

saccharide such as arabinose or glucose will help to develop an understanding of the origin of ROA spectra of carbohydrates at a fundamental level rather than simply relying on empirical correlations. Anticipated developments in the computation of the required optical activity tensor derivatives⁴⁴ should soon render

(44) Polavarapu, P. L., personal communication.

small carbohydrates accessible to such ab initio ROA studies.

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Influence of Alcohols on the β -Cyclodextrin/Acridine Complex

Jodi M. Schuette, Thilivhali T. Ndou,[†] Arsenio Muñoz de la Peña,[‡]
Srinivasan Mukundan, Jr.,[§] and Isiah M. Warner^{*•||}

Contribution from the Department of Chemistry, Emory University, Atlanta, Georgia 30322.
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Abstract: The apparent formation constant calculated for the β -cyclodextrin/acridine (β -CD/ACR) complex (287 M^{-1}) reveals a weak association in aqueous solution. An additional weakening of the binding strength between β -CD and ACR, and subsequent reduction in the previously reported quenching of ACR caused by β -CD, is observed upon interaction of the complex with selected straight-chain and branched alcohols. Nuclear magnetic resonance data suggest that the equilibrium involves the formation of ternary complexes. This equilibrium apparently leaves a greater number of ACR species accessible to the bulk aqueous environment, resulting in a consequent decrease in the total amount of ACR quenched. Specific mechanisms of interaction are further examined through information provided by steady-state fluorescence and fluorescence lifetime analysis.

Introduction

Several derivatives of acridine (ACR) are pharmaceutically active.¹ Acridine, therefore, provides a fundamental means of modeling the drug binding and biological transport properties of structurally similar compounds, specifically in aqueous solutions.^{2,3} ACR is generally categorized as a polynuclear nitrogen heterocycle and is characterized by sensitive deactivation mechanisms.⁴⁻¹⁰ Consequently, ACR exhibits weak fluorescence quantum yields. Deactivation of ACR is particularly prevalent in aprotic solvents and in the presence of nonbonding electrons. Most of the unusual properties of nitrogen heterocycles have been attributed to the specific interaction of proximal $n-\pi^*$ and $\pi-\pi^*$ transition states, whereby vibronic coupling facilitates radiationless deactivation through intersystem crossing to the triplet state.

Both fluorescence and phosphorescence measurements have been used to acquire detailed information concerning the quenching of ACR in cyclodextrin (CD) and various nucleosidic environments.^{5,11,12} Specific anchoring of ACR to sodium dodecyl sulfate (SDS) micelles was reported to occur through direct interaction of the nitrogen heteroatom with Ag^+ ions acting as external heavy atoms.¹² Enhanced room temperature phosphorescence was the observed result. The sensitivity of ACR to microenvironmental conditions, particularly to temperature and solvent effects,^{4,6-8,13,14} makes ACR an effective probe of the changes in microenvironment which occur upon complexation with CDs.¹¹

Cyclodextrins are cyclic oligosaccharides that have the ability to selectively incorporate various hydrocarbon guest molecules through size exclusion and hydrophobic interactions.¹⁵ Inner cavity diameters of 5.7, 7.8, and 9.5 Å for α -, β -, and γ -CD, respectively, enable CDs to discriminate between guest molecules on the basis of size. Alternatively, the slightly hydrophobic

character of the inner cavity provides a driving force for complexation with similarly apolar guest molecules. Together, the unique features of this system are often able to effect enhancements and/or perturbations of the photophysical and photochemical properties of included guest molecules.

Numerous investigators have examined the behavior of polycyclic aromatic hydrocarbons (PAHs) and azo dyes in the presence of alcohols and/or CDs.¹⁶⁻³⁰ Nelson et al.³¹⁻³⁴ have gathered

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* Author to whom correspondence should be addressed.

[†] Present address: Gillette Research Institute, Gaithersburg, MD 20879.

[‡] Present address: Department of Analytical Chemistry, University of Extremadura, Badajoz, Spain.

[§] Present address: Emory University School of Medicine, Atlanta, GA 30322.

^{||} Present address: Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803.

evidence for the formation of ternary complexes between alcohols, CDs, and various PAHs. A reduction in the quenching experienced by fluoranthene and pyrene in the presence of 2 M potassium iodide and β -CD upon addition of tertiary butyl alcohol was demonstrated.³¹ Muñoz de la Peña et al.²⁹ have investigated the effect of various straight-chain and branched alcohols upon the β -CD/pyrene complex. Zung et al.³⁰ have further studied the effect of cavity/guest dimension in achieving optimum association of alcohols with γ -CD/pyrene complexes. Using molecular modeling, they have also considered the influence of volume for individual alcohols as it affects their role of being a space-filling or space-regulating ternary component.

In this paper on acridine, steady-state fluorescence and fluorescence lifetime as well as nuclear magnetic resonance (NMR) techniques are employed as sensitive indicators of the role that alcohols play in the β -CD/ACR system.

Experimental Section

Apparatus. Steady-state fluorescence measurements were acquired with a Perkin-Elmer 650-10S fluorescence spectrophotometer equipped with a thermostated cell housing. Each sample was placed in an anaerobic cell and purged with nitrogen for 25 min prior to analysis. Fluorescence emission spectra were acquired using instrumental settings described previously.¹¹ A Photochemical Research Associates (PRA) 3000 fluorescence lifetime spectrophotometer was used to acquire lifetime information, which was then transferred to a Micro Vax II system for analysis. Excitation and emission wavelengths were set at 365 and 430 nm, respectively. The hydrogen-filled flash lamp was operated at a pressure of 14 mmHg and 5.5 kV with a repetition rate of 30 kHz. The lamp profile was approximately 2 ns full width at half-maximum (fwhm). Absorption spectra were obtained using a Cary 3 UV-vis spectrophotometer. The NMR spectra were collected using a General Electric GN-500 nuclear magnetic resonance spectrometer. Sample temperature for the NMR experiments was maintained at 22.0 ± 0.1 °C.

