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β-Cyclodextrin as a Photosensitizer Carrier: Effect on Photophysical Properties and Chemical Reactivity of Squaraine Dyes

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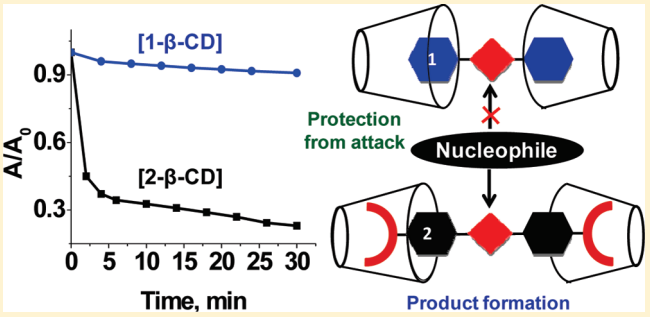
Supporting Information

ABSTRACT: With the objective of understanding the utility of β-cyclodextrin (β-CD) as a carrier system, we have investigated its interactions with a few near-infrared absorbing squaraine dyes (i.e., **1a,b** and **2a,b**) through absorption and steady-state and time-resolved fluorescence techniques. The addition of β-CD to the phloroglucinol dyes **1a,b** resulted in a significant bathochromic shift in absorption, together with a ca. 1.5−2.5-fold enhancement in fluorescence intensity, whereas for the aniline-based dyes **2a,b**, a hypsochromic shift in the absorption and a ca. 5−12-fold fluorescence enhancement were observed in a 10% (v/v) ethanol/water mixture. Benesi−Hildebrand analysis showed that both the dyes **1a,b** and **2a,b** form 2:1 stoichiometric complexes with β-CD. The complex formation was confirmed by competitive binding analysis employing adamantyl-1-carboxylic acid (ACA) and adamantyl-1-ammonium chloride (ADAC). The displacement of the dyes **1a,b** and **2a,b** from the [dye−β-CD] complex by ADAC and ACA unambiguously establishes the encapsulation of these dyes in the hydrophobic nanocavity of β-CD. Uniquely, the formation of the inclusion complexes with β-CD provides unusual protection from nucleophilic attack by aminothiols such as cysteine and glutathione for dyes **1a,b**, whereas negligible protection was observed for dyes **2a,b**. These results demonstrate the substituent-dependent encapsulation of potentially useful squaraine dyes in β-CD, thereby indicating its potential as a carrier system for the squaraine dyes **1a,b** useful in photodynamic therapy.



1. INTRODUCTION

The pharmacological activity of a bioactive molecule depends to a great extent on its protection from biodegradation. Such protection is often achieved by the utilization of a drug carrier system, which ensures not only the molecule's solubility but also its stability under physiological conditions.[1] In this context, cyclodextrins have emerged as carrier systems of choice and have been the subject of intense research in recent years.[2] Cyclodextrins (CDs) are cyclic oligomers of glucose characterized by their negligible toxicity and their ability to form inclusion complexes with several organic systems. CDs are thus widely used as delivery systems for drugs in pharmacological applications.[3] The supramolecular inclusion complexes formed between organic substrate and cyclodextrins have been used as models for protein−ligand interactions and enzyme catalysis.[4] Further, the study of the interactions of various compounds with cyclodextrins has been of enormous interest because CDs facilitate the solubilization of hydrophobic compounds in aqueous media and significantly modify the physicochemical and biological properties of active molecules.[5]

Squaraines are a class of dyes exhibiting sharp and intense absorption in the near-infrared region.[6] Because of the various applications of these dyes, their photochemical and photophysical aspects have been studied extensively.[7] These dyes have also been proposed to be useful in general applications as metal ion sensors[8] and in biological applications as long-wavelength fluorescent labels[9] and detectors of biorelevant thiols.[10] However, their potential applications as photosensitizers in photodynamic therapy (PDT) have not been attempted because of their poor intersystem crossing efficiency.[11] In this context, we have demonstrated that the substitution of these dyes with heavy atoms enhances their triplet quantum yield and, thereby, their ability to generate cytotoxic agents such as singlet oxygen.[12]

The effectiveness of a photosensitizer in PDT depends, to a large extent, on the photochemical and photophysical properties of the sensitizer. Although one can suitably tailor the photophysical properties of squaraine dyes, one principal drawback associated with these systems is stability, in particular, their propensity toward nucleophilic attack.[13] For example, the electron-deficient cyclobutene ring of a squaraine dye can undergo efficient nucleophilic attack by thiol-containing biomolecules such as cysteine, glutathione, and proteins, which would lead to the loss of the photophysical properties of these dyes. In addition, because of their low water solubility, these dyes are known to form aggregates in aqueous solutions,[14,15] which can

