Supramolecular Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713649759

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To cite this Article Dunbar, Richard A. and Bright, Frank V.(1994) 'Comparison of inter- and intramolecular cyclodextrin complexes', Supramolecular Chemistry, 3: 2, 93 — 99
To link to this Article: DOI: 10.1080/10610279408029847
URL: http://dx.doi.org/10.1080/10610279408029847

SUPRAMOLECULAR CHEMISTRY, Vol. 3, pp. 93–99
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Comparison of inter- and intramolecular cyclodextrin complexes

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(Received February 19, 1993)

We present the first comparative steady-state and time-resolved fluorescence studies of inter- and intramolecular cyclodextrin complexes. Specifically, we report equilibrium and kinetic results for dansyl-glycine complexed with β-cyclodextrin (intermolecular) and the dansyl-glycine-β-cyclodextrin adduct (intramolecular). The fluorescence intensity decay profile for the intermolecular system is best described by a discrete triple exponential decay law. This is consistent with stepwise 1:1 and 2:1 (β-cyclodextrin:guest) inclusion complexation. Equilibrium constants are in line with previous results on similar species. In contrast, we found that the intramolecular case was described by a doubly exponential decay law—consistent with a single intramolecular inclusion complex. Displacement experiments, with borneol, confirm the simplicity of the intramolecular complex. In all cases, continuous distribution models failed to fit the experimental data.

INTRODUCTION

Cyclodextrins (CDs) are water soluble, torroidally-shaped polysaccharides that can include (host) a variety of solutes within their central cavity. The hydrophobic nature and size variability of this cavity has prompted numerous studies.[1-16] They have been suggested as enzyme mimics[5] and as models for protein-substrate binding.[6] In addition, because of their semi-selective host–guest interactions, CDs have found numerous applications in industry and analytical chemistry.[1,7-16]

Many aspects of CD-guest chemistry have been explored and exploited over the years. As examples, ^{1}H NMR has been used to determine the host–guest stoichiometry and thermodynamics.[9,10] Kamiya and co-workers have used induced circular dichroism to determine the orientation and position of benzene derivatives within the CD cavity.[11] Hurtubise and co-workers have studied solid-phase CD-guest complexes.[12] Armstrong and his associates have exploited CD selectivity to develop a new generation of chromatographic stationary phases.[13,14] The Warner group has reported extensively on the steady-state and time-resolved fluorescence of intermolecular fluorophore-CD complexes.[15,16]

Our group has also been investigating the complexation of cyclodextrins with fluorescent guests. Initially we reported on the thermodynamics of inclusion complexes formed between β-cyclodextrin (β-CD) and a family of substituted anilinonaphthalene sulfonates (ANS).[2] More recently we extended this work and demonstrated that the time-resolved intensity decay for these same systems were best described by continuous distributions of lifetimes.[17,18] We also showed how surface-immobilization of β-CD could be used for development of simple chemical sensors[19] and that the probe decay kinetics were influenced by β-CD surface immobilization.[20]

In the past, CDs have been used in analytical chemistry to improve fluorescence and phosphorescence detection limits.[12,15,16,19] Unfortunately, such improvements are not realized if the guest (target analyte) does not undergo some measurable spectral change. For example, if the guest does not contain a convenient chromophore or if it binds to the CD, but there is no spectral change, one would not expect to see an improvement in detection limits. Thus, although CD are moderately selective[1-20] and may even complex with a particular analyte, one will not improve detection limits if the analyte is non-luminescent.

Ueno and co-workers recently synthesized an array of tethered cyclodextrins containing a fluorescent center.[21] These species are intriguing because they form presumably an intramolecular complex whereby the tethered fluorophore includes in its own CD cavity. This species can then be displaced selectively from the cavity. Thus, one has a means to quantify non-fluorescent species (the displacer) by following changes

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in the luminescence from the CD-tethered fluorescent center. (These results should be contrasted with recent work by Shinkai and co-workers using bichromophoric species.[22])

In this paper, we report the first comparative steady-state and time-resolved fluorescence studies on inter- and intramolecular cyclodextrin complexes. Specifically, we present new equilibrium and kinetic results for dansyl-glycine complexed with β-cyclodextrin adduct (intramolecular).

