Thermodynamic Study on the Effects of β-Cyclodextrin Inclusion with Anilinonaphthalenesulfonates

Gino C. Catena and Frank V. Bright*

Department of Chemistry, State University of New York at Buffalo, Buffalo, New York 14214

Thermodynamic parameters and stoichiometries for the binding of anilinonaphthalenesulfonates to β-cyclodextrin are obtained from steady-state fluorescence intensity and anisotropy measurements. Specifically, formation constant, enthalpy, and entropy values are obtained for complexes of β-cyclodextrin with eight different substrate molecules at five different temperatures and six different pH values, and their associated errors are given. We propose an explanation of the relative magnitudes of the values obtained with regard to the geometry of the substrate and the importance of the various noncovalent interactions responsible for the complexation.

INTRODUCTION

Cyclodextrins (CD's), or cycloamyloses, are toroidally shaped polysaccharides made up of six to eight D-glucose monomers connected at the 1 and 4 carbon atoms. The cavities of CD's are relatively hydrophobic and have an internal diameter of 4.7–8.3 Å (*1*). This difference in cavity size allows binding specificity to be tailored based on substrate size and geometry. Therefore, cyclodextrins are ideal molecules for the study of small ligand binding. Additionally, because of the hydrophobic character of the CD binding site, they are useful for studies directed at understanding, on a fundamental level, the binding of probe molecules to proteins and enzymes. Clearly, a thorough understanding of the stoichiometry and affinity of protein binding sites is integrally important in elucidation of protein function. Like protein–substrate binding, the primary factors in CD–guest complexes are hydrogen bonding and van der Waal's forces; however, other factors, such as strain-energy alleviation, may also be important in certain cases (*2*). Thus, one can see that cyclodextrin binding, like protein binding, is a complex phenomenon in which noncovalent molecular forces must be reconciled with conformational considerations in order that strong and specific binding results. As a result of their ability to host–guest complex, CD's have found numerous analytical and industrial applications. Several excellent review articles describing these increasingly important molecules have appeared (*1*, *3*). In addition, a number of papers have been published describing the analytical possibilities of this host–guest complexation. Indeed, the solubility enhancement of selected organic species of β-cyclodextrin (BCD) was reported as early as 1961 (*4*). ^{1}H NMR has been used to elucidate the exact conformation of cyclodextrins in solution (*5*). Armstrong and co-workers give a thorough review on the use of stationary-phase-bound CD's in the high-performance liquid chromatography (HPLC) separation of optical and structural isomers (*6*). Laufer and co-workers have used ^{13}C NMR to determine enthalpies and entropies of formation for some organic acids with α-cyclodextrin (*7*). A method for the fluorometric determination of trace amounts of scandium through the use of a complexing agent stabilized by BCD inclusion has recently appeared (*8*). Cline Love and co-workers employed cyclodextrins to enhance fluorescence and improve detection limits in the determination of licit and illicit drugs (*9*). A study of BCD–indole complexation has been performed by both absorbance and fluorescence lifetime measurements (*10*). In addition, Warner and co-workers (*11*, *12*) have studied the effects of alcohols on the inclusion complexation of cyclodextrins with polynuclear aromatic hydrocarbons. McGown and co-workers fluorometrically determined the thermodynamic constants for 4-amino-*N*-methylphthalimide–BCD complexation using phase-resolved fluorescence spectroscopy (*13*). Turro and Cox studied the effect of complex geometry on the formation constant in the case of methyl salicylate–cyclodextrin complexes (*14*). Weber et al. used poly(β-cyclodextrin–anilinonaphthalenesulfonate) complexes as a model for binding sites in protein complexes at high pressures (*15*).

Anilinonaphthalenesulfonates (ANS) are a family of substituted naphthalenes that have found extensive use as fluorescent probes for study of biologically active molecule structure (*16*). They have the advantage of intense visible fluorescence, with this fluorescence being very sensitive to the probe's local microenvironment. They are moderately soluble in water but would ideally prefer a more hydrophobic environment.

In this work, we use steady-state fluorescence spectroscopy to elucidate the effect upon BCD binding of slight changes in structural conformation within a family of these ANS probes. Results are shown for the determination of the various thermodynamic parameters (*K*, enthalpy, and entropy) and effects of pH on the aforementioned complexes presented. In addition, it is shown that the choice of probe molecule critically affects the stoichiometry of the resulting complex, and an attempt to correlate probe molecule geometry to the observed thermodynamic values is made.

