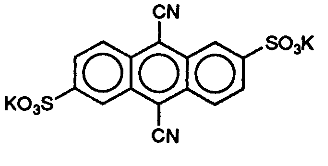
Static and Dynamic Fluorescence Quenching of a Dicyanoanthracene-capped β-Cyclodextrin

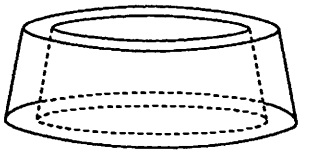
Michael F. Acquavella, Mary E. Evans, Steven W. Farraher, Cédrin J. Névoret and Christopher J. Abelt *
Department of Chemistry, College of William and Mary, Williamsburg, Virginia 23187-8795, USA

Fluorescence quenching of $6^A,6^{D(C)}$-(9,10-dicyanoanthracene-2,6-disulfonyl)-β-cyclodextrin (DCA-β-CD) by several amines (**1**–**6**) has been investigated. Apparent Stern–Volmer constants, which are the sum of dynamic and static (binding) components, have been determined. Quenching constants with potassium 9,10-dicyanoanthracene-2,6-disulfonate (DCA-DS) and binding constants with β-CD have been determined as approximations for the dynamic and static components, respectively. DCA-β-CD does not bind all amines more strongly than does β-CD despite its greater hydrophobic surface area. Dynamic quenching accounts for at least a fifth of the total even with strongly bound amines. Neither charge-transfer absorption nor exciplex emission is observed for any inclusion complex.

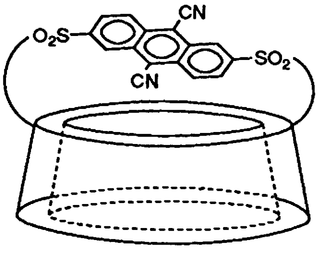
Cyclodextrins (CDs) are well known host molecules which find extensive use in complexation and catalysis studies.[1] Their well-defined cavities, small size, and ease of functionalization[2] make them ideal enzyme models.[3] We are interested in photochemically active CD derivatives. A number of photoreactive moieties have been attached to CDs including anthraquinone,[4] benzophenone,[5,6] flavin,[7] porphyrin,[8,9] and rose bengal.[10] Photo-reduction of quinones has been demonstrated with two different porphyrin–β-CD systems, whereas a flavin–β-CD host has provided for photo-oxidation of bound benzyl alcohols. Recently, we have reported the synthesis of a dicyanoanthracene-capped β-CD (DCA-β-CD) with the hopes that it would be a good host for photoinduced electron-transfer reactions.[11] In this paper we report fluorescence quenching results with a variety of guest amines as a probe for the host–guest relationship.



DCA-DS



β-CD



DCA-β-CD

Fluorescence quenching is a powerful technique with which to study cyclodextrin inclusion. Fluorescent guests (*e.g.*, naphthalenes,[12–18] pyrenes,[17–23] porphyrins[9]) are partially protected from solution quenchers such as ions[12,13,15,17,20] and poorly bound amines,[18,19,23] whereas binding quenchers either form ternary or higher-order complexes resulting in static quenching,[18,20,22] or they compete with the fluorophore for binding with CD resulting in protection by encapsulation of the quencher.[18] In some cases the fluorophore has been covalently bound to the CD, and the degree of encapsulation could be probed using solution quenchers.[16,23] The distinction between static and dynamic quenching has been made through fluorescence lifetime studies.[9,16–20,23] The appearance of a shorter-lived excited state component whose lifetime is invariant with added quencher is interepreted as arising from a complex with partial quenching. On the other hand, complete static quenching should result in two different Stern–Volmer (SV) constants. The lifetime-based constant reflects dynamic quenching, whereas the intensity-based SV constant is a combination of both static and dynamic quenching. In this paper we estimate the dynamic component through the intensity-based SV constant in a model system where complexation is negligible.