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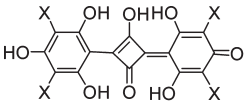
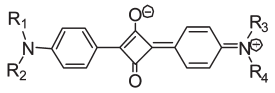
Chart 1



1a-b



2a-b

1a) X = Br
1b) X = I

2a) $R_1 = R_3 = CH_3$, $R_2 = R_4 = -(CH_2\text{-}CH_2\text{-}O)_4\text{-}CH_3$
2b) $R_1 = R_3 = CH_3$, $R_2 = R_4 = -(CH_2)_3\text{-}COOH$

significantly alter their photophysical properties, including the generation of cytotoxic agents. In this context, it is important to utilize suitable carrier systems that can not only control dye aggregation, but also prevent nucleophilic attack, thereby enhancing the therapeutic index of the photosensitizer.

In this regard, the present study aims to understand the ability of β-cyclodextrin (β-CD) to preserve the photophysical and chemical integrity of a few squaraine-based sensitizers and evaluate its utility as a sensitizer carrier system in PDT applications. With this objective, we have investigated the interactions of differently substituted squaraine dyes, both phloroglucinol-based (**1a**,**b**) and aniline-based (**2a**,**b**), with β-CD through various spectroscopic techniques (Chart1). Our results indicate that inclusion of the dyes in the β-CD cavity is able to protect, to a large extent, the phloroglucinol-based dyes **1a** and **1b** from the attack of nucleophiles such as aminothiols, thereby indicating the potential of β-CD as a carrier system for these PDT sensitizers.

2. EXPERIMENTAL SECTION

2.1. General Techniques. The equipment and procedures for melting point determination and spectral recordings have been described elsewhere.[16] Melting points were determined on a MEL-TEMPII melting point apparatus. Absorption was measured using Shimadzu model UV-3101PC UV−vis−NIR scanning and UV-2401PC UV−vis recording spectrophotometers. Fluorescence measurements were made on a Spex-Fluorolog F112X spectrofluorimeter. Fluorescence quantum yields were measured by the relative method using optically matched dilute solutions. Cresyl violet, with a quantum yield of 0.54 in methanol,[17] was used as the standard for dyes **1a**,**b**, whereas 4,4-[bis-(*N*,*N*-dimethylamino)phenyl]squaraine dye (0.70) in chloroform[18] was used for dyes **2a**-**b**. The quantum yields of fluorescence were calculated using the equation

$$\Phi_u = \frac{A_s F_u n_u^2}{A_u F_s n_s^2} \Phi_s \qquad (1)$$

where A_s and A_u are the absorbances of the standard and unknown, respectively; F_s and F_u are the areas of fluorescence peaks of the standard and unknown, respectively; and n_s and n_u are the refractive indices of the standard and unknown solvents, respectively. Φ_s and Φ_u are the fluorescence quantum yields of the standard and unknown dye, respectively. A solution of Rhodamine 6G in water was used as a quantum counter, which allows correction in the spectral range up to 750 nm. The values of $\Phi_{\beta\text{-CD}}$ were calculated using eq 1 and taking F_u to be the fluorescence intensity at the highest concentration of β-CD (3 mM for **1a**,**b** and 28 mM for **2a**,**b**). Figure 4 (below) was plotted by calculating the quantum yield of fluorescence upon each addition of β-CD.

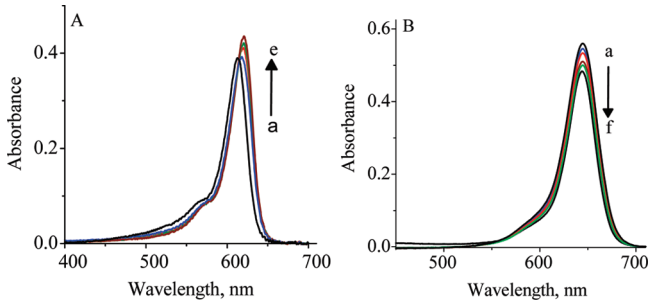


Figure 1. Changes in the absorption spectra of (A) **1a** (3.5 μM) and (B) **2a** (1.8 μM) in a 10% (v/v) ethanol/water mixture with increasing concentration of β-CD. [β-CD] = (a) 0, (e) 3, and (f) 28 mM.