EXPERIMENTAL SECTION

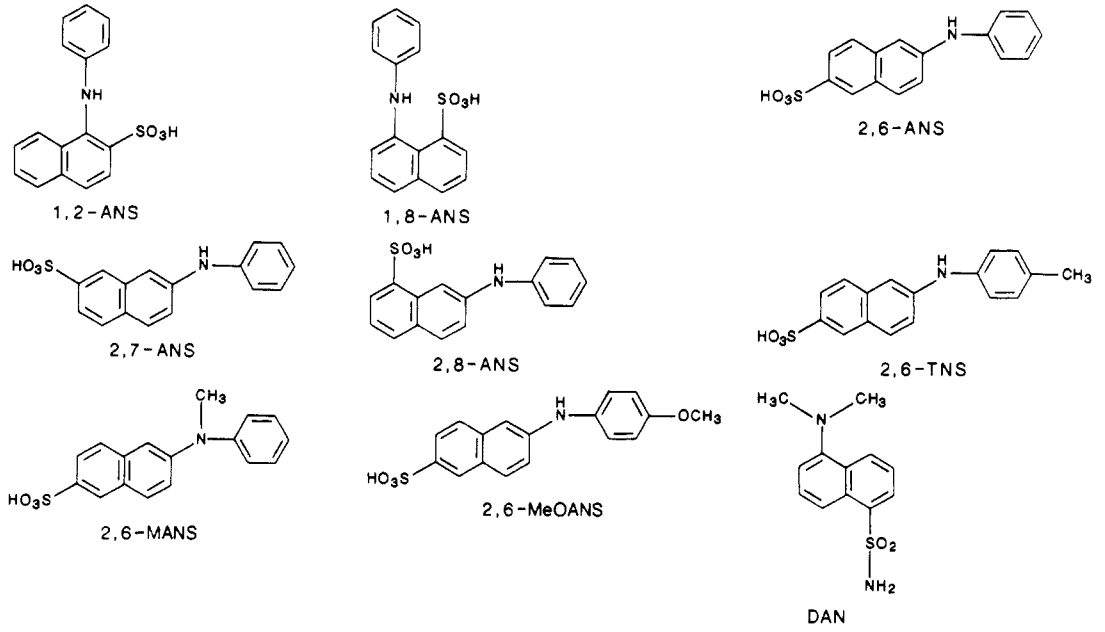
All steady-state fluorescence measurements were made with a Perkin-Elmer LS-3 fluorescence spectrometer or an SLM 48000. Excitation and emission wavelengths were optimally chosen for each probe; excitation wavelengths varied from 300 to 450 nm, while emission wavelengths varied from 400 to 550 nm. The sample chamber on the LS-3 was modified to accommodate an in-house-designed thermostated cuvette holder, controlled to ±0.1 °C via a Lauda RL6 temperature circulator.

All buffers utilized were 0.1 M and were used within 2 weeks of their preparation. These buffers included sulfate (pH 2.5), acetate (pH 4.0), phosphate (pH 6.0, 7.0), and carbonate (pH 9.5, 11.0). Stock solutions of BCD (Sigma) and fluorophores were prepared fresh each day, the former being prepared from the various buffer solutions. Dansylamide (DAN) was obtained from Aldrich; 2-(*p*-toluidinyl)naphthalene-6-sulfonic acid (2,6-TNS), 2-anilinonaphthalene-6-sulfonic acid (2,6-ANS), 2-anilinonaphtalene-7-sulfonic acid (2,7-ANS), 1-anilinonaphthalene-2-sulfonic acid (1,2-ANS), 1-anilinonaphthalene-8-sulfonic acid (1,8-ANS), 2-(*p*-anisidinyl)naphthalene-6-sulfonic acid (2,6-MeOANS), 2-(*N*-methylanilino)naphthalene-6-sulfonic acid (2,6-MANS), and 2-anilinonaphthalene-8-sulfonic acid (2,8-ANS) were obtained from Molecular Probes (Table I). All reagents were used as received.

Sample concentrations of BCD ranged from zero BCD up to 0.01 M. Fluorophore concentrations in each sample were always

0003-2700/89/0361-0905$01.50/0

Table I. Structures of the Probe Molecules Used in This Study



10^{-5} M. At this fluorophore concentration, the solutions were free of primary and secondary inter-filter affects. For the pH studies, a constant temperature of 25.0 °C was used in conjunction with solutions of pH values listed at the top of the previous paragraph. In the case of the temperature studies, temperatures of 5.0, 15.0, 25.0, 35.0, and 45.0 °C were used.