Experimental

The preparation of DCA-DS and DCA-β-CD is described elsewhere.[11] The DCA-β-CD was isolated as a 3:1 mixture of A,D- and A,C-regioisomers. β-CD was obtained from Amaizo and recrystallized twice from water. The amine quenchers were commercially available except 6-dimethylaminonaphthalene-2-sulfonate which was made by a modified literature method.[24] The aminonaphthalenesulfonates were recrystallized from aq. NaCl or aq. KCl, whereas the other amines were used without purification. UV–VIS spectra were obtained with a Beckman DU-70 spectrophotometer. Fluorescence spectra were obtained with a SLM-Aminco SPF-500C spectrofluorimeter thermostatted at 25 °C. For a typical binding determination 1 cm^3 aliquots of the fluorophore were combined with varying aliquots of quencher, and the mixture was diluted to 10 cm^3 with water. The blank was run first, then in order from the least concentrated to most concentrated to give ample equilibration time. Two excitation wavelengths were used for each binding study; intensities were obtained by integration of the emission spectra.

Theory

The fluorescence quenching results are interpreted in the context of a very simple system wherein the host fluorophore (F) which binds a guest quencher (Q) is completely quenched. Since

Q·F is a dark complex, only F emits. The free fluorophore concentration is governed by the association constant K, and it can be expressed in terms of F_0, the total initial concentration of the host fluorophore.

$$Q + F \rightleftharpoons Q{\cdot}F \Rightarrow K = \frac{[Q{\cdot}F]}{[Q]{\cdot}[F]} \Rightarrow [F] = \frac{F_0}{K[Q] + 1}$$

The Stern–Volmer relationship for a system with dynamic and static components has been derived previously,[25] and is given by eqn. (1). The derivation of eqn. (1) requires the

$$\frac{I_0 - I}{I} = [Q]\cdot(K + K_{SV} + K\cdot K_{SV}\cdot[Q]) \qquad (1)$$

standard assumptions of small absorption (<0.1) and irradiation at an isosbestic point for F and Q·F. The Stern–Volmer constant, K_{SV}, is the product of the fluorescence lifetime and the dynamic quenching rate constant, $\tau_f{\cdot}k_q$. This equation predicts that the limiting slope, as [Q] approaches zero, is $K + K_{SV}$ and that, at large [Q], the plot should deviate upward from the line intersecting the origin with this limiting slope.

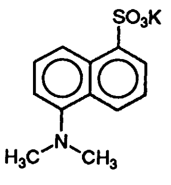
For a two-component system, *e.g.*, A,D- and A,C-regioisomers of DCA-β-CD, the Stern–Volmer relationship is more complicated, but it simplifies under certain conditions. If the various terms for each component are designated with a subscript 1 or 2, then the Stern–Volmer relationship is given by eqn. (2). Here x_1 and x_2 are the mole fractions of each

$$\frac{I_0 - I}{I} = [Q]\cdot\frac{x_1(K_1 + K_{SV1}) + x_2 f(K_2 + K_{SV2})}{x_1 + x_2 f} \qquad (2)$$

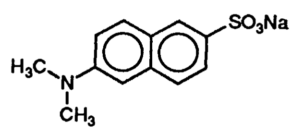
component and f is a factor which accounts for the differences in molar absorptivities (ε), fluorescence lifetimes in the presence of quencher $[\tau_{fQ} = (\tau_f^{-1} + k_q[Q])^{-1}]$, and association constants.

$$f = \frac{\varepsilon_2\tau_{fQ1}(K_1[Q] + 1)}{\varepsilon_1\tau_{fQ2}(K_2[Q] + 1)}$$

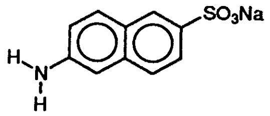
If the structure of the two components is nearly identical, as is the case with these regioisomers, then the extinction coefficients and lifetimes should be similar. The $K[Q] + 1$ terms approach one in the limit of small quencher concentrations. Thus, f is approximately one, and so the binding term simplifies to $x_1K_1 + x_2K_2$, the weighted sum of the binding constants for each component. Eqn. (2) can be generalized for several components. Since it is general, it applies to other situations, for example binding by different conformations of the same macrocyclic host.