Materials. The acridine (99% purity) was purchased from Aldrich. The β -CD was obtained from American Maize Products Co. (Hammond, IN) and recrystallized twice with water. Each NMR sample was prepared with deuterated water (99.9% atom D) purchased from Aldrich and was altered to a pD = 7.95 ± 0.08 using NaOD/DCl. The residual HOD resonance was used as an internal standard. Consequently, both pH, using a conventional pH meter, and temperature were carefully controlled. Absolute ethanol was purchased from Aaper Alcohol and Chemical Co. (Shelbyville, KY). Cyclopentanol (*c*-PeOH), cyclohexanol (*c*-HexOH), 1-propanol (1-PrOH), 2-propanol (2-PrOH), 1-butanol (1-BuOH), and 1-pentanol (1-PeOH) were purchased from Aldrich. The 2-methyl-2-propanol (*t*-BuOH) was purchased from Fisher. All chemicals other than β -CD were used as received.

Method. (a) Preparation of β -CD/Acridine/Alcohol Solutions for Fluorescence Measurement. An experimental (1.0×10^{-5} M) aqueous ACR solution was prepared as previously described.¹¹ Individual samples were prepared by adding the appropriate amounts of β -CD to each 10-mL flask and then measuring 100 μ L of the alcohol directly into the flask to give a solution which was 1% by volume in alcohol upon dilution to the mark with the 1.0×10^{-5} M aqueous ACR solution. Each sample was then shaken for 20 min with a wrist action shaker and allowed to equilibrate overnight.

(b) Preparation of β -CD/Acridine/Alcohol Blanks for Absorbance Measurement. Absorbance samples were prepared as described for the fluorescence samples above. Blanks contained the same concentrations

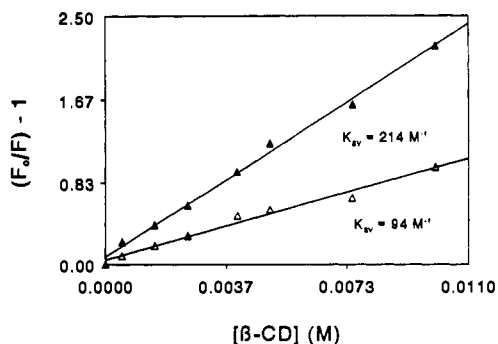


Figure 1. Stern-Volmer plot for β -CD/ACR in the presence (Δ) and absence (\square) of 1% (v/v) *t*-BuOH.

of β -CD and alcohol and were diluted with deionized water (Continental System, Atlanta).

Results and Discussion

Fluorescence Measurements. The quenching of ACR fluorescence by β -CD has been reported.¹¹ Quenching by CDs, however, is not limited to heterocycles. For example, Lin¹⁷ and Matsui and Mochida¹⁶ investigated the quenching phenomenon of methyl orange (MO) upon inclusion by α -CD. Hamai³⁵ reported the quenching of acenaphthene in the presence of increasing concentrations of β -CD. The ability of CDs, in general, to alter intrinsic fluorescence characteristics and quantum yields varies with the type of guest molecule. Nitrogen heterocycles appear to be particularly susceptible to quenching in aprotic solvents and CD media,^{5,11} while their polyaromatic hydrocarbon counterparts often exhibit enhanced fluorescence upon inclusion by appropriately sized CD molecules.^{28,31} The role of the nitrogen heteroatom as a symmetry interrupting entity has been cited as an important factor in the quenching mechanism for ACR.^{6,7} Through the incorporation of a heteroatom into the fused-ring system, the π -system is effectively destabilized. The fluorescence of acridine is strongly dependent on bulk solvent polarity, temperature, and pH. Nevertheless, it is this acute sensitivity that makes ACR an effective probe of the surrounding microenvironment.

The reduction in the quenching of ACR by β -CD through interaction with a series of individual straight-chain and branched alcohols has been examined. Figure 1 depicts a Stern-Volmer (S - V) plot of β -CD/ACR in the absence and presence of 1% (v/v) (0.11 M) *t*-BuOH. A lower slope and, correspondingly, lower S - V constant (94 vs 214 M^{-1} in the absence of alcohol) suggest the formation of a weaker complex for ACR and β -CD in the presence of *t*-BuOH. Matsui and Mochida¹⁶ observed a similar effect of alcohols upon the quenching of MO by α -CD and attributed the ensuing reduction in quenching to a disruption of the α -CD/MO complex in a competing equilibrium of MO with the particular alcohol. Although weak binding constants have been reported by others for CD/alcohol associations using spectroscopic,^{16,21,25} calorimetric,³⁶ and osmotic pressure³⁷ measurements, the concentration of alcohol used must also be considered in the final equilibrium which is favored and attained.³⁰ In the Matsui and Mochida study, 1-BuOH concentrations as small as 6.07 mM (0.0056% v/v) appear to induce an inhibitory type of interaction. Opallo et al.³⁸ examined the effect of concentrations as high as 0.8522 M (7.8% v/v) 1-BuOH upon the induced circular dichroism signal of the 2,3-anthracenedicarboxylate/ β - and γ -CD complexes. It was determined that the addition of alcohol at this percentage begins to affect the polarity and/or pH of the bulk solvent and thus alters the guest's affinity for the CD cavity. In the present study with ACR, the total alcohol concentration examined is 1%

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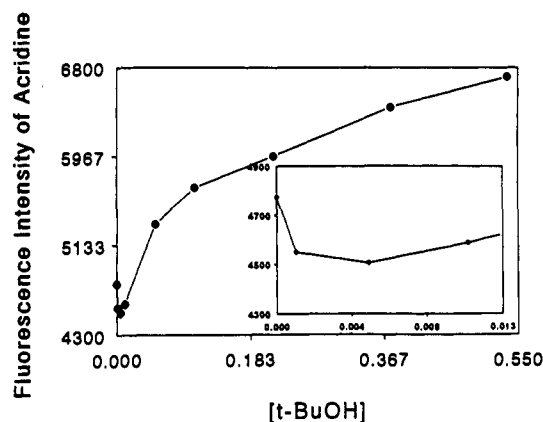


Figure 2. Plot of ACR fluorescence emission intensity vs $[t\text{-BuOH}]$. (Inset) Enlargement of region between 0.0 and 0.01 M alcohol.

