** (along with **V** and **VI**), **VIII**, and **IX** possess a group in the 2′ position preventing complete passage of the charged group through the cavity. As the size of this group gets larger going from methyl to t-butyl, the charged group is drawn closer to the low dielectric cavity. The expected decrease in stability is found.

The ΔH° and ΔS° values are also consistent with this interpretation. Based on the Born equation, ΔH° and ΔS° should both be zero if the carboxylate group is in the same dielectric environment in the free dye as in the complexed dye. As the charged group moves from a region of high to a region of lower dielectric constant, ΔH° becomes increasingly positive and ΔS° likewise becomes increasingly positive. An examination of these thermal parameters for dyes **III**, **V**, **VIII**, and **IX** shows the trend consistent with a carboxylate group established closer and closer to the low dielectric cavity. (Only the trend is significant. ΔH° and ΔS° would not actually be expected to be zero in the case of dye **III**, simply because other factors are also contributing to the absolute values of these quantities.)

Extending this simple picture to an explanation of the variation of the association constant with solvent composition meets with failure. In the case of dye **III**, which has no substituent in the 2′ position, the carboxylate group should be in the same dielectric environment in the free dye as in the complex; and so the binding constant should not be sensitive to variation in the dielectric medium. Dye **IX** experiences the greatest change in dielectric medium on going from the free to the complexed form, since the charged group is closer to the cavity and should show the greatest dependence on change in solvent composition. Dyes **V** and **VIII** should be intermediate in behavior. Examination of the data in Table 2 shows that the exact opposite effect is obtained, dye **III** showing the greatest sensitivity to solvent composition and dye **IX** the least. Clearly a simple electrostatic picture fails to rationalize all of the results.

A different picture of the results can be obtained as follows. Assume that the binding reaction involves water molecules, that is,

$$\mathrm{CD} + \mathrm{X} + n\mathrm{H_2O} \rightleftharpoons \mathrm{CD \cdot X \cdot} n\mathrm{H_2O}$$

with

$$K = K_a/C_{H_2O}^n$$

where K_a is the binding constant reported in Table 2. Note that n actually represents the addition of water to the already solvated cyclodextrin and dye molecules, that is, it represents an increase in bound water. (Competitive binding with DMSO is not included in this model for the simple reason that doing so demands the use of the unknown value of the DMSO binding constant. Since such a modification would affect all four systems in exactly the same way, the relative ordering of the increase in bound water would not be affected.) Using the results of the solvent variation study, a plot of ln K_d vs ln C_{H_2O} gives a value of n from the slope. The results are **III**, $n = 9$; **V**, $n = 5.2$; **VIII**, $n = 3.5$; and **IX**, $n = 0.3$. Thus the binding of **III** to α-cyclodextrin is accompanied by a net increase in the number of water molecules bound to the complex over the number bound to the free dye and free cyclodextrin. The same is true but by a diminishing amount for the other dyes. Since the increase in bound water molecules should be accompanied by an increase in the number of hydrogen bonds, the ΔH° for the series should decrease as the number of bound water molecules decreases and the ΔS° should become less negative. The data in Table 2 clearly behave this way.

These limited results lead us to propose the following picture of the binding process. Evidence exists for hydrogen-bonded interactions of water with carbohydrates (*7*). The hydration number of glucose is about 6. It is expected that the hydration number of cyclodextrin will be about 30 to 40. The interaction of a specific sugar with solvent is a function of the position of the hydroxyl groups (*8*), hydration being enhanced by conformations which place the hydroxyls into positions compatable with the structure of solid water. Since cyclodextrin has been reasonably shown to go from a configuration with considerable twisting of glucose units inward toward the cavity to a more symmetrical form upon formation of a complex (*9*), it is expected that the orientation of the primary hydroxyl groups will be quite different in the complexed sugar from that in the free form. When a dye enters the cavity, the carboxylate protrudes into the region of the primary hydroxyl groups incorporating itself into the hydration structure in that region. As the carboxylate is drawn toward the cavity by increasing the size of the group in the 2′ position, its ability to incorporate itself into this hydration structure is affected adversely, reflected in the decreasing number of water molecules bound and the lower value for the association constant.