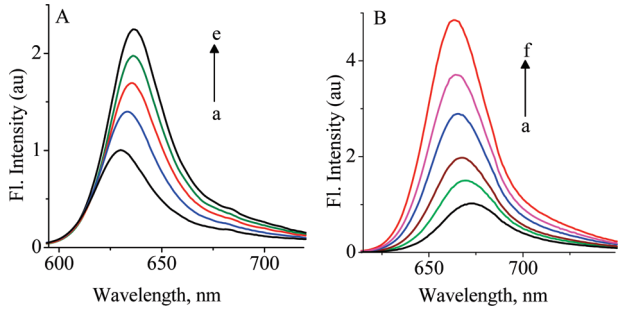


Figure 2. Changes in the emission spectra of (A) **1a** (3.5 μM) and (B) **2a** (1.8 μM) in a 10% (v/v) ethanol/water mixture with increasing concentration of β-CD. [β-CD] = (a) 0, (e) 3, and (f) 28 mM. The excitation wavelength was 575 nm for **1a** and 600 nm for **2a**.

Fluorescence lifetimes were measured on an IBH picosecond single photon counting system using a 635-nm IBH NanoLED source and a Hamamatsu C4878-02 microchannel plate (MCP) detector. The fluorescence decay profiles were deconvoluted using IBH data station software V2.1, fitted with a mono- or biexponential decay and minimizing the χ^2 values of the fit to 1 ± 0.1. Solutions for optical measurements were prepared from freshly prepared stock solutions of the dye in ethanol. Measurements in the presence of additives were made by adding the dye solution in ethanol to the already prepared solution of β-CD. The concentrations of the dyes were chosen in the micromolar range for all studies, so that the dye molecules exist as monomers as observed from their absorption spectra. The solvents used were purified before use. Doubly distilled water was used in all cases. All experiments were carried out at room temperature (25 ± 1 °C), unless otherwise mentioned. All experiments were carried out more than three times for consistency.

2.2. Materials. β-Cyclodextrin (β-CD), glutathione, and cysteine were purchased from Aldrich and were used without further purification. The squaraine dyes **1a** [mp 314−316 °C (lit. mp 315 °C)],[11] **1b** [mp 269−270 °C (lit. mp 270 °C)],[11] **2a** [mp 100−101 °C (lit. mp 100−102 °C)],[13] and **2b** [mp 237−239 °C (lit. mp 238−240 °C)][13] were prepared by following the reported procedures.

3. RESULTS

3.1. Effect of β-CD on Absorption and Emission Properties. To examine the effect of the hydrophobic environment on the photophysical properties of the squaraine dyes, we monitored

Table 1. Absorption, Fluorescence Emission, and Fluorescence Quantum Yields of the Squaraine Dyes 1a,b and 2a,b in the Presence and Absence of β-CD[a,b]

dye	λ_{ab}[b] (nm)	$\lambda_{ab,\beta\text{-CD}}$[b] (nm)	λ_{em}[b] (nm)	$\lambda_{em,\beta\text{-CD}}$[b] (nm)	Φ_f[b] (10^{-2})	$\Phi_{\beta\text{-CD}}$[c] (10^{-2})
1a	613	620	630	636	0.22	0.38
1b	617	625	634	640	0.09	0.15
2a	645	644	674	663	1.8	10
2b	647	649	673	666	1.5	19

[a] Averages of more than two experiments. Error in Φ_f values of ca. ±5%. [b] λ_{ab}, λ_{em}, $\lambda_{ab,\beta\text{-CD}}$ and $\lambda_{em,\beta\text{-CD}}$ denotes absorption and emission maxima of the dyes in the absence and presence of β-CD. [c] β-CD concentration: 3 mM for **1a,b** and 28 mM for **2a,b**. Excitation wavelength: 575 nm for **1a,b** and 600 nm for **2a,b**.

the changes in the absorption and emission spectra of **1a,b** and **2a,b** in the presence of β-CD. Figure 1A shows the change in the absorption spectrum of dye **1a** in a 10% (v/v) ethanol/water mixture with increasing concentration of β-CD. With the addition of β-CD, an increase in the extinction coefficient at 613 nm and a bathochromic shift of about 7 nm were observed. In contrast, the aniline-based squaraine dye **2a** showed a marginal decrease in the extinction coefficient at 645 nm, with negligible changes in its absorption maximum (Figure 1B). Similar observations were made for dyes **1b** and **2b**, with **1b** exhibiting a bathochromic shift of 8 nm and **2b** showing no changes in its absorption maximum in the presence of β-CD (Figure S1, Supporting Information).