RESULTS AND DISCUSSION

The underlying theory for employing steady-state fluorescence intensities to calculate equilibrium constants has been previously described (*13*). Briefly, for a simple 1:1 (host:guest) complex, S is taken to represent the fluorescent substrate, and B is the binder (BCD), then the equilibrium can be written

$$S + B = SB \quad (1)$$

The equilibrium constant, K, is then expressed as

$$K = [SB]/[S][B] \quad (2)$$

Substituting mass balance expressions for S and B, one obtains

$$K = [SB]/\{(C_S - [SB])(C_B - [SB])\} \quad (3)$$

where C_S is the analytical concentration of S and C_B is the analytical concentration of B. Because we always employ a large excess of BCD relative to substrate, we can assume that $C_B \gg [SB]$, thus, eq 3 becomes

$$K = [SB]/\{(C_S - [SB])(C_B)\} \quad (4)$$

The quantum yield expression for the complex is given by

$$Q_{SB} = F_{SB}/\{k_{SB}[SB]\} \quad (5)$$

where Q_{SB} is the quantum yield for SB, F_{SB} is the fluorescence intensity of SB, and k_{SB} is an instrumental constant. Multiplication of the latter equation by the former yields

$$C_S/F_{SB} = \{(Kk_{SB}Q_{SB})^{-1}(C_B)^{-1}\} + (k_{SB}Q_{SB})^{-1} \quad (6)$$

Thus, a reasonable estimate of K can be obtained from a plot of C_S/F_{SB} versus $1/C_B$, by simply dividing the intercept by the slope. Plots of this type are referred to as double reciprocal plots. Figure 1 illustrates a double reciprocal plot for 2,6-ANS complexed to BCD. The completely linear region for this plot is indicative of 1:1 complexation.

In the case of successive 2:1 complexation, we also have the additional stepwise equilibrium to consider

$$SB + B = SB_2 \quad (7)$$

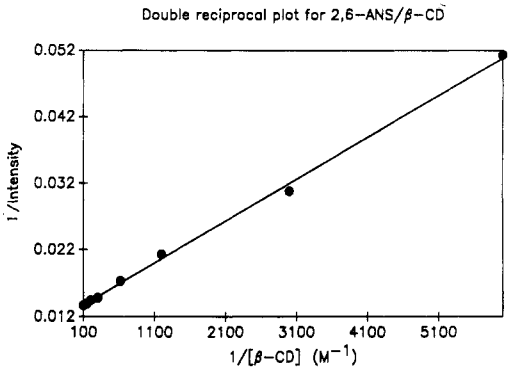


Figure 1. Double reciprocal plot for the 2,6-ANS/BCD complex. The plot is linear, indicating 1:1 complexation only throughout the concentration range of BCD used (0.0–0.01 M).

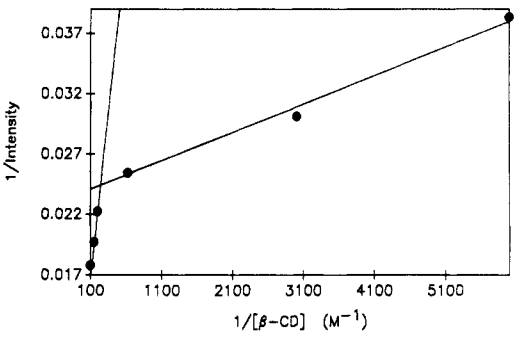


Figure 2. Double reciprocal plot for the 2,6-MANS/BCD complex. The plot exhibits two distinct linear portions, indicating 2:1, as well as 1:1 complexation.

In an analogous fashion to Figure 1, Figure 2 shows a double reciprocal plot for 2,6-MANS complexed to BCD. Clearly, the plot is not well described as a single straight line but is best described by two linear segments. The initial linear portion contains K_2 for the 2:1 (BCD_2–2,6-MANS) complex, while the final linear portion contains K_1 for the 1:1 (BCD–2,6-MANS) complex. The calculation of K_2 from the aforementioned double reciprocal plots is analogous to that for K_1 (*17*).

