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Supramolecular Host–Guest Interactions of Oxazine-1 Dye with β- and γ-Cyclodextrins: A Photophysical and Quantum Chemical Study

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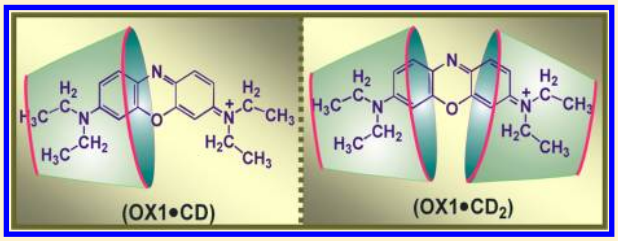
Supporting Information

ABSTRACT: Supramolecular host–guest interactions of oxazine-1 dye with β- and γ-cyclodextrins (βCD and γCD, respectively) have been investigated in neutral aqueous solution (pH ∼ 7) at ambient temperature (∼25 °C) following absorption, fluorescence, and circular dichroism measurements. The dye forms inclusion complexes with both CDs, causing significant changes in its photophysical properties. Whereas fluorescence titration data for lower dye concentrations fit well with 1:1 stoichiometric complexes, the time-resolved fluorescence results indicate formation of a small extent of 1:2 (dye–host) complexes as well, especially at higher CD concentrations. The moderate range of the binding constant values for the present systems indicates the weaker hydrophobic interaction as responsible for the inclusion complex formation in these systems. It has also been observed that γCD facilitates dimerization of the dye, prominently indicated at the higher dye concentrations. On the contrary, βCD always assists deaggregation of the dye, even at very high dye concentrations. Time-resolved fluorescence anisotropy results qualitatively support the inclusion complex formation in the present systems. Results from quantum chemical calculations also nicely corroborate with the inferences drawn from photophysical studies. Observed results demonstrate that the size compatibility of the guest and the host cavity mainly determines the host–guest interaction in the present systems, much similar to the substrate–catalyst binding in many biological systems.



1. INTRODUCTION

Host–guest complexation of chromophoric dyes with macrocyclic hosts often leads to increased photostability of the guest dyes along with significant changes in their photophysical and other properties.[1,2] Studies on such systems have gathered tremendous interest for a long time aiming to explore their potential applications in different areas.[1−12] Macrocyclic hosts are unique cagelike molecules that possess hydrophobic cavities where suitable guest molecules can be encapsulated partially or fully.[1−3,8,9,13] Although, in most host–guest systems, the hydrophobic interaction plays a major role,[1−11] in some of the systems, depending upon the nature of the host and guest molecules, H-bonding,[1,12,14,16] ion–dipole,[1,2,4−7] or charge–dipole[2,14] interactions can also render additional binding strengths to the host–guest complexes. In fact, in the host–guest complexes involving cucurbituril hosts and cationic guests, the ion–dipole interaction actually plays the dominant role in providing much stronger binding for the host–guest complexes than those normally formed using only the hydrophobic interaction.[1−11]

In supramolecular chemistry, the cyclodextrin (CD) hosts have attracted enormous interest due to their potential applications in the areas such as drug formalisms, drug delivery, sensors, catalysis, functional materials, etc.[8−10,14−19] Structurally CDs are the truncated cone-shaped cage molecules formed by the joining of the D-glucopyranose units through ether linkages.[8−10,13−19] While the interior of the CD cavities are hydrophobic in nature, their wider rim contains secondary hydroxyl groups and the narrower rim is laced with the primary hydroxyl groups. Depending on the number of D-glucopyranose units present, different CDs of varying cavity sizes are available, the most common homologues being the αCD, βCD, and γCD, having 6, 7, and 8 monomer units, respectively. For many organic dyes, the CDs show significant binding interactions whereby the photophysical properties of the guest dyes are considerably modulated, which is one of the subjects of intense research in the host–guest chemistry, aiming for their potential applications.[1,3,8−10,13−19]

Oxazine dyes are planar fluorescent molecules, belonging to the subclass of quinone imines, and are structurally related to the *p*-benzoquinone imine or diimine scaffold.[20] These dyes are known for their applications as laser dyes, optical sensors, dying fabrics and fibers, fluorescent labeling of biomacromolecules, etc.[20−24] They also have applications as fluorescent probes in investigating processes, such as rotational relaxation,[25] proton transfer,[26] aggregation,[27] solvatochromism,[27,28] photoinduced electron transfer,[29,30] etc. Oxazine-1 (OX1) dye investigated in