The changes observed in the emission spectra of **1a,b** and **2a,b** upon the addition of β-CD are shown in Figure 2. For example, **1a** alone in a 10% (v/v) ethanol/water mixture showed an emission maximum at 630 nm (Figure 2A). The addition of β-CD to the **1a** solution caused an increase in the fluorescence intensity along with a 6-nm red shift of the peak to 636 nm. The fluorescence changes reached saturation at about 3 mM β-CD. As observed for **1a**, **1b** showed a 6-nm bathochromic shift in the emission maxima in the presence of β-CD (Figure S2A, Supporting Information). Surprisingly, aniline-based dye **2a** showed an enhancement in fluorescence emission intensity along with a hypsochromic shift from 674 to 663 nm in the presence of β-CD. Similar results were observed in the case of the dye **2b** (Figure S2B, Supporting Information). The photophysical properties of the dyes **1a,b** and **2a,b** in the presence and absence of β-CD are summarized in Table 1. Of the various squaraine dyes, **2b** was found to show the maximum enhancement in fluorescence quantum yield of ca. 12-fold and reached saturation at a 25 mM concentration of β-CD (Figure S3, Supporting Information).

3.2. Stoichiometry and Association Constants of β-CD Inclusion Complexes. To evaluate the stoichiometry of the complexation, a Jobs plot analysis was carried out for the representative squaraine dye **1a**. The plot of fluorescence enhancement versus mole fraction of β-CD showed an inflection point at a mole fraction of 0.67, which indicates the formation of a 2:1 complex (Figure S4, Supporting Information). The association between the dyes **1a,b** and **2a,b** with β-CD was calculated for a 2:1 stoichiometry using fluorescence changes through the Benesi–Hildebrand equation[19]

$$\frac{1}{(\Phi_{ob}-\Phi_f)} = \frac{1}{(\Phi_{fc}-\Phi_f)} + \frac{1}{K(\Phi_{fc}-\Phi_f)[\beta\text{-CD}]^2} \qquad (2)$$

where K is the association constant, Φ_f is the fluorescence quantum yield of the free dye, Φ_{ob} is the observed quantum yield at each β-CD concentration, and Φ_{fc} is the quantum yield of emission of the [dye–β-CD] complex. Figure 3 shows the Benesi–Hildebrand plot for dyes **1a** and **2a** using the fluorescence changes. The observation of linear plots indicates that both **1a** and **2a** form 2:1 stoichiometric complexes with β-CD with association constants (K) of 2.7 ± 0.1 × 10^3 and 4.8 ± 0.2 × 10^4 M^{-1}, respectively. It might be mentioned that, although the double reciprocal plots allow for the establishment of the stoichiometry of the inclusion complex, the K values determined from these plots give only an estimated value of the association constants because the emphasis was placed on lower CD concentrations rather than higher concentrations.

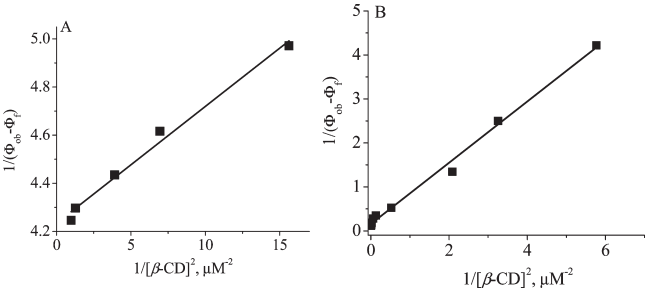


Figure 3. Benesi–Hildebrand plots for complexation between β-CD and the squaraine dyes (A) **1a** and (B) **2a** in a 10% (v/v) ethanol/water mixture.

3.3. Effect of Solvent Polarity on Complex Formation. Because the absorption and emission maxima of squaraine dyes are known to vary with solvent polarity,[20] attempts were made to estimate the polarity of the environment experienced by the dyes in the β-CD cavity by correlating the spectral shifts with different solvent polarity scales such as the Dimorth–Reichardt solvatochromic parameter [$E_T(30)$]. The solvent polarity scale $E_T(30)$ is based on the solvent-induced shift of the electronic absorption transitions of the negatively solvatochromic dye pyridinium *N*-phenoxide betaine 30.[21] A plot of the Stokes shift against the $E_T(30)$ values of solvents of varying polarity gave a linear correlation for the dye **2a** (Figure S5, Supporting Information).[9c–e] Extrapolation of this plot gave an $E_T(30)$ value of 39.4 for the β-CD cavity, indicating that the polarity of the cyclodextrin interior is close to that of *t*-butanol.[22] The lower polarity of the β-CD cavity compared to water accounts for the enhancement in fluorescence quantum yield of the dyes. However, we could obtain no correlation for dyes **1a,b**, because these dyes are known to exist in different forms in different solvents and each of these forms has different absorption and emission properties.[14]