Unfortunately, while the more classical approach described above does work and often yields very good estimates of the equilibrium constants, it does not weight the data properly

Table II. Equilibrium Constants and Uncertainty (in Parentheses) Values at Five Different Temperatures for the Probe/BCD Complexes Studied (pH 7.0)

probe molecule	5 °C	15 °C	25 °C	35 °C	45 °C
2,6-ANS[a]	3010 (130)	2570 (30)	2080 (20)	1610 (100)	1260 (50)
2,7-ANS[a]	2080 (110)	1510 (90)	1290 (90)	970 (30)	760 (40)
2,8-ANS[a]	200 (10)	190 (9)	130 (8)	120 (10)	100 (10)
dansylamide[a]	120 (2)	100 (7)	60 (4)	40 (3)	30 (5)
1,8-ANS[a]	135 (11)	125 (8)	110 (4)	100 (6)	95 (7)
1,2-ANS[a,b]	nb	nb	nb	nb	nb
2,6-MeOANS[b]	1065 (81)	975 (63)	945 (24)	855 (35)	810 (51)
2,6-TNS[a,c]	2310 (101)	2220 (93)	1980 (84)	1700 (62)	1390 (83)
	600 (105)	590 (100)	600 (95)	680 (64)	670 (29)
2,6-MANS[a,c]	9290 (81)	7820 (97)	7360 (59)	6860 (104)	6330 (72)
	320 (90)	310 (61)	300 (43)	290 (28)	270 (51)

[a] Determined using steady-state intensity measurements. [b] Determined using steady-state anisotropy measurements. [c] Species which form 2:1 as well as 1:1 complexes are indicated as K_1 with the corresponding K_2 below it.

Table III. Enthalpies, Entropies, and Associated Uncertainties (in Parentheses) for the Formation of the Probe/BCD Complexes

probe molecule	10^3H, J/mol	S, J/(K mol)
2,6-ANS[a]	−16.2 (1.8)	8.8 (1.0)
2,7-ANS[a]	−18.0 (1.2)	−1.3 (0.2)
2,8-ANS[a]	−13.2 (1.4)	−3.0 (0.4)
dansylamide[a]	−26.3 (2.1)	54.2 (5.9)
1,8-ANS[a]	−6.9 (1.2)	16.1 (3.6)
1,2-ANS[a,b]	nb	nb
2,6-MeOANS[b]	−4.9 (0.5)	40.2 (0.5)
2,6-TNS[a,c]	−9.3, 29.9 (0.6), (12.5)	31.4, 63.6 (3.8), (28.1)
2,6-MANS[a,c]	−6.7, −1.1 (0.5), (2.8)	51.8, 43.8 (7.1), (28.2)

[a] Determined from steady-state intensity measurements. [b] Determined from steady-state anisotropy measurements. [c] Species which form both 2:1 and 1:1 are written as 1:1, 2:1.

(*18*). Specifically, double reciprocal plots place more weight on the lower (1/intensity) values than the higher ones. Thus, in order to accurately weight the data, it is necessary to employ a nonlinear regression package (*18*) and fit the experimental data directly. That is, we did not "rework" the data as done for the double reciprocal plots but instead directly fit the experimental data by using the equilibrium constants determined from the double reciprocal plots as "seed" values for the fits. It should be noted that the use of the nonlinear least-squares approach did not alter the values of the originally recovered equilibrium constants by more than ±5–15%. However, the nonlinear approach provided estimates of the equilibrium constants that were subsequently incorporated in the determinations of the enthalpy and entropy.

Of course, it is possible that an ANS probe molecule could bind to BCD and not induce any change in the spectral character of the probe. Thus, measurement of the intensity would not provide any insight into the thermodynamics of the complexation. However, if binding were to occur, a marked change in the fluorescence anisotropy (r) must always be noted (*19*). From measuring the anisotropy of the free probe in the absence of BCD (r_{free}), the bound probe with an excess of BCD (r_{bound}), and the anisotropy (r) at an intermediate BCD level, one can easily determine the equilibrium constant (*19*). Again, we employed this approach to generate seed values for the nonlinear least-squares analysis (*18*).

Table I illustrates the chemical structures for each of the fluorophores used in this study. A summary of the values of K and associated errors (95% confidence) at five different temperatures for each complex is given in Table II, and the enthalpies and entropies calculated from these experiments are subsequently listed in Table III. These were obtained via the classical method of plotting ln K versus $1/T$. In this case, the corresponding enthalpy and entropy are contained in the slope and intercept, respectively. In an analogous fashion to Table II, Table IV summarizes the K values for the complexes at five different pH values.