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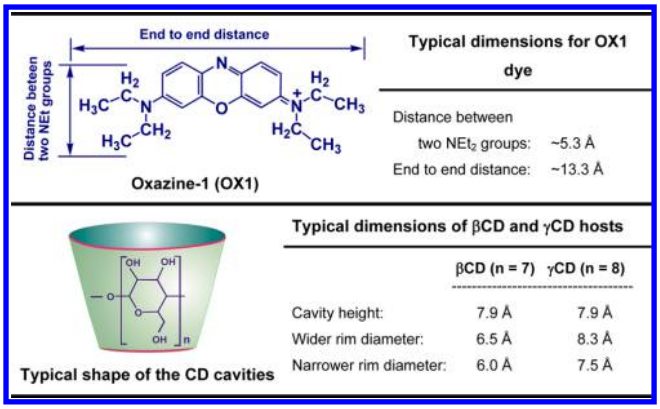
the present study is a symmetric cationic dye in the oxazine series.[31,32] In aqueous solution, OX1 exists in its monomeric form up to about a 10 μM concentration.[31,32] Though a few studies are reported on the effect of the host−guest interaction on the aggregation behavior of OX1 dye in aqueous solution,[32−35] no detailed study is reported on the effect of such an interaction on the photophysical behavior of the dye. In the present study, we have thus systematically investigated the interaction of OX1 dye with two CD hosts, namely, βCD and γCD, to understand its effect on the photophysical properties of the dye. Quantum chemical calculations have also been carried out to substantiate the results obtained from photophysical studies. Our aim in the present study is to understand how the differences in the cavity size of the two CD hosts affect the interaction and influence the photophysical properties of the studied dye. The chemical structure of the OX1 dye and the schematic of the CD host cavities are shown in Chart 1. Important dimensions of the guest molecule and the host cavities are also listed in Chart 1 for a quick visualization.

Chart 1. Chemical Structure of Oxazine-1 (OX1) Dye and the Typical Shape of the βCD and γCD Cages Are Shown (Not in Actual Scales)[a]



[a]Typical dimensions of the OX1 dye estimated from the geometry-optimized *cis* and *trans* structures (cf. section 3.4) and those reported in the literature for βCD and γCD host cavities[8−11] are also given in this chart.

2. MATERIALS AND METHODS

Laser-grade oxazine-1 (OX1) was obtained from Exciton and used as received. The purest-grade βCD (purity > 99%) and γCD (purity > 98%) samples were obtained from TCI (Tokyo, Japan) and used without further purification. Nanopure water used for solution preparation was obtained from a Millipore Elix 3/A10 water purification system (conductivity less than 0.1 μS cm^{-1}). Interaction of OX1 with βCD and γCD was studied following the changes in the absorption and fluorescence properties of the dye on gradual addition of the weighed amounts of hosts to a known volume (2 mL) of the dye solution. Except for some specific cases, all other measurements were carried out with an exceedingly low dye concentration, only about 1 μM, to avoid dye aggregation.[34,35] All the studies were carried out in neutral aqueous solutions (pH ∼ 7) at ambient temperature (∼25 °C).

Ground-state absorption, steady-state fluorescence, and circular dichroism measurements were recorded using a Shimadzu UV−visible spectrophotometer (model UV-160A; Kyoto, Japan), Hitachi spectrofluorimeter (model F-4500; Tokyo, Japan), and a JASCO CD spectrometer (model J-815; Tokyo, Japan), respectively. In fluorescence measurements, samples were excited at 636 nm, a wavelength similar to that of the diode laser used in the time-resolved fluorescence studies. The observed fluorescence intensity was corrected by OD normalization for the small absorbance changes at the excitation wavelength with the increasing host concentrations.[14] Time-resolved fluorescence measurements were carried out using a time-correlated single-photon-counting (TCSPC) spectrometer,[36,37] obtained from IBH (Glasgow, U.K.), where a 636 nm diode laser (pulse width ∼ 100 ps, repetition rate = 1 MHz) was mainly used as the excitation source and a MCP-PMT was used for fluorescence detection. The instrument response function for the setup was obtained by substituting the sample cell with a light scatterer (suspension of TiO_2 particles in water). Observed decays were fitted as a sum of exponentials, in general, following a reconvolution procedure[36,37]

$$I(t) = \sum B_i \exp(-t/\tau_i) \quad (1)$$

where B_i and τ_i are the pre-exponential factor and the fluorescence lifetime of the ith component of the decay, respectively. For the accepted fits, the χ^2 values were close to unity (1.00−1.20) and the weighted residuals were randomly distributed around zero among the data channels.[41,42] For anisotropy measurements, fluorescence decays with parallel $I_{\parallel}(t)$ and perpendicular $I_{\perp}(t)$ emission polarizations with respect to the vertically polarized excitation light were collected and the anisotropy decay function $r(t)$ was constructed as[36,37]

$$r(t) = \frac{I_{\parallel}(t) - GI_{\perp}(t)}{I_{\parallel}(t) + 2GI_{\perp}(t)} \quad (2)$$

where G is the correction factor for the polarization bias of the detection setup. The G factor was obtained independently by measuring the two perpendicularly polarized fluorescence decays while the sample was excited with the horizontally polarized excitation light.[36,37]