Table I. Lifetime Results for $\beta\text{-CD}/\text{ACR}$ in the Presence of 1% (v/v) Alcohol

alcohol	τ_1 , ^a ns	τ_2 , ns	χ_R ^{2b}	K_{DYN} , M^{-1}
	11.26	6.94	1.17	31.37
1-pentanol	10.79	4.80	1.43	372.68
cyclopentanol	10.56	4.82	1.38	178
1-propanol	10.41	3.13	1.63	427.76

^a τ_1 denotes the longer-lived of two resolved components. ^b χ_R^2 values of this magnitude are usually not considered acceptable (0.8–1.2); however, they are reported for lack of a more appropriate physical model.

(v/v). This percentage corresponds to slightly different concentrations depending on the density of the alcohol examined. At these concentrations, the alcohol does not significantly alter the bulk solvent hydrophobicity. Moreover, this concentration enables and assures optimum interaction of the alcohol with $\beta\text{-CD}$ since the alcohol concentration, at 0.10 M, is in excess of the highest $\beta\text{-CD}$ concentration examined. Since typical binding constants for the alcohols examined here are only about $10\text{--}100\text{ M}^{-1}$,¹⁶ the concentration of alcohol must govern the extent of CD/alcohol complexation. It is interesting to note that the change in fluorescence intensity for ACR in the absence of $\beta\text{-CD}$ is negligible in the presence of 1% (v/v) alcohol. This suggests that, for the most part, reduction in quenching experienced by ACR when $\beta\text{-CD}$ is present does not originate from the direct interaction of the uncomplexed ACR molecules with the alcohol moiety. Also evaluated was the influence of increasing alcohol concentration. A plot of ACR fluorescence intensity vs $t\text{-BuOH}$ concentration is presented in Figure 2. The inset depicts the initial decrease in intensity between 0 and 0.01 M alcohol, suggesting the ability of the alcohol to intensify the $\beta\text{-CD}/\text{ACR}$ interaction and eventually extract the ACR molecule into the increasingly nonpolar bulk phase at higher alcohol concentrations.

Mechanism of Quenching for $\beta\text{-CD}/\text{ACR}$ in the Presence of an Alcohol. Additional information concerning the interaction of the alcohol is gained from changes in fluorescence lifetimes which provide a sensitive indication of the microenvironment experienced by a probe molecule. Using such information, the quenching of ACR by $\beta\text{-CD}$ has been determined to be predominantly static, although a small dynamic component also exists.¹¹ Furthermore, pH studies demonstrated a stronger interaction and, consequently, greater quenching for the unprotonated ACR species. Global analysis of ACR lifetime decays in the presence of increasing concentrations of $\beta\text{-CD}$ reveals an underlying two-exponential decay (Table I). Two component lifetimes have been resolved for ACR and have been attributed to a free ($\tau_1 = 11.26\text{ ns}$) and complexed ($\tau_2 = 6.94\text{ ns}$) species. A three-exponential decay function was also examined. Analysis of the A factor attributed to the third component, however, revealed a physically inexplicable negative contribution to the overall decay. Therefore, although this model provided better χ^2 values, the double-exponential decay function better accounted for the contribution of

free and complexed components to the overall decay.

A dramatic decrease ($\approx 31\text{--}55\%$) in the lifetime of the complexed ACR occurs upon addition of 1-PeOH, *c*-PeOH, and 1-PrOH. Additionally, the free ACR species is also slightly affected by the presence of 1% (v/v) alcohol in the bulk aqueous solution. A 4.2–7.5% decrease in the lifetime of free ACR resulted with addition of these alcohols. Stern–Volmer plots of $(\tau^0/\tau - 1)$ vs $[\beta\text{-CD}]$ reveal a 3–7-fold increase in the dynamic quenching contribution. The increase in the dynamic quenching constant, K_{DYN} , parallels the decreased lifetime of the complexed ACR species. Smaller volume and long-chain alcohols appear to have the most pronounced effect on the dynamic quenching constant. Since both free and complexed ACR lifetimes are observed to decrease in the presence of the three alcohols examined, some direct interaction of each ACR species (i.e., both free and complexed) with the alcohol is implied even though the steady-state fluorescence data do not support the interaction of free ACR with the alcohols located in the bulk environment. Apparently, then, the decrease in quenching observed through steady-state fluorescence data actually reflects a decrease in the static component of the quenching since lifetime data reveal an increased contribution from dynamic quenching. Thus, both quenching mechanisms are required to fully explain the interaction of alcohols with the $\beta\text{-CD}/\text{ACR}$ complex.

The ability of longer-chained alcohols such as 1-PeOH to interact more deeply within the cavity than the bulkier cyclic counterpart results in a greater weakening of the specific binding regions between the nitrogen heteroatom of ACR and the glycosidic oxygens. It is this ternary interaction that results in increased dynamic quenching of the excited complex as the complexed ACR molecule encounters the alcohol moiety. Conversely, a shallow inclusion of ACR within $\beta\text{-CD}$ might involve interaction with only the primary or secondary hydroxyl groups. The alcohol could conceivably weaken this interaction as well, particularly since the binding of alcohols to CDs has been reported to depend on the hydroxyl groups crowning the primary and secondary edges of the CD molecule.²³ The decreased static quenching and weaker association constants may simply reflect this ability of the various alcohols to regulate the interaction of ACR with these hydroxyl groups, rather than simply fill the void regions left by ACR. Consequently, although dynamic quenching is increased in the presence of alcohols as evidenced by lifetime results, it is the reduction in the number of static ground-state complexes which is suggested through steady-state fluorescence measurements.