When the relative K values are viewed as a whole, taking into account substrate structure, it becomes clear that for binding to occur to even a slight degree, the naphthalene moiety must be sterically "free" to slip into the BCD cavity. Any substituent groups that tend to retard this process descrease binding affinity markedly. Furthermore, binding to BCD is especially facile when the BCD approaches the naphthalene moiety along an axis bisecting the carbon–carbon bond fusing the two rings. We will refer to this mechanism as "equatorial approach", and this is illustrated in Figure 3A. The other binding approach possible on the naphthalene moiety is illustrated in Figure 3B. In this scheme, which we refer to as "axial approach", the BCD approaches along the axis of the aforementioned bond. This complexation scheme

Table IV. Equilibrium Constant and Associated Uncertainty Values (in Parentheses) at Five Different pH Values for the Probe/BCD Complexes

probe molecule	pH 2.5	pH 4.0	pH 6.0	pH 9.5	pH 11.0
2,6-ANS[a]	2650 (125)	2560 (92)	2500 (84)	2270 (61)	1860 (75)
2.7-ANS[a]	2020 (201)	1370 (19)	1570 (144)	1480 (78)	1820 (61)
2,8-ANS[a]	260 (17)	310 (21)	200 (42)	230 (23)	70 (11)
dansylamide[a]	130 (8)	200 (12)	180 (17)	340 (21)	440 (15)
1,8-ANS[a]	110 (12)	120 (10)	130 (7)	120 (9)	210 (4)
1,2-ANS[a,b]	nb	nb	nb	nb	nb
2,6-MeOANS[b]	890 (46)	970 (81)	955 (34)	890 (39)	940 (62)
2,6-TNS[a,c]	2570 (125)	1920 (116)	2500 (100)	2250 (135)	2260 (96)
	240 (12)	350 (15)	340 (23)	270 (19)	340 (25)
2,6-MANS[a,c]	7970 (240)	7800 (310)	7700 (105)	7910 (196)	7730 (81)
	430 (28)	370 (74)	320 (16)	290 (82)	320 (14)

[a] Determined from steady-state intensity measurements. [b] Determined from anisotropy measurements. [c] Species which form 2:1 as well as 1:1 complexes are indicated as K_1 with the corresponding K_2 below it.

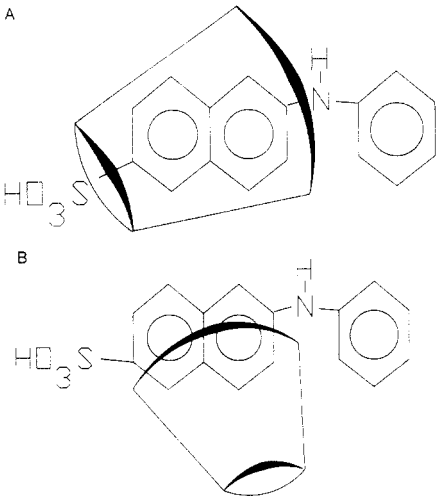


Figure 3. (A) "Equatorial" approach of an incoming BCD molecule to a 2,6-ANS molecule. This appears to be the favored mode of complexation. (B) "Axial" approach of an incoming BCD molecule to a 2,6-ANS molecule. This seems to result in a complex that is too "tight" to be energetically favored.

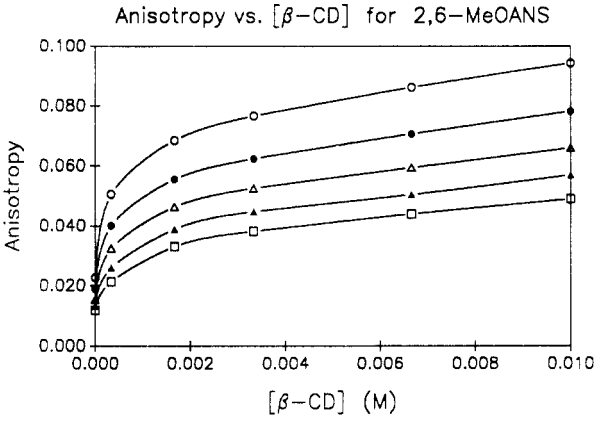


Figure 4. Steady-state anisotropy versus added BCD for 2,6-MeOANS at pH 7.0 and 5 °C (○), 15 °C (●), 25 °C (△), 35 °C (▲), 45 °C (□).

would result in a binding fit that is quite snug and, based on the dimensions of the naphthalene moiety and the BCD cavity, may strain the cyclodextrin backbone. We believe that the former mode is much more thermodynamically favored than the latter.