Because of computational limitations imposed by the large size and complexity of the present systems, the quantum chemical calculations were mainly carried out using the semiempirical molecular orbital method,[38,39] where the PM6 Hamiltonian corrected for hydrogen bonding and dispersion (DH2) was used, as implemented in MOPAC2009.[39] To compare the results from the PM6-DH2 method, benchmark quantum chemical calculations were carried out for the OX1−βCD system using dispersion-corrected density functional theory (DFT-D), where the BP86 functional in conjunction with the def2-SV(P) basis set was used for all the atoms, as implemented in TURBOMOLE.[39] To speed up the geometry optimizations at the DFT-D level, a resolution of identity (RI) approximation was invoked by using the def2-SV(P) auxiliary basis for Coulomb exchange terms. For the calculation of the energies, the B3LYP-D functional (dispersion-corrected) was used with the same def2-SV(P) basis sets for both semiempirical and BP86-optimized structures. Further, the energies were corrected for the solvent effects using the dielectric constant of water in the framework of the continuum COSMO solvation model as implemented in TURBOMOLE.[39] For the host−guest complexes, the starting structures for β- and γ-CDs were their crystallographic

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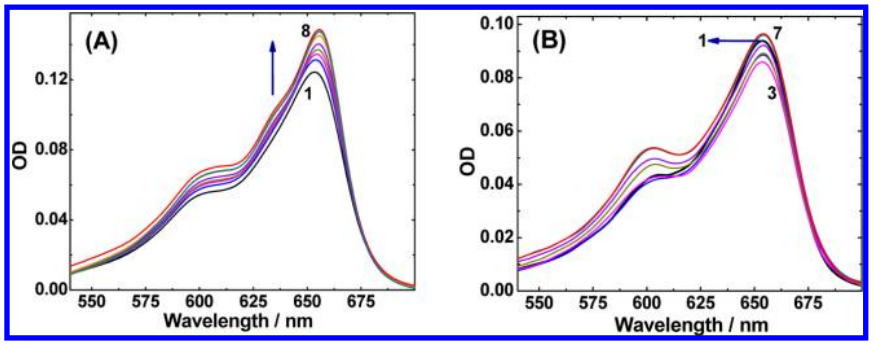

Figure 1. Changes in the absorption spectra for (A) OX1−βCD and (B) OX1−γCD systems. The dye concentrations were 1.2 and 0.9 μM, respectively, in the two cases. In panel A, the βCD concentrations were 0.0, 0.22, 1.25, 1.93, 3.6, 6.1, 10.5, and 18 mM, respectively, for the spectra 1−8. In panel B, the γCD concentrations were 0.0, 2.1, 3.9, 10, 13, 20, and 30 mM, respectively, for the spectra 1−7.