THEORY

Assume that an environmental sensitive fluorophore, like dansyl,[23] is located simultaneously in a finite number (n) of discrete microenvironments. The corresponding time-resolved fluorescence intensity ($I(t)$) can be described by a multiple exponential decay of the form:[24-26]

$$I(t) = \sum_{i}^{n} \alpha_i e^{-t/\tau_i} \tag{1}$$

where α_i is a pre-exponential factor for component i having excited-state lifetime τ_i. (Under conditions where the absorbance spectra change little α_i is a direct measure of the mole fraction of component i). In the current experiments, the measurables are the frequency-dependent (ω) phase shift ($\theta_m(\omega)$) and modulation ($M_m(\omega)$).[24-26] These are related to the model kinetic parameters (α_i and τ_i in eqn. (1)) by the sine and cosine Fourier transforms:

$$S(\omega) = \int_0^\infty I(t) \sin(\omega\tau)\, dt \tag{2}$$

$$C(\omega) = \int_0^\infty I(t) \cos(\omega\tau)\, dt \tag{3}$$

where the calculated values for $\theta_c(\omega)$ and $M_c(\omega)$ are given by:

$$\theta_c(\omega) = \tan^{-1} \frac{S(\omega)}{C(\omega)} \tag{4}$$

$$M_c(\omega) = \sqrt{S(\omega)^2 + C(\omega)^2} \tag{5}$$

The goodness of fit between the experimental measurables ($\theta_m(\omega)$ and $M_m(\omega)$) and the model eqn. (1) are determined by nonlinear least squares minimization of the chi-squared (χ^2) function:

$$\chi^2 = \frac{1}{D}\sum_{\omega}\left(\frac{\theta_m(\omega) - \theta_c(\omega)}{\sigma_\theta}\right)^2 + \frac{1}{D}\sum_{\omega}\left(\frac{M_m(\omega) - M_c(\omega)}{\sigma_M}\right)^2 \tag{6}$$

where σ_θ and σ_M are the standard deviations in phase and modulation, respectively, and D is the number o degrees of freedom. If the standard deviation in phas and modulation reflect the actual uncertainty, th goodness of fit between the experimental data an model is judged by the closeness of χ^2 to unity an random distribution of the residuals about zero.

RESULTS AND DISCUSSION

Dansyl-glycine/β-cyclodextrin intermolecular complexation steady-state fluorescence

Steady-state fluorescence is commonly used to study host–guest binding. Figure 1 shows typical static emission spectra for dansyl-glycine in water (lower trace) and in the presence of 14 mM β-CD (upper trace). These results show there is significant increase in the fluorescence due to association of the dansyl-glycine within the CD.[12,15,16,19,20] In addition, there is a significant blue shift upon association. These results are indicative of the dansyl-glycine moving from a hydrophilic (water) to hydrophobic environment (β-CD cavity).[12,15,16,19,20,27]

To more clearly illustrate the binding of dansyl-glycine to β-CD, Fig 2 shows the integrated fluorescence as a function of added β-CD. In simple cases, this type of data can be used to estimate the system equilibrium constants.[2,15,16] Qualitatively, one can see that the average equilibrium constant is fairly small. For example, at 15 mM β-CD the concentration ratio of β-CD to dansyl-glycine is 30,000; however, not all the dansyl-glycine is complexed by β-CD (i.e. the intensity has not levelled off). Thus a complete saturation curve is not obtained. Fortunately, Deranlequ[28] has shown that the most important data for recovering small equilibrium constants lies between the 20 and 80% of the saturation curve.

A double reciprocal or Benesi-Hildebrand plot is commonly used to estimate simple (1:1) equilibrium

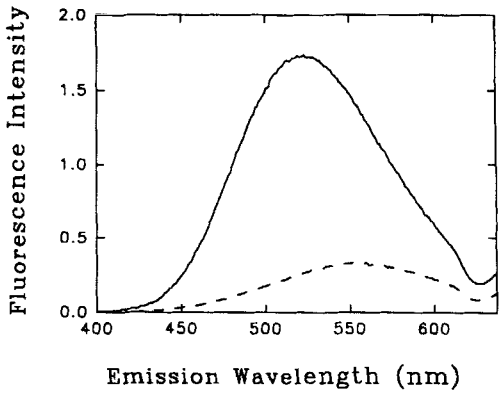

Figure 1 Steady-state fluorescence spectra of 0.5 μM dansyl-glycine with (——) and without (– – –) 14.0 mM β-CD.