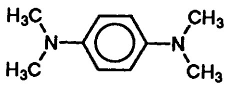
1



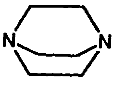
2



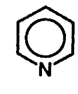
3



4



5



6

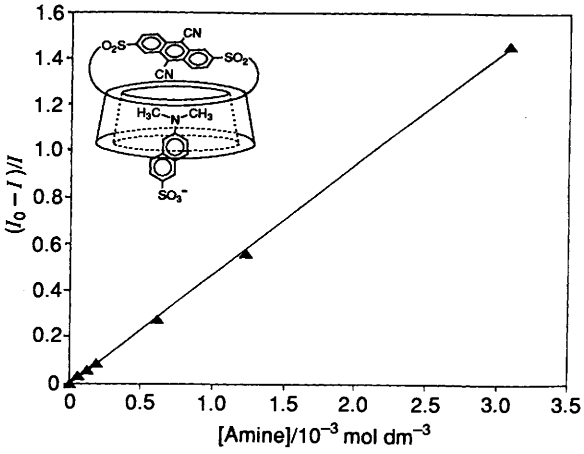


Fig. 1 Stern–Volmer plot for the fluorescence quenching of DCA-β-CD with **2** at 25 °C

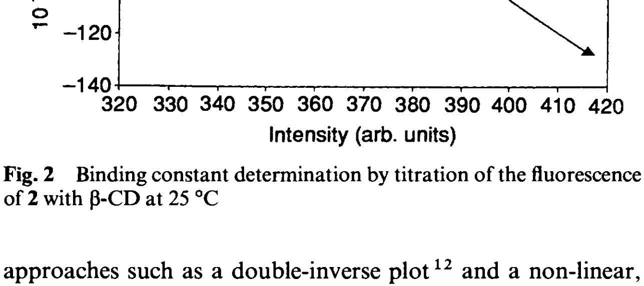


Fig. 2 Binding constant determination by titration of the fluorescence of **2** with β-CD at 25 °C

Results

The interaction between DCA-β-CD and several amine guests was studied by fluorescence spectroscopy. For comparison purposes, the interaction of these amines with β-CD and DCA-DS was included. Amines quench the fluorescence of the DCA moiety, whereas β-CD enhances the fluorescence of bound amines. Apparent Stern–Volmer (SV) quenching constants for DCA-β-CD and DCA-DS were obtained by titrating the DCA fluorescence with added amines **1**–**6**. Plots of $(I_0 - I)/I$ *vs.* [amine], where I_0 is the intensity in the absence of added agent, gave good linear correlations whose slope defines the SV constant (Fig. 1). Association constants between β-CD and fluorescent amines **1**–**3** were determined by titration with β-CD. Here, plots of $(I - I_0)/[\beta\text{-CD}]$ *vs.* I were linear, and the slope of this line is the negative of the binding constant (Fig. 2). Other approaches such as a double-inverse plot[12] and a non-linear, parametrized general treatment[21] gave similar results. The Stern–Volmer and the association constants are reported in Table 1. The spectrometric titrations were conducted in non-buffered solutions because DCA-β-CD is not stable at pH 10. In

Table 1 Stern–Volmer constants for quenching of DCA-β-CD and DCA-DS with several amines and binding constants with β-CD[a,b]

Amine	DCA-β-CD	β-CD	DCA-DS
1	280 ± 10	110 ± 6	59 ± 2
2	475 ± 3	1270 ± 30	182 ± 4
3	254 ± 10	358 ± 22	126 ± 5
4	642 ± 8	—	204 ± 5
5	86 ± 2	—	94 ± 1
6	60 ± 1	—	29 ± 1

[a] The units are $dm^3\ mol^{-1}$. [b] The reported error is the standard error of the linear regression.

fact, at pH 10 its visible absorption spectrum changes over several minutes and eventually becomes identical with that of DCA-DS. Of the amines studied, only DABCO (**5**, pK_{a1} 3.0[26]) exists mainly as its conjugate acid in neutral solution.

Discussion

The photophysical behaviour of DCA-β-CD in the presence of amines was studied to provide insight into the bind strength and photo-oxidation capabilities of this host. The aminonaphthalenesulfonates (**1–3**) were chosen because the naphthalene nucleus fits well into β-CD and the sulfonates will direct the amino group inside the CD cavity. Tetramethylphenylenediamine (**4**) has only one axial binding mode, and without a water-soluble sulfonate group, it should bind strongly. DABCO and pyridine (**5** and **6**, respectively) should not bind strongly to the host.