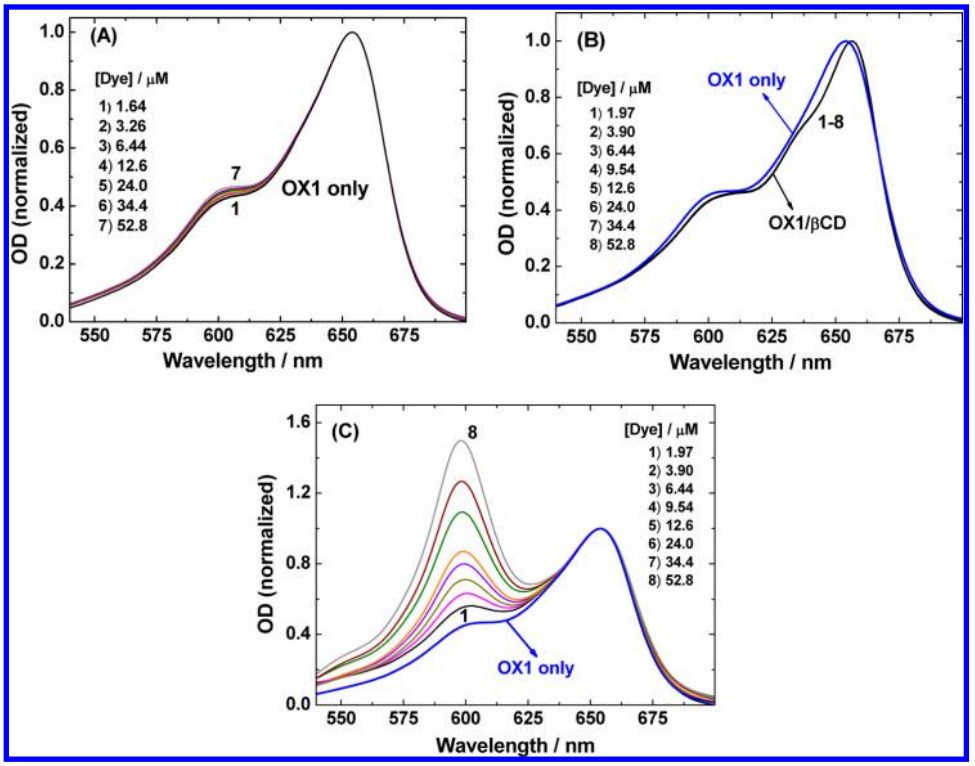

Figure 2. Changes in the absorption spectra for OX1 dye with the changing dye concentration. All the spectra were normalized at 654 nm. The spectra in different panels are (A) in the absence of any host, (B) in the presence of 18 mM βCD, and (C) in the presence of 30 mM γCD. The dye concentrations are as indicated in the panels. In panels (B) and (C), the spectrum indicated as OX1 only represents the spectrum for 52.8 μM dye solution in the absence of any CD host.

geometries and that of OX1 were its geometry-optimized two conformational structures, as is discussed latter in section 3.6.

3. RESULTS AND DISCUSSION

3.1. Ground-State Absorption Studies on the OX1−CD Systems. In aqueous solution, OX1 shows its absorption peak at 654 nm along with a shoulder at around 600 nm.[20,21,31−35] OX1 does not undergo any appreciable aggregation in aqueous solution below a concentration of ~10 μM.[32−35] In most of the present study, the OX1 concentration was thus kept significantly low, to avoid any aggregation. The host−guest interaction was systematically studied by gradually increasing the host concentration in a fixed concentration (~1 μM) of the dye solution. On addition of βCD to the OX1 solution (~1.2 μM), there is a small, but systematic, increase in absorbance, as shown in Figure 1A. The absorption peak undergoes only a marginal red shift (~2 nm), but there is the appearance of a small shoulder-like feature at around 636 nm at higher βCD concentrations. Observed changes are attributed to the dye inclusion into the less-polar βCD cavity.

As shown in Figure 1B, the changes in the absorption spectra of OX1 (0.9 μM) with increasing γCD concentration are somewhat different than those observed for the OX1−βCD system. In this case, the absorbance of the main 654 nm band initially decreases at the lower γCD concentrations, but increases later, though to a small extent, as the γCD concentration is increased further, without showing any observable change of the 654 nm peak position. There is also no appearance of the shoulder-like feature at ~636 nm, which is otherwise observed in the case of βCD. A critical inspection further indicates that, unlike the OX1−βCD system, where

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absorbance changes occur very similarly throughout the whole spectral region, for the OX1−γCD system, there is a continuous increase in the absorbance at the 600 nm shoulder band with added γCD, leading finally to the development of a clear peak at ∼603 nm, though the main 654 nm absorption band shows an initial decrease, followed by an increase in absorbance with increased γCD concentration. These observations suggest that the interaction of OX1 with γCD is somewhat more complex than that with βCD. In the literature, it is reported that the OX1 dimer has its characteristic absorption peak at ∼600 nm.[32−35] Though, with the ∼1 μM dye concentration, no dimerization is expected as such,[32−35] it is possible that the larger cavity of γCD might promote incorporation of two OX1 molecules simultaneously, leading to the appearance of the 600 nm dimer absorption band for the system. With a smaller cavity, such a dimeric dye−host complex formation is, however, not possible with the βCD host.