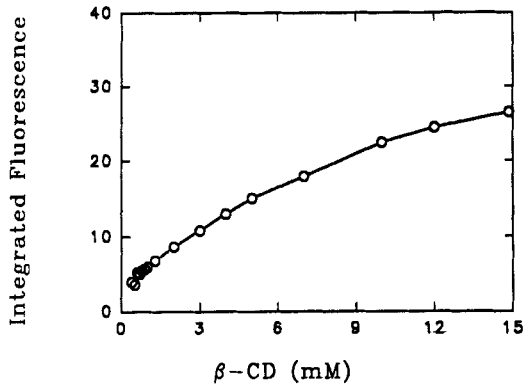

Figure 2 Integrated fluorescence intensity (515–525 nm) of 0.5 μM dansyl-glycine as a function of added β-CD.

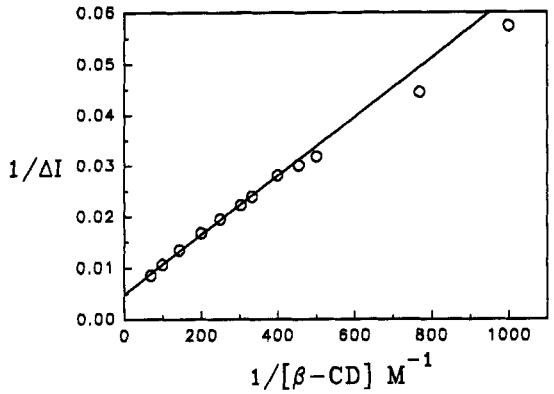

Figure 3 Double reciprocal plot of 0.5 μM dansyl-glycine titrated with β-CD.

constants.[2,29] Figure 3 shows such a plot for the complexation of dansyl-glycine with β-CD (T=25 °C). Our data do not fall on a straight line, indicating the equilibrium is more complicated than a simple 1:1 complex.

In an effort to determine the system stoichiometry, we carried out a continuous variation or Job's analyses[30] on the dansyl-glycine/β-CD system (Fig 4). In this approach, the fluorescence intensities are measured for a series of solutions in which the sum of the analytical concentrations of dansyl-glycine and β-CD is constant, and their ratio is varied continuously. If one forms a simple 1:1 complex, the Job's plot would peak at 0.5. Similarly, if the complex was 2:1 (host:guest), a peak would be seen at 0.33. Our experimental results peak somewhere between the fraction 0.33 and 0.5 (Fig 4). This is indicative of a more complex equilibrium.

Time-resolved fluorescence

Steady-state fluorescence provides information on the average emission. Unfortunately, it cannot be used routinely to extract information on the individual species contributing to the observed emission. Time-resolved fluorescence is well suited to this task. Thus, in an effort to better understand the system equilibria, we carried out a series time-resolved fluorescence experiments on dansyl-glycine as a function added β-CD.

Figure 5 shows a typical multifrequency data set for 0.50 μM dansyl-glycine in the presence of 14.5 mM β-CD. (The actual data file consists of 15 such traces acquired as a function of added β-CD.) The solid points are the experimental data and the traces represent the best fits to various kinetic models.[17,18,24,31] Table 1 summarizes the fits of the multifrequency data to the

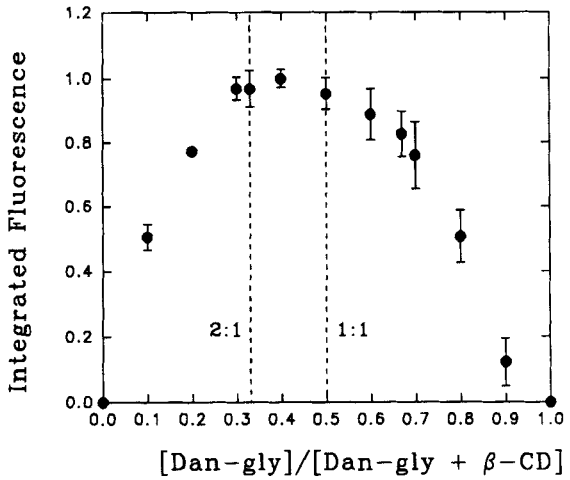

Figure 4 A Jobs plot for the intermolecular dansyl-glycine/β-CD system. Concentrations of dansyl-glycine and β-CD ranged from 0 to 100 μM to yield fractions 0 to 1.0. The fluorescence spectra were integrated from 400–500 nm.