Orientation of Interacting Species. It is important that the involvement of H_2O with the dyes be determined to assess the interaction of alcohols. Pines et al.³⁹ and Gafni and Brand⁴⁰ have reported the tendency of excited heterocyclic compounds to abstract protons from water molecules, forming positively charged species. In fact, the fluorescence of the acridine molecule is attributed to an entity which arises in the first excited state upon H-bonding to water.⁴¹ Both the alcohol and ACR will preferentially interact with $\beta\text{-CD}$ in such a way as to optimize contact within the cavity. Corey–Pauling–Koltun (CPK) models indicate that a diagonally skewed arrangement of ACR, within the cavity, would actually allow ACR to optimize interaction with the $\beta\text{-CD}$ cavity, leaving residual pocket voids at each CD opening for the alcohol to occupy. The void space proximal to the primary hydroxyl edge would tend to be more compatible with bulkier or cyclic alcohols, providing optimal contact with the cyclodextrin cavity. In contrast, the ideal arrangement of straight-chain alcohols is shown by CPK models to be an association alongside ACR within the cavity. In this situation, where the alcohol assumes a space-filling role, the shifting or rotation of ACR inside the $\beta\text{-CD}$ cavity is more restricted, leaving ACR less accessible to external water molecules. Bergmark et al.⁴² attributed the

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enhanced quantum yields for CD/aminocoumarin complexes to the favorable *expulsion* of water molecules normally included within the cyclodextrin cavity along with the coumarin dye by the organic solvent modifier. The influence of ethanol was examined for both β - and γ -CD systems. Given the established H_2O dependence of ACR fluorescence, a similar driving force for the β -CD/ACR/alcohol system should result in enhanced quenching. However, it is a decrease in quenching that is actually observed in the presence of every alcohol examined in the present study. As a consequence of the necessary, favorable expulsion of highly ordered H_2O from the CD cavity upon inclusion of a guest molecule of ACR, ACR is likely prevented from associating with formerly co-included water molecules. Partial protrusion of ACR into the bulk aqueous phase through a competitive equilibria with the alcohol, however, would reinitiate some interaction with the H_2O molecules and, thus, reduce static quenching. Hamai^{25,43} suggested a similar mechanism for the accommodation of pyrene or fluorene within the β -CD cavity when the cavity already included a straight-chain alcohol with greater than three carbons. They also reported the tendency of pyrene molecules already occupying the β -CD cavity to facilitate the subsequent association of alcohols with the cavity. Alcohols, therefore, which are able to achieve the most compact interaction with β -CD, shift the equilibrium in favor of such weaker complexes.

The actual manifestation of the reduction in quenching depends on the site of binding between β -CD and ACR, i.e., whether the quenching of ACR is the result of interaction of the nitrogen heteroatom with the glycosidic oxygens or the hydroxyl groups. It is not clear from fluorescence and fluorescence lifetime studies which arrangement provides the most favorable interaction. Therefore, this particular point has been investigated in more depth using NMR techniques.

NMR Studies of β -CD/ACR Containing *c*-PeOH. Additional evidence for the formation of a ternary complex is derived from changes in chemical shift and line shape of β -CD NMR proton signals. The NMR spectrum of β -CD in D_2O is depicted in Figure 3b. Peak assignment has been reported elsewhere.¹¹ Figure 3c illustrates the qualitative features concerning the interaction of 1% (v/v) (0.10 M) *c*-PeOH with β -CD. Cyclopentanol induces a dramatic upfield shift (0.053 ppm) of the H-3 signal. There is also a significant upfield shift of the H-5 signal (to δ 3.787) from the region where the H-5 and H-6 signals usually overlap in the pure β -CD spectrum. This is accompanied by a substantial change in line shape for the H-6 proton signal. The H-2 and H-4 proton resonances also appear to be relatively unaffected by the addition of 0.10 M *c*-PeOH, exhibiting ca. 0.004 ppm shifts. The H-3 and H-5 protons are located in the interior of the β -CD cavity; therefore, it is likely that the H-3 and H-5 protons would shift as a result of proximal or direct interactions with the alcohol or ACR guest molecules. While ACR alone induces almost no observable change in spectral shape or chemical shift of the β -CD spectrum (Figure 3a), several distinct changes were noted for H-3 after addition of ACR to the β -CD/*c*-PeOH solution (Figure 3d). The presence of ACR in the β -CD/*c*-PeOH system induces additional 0.006 and 0.010 ppm upfield shifts in the H-3 and H-5 signals with respect to where these protons are positioned in the presence of 0.11 M *c*-PeOH alone. While no further change in spectral shape is noted for the H-6 signal, a 0.010 ppm upfield shift of the H-6 signal is observed for β -CD/*c*-PeOH in the presence of ACR. These data suggest that the strongest interaction of ACR occurs in the vicinity of the H-5 and H-6 protons. Interaction of ACR with the internal H-5 protons and the H-6 protons crowning the primary hydroxyl edge of the cavity suggests that the three molecular species interact optimally near the smaller opening of the cavity. Consequently, it is inferred that the induced NMR shifts observed for H-3, H-5, and H-6 of β -CD result from the interaction of both ACR and *c*-PeOH within the β -CD cavity. Recently, Muñoz de la Peña et al.²⁹ reported evidence for inclusion