To investigate the dimeric dye−host complexes further, we measured the absorption spectra of the dye in the presence of a fixed host concentration, but increasing the dye concentration substantially, as shown in Figure 2. Concentration-dependent changes in the absorption spectra of the dye in the absence of any host (Figure 2A), after their normalization at the 654 nm peak, do not show any appreciable change except a marginal increase in absorbance around 600 nm, due to a small extent of dimerization at higher dye concentrations. Figure 2B shows the similarly normalized absorption spectra for OX1 at different dye concentrations in the presence of 18 mM βCD. As is seen, there is no effective change in the spectral shape for the whole dye concentration range used. The shapes of these spectra are, however, distinctly different than that of the free dye, also shown in Figure 2B for a comparison. Present observations clearly suggest that not only there is no dimeric dye−βCD complex formation but also there is a deaggregation of the dimers that were otherwise present for the free dye, due to the formation of the monomeric dye−βCD complexes. Figure 2C shows the concentration-dependent changes in the absorption spectra for the OX1 dye in the presence of 30 mM γCD, plotted after their normalization at the 654 nm peak. It is clearly seen that there is a large increase in absorbance for the 600 nm band on increasing the dye concentration, suggesting an obvious γ-CD-assisted dimerization of the OX1 dye.[40] It is important to note that, while γCD promotes aggregation of OX1, especially at higher dye concentration, βCD causes a complete deaggregation of the dye. This contrasting behavior is certainly due to the large γCD cavity that can simultaneously host two OX1 molecules, which is not possible with the smaller βCD cavity.

Induced circular dichroism (ICD) measurements often provide valuable information on the host−guest complexes involving CDs, as these host molecules are achiral in nature.[41−44] Though no reasonable ICD spectra could be obtained on using very low dye concentrations, the spectra were quite reasonable for both OX1−βCD and OX1−γCD systems on using higher dye concentrations. As shown in Figure 3, observed ICD spectra for the OX1−γCD system are distinctly different from those of the OX1−βCD system, though the signals are quite noisy in both cases for any quantitative analysis. As indicated from Figure 3, for the OX1−βCD system, there is a reasonably strong positive Cotton effect for the main 654 nm absorption band along with a weaker positive effect around the 600 nm shoulder band, suggesting a 1:1 host−guest complex formation in this system

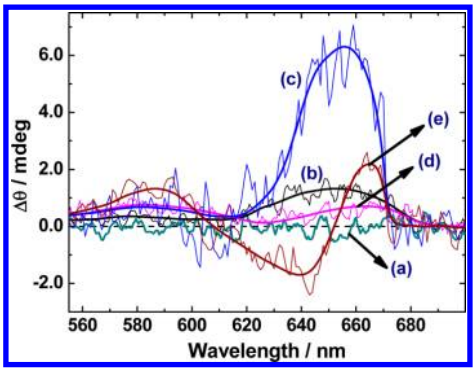

Figure 3. Circular dichroism spectra of OX1, OX1−βCD, and OX1−γCD systems: (a) 4.8 μM OX1 dye only, (b) 4.8 μM OX1 and 18 mM βCD, (c) 14 μM OX1 and 18 mM βCD, (d) 4.8 μM OX1 and 30 mM γCD, and (e) 14 μM OX1 and 30 mM γCD, respectively.

with axial orientation of the dye with respect to the CD cavity.[41−44] For the OX1−γCD system, the 654 nm absorption band shows a positive effect at the red edge and a negative effect at the blue edge, indicating an exciton splitting and, hence, the formation of dimeric dye−γCD complexes.[41−44] For the spectral region below ∼610 nm, the OX1−γCD system shows a weak positive effect, somewhat higher than that of the OX1−βCD system, possibly also due to the contributions of the dimeric dye−CD complexes. In brief, the ICD results are qualitatively in support of our inference that there is a dimeric dye−CD complex formation with the γCD host, which is not the case with βCD. It is, however, important to mention here that, at very low dye concentrations (∼1 μM), as are the conditions used in most of the photophysical studies (cf. Figure 1 and forthcoming sections), the extent of the dimeric dye−host complex formation would be exceedingly low, and accordingly, their effect can be neglected in the subsequent analysis.

3.2. Steady-State Fluorescence Studies on the OX1−CD Systems. Steady-state fluorescence characteristics of the OX1 dye were investigated as a function of βCD and γCD concentrations to understand the interaction of the dye with the CD hosts. In aqueous solution, OX1 shows its fluorescence peak at ∼672 nm along with a shoulder at around 740 nm.[20,21,31−35] The fluorescence intensity of the dye systematically decreases on increasing βCD and γCD concentrations in the solution, as shown in Figure 4A,B, respectively. Generally, dye binding to the host cavity causes an enhancement in fluorescence intensity, as the rotational and vibrational motions of the bound dye are retarded by the steric hindrance of the host cavity, causing a reduction in the nonradiative deexcitation rates.[1,2,5−7,14−19] Fluorescence quenching in the present systems is thus an apparently unusual observation, and we attribute this to a kind of specific interaction, most likely involving hydrogen bonding between the encapsulated dye and the portal OH groups of the hosts. It should be mentioned that, though fluorescence enhancement is a more general observation in the host−guest systems, there are examples where fluorescence quenching is observed, and the effect is attributed mainly to specific interactions, such as hydrogen bonding, charge transfer, etc.[45−47] The steady-state fluorescence results for the present systems clearly indicate that the OX1 dye interacts quite significantly with both βCD and γCD hosts. The observation that a relatively lower βCD concentration (∼18 mM) is required to reach the saturation effect in the