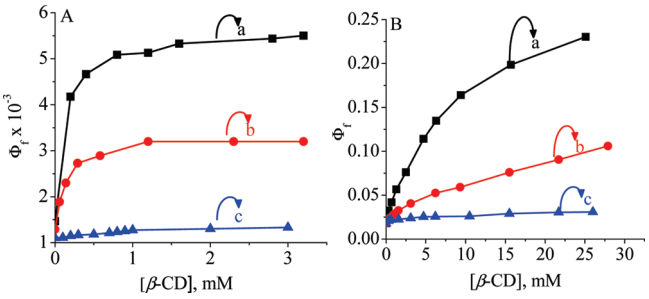


Figure 4. Plots of fluorescence quantum yields (normalized) of (A) **1a** (3.5 μM) and (B) **2a** (1.8 μM) in ethanol/water mixtures of varying percentages (v/v) versus β-CD concentration. Ethanol/water mixture (v/v): (a) 2%, (b) 10%, and (c) 30%.

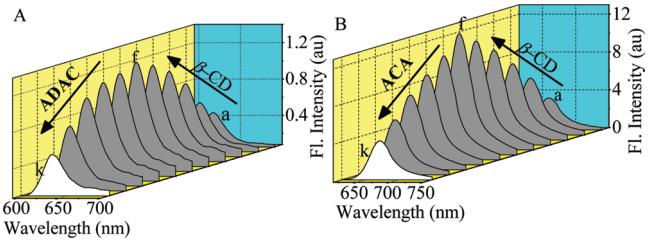


Figure 5. Fluorescence spectra upon the addition of β-CD to a 10% (v/v) ethanol/water mixture followed by gradual addition of adamantyl-1-ammonium chloride (ADAC)/adamantyl-1-carboxylic acid (ACA). (A) **1a** (3.5 μM); [β-CD] = (a) 0 and (f) 3 mM, [ADAC] = (f) 0 and (k) 11.2 mM. (B) **2a** (1.8 μM); [β-CD] = (a) 0 and (f) 28 mM, [ACA] = (f) 0 and (k) 11.2 mM. The excitation wavelength was 575 nm for **1a** and 600 nm for **2a**.

Further, to determine the influence of the organic solvent ethanol on the complexation of the dyes with β-CD,[23] we measured the absorption and emission changes of dyes **1a** and **2a** with β-CD in ethanol/water mixtures of various proportions. Figure 4 shows plots of the fluorescence quantum yields of **1a** and **2a** as a function of the β-CD concentration in various percentages of ethanol and water. As can be seen from the figure, the fluorescence quantum yields of both of the dyes increase with β-CD concentration, but to different extents. In a 2% (v/v) ethanol/water mixture, dye **1a** exhibited a 4-fold enhancement in fluorescence quantum yield. Interestingly, when the ethanol concentration was increased to 10% and 30%, we observed decreases in the enhancements to 2.5- and 0.8-fold, respectively. Similarly, for the aniline-based dye **2a**, the enhancement in fluorescence quantum yield was found to be 1.7-fold in a 30% (v/v) ethanol/water mixture, whereas it was 5- and 13-fold in 10% and 2% (v/v) ethanol/water mixtures, respectively. This difference in the magnitudes of the fluorescence quantum yield enhancements with variations in the ethanol proportion can be attributed to the preferential occupation of the β-CD cavity by the hydrophobic ethanol molecules, which, thereby, restrict the formation of the [β-CD−dye] complexes.

3.4. Confirmation of β-CD Encapsulation. To confirm the inclusion of the squaraine dye inside the β-CD cavity, we carried out displacement studies using the well-known β-CD binding agents adamantyl-1-ammonium chloride (ADAC) and adamantyl-1-carboxylic acid (ACA). Figure 5 shows the changes in fluorescence intensity of the [dye−β-CD] complex upon addition of ADAC or ACA. As mentioned previously, we observed an increase in the fluorescence intensity of the dye with increasing concentration of β-CD, as seen from the upward curve (traces a−f, Figure 5). Interestingly, the subsequent addition of increasing amounts of ADAC or ACA resulted in a decrease in the fluorescence intensity of the dye, until finally, the fluorescence yields corresponding to the free dye molecules were observed (traces f−k, Figure 5). The reversal in the fluorescence intensity upon the addition of β-CD encapsulating agents further confirms that the enhancement in the fluorescence quantum yield of the squaraine dyes upon interaction with β-CD is due to their encapsulation.[24]