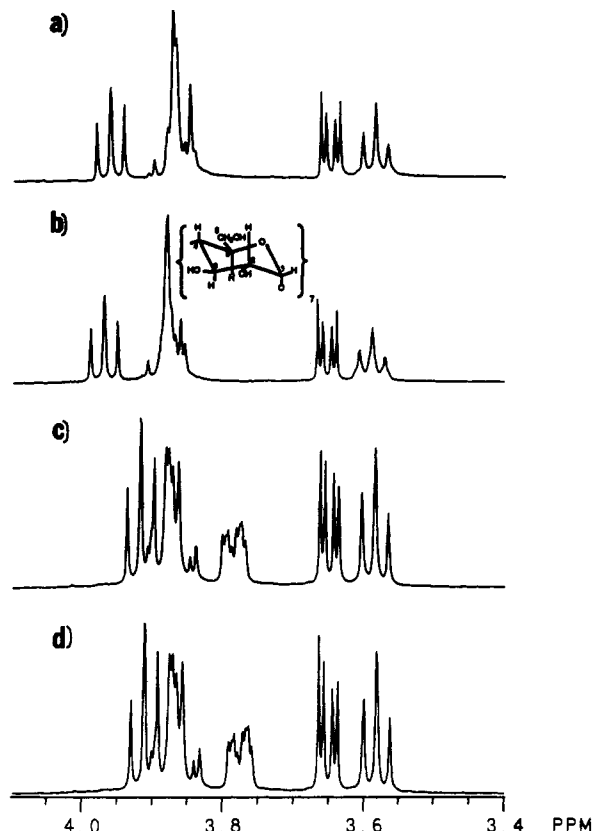


Figure 3. Nuclear magnetic resonance spectra of (a) 5.0×10^{-3} M β -CD with 2.6×10^{-4} M ACR in D_2O , (b) 5.0×10^{-3} M β -CD in D_2O , (c) same as (b) with 1% v/v (0.10 M) *c*-PeOH, and (d) same as (c) with 2.6×10^{-4} M ACR. Spectra were acquired with a 500-MHz General Electric spectrometer.

complexes formed by interaction of 2-PrOH with pyrene inside the β -CD cavity. Furthermore, they established that the alcohol comes into closer proximity with the H-3 and H-5 protons and is situated near the primary edge. Given the larger volume of pyrene with respect to acridine, this type of arrangement not only optimizes the alcohol interaction but is the only site available for interaction with the 2:1 β -CD/pyrene system. Finally, the resonances for the H-1, H-2, and H-4 protons located at the exterior of the β -CD torus are relatively unaffected by the addition of ACR, suggesting that the association does not take place at the exterior of the torus.

Figure 4b depicts the spectral shifts for a 2.6×10^{-4} M aqueous ACR solution. Significant insight can be gained from interpretation in this region. The most dramatic shifts occur for the H_a proton of ACR which is located opposite the nitrogen heteroatom of ACR. The addition of β -CD results in a 0.141 ppm upfield shift for this singlet (Figure 4c). Further addition of 0.11 M *c*-PeOH induces a 0.123 ppm downfield shift of the H_a singlet (Figure 4d) from the position of the H_a proton in the β -CD/ACR spectrum. This suggests the influence of the alcohol in terms of deshielding the H_a proton of ACR from the effects of β -CD. The addition of β -CD to ACR also results in a downfield shift of 0.020 ppm and an upfield shift of 0.040 ppm for the H_b and H_d protons, respectively (Figure 4c). The overall effect is a divergence of the H_a and H_d doublets. H_a and H_d are not microenvironmentally equivalent as far as their interactions with the CD are concerned. While the H_a protons are deshielded in the presence of β -CD, the H_d protons become shielded. This may suggest a skewed arrangement of the ACR molecule inside the CD, whereby the H_d protons interact most strongly. Interestingly, the alcohol by itself does not significantly affect the location of the H_a or H_d doublets (Figure 4a) as compared to the spectrum of ACR alone; however, the addition of *c*-PeOH to the β -CD/ACR system results in a further 0.011 ppm downfield shift in the H_a proton signal and a dramatic 0.0925 ppm downfield shift of the H_d resonance. As

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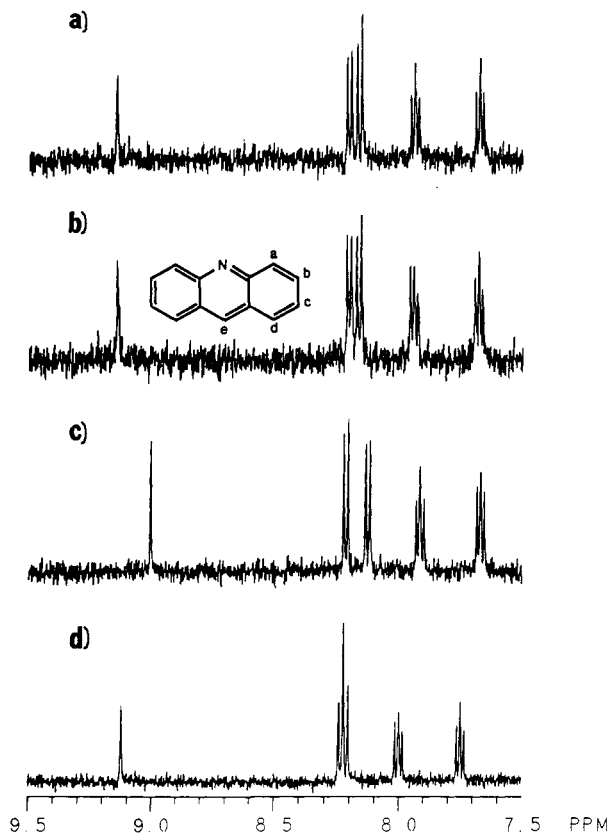


Figure 4. Nuclear magnetic resonance spectra of (a) 2.6×10^{-4} M ACR with 1% v/v (0.10 M) *c*-PeOH in D_2O , (b) 2.6×10^{-4} M ACR in D_2O , (c) same as (b) with 5.0×10^{-3} M β -CD, and (d) same as (c) with 1% v/v (0.10 M) *c*-PeOH.

a result, the H_a and H_d doublets nearly overlap, giving the appearance of a triplet (Figure 4d). Binding of the alcohol with the CD occurs through interaction with the hydroxyl groups of the CD. Thus, the deshielding of the H_d signal upon addition of the alcohol lends support to the premise of ACR interaction with the hydroxyl groups lining the cavity. The ACR H_b and H_c triplets are shifted downfield 0.060 and 0.073 ppm in the presence of β -CD and *c*-PeOH, with respect to their positions in the pure ACR spectrum. The H_b protons, which exhibit a 0.028 ppm upfield chemical shift, appear to be more strongly influenced by addition of β -CD than the H_c protons. It is apparent from this information that the interaction among all three components inside the β -CD cavity results in the most dramatic changes in chemical shift and loss of spectral detail in the ACR proton signals as observed in the downfield shift of both the H_b and H_c proton signals in Figure 4d. Additional NMR studies conducted by varying the ACR concentration revealed that the observed chemical shifts were not due to dimerization. Consequently, a ternary interaction is consistent with the above data.