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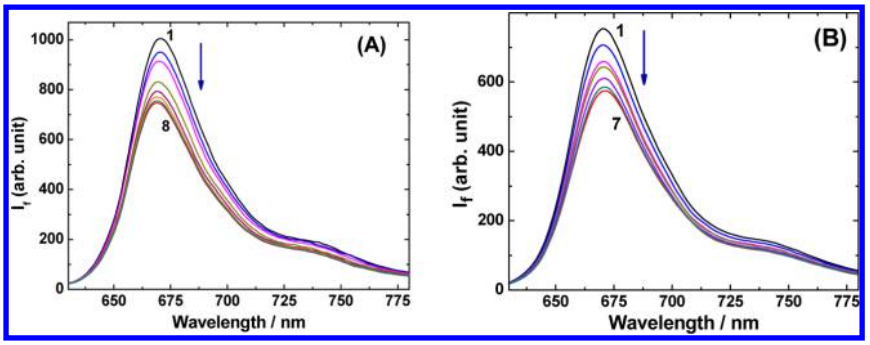

Figure 4. Changes in the steady-state fluorescence spectra of OX1 dye in the presence of (A) βCD and (B) γCD hosts. The dye concentrations for the OX1−βCD and OX1−γCD systems were 1.2 and 0.9 μM, respectively. In panel A, the βCD concentrations were 0.0, 0.22, 1.25, 1.93, 3.6, 6.1, 10.5, and 18 mM, respectively, for the spectra 1−8. In panel B, the γCD concentrations were 0.0, 2.1, 3.9, 10, 13, 20, and 30 mM, respectively, for the spectra 1−7.

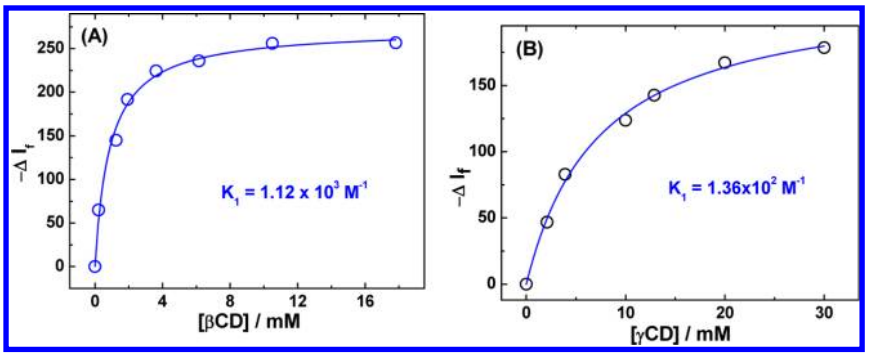

Figure 5. Changes in the fluorescence intensity (ΔI_f) for OX1 dye in aqueous solution with varying concentrations of (A) βCD and (B) γCD. The dye concentrations in the two respective cases were 1.2 and 0.9 μM. Open circles are the experimental data points, and the continuous lines are the fitted curves following eq 5.

fluorescence changes than the γCD concentration (~30 mM) suggests that the interaction is relatively stronger in the former case than in the latter.[14−19] This is necessarily due to the tighter binding of the dye with the smaller βCD cavity than the larger γCD cavity. As indicated from Figure 4A,B, there is no observable change in the shape and peak position of the fluorescence spectra on interaction of the dye with the CD hosts. This is because the fluorescence spectra of the OX1 dye are not that sensitive to the solvent polarities[20,21,31−34] and, hence, cannot respond to the reduced micropolarity inside the CD cavities.