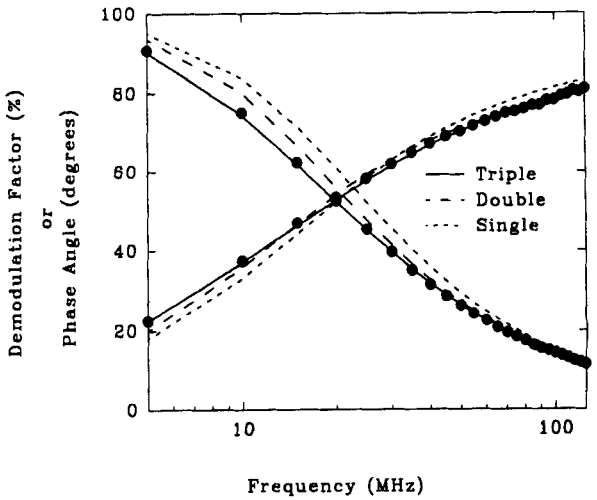

Figure 5 Typical multifrequency phase and modulation data for 0.5 μM dansyl-glycine with 14.5 mM β-CD (T=25 °C). Fits to a single, double, and triple exponential decay laws are shown. Laser excitation 363.8 nm. Emission monitored using a 420 nm LP filter.

different decay models. (Note: We have purposely omitted the recovered pre-exponential factors, 15 per set, for clarity.) These particular data sets were analyzed globally[18,31] to obtain a self-consistent set of kinetic parameters. That is, the excited-state lifetimes were linked throughout the analysis and the pre-exponential factors allowed to float. The χ^2 term reported in Table 1 represents the global (overall) values for the simultaneous analysis of 15 data files (717 degrees of freedom).

Table 1 Recovered lifetime parameters for dansyl-glycine/β-CD[a]

Model[b]	τ_1 *(ns)*	τ_2 *(ns)*	τ_3 *(ns)*	*W*[c] (ns)	*Global*[d] χ^2
D	10.0				29.6
DD	2.3[e]	11.9			3.00
DDD	2.3[e]	7.1	16.9		0.48
GD	2.3[e]	8.3		6.2	2.74
LD	2.3[e]	9.3		5.2	2.69

[a] The recovered pre-exponential factors are omitted for clarity of presentation.
[b] Model type: D—single discrete; DD—double discrete; DDD—triple discrete; GD—Gaussian with a discrete component; LD—Lorentzian with a discrete component.
[c] Width term associated with a continuous Gaussian or Lorentzian lifetime distribution.[16,17]
[d] Global χ^2 from the simultaneous analysis of 15 multifrequency data sets at various β-CD concentrations.
[e] Determined from separate experiment without β-CD.

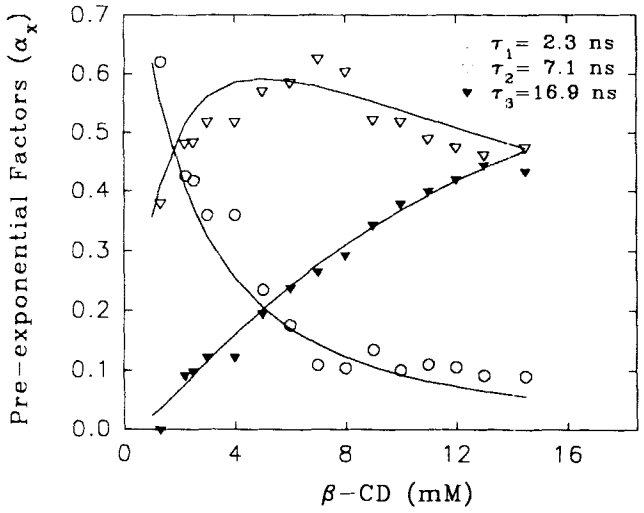

Figure 6 Pre-exponential factor of each lifetime component in a triple exponential decay law as a function of added β-CD. The concentration of dansyl-glycine was 0.5 μM. The traces represent fits to a stepwise 1:1 and 2:1 (β-CD:dansyl-glycine) equilibria.