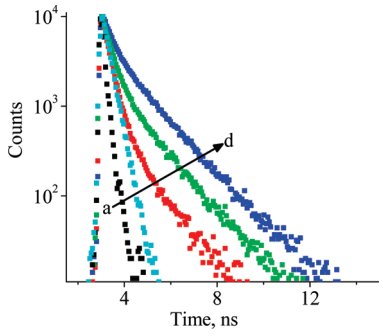


Figure 6. Fluorescence decay profiles of **2a** (1.6 μM) in 10% (v/v) ethanol/water mixtures. [β-CD] = (a) 0 and (d) 28 mM. Excitation was at 635 nm, and emission was collected at 670 nm.

The interaction of the dye molecules with the saccharide unit in β-CD might also alter the spectral properties of the dyes. To demonstrate that the observed spectral changes are due to encapsulation in the hydrophobic cavity rather than interaction with the saccharide unit on the surface, we recorded the absorption and emission spectra of representative dye **1a** in the presence of the noncyclic saccharide D-(+)-glucose instead of β-CD. We observed negligible changes in the fluorescence quantum yield of the dye molecules upon interaction with D-(+)-glucose (Figure S6, Supporting Information).

3.5. Picosecond Lifetime Measurements. To obtain a better understanding of the fluorescence changes observed in the presence of β-CD, we analyzed the interaction of β-CD with dyes **1a**,**b** and **2a**,**b** in 10% (v/v) ethanol/water mixtures through the picosecond time-resolved fluorescence technique. For example, Figure 6 shows the fluorescence decay profiles of dye **2a** in the presence and absence of β-CD. **2a** alone exhibited a monoexponential decay with a lifetime of 160 ps. As can be seen from the figure, at the lowest concentration of β-CD (1.9 mM), the short-lived component was the major species (80%), with a lifetime of 0.324 ns, whereas the minor component (20%) exhibited a lifetime of 1.34 ns. Interestingly, as the concentration of β-CD increased, we observed an increase in the percentage of the long-lived component. Thus, the amplitude of the long-lived species was 42% at 6.2 mM β-CD and increased to 70% at 18.6 mM β-CD. The observed enhancement can be attributed to the effective interaction of the dye with the β-CD. This is in conjunction with our observation of enhanced fluorescence quantum yield due to restricted rotation inside the β-CD cavity, which, in turn, results in reduced nonradiative decay processes and an increased lifetime. Moreover, the environment experienced by the encapsulated dye molecule is less polar than the free solution environment, and it is known that squaraine dyes have higher fluorescence quantum yields and, in turn, longer lifetimes

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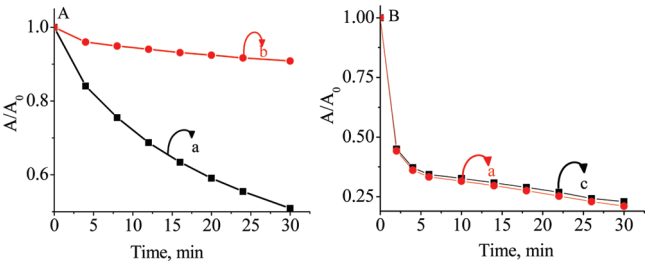

Figure 7. Change in absorbance of (A) **1a** (3.6 μM) and (B) **2a** (1.8 μM) versus time with addition of cysteine (54 μM) in the presence of β-CD in a 10% (v/v) ethanol/water mixture. [β-CD] = (a) 0, (b) 3.4, and (c) 28 mM.

in less polar environments. The species with the longer lifetime can be assigned to dye molecules encapsulated in the β-CD cavity, whereas the short-lived species could be due to free dye molecules. As the concentration of β-CD increased, we observed a corresponding increase in the percentage of long-lived species (i.e., a higher percentage of the dye molecules became encapsulated within the cavity of the β-CD) and a concomitant decrease in the percentage of short-lived species (i.e., free dye molecules). The observed increase in the lifetimes of the short-lived species, when compared to the dye molecules in the absence of β-CD, indicates that the macroscopic environment has an influence on the lifetimes of these dye molecules in the presence of β-CD.