3.3. Estimation of the Binding Constant Values for the OX1−CD Complexes. For the low dye concentrations, as the changes in the absorption characteristics in the presence of CD hosts are not substantial (cf. Figure 1A,B), the OX1−CD binding constants were determined by the fluorescence titration method.[5,6,14,15,19] The titration curves thus obtained for OX1−βCD and OX1−γCD systems are shown in Figure 5A,B, respectively. For the sufficiently low dye concentrations used in the titration measurements, the monomeric dye−host complexes would mainly dominate in the systems (cf. section 3.1). Accordingly, the fluorescence titration curves were analyzed following a 1:1 stoichiometric equilibrium (eq 3)[19,48]

$$D + H \overset{K_1}{\rightleftharpoons} D{\cdot}H \tag{3}$$

where D, H, and D·H stand for the free dye, free host, and dye−host complex, respectively, and K_1 is the binding constant. Since binding kinetics (microseonds) is much slower than the fluorescence kinetics of the dye (nanoseconds), it is expected that there will be hardly any dye exchange between the CD cavity and the bulk water during the fluorescence lifetime of the dye.[13,14] Thus, the ground-state binding equilibrium can satisfactorily be applied in correlating the fluorescence titration data, where the observed fluorescence intensity I_f at any host concentration $[H]_0$ can be expressed as

$$I_f = I_D^0 \frac{[D]_{eq}}{[D]_0} + I_{D\cdot H}^{\infty} \frac{[D{\cdot}H]_{eq}}{[D]_0} \tag{4}$$

where I_D^0 and $I_{D\cdot H}^{\infty}$ are the initial (only dye) and the final (complete complexation) intensity, $[D]_{eq}$ and $[D{\cdot}H]_{eq}$ are the equilibrium concentrations of the respective species, and $[D]_0$ is the total dye concentration used. For the present systems, as the host concentrations used are much larger than $[D]_0$, the $[H]_{eq}$ is effectively equal to $[H]_0$, the total host concentration used. Accordingly, the changes in the observed fluorescence intensity, $\Delta I_f = I_f - I_D^0$, can be expressed as[19,48]

$$\Delta I_f = (I_f - I_D^0) = \left(\frac{K_1[H]_0}{1 + K_1[H]_0}\right)(I_{D\cdot H}^{\infty} - I_D^0) \tag{5}$$

For the present systems, the fluorescence titration data fit reasonably well with eq 5 (cf. Figure 5A,B), giving K_1 values as 1.12×10^3 and 1.36×10^2 M^{-1}, respectively, for OX1−βCD and OX1−γCD systems (R^2 = 0.992 and 0.996, respectively), which are very similar to those reported in the literature.[32,35] Since these K_1 values are only in the moderate range, the weak hydrophobic interaction is suggested to be mainly responsible for the formation of the host−guest complex in these systems.[13−19] That the K_1 value of the OX1−βCD system is about 1 order of magnitude higher than that of the OX1−γCD

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system suggests that tighter binding of OX1 with the smaller βCD cavity provides better van der Waals contacts and, hence, stronger hydrophobic interaction than that with the larger γCD cavity. Present results thus suggest that the size compatibility of the guest and the host cavities largely determines the strength of interaction in the present systems.

Though the Job's plot method is often useful in determining the stoichiometry of the host−guest complexes,[49] for the present systems, it could not be applied conclusively as the absorbance changes were not significant. Using the Job's plot method following fluorescence intensity changes was also not successful for the present systems, as the necessary corrections for the changing dye concentrations in the measurements were difficult to apply. Nevertheless, on the basis of the absorption and ICD results and the reasonably good fit of the fluorescence titration data following eq 5, it is inferred that, at sufficiently low dye concentrations, the overall changes in the photophysical properties in the presence of the CD hosts are mainly due to the formation of the 1:1 complexes. Since time-resolved fluorescence is more sensitive in identifying multiple emissive species present in a system, we carried out these measurements to get a better insight into the host−guest complexes formed in the present systems.