The data in Table 1 reveal several properties of this host. First, DCA-β-CD is not necessarily a better binding than β-CD despite its increased hydrophobic surface area.[27] Both 2,6-substituted naphthyl derivatives bind more strongly to β-CD than to DCA-β-CD. This difference is more dramatic with the dimethylamino compound **2** than the simple amino compound **3**. One possible explanation is that the guest can fully penetrate β-CD, whereas in DCA-β-CD the cap blocks full inclusion, thus forcing a portion of the guest outside the cavity. Support for this idea comes from the observation that dansyl ion **1**, which cannot fully penetrate β-CD owing to its substitution pattern, is bound more strongly by DCA-β-CD than β-CD.

The observed quenching is likely a result of a static process due to complexation and a dynamic process from random encounters between excited fluorophore and quencher. Since β-CD is known to form complexes (*vide supra*) with several of the amines, it is reasonable to assume that DCA-β-CD behaves similarly. Quenching in the DCA-β-CD/amine complex can be partial or complete. The results suggest that static quenching must be complete; otherwise, the SV-plots would deviate significantly from linearity.[9,20] Quenching constants of DCA-DS were determined as estimates for the dynamic quenching component of DCA-β-CD. DCA-DS does not suffer static quenching;† therefore, it offers a reasonable estimate of the dynamic component. The only significant differences between DCA-β-CD and DCA-DS with respect to dynamic quenching lie in the electrostatic interactions and quenching cross-sections. DCA-β-CD is neutral, whereas DCA-DS is a dianion. The naphthalenesulfonates are anions, so they will encounter a greater degree of repulsion with DCA-DS than with DCA-β-CD. On the other hand, DABCO is protonated at pH 7, so it will be attracted to DCA-DS. Indeed, the SV constant with DCA-DS is greater than the observed SV constant with DCA-β-CD. The presence of the large β-CD moiety in DCA-β-CD will result in less efficient quenching of the excited DCA group. Collision with DCA-DS will always bring the donor and acceptor orbitals close together, whereas collisions with DCA-β-CD may not.

The dynamic quenching component, as estimated by the SV constant with DCA-DS, is significant for all amines studied. It is least significant with the dansyl ion **1**, where it still accounts for 21% of the observed constant ($K + K_{SV} = 280$, $K_{SV} = 59$). Diamine **4** which shows the greatest observed SV constant with DCA-β-CD (642) also has a large SV constant with DCA-DS (204). Although these conclusions were drawn from data obtained from a regioisomeric mixture, the relative differences in the binding constants and dynamic quenching rates for the A,D- and A,C-isomers are likely to be small, so the general conclusion that dynamic quenching is significant can be applied to both the A,D- and A,C-isomers.

The existence of the dynamic quenching process will impact photo-oxidation studies. Not all of the photo-oxidized substrate will reside in the CD cavity. Thus, the observed reaction will be the product of complexed and uncomplexed processes. In this respect, the sandwiched porphyrin structure reported by Kuroda[9] is ideal because the only approach by a quencher is through a β-CD cavity.

Finally, we note that neither charge-transfer (CT) absorption nor exciplex emission was observed. This result stands in contrast with a number of examples of complexation-induced exciplex formation. In all of these β-CD forms multicomponent complexes with the donor and acceptor molecules. Donor–acceptor pairs include 2-methoxynaphthalene/1,2-dicyanobenzene,[12] anisole/1-cyanonaphthalene,[15] pyrene/aniline,[22] and naphthalene/trimethylamine.[28] The present system differs in that the acceptor is covalently attached to the β-CD in a lid-type structure. The axial-lid inclusion complex is probably neither tight enough nor close enough for a CT complex to form. These results are guiding our continued investigation into other dicyanoanthracene-derivatized β-cyclodextrins.

Acknowledgements

This work was supported by the National Science Foundation (CHE-8920447). The SPF-500C spectrofluorimeter was purchased through a Research Corporation grant. We thank American Maize Products for a gift of β-cyclodextrin.