This mechanism is clearly supported by the behavior of 1,2-ANS and 2,6-MeOANS. Neither of these species showed evidence of binding based on steady-state intensity measurements alone. However, 2,6-MeOANS does indeed exhibit significant binding based upon steady-state anisotropy measurements (Figure 4). One can see from the structure of this compound (Table I) that no stearic hindrances exist which preclude the equatorial approach. The BCD fits over the naphthalene group nicely, with the charged sulfonate group extending into the bulk solution. The contrast, in the case of 1,2-ANS, equatorial approach is precluded by the presence of the large anilino group. Also, the energetically less favored axial approach is hindered by the positioning of the sulfonate group. It is for this reason, we suspect, that binding does not occur in the case of 1,2-ANS, as we observe no discernible effect of BCD concentration on anisotropy (Figure 5). However, as temperature increases, anisotropy decreases, reflecting the increased rotational motion of the free probe as solvent viscosity decreases.

Of the species studied, only 2,6-MANS (Figure 2) and 2,6-TNS (Table I) clearly exhibited both 1:1 and 2:1 complexation. Our observation of the formation of both a 1:1 and 2:1 complex for 2,6-TNS is in agreement with earlier publi-

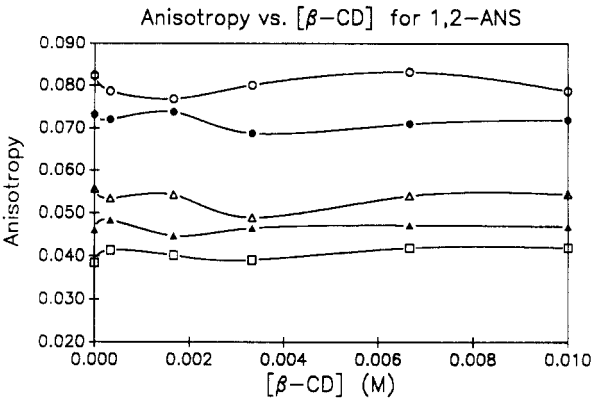


Figure 5. Steady-state anisotropy versus added BCD for 1,2-ANS at pH 7.0 and 25 °C, same symbolism as Figure 4.

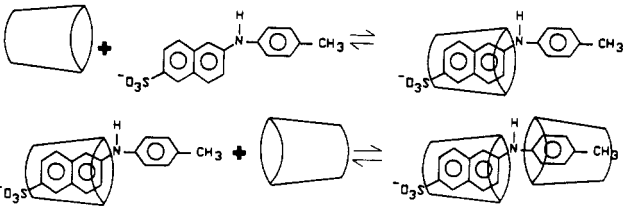


Figure 6. Schematic depiction of the route of BCD complexation with 2,6-TNS. Top equilibrium illustrates the formation of the 1:1 complex (K_1) via the favored "equatorial" approach. Bottom equilibrium illustrates the formation of the 2:1 complex (K_2) when appropriate.

cations on 2,6-TNS (*20*, *21*). In contrast, our results with 2,6-MANS do not agree with earlier literature reports (*22*) stating only 1:1 complexation. However, one can clearly see that the double reciprocal plot (Figure 2) exhibits two discrete linear regions. These experiments have been repeated at least a dozen times, using different batches of reagents, and 1:1 and 2:1 complexation is always evident. Moreover, any attempt to fit the data to a single equilibrium constant model resulted in systematically deviating residual errors between the experimental data and the calculated fit. The experimental details of the earlier results (*22*) are sketchy at best and do not indicate the BCD concentration range studied nor the concentration of 2,6-MANS employed, but inspection of Figure 1 from ref 22 implies that these earlier authors employed 6.5-fold less BCD than employed in the present study. Thus, there is the distinct possibility that these authors never used enough BCD to form the less favored 2:1 complex.