From the observed fluorescence quantum yield (Φ_f) and fluorescence lifetime (τ_f) of dye **2a**, the rate constants for radiative decay (k_r), nonradiative internal conversion (k_{nr}), and intersystem crossing (k_{isc}) can be calculated using the equations

$$\Phi_f = \frac{k_f}{k_r + k_{isc} + k_{nr}} \quad (3)$$

$$\tau_f = \frac{1}{k_r + k_{isc} + k_{nr}} \quad (4)$$

Because the triplet quantum yield (Φ_T) of the dye is negligible, $k_{isc} \ll k_r + k_{nr}$, so eqs 3 and 4 can be simplified to give

$$k_r = \frac{\Phi_f}{\tau_f} \quad (5)$$

$$k_{nr} = k_r\left(\frac{1}{\Phi_f} - 1\right) \quad (6)$$

Substituting the values for the quantum yield (0.018) and lifetime (160 ps) of **2a** in a 10% (v/v) ethanol/water mixture, we obtained the estimates $k_r = 1.1 \times 10^8\ s^{-1}$ and $k_{nr} = 6.0 \times 10^9\ s^{-1}$. However, in the presence of β-CD, the fluorescence quantum yield and lifetime increased to 0.1 and 1.6 ns (average lifetime),[25] respectively. Analysis of these data using eqs 5 and 6 gives radiative and nonradiative decay rate constants of $k_r = 6.3 \times 10^7\ s^{-1}$ and $k_{nr} = 5.7 \times 10^8\ s^{-1}$ respectively. The decrease in the k_{nr} value in the presence of β-CD shows the suppression of the nonradiative decay processes in the deactivation of the excited singlet of **2a**, as discussed earlier.

3.6. Evaluation of β-CD as a Carrier System. The potential utility of the squaraine dyes in various applications is limited by certain drawbacks associated with them, namely, their susceptibility to chemical attack[26a] and their tendency to form aggregates. These two drawbacks can lead to the loss of the intense absorption of the dyes, eventually affecting their potential as sensitizers in PDT. The problem of aggregation could be prevented by encapsulating the dye molecules in a macrocyclic host such has β-CD.[27] Recently, Smith and co-workers encapsulated a squaraine dye as a rotaxane, thereby preventing its aggregation as well as protecting the chromophore from nucleophilic attack.[26] Because the dye molecules are effectively and efficiently encapsulated inside the β-CD cavity, we studied the interaction of the dyes with amino acids such as cysteine (Cys) and the tripeptide glutathione (GSH) in the absence and presence of β-CD to understand the efficiency of protection from chemical attack and also the potential of β-CD as a carrier system.

Figure 7A shows the changes in the absorbance of dye **1a** with time upon addition of Cys in the presence and absence of β-CD in a 10% (v/v) ethanol/water mixture. Upon addition of 5.4×10^{-5} M of Cys to a solution of **1a**, we observed a decrease in the absorbance of the dye with time, indicating nucleophilic attack of the thiol group of the cysteine at the squaryl ring and formation of a squaraine−cysteine adduct. Interestingly, in the presence of β-CD, a negligible decrease in the absorbance of **1a** was observed, suggesting that the encapsulation of the dye in the β-CD cavity protects the dye against nucleophilic attack. Similar observations were made with the addition of the tripeptide glutathione, indicating that the β-CD-encapsulated **1a** is protected from glutathione attack. In contrast, we observed a significant decrease in the absorbance of **2a** upon the addition of cysteine in both the absence and the presence of β-CD (Figure 7B). The decrease in the absorbance of **2a** with cysteine in the presence of β-CD indicates that, even though the dye is encapsulated within the hydrophobic cavity of β-CD, encapsulation provides less protection from nucleophilic attack in this case. Similar observations were made with the aniline-based dye **2b** in the presence of cysteine and glutathione, thereby indicating that β-CD is an inefficient carrier system for these aniline-based dyes (Figure S7, Supporting Information).

4. DISCUSSION

Cyclodextrins (CDs) are cyclic oligosaccharides that have a central cavity capable of accommodating guest molecules in aqueous solution. These molecules contain six (α-CD), seven (β-CD), and eight (γ-CD) glucose units, giving diameters of approximately 4.5, 6.5, and 8.5 Å, respectively. The interiors of the cavity of CDs are encircled by ether oxygens and provide a hydrophobic microenvironment in aqueous solutions. The guest molecules that are accommodated in these cavities are relatively isolated from the bulk water environment and often have enforced and constrained conformations.[28] A hypsochromic or bathochromic shift of the fluorescence spectrum with progressively increasing β-CD concentration suggests an interaction of β-CD with the dye. The cavity within β-CD is hydrophobic and also less polar than the surrounding water medium, which makes the dye molecule preferentially bind within the relatively nonpolar β-CD cavity.