Naturally, this picture does not explain every detail of the inclusion process. Electrostatics must be important for it has been shown in kinetic studies that the passage of a charged group through the cavity takes place about a 1000 times more slowly than the corresponding neutral species (*5*, *6*). The van der Waals interactions (*10*) and solvation changes in the regions of the secondary hydroxyl and within the cavity itself must also be important in determining the overall stability. Certainly, the dimethyl sulfoxide cannot merely be viewed as a diluent. Its specific solvation properties must be taken into account. What is suggested here is that specific interactions in the region of the primary hydroxyls may be significant in explaining differences in stability. Advantage will be taken of the diverse number

of dyes which can be prepared and used as probes of the interactions exterior to the cavity.

EXPERIMENTAL

α-Cyclodextrin was purchased from Aldrich Chemicals and purified by precipitating the sugar as the cyclohexane complex from a water–cyclohexane two-phase mixture, redissolving and boiling to drive off the cyclohexane and crystallizing from water. The azo dyes were synthesized using standard azo coupling methods from readily available phenols and anilines. Dyes were recrystallized from ethanol–water mixtures. Purity was checked by thin-layer chromatography using activated silica gel as the stationary phase and a solvent of pH 4.7 acetate buffer, methanol, chloroform, and ethylacetate in a ratio of 2.5:9:61.5:27. A single colored spot was observed which also was the only spot under uv light. The R_f values ranged from 0.32 to 0.52. Dyes were dried at 120°C for 10 to 15 hours. C, H, and N analyses were performed by Galbraith Laboratories. *Anal.* Calcd for **I**: C, 64.46; H, 4.16; N, 11.56. Found: C, 64.54; H, 4.20; N, 11.55. Calcd for **II**: C, 50.19; H, 2.59; N, 9.00. Found: C, 50.32; H, 2.66; N, 8.90. Calcd for **III**: C, 66.66; H, 5.22; N, 10.36. Found: C, 66.65; H, 5.42; N, 10.21. Calcd for **IV**: C, 65.62; H, 4.72; N, 10.93. Found: C, 65.80, H, 4.86; N, 11.03. Calcd for **V**: C, 66.66; H, 5.22; N, 10.36. Found: C, 66.56; H, 5.37; N, 10.32. Calcd for **VI**: C, 67.59; H, 5.67; N, 9.85. Found: C, 67.53; H, 5.79; N, 9.69. Calcd for **VII**: C, 55.82; H, 3.68; N, 13.95. Found: C, 55.94; H, 3.66; N, 13.60. Calcd for **VIII**: C, 66.66; H, 5.22; N, 10.36. Found: C, 66.67; H, 5.26; N, 10.10. Calcd for **IX**: C, 68.44; H, 6.08; N, 9.39. Found: C, 68.04; H, 6.02; N, 9.69. Calcd for **X**: C, 68.44; H, 6.08; N, 9.39. Found: C, 68.63; H, 6.18; N, 9.07. NMR spectra taken on a Perkin–Elmer Model R32 in d_6 acetone or d_6 acetone–d_6 dimethyl sulfoxide were consistent with expected spectra.

The aggregation of the dyes was studied by measuring the spectra of the dyes at wavelengths between 600 and 300 nm over a concentration range of 1.0×10^{-6} *M* to 1.0×10^{-4} *M*. The dimerization constants were determined at the lower concentrations. The failure of the equation for dimerization to explain the data over the entire concentration range suggests that higher aggregates are formed. Having established that the monomer predominates at the lowest concentration, this aspect of the dye behavior was not pursued in depth.

The association constants were determined by measuring the effect of the concentration of α-cyclodextrin upon the spectrum of the dye. The dye concentration was about 1.0×10^{-6} *M*. The cyclodextrin concentration was varied from 0.0 to 0.01 *M*. The change in the absorbance of the dye at the wavelength of its maximum absorbance (~350 nm) was used to determine the equilibrium constant. Calculations were performed using the Hildebrand–Benesi method (*11*). The pH was maintained at 7.0 and the ionic strength at 0.15 *M* by means of a phosphate buffer. (These conditions were also maintained in the aggregation study.) Spectra were measured in the thermostated cell block (±0.02) on a Cary 14 spectrophotometer. Dimethyl sulfoxide concentrations are stated as percent DMSO by volume.

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