Invariably, the 2:1 complex must form on the anilino moiety. From this work, the key factor in the formation of 2:1 complex appears to be a methyl substituent on the anilino portion of the molecule. In the case of 2,6-TNS, the methyl group is located in the para position on the aniline moiety, while in the case of 2,6-MANS, the methyl is N-substituted. Thus, it would appear that the protection of the methyl substituent from the aqueous solvent afforded by BCD provides the added stabilization required for 2:1 complex formation. This leads us to believe that complexation in the more usual 1:1 case occurs over the naphthalene moiety rather than on the aniline portion, which is in agreement with the relative degree of hydrophobicity of the two functional groups.

Interestingly, for both 2,6-TNS and 2,6-MANS, the dependence of K on temperature is not as great in the case of the 2:1 complex as it is for the 1:1 complex. For this reason, we suspect that hydrogen bonding is not nearly as important in stabilizing the 2:1 complex as it is in the 1:1 case. If these complexes form as we propose, i.e., 1:1 complex forming first on the naphthyl group followed by 2:1 complex formation on the anilino functionality (Figure 6), then this is understandable. Hydrogen bonding from the tapered rim of the BCD to the deprotonated sulfonate group will help to stabilize the

complex more; however, this effect is absent in the case of the aniline moiety, as the methyl group is only weakly hydrogen bonding at best. Thus, in the case of these two species, both hydrogen bonding and the hydrophobic effect are important in the formation of the 1:1 complex, while hydrogen bonding plays only a minor role in the formation of the 2:1 complex. With 2,6-TNS, hydrogen bonding may still occur between the front end of the BCD to the amine function. In the case of 2,6-MANS, this may be sterically retarded by the methyl group on the nitrogen atom. This explanation would account for the slightly unfavorable enthalpy term for the 2:1 complex formation for 2,6-MANS.

Neither 2,6-TNS nor 2,6-MANS showed any clear trend in binding to BCD as a function of pH for either the 2:1 or 1:1 case. Apparently, an excess or deficiency in protons of the magnitudes which we studied (8.5 orders of magnitude) is not a sufficient perturbation to offset the other factors mentioned above. This is expected in the case of the acidic sulfonate but is somewhat puzzling in the case of the amine function. The reason for this is presently unclear.

2,6-ANS, 2,7-ANS, and 2,8-ANS are geometric isomers which differ only in that the sulfonate group is shifted between the 6, 7, and 8 position on the naphthalene portion of the molecule (Table I), respectively. Nonetheless, these three species exhibited strikingly different binding characteristics, as the position of the sulfonate group critically affects the extent of binding. In the case of 2,6-ANS, the sulfonate group is positioned such that the BCD can easily slip over it, onto the naphthalene, thus leaving the charged sulfonate in the bulk solution. The binding is most complete for 2,6-ANS and becomes successively poorer for 2,7-ANS and 2,8-ANS. This again supports our proposition that the BCD molecule prefers to approach the substrate equatorially (Figure 3A) as opposed to axially (Figure 3B). In the case of 2,6-ANS, the molecule is clearly more "linear", facilitating equatorial approach. As one moves on to 2,7-ANS, the sulfonate group is now in a position to somewhat hinder the equatorial approach. In the case of 2,8-ANS, one can see how the position of the sulfonate tends to fight and repel the equatorially approaching BCD. Thus, the only binding mechanism available in this case is the axial approach, and this is evidenced by the relatively low binding constants for 2,8-ANS. There is no alkyl substituent present on the aniline portion in this series, and accordingly, there is no evidence of 2:1 complexation throughout the BCD concentration range studied.

The enthalpies of formation are all comparable for each of these species; however, the entropy is favorable only in the case of 2,6-ANS. Apparently, it seems that the "linear" shape of the molecule allows for a stronger, more ordered solvent shell while the molecule is free in solution. Upon binding, this solvent shell is broken up, leading to the favorable entropy term. The entropy increase for this process is great enough to offset the entropy loss associated with the binding itself. In the case of 2,7-ANS and 2,8-ANS, the solvent shell may not be nearly as efficiently ordered due to the geometry of these species. Thus, the entropy gain is disrupting the solvent shell may not be sufficient to overcome the entropy loss which results from the binding.

With the exception of 2,7-ANS, these three isomeric species generally exhibited a decrease in K with increasing pH. As pH increases, the amine function becomes ionized to a greater extent, giving the species a formal charge of −2. This may tend to partially offset the desire on the part of the hydrophobic portion of the molecule to reside in BCD. In the case of 2,7-ANS, this trend was not so apparent. It should be noted that the sulfonate and amine functions are farthest apart in the case of 2,6-ANS. This may allow BCD to slip between the two localized charges and protect the naphthalene moiety in the process. This scenario clearly becomes increasingly unlikely as we move to 2,7-ANS and 2,8-ANS.