Stoichiometry. A foundation for understanding both the mechanism and orientation of the interacting molecules requires a determination of the overall stoichiometry of the association. This is particularly important to know before the formation constants of ACR with β -CD in the presence of an alcohol are assessed. It appears likely that some alcohols, through such an interaction, might induce 2:1 or 2:2 β -CD/ACR complexation schemes. Benesi-Hildebrand (B-H) plots and information gained from absorbance and NMR experiments were used to determine if just such an arrangement occurred for any of the β -CD/ACR/alcohol associations studied. We have previously reported a 1:1 stoichiometry for β -CD/ACR,¹¹ and this appears to be consistent with NMR and absorbance results which indicate no apparent formation of dimers. Our data suggest that a small part of ACR is still exposed to the aqueous environment; yet, it is likely not amenable to complexation with a second host molecule. Nevertheless, the potential for partial displacement of ACR from

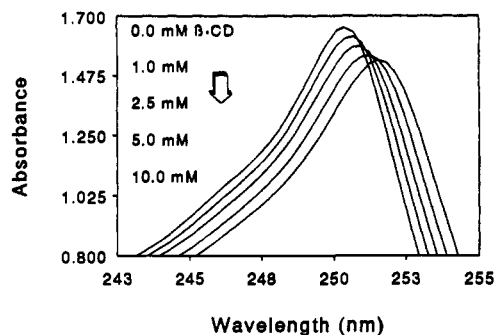


Figure 5. Absorbance plot of ACR in the presence of increasing $[\beta\text{-CD}]$. The region presented represents an enlarged view of the band centered ≈ 249 nm.

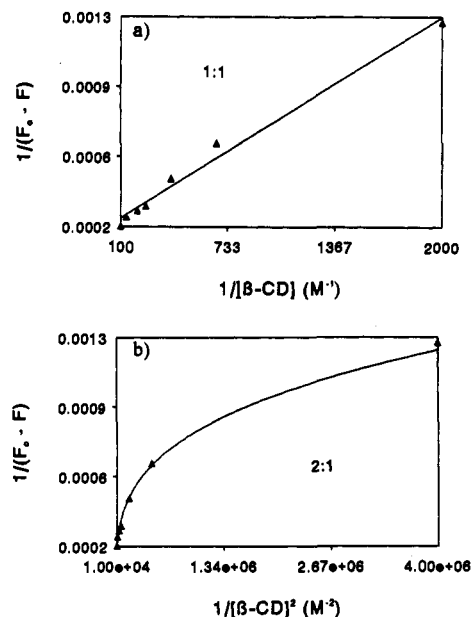


Figure 6. Benesi-Hildebrand plot for β -CD/ACR/*t*-BuOH depicting (a) 1:1 β -CD/ACR and (b) 2:1 β -CD/ACR stoichiometric fits.

the cavity, as a result of complexation with the longer-chained ternary components, makes complexation with more than one β -CD molecule a distinct possibility. The absorbance spectrum of β -CD/ACR in the presence of *t*-BuOH (Figure 5) displays no single isosbestic point. This may signal the existence of several equilibrium species in solution. Nevertheless, Benesi-Hildebrand plots of the fluorescence data fail to substantiate this result (Figure 6). The curvilinear nature of a 2:1 plot suggests that the most appropriate model for β -CD and ACR in the presence of *t*-BuOH is 1:1. The number of alcohols that interact in this system, however, is unclear.

The method of "continuous variation" has been employed as a means of verifying the stoichiometry of CD/guest associations.²⁹ This method involves the adjustment of the molar fraction of CD and guest such that the sum of the CD and guest concentration remains constant. Contributions from the guest are subtracted, and the resulting intensity is plotted against CD molar fraction. The application of this method to the β -CD/ACR system produced inconsistent results. This is attributed to the fact that the highest attainable aqueous concentration of ACR is 2.6×10^{-4} M. The effect of β -CD at this and necessarily lower concentrations, as required by the method, is not discernible. The method also produced inconsistent results for determining the alcohol stoichiometry due to the quenched nature of the β -CD/ACR complex. The continuous variation method relies on the countering effects of increasing $[\text{CD}]$ vs decreasing guest concentration or vice versa. Usually, increasing the CD concentration results in an enhancement in fluorophore intensity until the decreasing ratio of the fluorophore results in a larger contribution to the measured

Table II. Apparent Formation Constants of β -CD/ACR in the Presence of Alcohols (1% v/v)

straight chain	log K'	branched and cyclic	log K'
none	2.46		
ethanol	2.32	2-propanol	2.28
1-propanol	2.23	<i>tert</i> -butanol	2.25
1-butanol	1.89	cyclopentanol	2.16
1-pentanol	1.75	cyclohexanol	1.37

fluorescence—the result being an eventual decrease in measured intensity. However, for ACR, an increase in CD concentration results in a reduction in ACR fluorescence which is only further enhanced by the decrease in the ratio of ACR as required by the method. If ACR is used as a probe for the interaction of the alcohol, the increase in alcohol and simultaneous decrease in β -CD both result in a higher measured ACR fluorescence.

It is apparent from B-H plots that the interaction of *t*-BuOH with the β -CD/ACR system is minimal and does not entirely expel ACR from the cavity or enhance formation of a 2:1 species. Similarly, EtOH, a comparatively smaller volume alcohol and the structurally smallest alcohol examined in this study, as well as *c*-PeOH, which is a larger volume alcohol, did not alter the 1:1 stoichiometry of β -CD and ACR. This is logical in light of the potential for more than two EtOH moieties to fit inside the cavity with ACR. In addition, the weak binding of EtOH to β -CD makes EtOH less effective in its ability to alter the β -CD/ACR interaction. While longer-chained alcohols such as 1-PeOH might be expected to induce complexation with a second host molecule by protruding from the β -CD cavity,¹⁶ this was not observed to be the case. The 1:1 β -CD/ACR complex persisted even in the presence of 1-PeOH.