An interesting observation made in this work was the contrasting behaviors of dyes **1a**,**b** and **2a**,**b** in the presence of β-CD. Whereas the phloroglucinol dyes **1a**,**b** showed a bathochromic shift in absorption and emission with increasing concentration of β-CD, the aniline-based dyes **2a**,**b** showed a hypsochromic shift. This can be explained based on the behaviors of these dyes in environments with different polarities and their tendency to exist in different forms in different solvents. It has been proposed that

the major nonradiative decay of the excited-state squaraine chromophore is by the rotation of the C−C bond between the phenyl ring and the C_4O_2 unit. As the twist angle increases, the rate of rotation of the C−C bond increases, which results in increased nonradiative deactivation.[29] Moreover, the existence of hydrogen bonding between water molecules and the squaraine moiety can also increase the twist angle and distort the coplanarity of the D−A−D structure, thereby resulting in a reduced fluorescence quantum yield. Because the cavity of β-CD is hydrophobic in nature, the encapsulation of these dye molecules is likely to prevent intermolecular hydrogen bonding with the surrounding water molecules, thereby making the molecules more planar. The enhancement in the fluorescent quantum yields of the dyes **1a**,**b** and **2a**,**b** can thus be attributed to the decrease in nonradiative decay processes brought about by the restriction of the rotational freedom of the encapsulated molecules and the elimination of quencher water molecules from the immediate surroundings.

The fluorescence decay profiles in the presence of β-CD indicate the existence of two spectroscopically distinct species, of which the long-lived component can be attributed to the encapsulated dye molecules and the component with the shorter lifetime is assigned to the unbound dye molecules. Our observation of an increased percentage of the long-lived species (i.e., encapsulated dye molecules) together with a decrease in the percentage of the short-lived species as the concentration of β-CD increases supports this argument. However, the second component having the short lifetime showed a marginally longer lifetime than that observed for the dye in a 10% (v/v) ethanol/water mixture. This might be due to the variation in the macroscopic environment experienced by the dye molecule in the presence of β-CD solution, which is expected to be quite different from the pure solvent mixture. On the basis of these experiments, we propose that dye molecules are encapsulated within the β-CD cavity.

The interaction of the squaraine dyes with β-CD was found to depend on the percentage of ethanol in the solvent medium. This variation can be explained on the basis of the competition between the ethanol and dye molecules for the β-CD cavity. The encapsulation of the dye inside the cavity was further confirmed by titration with noncyclic saccharide D-(+)-glucose and displacement studies employing β-CD encapsulating agents such as ACA and ADAC.

The squaraine dyes **1a**,**b** and **2a**,**b** can, in principle, undergo interactions with β-CD along its long molecular axis; however, the long alkyl/alkoxy chains in the phenyl ring prevent the complete encapsulation of the dye in the case of the aniline dyes **2a**,**b**. This can be readily understood from the 12-fold fluorescence enhancement observed for dye **2b** with the shorter alkyl chain as compared to the 5-fold enhancement observed for **2a** with the longer alkoxy chain, thereby indicating that the substituents present in the squaraine dye play a crucial role in the formation of the inclusion complex. It is plausible that the presence of these alkyl chains might prevent the encapsulation of the aromatic moiety of **2a**,**b** inside the β-CD cavity, leaving the squaryl chromophore open to nucleophilic attack. In contrast, the complete entrapment of the phloroglucinol-based dyes **1a** and **1b** in the interior of the cyclodextrin cavity protects these dyes from the attack of cysteine and glutathione.

5. CONCLUSIONS

In conclusion, we have demonstrated the utility of β-cyclodextrin as an efficient carrier system for squaraine dyes having specific substitutions. These dyes showed enhanced fluorescence emission and lifetimes in the presence of β-CD, indicating the effective interaction between the dye and β-CD. The increase in fluorescence quantum yield is attributed to a decrease in rotational freedom of motion, which decreases nonradiative decay processes. Moreover, the encapsulation of the dyes by β-CD provides protection to the squaraine moiety from nucleophlic attack by low-molecular-weight aminothiols such as cysteine and glutathione thereby revealing that β-cyclodextrin can function as an efficient delivery system for the squaraine dyes **1a**,**b** useful in PDT applications.

■ ASSOCIATED CONTENT

Supporting Information. Figures S1−S7 showing the changes in the photophysical properties of the dyes alone and in the presence of β-CD, D-glucose, and nucleophiles such as cysteine and glutathione. This material is available free of charge via the Internet at http://pubs.acs.org/

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