The last two structurally analogous compounds studied were dansylamide and 1,8-ANS. On the basis of their structure, it would appear that equatorial approach is sterically hindered in both of these cases. The relatively low K values for each of these species appears to support this observation. In the case of 1,8-ANS, we see that axial approach is sterically allowed, and this is most likely the mode of complexation. For dansylamide, both equatorial and axial mechanisms appear to be hindered; however, we do observe weak binding. In such a case, we assume that the dimethylamino function would be partially housed in BCD, as the BCD axially approaches the substrate from this side of the molecule. Neither compound changed its binding characteristics to any great extent upon varying pH.

ACKNOWLEDGMENT

We thank Kevin S. Litwiler for his contribution of Figure 3 and Gary Sagerman and Steve Palistrant for the construction of the thermostated cuvette holder used in this work.

LITERATURE CITED

(1) Saenger, W. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 344–362.
(2) Bender, M. L.; Komiyama, M. *Cyclodextrin Chemistry*; Springer-Verlag: Berlin, 1978.
(3) Pagington, J. S. *Chem. Brit.* **1987**, *23*, 5, 455–458.
(4) Schlenk, H.; Sand, D. M. *J. Am. Chem. Soc.* **1961**, *83*, 2312–2320.
(5) Glass, C. A. *Can. J. Chem.* **1965**, *43*, 2652–2659.
(6) Armstrong, D. W.; Alak, A.; Bui, K.; DeMond, W.; Ward, T.; Riehl, T. E. *J. Inclusion Phenom.* **1984**, *2*(3-4), 533–545.
(7) Laufer, D. A.; Johnson, R. F.; Fuhrman, H. S.; Cardelino, B.; Schwartz, L. M.; Gelb, R. I. *J. Am. Chem. Soc.* **1981**, *103*, 1750–1757.
(8) Gomez, J. C. M.; Lopez, M. H.; Sanchez, F. G. *Analyst* **1987**, *112*, 1037–1040.
(9) Cline-Love, L. J.; Grayeski, M. L.; Noroski, J.; Weinberger, R. *Anal. Chim. Acta* **1985**, *170*, 3–12.
(10) Ross, J. B. A.; Orstan, A. *J. Phys. Chem.* **1987**, *91*, 2739–2745.
(11) Warner, I. M.; Patonay, G.; Nelson, G. *Anal. Chem.* **1988**, *60*, 274–279.
(12) Nelson, G.; Neal, S. L.; Warner, I. M. *Spectroscopy* **1988**, *3*, 24–28.
(13) Bright, F. V.; Keimig, T. L.; McGown, L. B. *Anal. Chim. Acta* **1985**, *175*, 189–201.
(14) Turro, N. J.; Cox, G. S. *Photochem. Photobiol.* **1984**, *40*, 185–188.
(15) Weber, G.; Drickamer, H. G.; Torgerson, P. M. *Biochemistry* **1979**, *18*, 3079–3083.
(16) Haugland, R. P. *Molecular Probes Inc. Handbook of Fluorescent Probes and Research Chemicals* April, 1985.
(17) Hume, D. N.; Newman, L. *J. Am. Chem. Soc.* **1957**, *79*, 4571–4576.
(18) Leatherbarrow, R. J. *Enzfitter: A Non-linear Regression Data Analysis Program*; Elsevier Biosoft: Cambridge, UK, 1987.
(19) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*; Plenum: New York, 1983.
(20) Nazakura, S.; Furue, M.; Harada, A. *Polym. J.* **1980**, *12*, 29–33.
(21) Jobe, D. J.; Verrall, R. E.; Palepu, R.; Reinsborough, V. C. *J. Phys. Chem.* **1988**, *92*, 3582–3586.
(22) Sellskar, C. J.; Brand, L. *Science* **1971**, 799–800.

RECEIVED for review August 9, 1988. Accepted January 4, 1989. This work was supported by BRSG S07 RR 07066 awarded by the Biomedical Research Support Grant program, Division of Resources, National Institutes of Health, the donors of the Petroleum Research Fund, administered by the American Chemical Society, a New Faculty Development Award from the New York State/United University Professions, a Non-Tenured Faculty Grant from 3M, Inc., and a grant from the Health Care Instruments and Devices Institute at SUNY—Buffalo.