Inspection of the results presented in Table 1 allow us to eliminate the single exponential decay (D) law as an accurate description of the system decay kinetics ($\chi^2 = 29.6$). Similarly, we can eliminate the double discrete (DD) and continuous distributions (GD and (LD) based on the large χ^2. Based on these results we conclude that the dansyl-glycine/β-CD system is best described by the discrete triple exponential decay law (DDD).

Recovery of equilibrium constants

The time-resolved fluorescence results demonstrate that the dansyl-glycine/β-CD system consists of three discrete emissive centers which contribute to the total fluorescence. This could easily be explained by two different 1:1 complexes or a stepwise 1:1 followed by a 2:1 complex (β-CD:dansyl-glycine) and the free dansyl-glycine. Thus, it becomes important to distinguish between these two different schemes. Fortunately, because the molar absorptivity of dansyl-glycine is unaffected by changes in the physicochemical properties of its surrounding environment, the recovered pre-exponential factors (α_i) associated with each lifetime (τ_i) are related directly to the mole fraction of each species.[32,33] That is, α_i is a direct measure of the distribution of the dansyl-glycine species. Therefore, the time-resolved data can be used to: (1) distinguish between the possible stoichiometries and (2) extract the associated equilibrium constants.[32,33]

Figure 6 presents the recovered pre-exponential factors for as a function of added β-CD. The symbols denote the experimental data and the traces represent the theoretical fit based on the stepwise model given in Fig 7. The corresponding formation constants are written:[32,33]

$$K_1 = \frac{\alpha_2 C_{dan-gly}}{\alpha_1 C_{dan-gly}[[\beta\text{-CD}] - \alpha_1\alpha_2 C_{dan-gly}]} \quad (7)$$

$$K_2 = \frac{\alpha_3 C_{dan-gly}}{\alpha_1 C_{dan-gly}[[\beta\text{-CD}] - \alpha_1\alpha_2 C_{dan-gly}]} \quad (8)$$

where α_i is the mole fraction of dansyl-glycine in form i and $C_{dan-gly}$ represents the analytical concentration

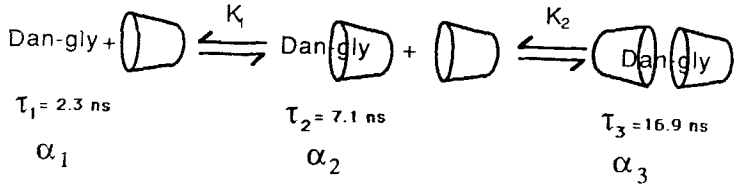

Figure 7 Equilibrium model for the intermolecular dansyl-glycine/β-CD system.

Table 2 Recovered lifetime parameters for the dansyl-glycine appended β-cyclodextrin

Model[a]	τ_1 *(ns)*	τ_2 *(ns)*	τ_3 *(ns)*	α_1	α_2	α_3	χ^2
D	13.5			1.00			44.6
DD	5.6	16.9		0.02[b]	0.98[b]		0.38
				0.57[c]	0.43[c]		
DDD	13.4	21.4	40.1	0.42[b]	0.64[b]	−0.06[b]	0.39
				0.65[c]	0.39[c]	−0.04[c]	

[a] Model type: D—single discrete; DD—double discrete; DDD—triple discrete. The continuous distribution models exhibited χ^2 values in excess of 2.5.
[b] 1 μM dansyl-glycine-β-CD without borneol.
[c] 1 μM dansyl-glycine-β-CD with 1 mM borneol.

of dansyl-glycine. Because $[\beta\text{-CD}] \gg C_{dan-gly}$, eqns (7) and (8) can be further simplified to:

$$K_1 = \frac{\alpha_2}{\alpha_1 C_{\beta\text{-}CD}} \tag{9}$$

$$K_2 = \frac{\alpha_3}{\alpha_1 C_{\beta\text{-}CD}} \tag{10}$$

where $C_{\beta\text{-}CD}$ represents the analytical concentration of β-CD. Upon rearrangement:

$$\alpha_1 = \frac{K_1 C_{\beta\text{-}CD}}{1 + K_1 C_{\beta\text{-}CD} + K_1 K_2 C_{\beta\text{-}CD}^2} \tag{11}$$