References

1 M. L. Bender and M. Komiyana, *Cyclodextrin Chemistry*, Springer-Verlag, New York, 1978; W. Saenger, *Angew Chem., Int. Ed. Engl.*, 1980, **19**, 344.
2 A. P. Croft and R. A. Bartsch, *Tetrahedron*, 1983, **39**, 1417.
3 R. Breslow, *Science*, 1982, **218**, 532; I. Tabushi, *Acc. Chem. Res.*, 1982, **15**, 66; V. T. D'Souza and M. L. Bender, *Acc. Chem. Res.*, 1987, **20**, 146.
4 A. M. Aquino, C. J. Abelt, K. L. Berger, C. L. Darragh, S. E. Kelley and M. V. Cossette, *J. Am. Chem. Soc.*, 1990, **112**, 5819.
5 I. Tabushi, K. Fujita and L. C. Yuan, *Tetrahedron Lett.*, 1977, 2503.
6 K. L. Berger, A. L. Nemecek and C. J. Abelt, *J. Org. Chem.*, 1991, **56**, 3514.
7 H. Ye, W. Tong and V. T. D'Souza, *J. Am. Chem. Soc.*, 1992, **114**, 5470.
8 M. C. Gonzalez, A. R. McIntosh, J. R. Bolton and A. C. Weedon, *J. Chem. Soc., Chem. Commun.*, 1984, 1138.
9 Y. Kuroda, M. Ito, T. Sera and H. Ogoshi, *J. Am. Chem. Soc.*, 1993, **115**, 7003.
10 D. C. Neckers and J. Paczkowski, *J. Am. Chem. Soc.*, 1986, **108**, 291; D. C. Neckers and J. Paczkowski, *Tetrahedron*, 1986, **42**, 4671.
11 M. F. Acquavella, M. E. Evans, S. W. Farraher, C. J. Névoret and C. J. Abelt, *J. Org. Chem.*, 1994, **59**, 2894.

---

† Association of DCA-DS with amines should show charge-transfer absorption, but none is observed. Since DCA-DS is a dianion, it should be fully solvated in water and show little tendency towards association.

12 S. Hamai, *Bull. Chem. Soc. Jpn.*, 1982, **55**, 2721.
13 T. Yorozu, M. Hoshino, M. Imamura and H. Shizuka, *J. Phys. Chem.*, 1982, **86**, 4422.
14 A. Ueno, F. Moriwaki and T. Osa, *Tetrahedron*, 1987, **43**, 1571.
15 S. Hamai, *J. Phys. Chem.*, 1990, **94**, 2595.
16 D. M. Gravett and J. E. Guillet, *J. Am. Chem. Soc.*, 1993, **115**, 5970.
17 G. Nelson and I. Warner, *J. Phys. Chem.*, 1990, **94**, 576.
18 K. Kano, I. Takenoshita and T. Ogawa, *J. Phys. Chem.*, 1982, **86**, 1833.
19 W. G. Herkstroeter, P. A. Martic and S. Farid, *J. Chem. Soc., Perkin Trans. 2*, 1984, 1453; W. G. Herkstroeter, P. A. Martic, T. R. Evans and S. Farid, *J. Am. Chem. Soc.*, 1986, **108**, 3275.
20 S. Hashimoto and J. K. Thomas, *J. Am. Chem. Soc.*, 1985, **107**, 4655.
21 G. Patonay, A. Shapira, P. Diamond and I. M. Warner, *J. Phys. Chem.*, 1986, **90**, 1963.
22 S. Hamai, *J. Phys. Chem.*, 1988, **92**, 6140.
23 A. Ueno, I. Suzuki and T. J. Osa, *J. Am. Chem. Soc.*, 1989, **111**, 6391.
24 D. J. R. Laurence, *Methods Enzymol.*, 1957, **4**, 208.
25 N. J. Demas, *Excited State Lifetime Measurements*, Academic Press, New York, 1983, p. 53.
26 *Dictionary of Organic Compounds*, 5th edn., Chapman and Hall, New York, 1982, Vol. 2, p. 1582.
27 J. Emert and R. Breslow, *J. Am. Chem. Soc.*, 1975, **97**, 670; I. Tabushi, K. Shimokawa, N. Shimizu, H. Shirakata and K. Fujita, *J. Am. Chem. Soc.*, 1976, **98**, 7855.
28 K. Kano, S. Hashimoto, A. Imai and T. Ogawa, *J. Inclusion Phenom.*, 1984, **2**, 737; Turro and Yang have reported that an intramolecular exciplex forms with naphthalene tethered to an amine: G. S. Cox, N. J. Turro, N. C. Yang and M. J. Chen, *J. Am. Chem. Soc.*, 1984, **106**, 422.

Paper 4/03285C
Received 2*nd June* 1994
Accepted 21*st September* 1994