Strength of Association. Information about the possible mechanism governing the β -CD/ACR system in the presence of alcohols can also be gained by assessing the pattern of variation in association constants of ACR with β -CD as a function of the alcohol type. Table II depicts the apparent formation constants for ACR with β -CD in the presence of various alcohols. In this study, formation constants were calculated using the equations

$$\beta\text{-CD/ACR} + nA = \beta\text{-CD/ACR}/A_n \quad (1)$$

$$K_1 = [\beta\text{-CD/ACR}/A_n]/[\beta\text{-CD/ACR}][A]^n \quad (2)$$

where $[\beta\text{-CD/ACR}/A_n]$ is the equilibrium concentration of the $\beta\text{-CD/ACR}/A_n$ complex at a specific concentration of β -CD. The equilibrium concentrations of the $\beta\text{-CD/ACR}$ complex and alcohol are represented by $[\beta\text{-CD/ACR}]$ and $[A]$, respectively. The overall formation constant is given by K_2 .

$$K_2 = [\beta\text{-CD/ACR}/A_n]/[\beta\text{-CD}][\text{ACR}][A]^n \quad (3)$$

However, the alcohol concentration at 1% (v/v) is in large excess of both ACR and β -CD over the range of β -CD concentrations examined and induces no discernible medium effects. Therefore, an apparent equilibrium constant is given by

$$K' = [\beta\text{-CD/ACR}/A_n]/[\beta\text{-CD}][\text{ACR}] \quad (4)$$

Given that $[\beta\text{-CD}] \gg [\beta\text{-CD/ACR}/A_n]$, we approximate $[\beta\text{-CD}] = [\beta\text{-CD}]_0$, i.e., the initial concentration of β -CD added. The final nonlinear equation, eq 5, used to calculate the apparent formation constant is then identical to the equation used to calculate $K'_{\beta\text{-CD/ACR}}$.¹¹

$$F - F_0 = K'[\beta\text{-CD}]_0/(1 + K'[\beta\text{-CD}]_0) \quad (5)$$

The only difference is that K' now refers to $K'_{\beta\text{-CD/ACR}/A_n}$.

The formation constant for ACR/ β -CD using nonlinear regression analysis is 287 M⁻¹. The A factors determined from lifetime measurements are often used to give an indication of the contribution of free and complexed components to the overall lifetime decay. They can also be used to estimate the formation constant if the ratio of A factors, plotted against the β -CD concentration, is linear. Association constants calculated in this manner from A_2/A_1 plots are in very good agreement with those calculated by nonlinear regression of the fluorescence data for

the β -CD/ACR/alcohol systems.

The magnitudes of the apparent binding constant appear to be a function of alcohol structure as well as polarity. Hamai⁴³ showed that the tendency of alcohols to associate with the β -CD/pyrene complex increased monotonically with alkyl chain length of the alcohol. A similar trend is reflected through a decrease in the apparent binding constant of the β -CD/ACR complex with increasing alcohol chain length as depicted in Table II. Here, it is observed that EtOH is almost ineffectual in reducing the quenching of ACR, while *c*-HexOH exhibits the greatest effect in terms of weakening the binding of ACR to β -CD. This is in contrast to the study of Muñoz de la Peña et al.,²⁹ where it was determined that *c*-PeOH exhibited the greatest enhancement on β -CD/pyrene binding. Such a result is expected for their system since incorporation of pyrene necessarily placed more stringent requirements on the optimum spatial volume remaining available for occupation by the co-included alcohols. The smaller void volumes were also due to the proposed 2:1 β -CD/pyrene barrel-type arrangement which leaves only the primary ends accessible to the alcohol.

Interestingly, branching and the location of the hydroxyl group appear to affect the extent of quenching of ACR as well. Apparently, positioning of the hydroxyl group at the terminus of the propanol, as in 1-PrOH, reduces the quenching of ACR more than positioning it at the center methylene group as with 2-propanol. Barone et al.³⁶ determined through calorimetric measurements that the straight-chain alcohols preferentially enter the cavity through the alkyl chain, followed by the hydroxyl end. Branching of butanol evidently reduces the effective interaction of butanol in the specific binding regions of ACR to β -CD. Consequently, a higher apparent formation constant is observed for ACR/ β -CD in the presence of *t*-BuOH vs 1-BuOH. The same is true with cyclization of a particular alcohol as observed with 1-PeOH and *c*-PeOH. Matsui and Mochida¹⁶ performed an extensive study on the interaction of various alcohols with α - and β -CD. They reported that the bulkiness of the alcohol affected the stability of the CD/alcohol adduct with respect to relative sizes of the alcohol and CD moieties. Branched and cyclic alcohols, while too bulky to be deeply included within the α -CD cavity, achieved maximal interaction with the larger-diameter β -CD. Similarly, straight-chain alcohols associated more strongly with α -CD. As suggested earlier in this paper, the apparent formation constant also depends on the alcohol concentration. The log association constant of β -CD/ACR decreases dramatically with increased concentrations of *t*-BuOH (i.e., 1.85 ± 0.32 at 0.214 M, 2.03 ± 0.06 at 0.375 M, and 1.85 ± 0.39 at 0.535 M), although the values do not appear to reflect a particular trend. This information lends credence to the tendency of large concentrations of alcohol to extract the hydrophobic guest from the cavity by effectively eliminating the polarity differences between the interior and exterior of the cavity.

Due to their greater accessibility to the internal cavity of the β -CD, longer hydrocarbon or small-volume alcohols such as 1-BuOH (88.6 Å³) or 1-PrOH (72 Å³), respectively, are expected to and, in fact, do exhibit stronger reductions on the quenching of ACR than *t*-BuOH (88.2 Å³), for example. Zung et al.³⁰ attempted to correlate the enhancement in γ -CD/pyrene binding to the alcohol polarity and/or the volume and its consequent ability to fill spatial voids created through the association. At some point, however, spatial volume becomes a more significant parameter than chain length. For example, a stronger interaction is observed for *c*-HexOH than for 1-PeOH. Thus, the incorporation of the alcohol moiety depends on the structure and spatial volume of the alcohol as well as the volume of the void regions available in the CD molecule after complexation with a guest moiety.