$$\alpha_2 = \frac{K_1 K_2 C_{\beta\text{-}CD}}{1 + K_1 C_{\beta\text{-}CD} + K_1 K_2 C_{\beta\text{-}CD}^2} \tag{12}$$

$$\alpha_3 = 1 - \alpha_2 - \alpha_3 \tag{13}$$

Therefore, by fitting the experimental pre-exponential factors vs. β-CD one can extract K_1 and K_2 using nonlinear least squares (e.g. Sigmaplot). The estimates of K_1 and K_2 were 567 ± 36 and $68 \pm 4\ M^{-1}$, respectively, at 25 °C. The calculated formation constant for the 2:1 complex is close to the literature value reported for other dansyl derivatives complexed with β-CD.[2,34] The agreement between the experimental data and the stepwise equilibria model is a strong indication that this scheme correctly models the system.

The recovered lifetimes for the triple exponential decay law are 2.3, 7.1, and 16.9 ns (Fig 6). The 2.3 ns component is identical to the value recovered for dansyl-glycine alone in water, and represents the free component. When we increase the concentration of β-CD, we observe that the amount for free dansyl-glycine (α_1,○) decreases due to complexation. As the free component decreases we see a concomitant increase in the 7.1 and 16.9 ns components. However, the 7.1 ns component (α_2,▽) increases initially then begins to decrease. In contrast, the 16.9 ns component (α_3,▼) increases continuously with added β-CD. We also note that the 16.9 ns lifetime is very close to the mean lifetime recovered for dansylamide complexed with β-CD.[18] Based on these results we conclude that the 7.1 ns component represents the inclusion of the glycine moiety within the β-CD cavity. Its lifetime is longer than the free component due to some shielding or restriction of the twisted intramolecular charge transfer process.[27] The 16.9 ns component corresponds to inclusion of the actual dansyl moiety. Figure 7 summarizes these equilibria and identifies all the species.

Dansyl-glycine-β-CD intramolecular complexation

With an understanding of the photophysics of the intermolecular complex, we set out to determine the behaviour of the intramolecular complex. Toward this end, we carried out a series of multifrequency phase and modulation experiments on dansyl-glycine-β-CD in solution. A summary of these results is presented in Table 2; they differ significantly from the intermolecular case and merit additional discussion. First, based on the quality of the fit (χ^2) we find that the fluorescence intensity decay is described by a two-component model. (One might argue for the triple exponential decay law, but the negative pre-exponential factor and exceedingly long decay time for dansyl are not expected nor reasonable.) The recovered lifetimes are 5.6 and 16.9 ns. Second, in water the mole fraction of the longer-lived component is 0.98; it dominates the emission. Third, the 16.9 ns lifetime is identical to the value recovered for the dansyl-glycine/β-CD system (*vide supra*). This is consistent with the dansyl-included species.[18] Fourth, the 5.6 ns component lifetime is between the lifetime for free dansyl-glycine and dansyl-glycine (glycine end) complexed with β-CD (*vide supra*). This result is consistent with the tethered dansyl-glycine displaced from the cavity. The tethered dansyl group would be restricted and shielded somewhat, but not to the extent of the actual glycine-included species (*vide supra*). Therefore, one would expect its lifetime to be intermediate. Finally, we see that addition of the non-fluorescent species, borneol (a non-fluorescent displacer),[21] leads to a decrease in the relative contribution of the longer-lived species, but the actual decay times remain unaffected. This result demonstrates that borneol does not affect the system photophysics per se; it only changes the relative contribution of the two fluorescing species. Based on these results we present a simple equilibrium model describing the dansyl-glycine-β-CD/borneol system (Fig 8). The upper panel illustrates the situation in neat water (98% bound). The lower panel summarizes the time-resolved fluorescence results when we added borneol.

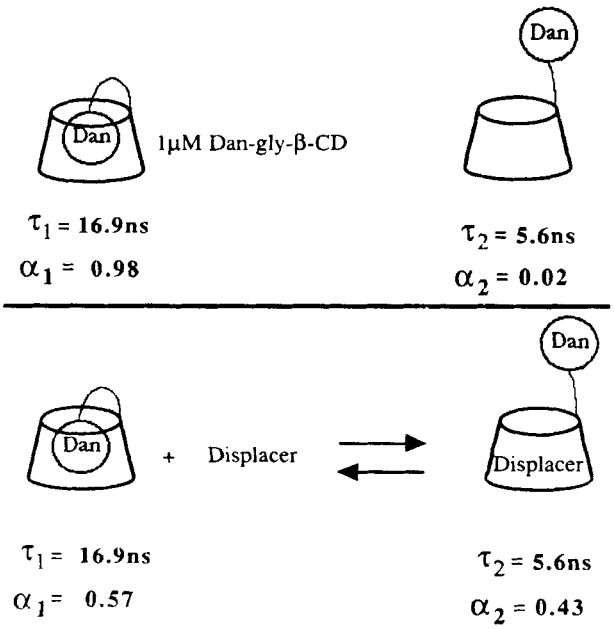

Figure 8 Model of the tethered dansyl-glycine-β-CD intramolecular complex. The displacer in these experiments was borneol.

CONCLUSIONS

We present the first detailed results comparing the inter- and intramolecular complexation in cyclodextrin systems. We report on the stoichiometry and equilibrium constants for the dansyl-glycine/β-CD system (intermolecular case) and demonstrate that it is stepwise in nature. Time-resolved fluorescence data are used to recover the actual equilibrium constants (Fig 7) and they are in agreement with previous work on similar systems. The fluorescence lifetime of the dansyl group included in the CD cavity is the same for the inter- and intramolecular system. The pre-exponential factors associated with the intramolecular complex change when one adds non-fluorescent species that strongly complex with β-CD. The fact that the decay times do not change upon complexation argues for a simple displacement mechanism (Fig 8). We also see that the process of tethering the fluorescent center to the cyclodextrin ring (locking) decreases the complexity of the photophysics. Finally, unimodal continuous distribution models[17,18,20,31] failed to describe the present fluorescence intensity decay kinetics. This is probably a consequence of several factors: (1) the distributions are not an accurate model for these particular systems; (2) fluorescence from the free probe is moderate and contributes to the observed emission; (3) the excited-state lifetimes for the free and bound probes are relatively close compared to the previous work; (4) the equilibria are more complex compared to the simple 1:1 situations seen previously; and (5) the tether between the CD and the dansyl limits slow mobility within the CD cavity.

EXPERIMENTAL SECTION

Materials

Dansyl-glycine (Sigma); N,N-dicyclohexylcarbiimide (Sigma); 6-dioxy-6-amino-β-cyclodextrin (Advanced Separation Technologies); β-cyclodextrin (Advanced Separation Technologies).

Synthesis of appended-cyclodextrin

A solution of dansyl-glycine (0.8328 g, 2.7 mmol) and N,N,-dicyclohexylcarbodiimide (618 mg, 3.0 mmol) in DMF (15 mL) was stirred at 0 °C for 40 min. To this mixture was added 6-dioxy-6-amino-β-CD (0.777 g, 0.6 mmol) and this mixture was stirred at the same temperature for 30 min and then at room temperature for an additional 5 hrs. The reaction mixture was then filtered using a fine glass frit. The reaction mixture was then poured into ice cold acetone (400 mL) and the precipitate collected. The precipitate was dissolved in a minimum volume of DMF:water mixture (35:65 v/v), then loaded onto a Sephadex LH-20 column (25 mm × 1000 mm) and eluted with the same DMF:water solvent. Fractions were collected, and those containing the product were pooled together. The traction were concentrated and poured into acetone (200 mL), and the precipitate was collected, washed with acetone, and stored in a desiccator placed in a freezer. The purity of the product was checked by reverse phase HPLC and determine to be >96%.

Sample preparations

All materials were used as received. Distilled deionized water was used to prepare all solutions. Samples were prepared fresh daily and run immediately after preparations.

Instrumentation

All steady-state and time-resolved fluorescence measurements were made with an SLM 48000 MHF spectrofluorimeter. The majority of instrumentation used for this work has been described in detail elsewhere.[35-38] For lifetime measurements an argon-ion laser operating at 363.8 nm. The reference lifetime standard was Me_2POPOP in ethanol; it was assigned a value of 1.45 ns.[38]

ACKNOWLEDGMENTS

This work was supported by the National Science Foundation CHE-8921517 and CHE-9300694.

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