Conclusions. The information from NMR and fluorescence lifetime experiments suggests a predominantly space-regulatory role for those alcohols having an appropriate physical structure to accommodate the remaining sites of interaction. For example, *c*-HexOH has been reported to exhibit both space-regulatory and competitive properties in association with γ - and β -CD/ α -naphthylxyacetic acid complexes, respectively.¹⁸ It also appears

that the nitrogen heteroatom of ACR plays an important role in the destabilization of the complex by the alcohol since polynuclear aromatic counterparts of ACR behave much differently in both the magnitude of their formation constants and interaction with the alcohols.

Finally, the cooperative effects of two quenching mechanisms appear to be in operation. Upon addition of the alcohol, the static interaction of ACR with β -CD is reduced and an increase in the contribution from the free ACR species is observed. This suggests a partial disruption of the complex, since NMR data provide evidence for the existence of some remaining ternary associations. Additionally, the increase in the collisional quenching, as deter-

mined from dynamic quenching constants, supports the weaker nature of the existing ternary associations.

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Subpicosecond $^1\text{MLCT} \rightarrow ^5\text{T}_2$ Intersystem Crossing of Low-Spin Polypyridyl Ferrous Complexes

James K. McCusker, Kevin N. Walda, Robert C. Dunn, John D. Simon,*
Douglas Magde,* and David N. Hendrickson*

Contribution from the Department of Chemistry-0506, University of California at San Diego, La Jolla, California 92093-0506. Received July 24, 1992

Abstract: Two different $\Delta S = 2$ intersystem crossing processes, the $^1\text{MLCT} \rightarrow ^5\text{T}_2$ and $^5\text{T}_2 \rightarrow ^1\text{A}_1$ conversions which follow the photoexcitation of low-spin Fe^{II} complexes, are examined on the nanosecond, picosecond, and subpicosecond time scales. Aqueous solutions of the complexes $[\text{Fe}(\text{tpen})](\text{ClO}_4)_2$, $[\text{Fe}(\text{tptn})](\text{ClO}_4)_2$, $[\text{Fe}(\text{t-pchxn})](\text{ClO}_4)_2$, $[\text{Fe}(\text{bpy})_3](\text{ClO}_4)_2$, $[\text{Fe}(\text{phen})_3](\text{ClO}_4)_2$, and $[\text{Fe}(\text{terpy})_2](\text{ClO}_4)_2$ are studied, where in the first three complexes the hexadentate ligands are tetrakis(2-pyridylmethyl)ethylenediamine (tpen), tetrakis(2-pyridylmethyl)-1,3-propylenediamine (tptn), and tetrakis(2-pyridylmethyl)-*trans*-cyclohexane-1,2-diamine (t-pchxn). The $[\text{Fe}(\text{tpen})]^{2+}$ complex is a spin-crossover complex with a $^1\text{A}_1$ ground state and a thermally accessible $^5\text{T}_2$ excited state. In H_2O this complex exhibits an isosbestic point in its electronic absorption spectrum at 302 ± 2 nm. Picosecond laser-flash instrumentation is used with probe wavelengths from 266 to 460 nm to determine the nature of the (18 ± 2) -ns relaxation seen for $[\text{Fe}(\text{tpen})]^{2+}$. In the transient difference spectrum, there is a change from bleach to absorption response at ~ 300 nm. This definitively establishes the (18 ± 2) -ns relaxation as being associated with the $^5\text{T}_2 \rightarrow ^1\text{A}_1$ conversion. The related complex $[\text{Fe}(\text{tptn})]^{2+}$, which is all low-spin in solution throughout the temperature range examined, also shows a transient difference spectrum with an isosbestic at ~ 300 nm, suggesting that formation of $^5\text{T}_2$ is a general feature. Laser instrumentation with a ~ 500 -fs pulse ($\lambda_{\text{pump}} = 314$ nm) is used to measure the rate of formation of the $^5\text{T}_2$ state following $^1\text{MLCT} \leftarrow ^1\text{A}_1$ excitation of low-spin Fe^{II} complexes. All of the above complexes were examined, and the observed kinetics were found to be independent of probe wavelength in the 385–510-nm range. A plot for each complex of ΔOD versus time of data collected in increments of 167 fs over the first 10–15 ps following $^1\text{MLCT} \leftarrow ^1\text{A}_1$ excitation clearly shows that the $^5\text{T}_2$ state of these Fe^{II} complexes is formed within ~ 700 fs. Intersystem crossing is at least as fast as internal conversion and vibrational cooling in these complexes. Transient absorption spectra (333-fs increments) suggest, furthermore, that vibrational cooling in the $^5\text{T}_2$ excited state is complete within ~ 2 –3 ps. The reasons for the surprisingly fast ($< \sim 700$ fs) $\text{MLCT} \rightarrow ^5\text{T}_2$ intersystem crossing where $\Delta S = 2$ are discussed.

Introduction

Molecules which exhibit the phenomenon of thermal spin-crossover have been of interest since 1932, when Cambi first noted anomalous magnetic behavior in certain ferric dithiocarbamates.¹ Since the first full review of the subject by Martin and White in 1968, a considerable amount of effort has been put forth to understand these types of complexes.² Aside from providing fundamental information about spin-state interconversions, the spin-crossover transformation, as it occurs in complexes of Fe^{II} in particular, is intimately related to oxygenation and carbonylation

of myoglobin and hemoglobin and as well of the electron-transport chains associated with heme proteins such as P450.³ Thus, the most widely studied of spin-crossover complexes are those containing iron. Most of the research on spin-crossover systems has concentrated on studies in the solid state. However, the benchmark paper by Sorai and Seki⁴ establishing that cooperativity can have an overriding influence on the nature and mechanism of spin-crossover transformations serves to illustrate that understanding the intramolecular phenomenon of spin-crossover in the solid state is a complex problem.⁵

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