3.4. Time-Resolved Fluorescence Studies on OX1−CD Systems. Typical fluorescence decays of the OX1 dye (measured at emission peak) both in the absence and in the presence of CD hosts are shown in Figure 6. In the absence of CD, the decay follows a single-exponential kinetics with the fluorescence lifetime as 0.46 ± 0.02 ns. In the presence of 18 mM βCD or 30 mM γCD, the observed decays become biexponential in nature, giving lifetime components as 0.34 ± 0.02 ns (~98%) and 1.30 ± 0.03 ns (~2%) for the former and as 0.39 ± 0.02 ns (90%) and 0.80 ± 0.03 ns (~10%) for the latter systems. As discussed in the previous sections, under the present experimental condition (very low dye concentration), the 1:1 host−guest complexes would mainly dominate in the solution. For the present systems, since there is a significant steady-state fluorescence quenching observed, we infer that the shorter 0.34 and 0.39 ns components for OX1−βCD and OX1−γCD systems are due to the 1:1 inclusion complexes, where specific hydrogen-bonding interaction of the bound dye simultaneously with several OH groups at the CD portals causes a reduction in its fluorescence lifetime.[45−47] For the longer 1.30 and 0.80 ns components of the two respective systems, as these lifetimes are 2−3 times longer than that of the free dye, we assign it to the formation of a small extent of 1:2 (dye−host) inclusion complexes at higher host concentrations. Since the length of OX1 is much larger than the height of the CD cavities (cf. Chart 1), two CD molecules can easily encapsulate two opposite ends of the dye, forming 1:2 complexes. In these 1:2 complexes, as the dye is quite protected from both outside solvents and the specific interactions of portal OH groups of the hosts, and there will also be a significant retardation in the rotational and vibrational motions due to the steric restrictions imposed by the host cavities, the fluorescence lifetime of the dye will be significantly longer than that of the free dye. The point to be considered here is, with OX1 being a cationic dye, whether it would allow binding of two CD molecules from both of its ends, forming the 1:2 complexes. Since the positive charge of OX1 is expected to be highly delocalized (cf. section 3.6, quantuam chemical calcualtions), we feel that binding of a second CD to a 1:1 complex would be quite possible, as indicated from the observed time-resolved results.

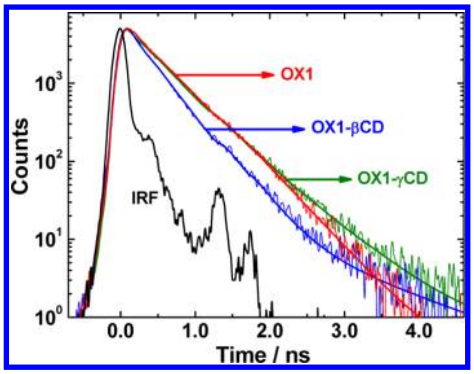

Figure 6. Fluorescence decays of OX1 dye in aqueous solution in the absence and in the presence of βCD (18 mM) and γCD (30 mM) hosts. The dye concentrations in the cases of βCD and γCD hosts were 1.2 and 0.9 μM, respectively. The instrument response function (IRF) is also shown in the figure. Excitation and emission wavelengths were 636 and 672 nm, respectively.

In relation to the above time-resolved fluorescence results, one can also think of the formation of a small extent of exclusion complexes in the present systems. We, however, feel that, in the present systems, formation of exclusion complexes will be extremely weak because hydrophobic interaction will be much less effective in these complexes than in the inclusion complexes. This proposition is, in fact, supported from the quantum chemical calculations, discussed in section 3.6. In the present context, however, we also measured fluorescence decays of OX1 in the presence of additives, such as D-glucose, ethylene glycol, and ethanol. In all the cases, the fluorescence lifetime gradually increases (cf. Figure S1 and Table S1, Supporting Information), instead of the reduction, which was otherwise observed for 1:1 OX1−CD complexes. In the steady-state measurements also, there was an increase in the fluorescence intensity in the presence of the above additives. The increase in the fluorescence intensity and lifetime is possibly due to the combined effect of increased viscosity (for D-glucose, ethylene glycol) and reduced polarity (for all the additives) of the solvent systems in the presence of the additives. Present results further indicate that the presence of several OH groups at the portals of the CDs would mainly be responsible for the observed fluorescence quenching in the present dye−CD systems.

That the contributions of 1:2 complexes in the OX1−CD systems are very small certainly suggests that the binding constant values (K_2) for these complexes are much lower than those (K_1) of the 1:1 complexes. In the present context, we also tried to analyze the fluorescence titration data following simultaneous involvement of 1:1 and 1:2 (dye−host) equilibria (cf. section II, Supporting Information). Surprisingly, however, such an analysis does not give any improvement in the fits (cf. Figure S2, Supporting Information) compared to that with the 1:1 equilibrium alone (cf. eq 5 and Figure 4). This is certainly due to the exceedingly small contributions of the 1:2 complexes for most of the titration data points where CD concentrations are lower than those used in Figure 6. The marginally higher lifetime for the 1:1 complex in OX1−γCD than in OX1−βCD suggests that OX1 binding with the larger γCD cavity is relatively loose, and hence, there is a less quenching interaction by the portal OH groups of the host. A relatively shorter

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lifetime for the 1:2 complex involving γCD than βCD is also attributed to the loose binding of the dye to the larger γCD cavities so that the nonradiative rates are retarded much less than in the case of the βCD host.

Recall here that, for the OX1−γCD system, there was some dimeric dye−γCD complex formation indicated from absorption and ICD studies (cf. section 3.1). In the time-resolved fluorescence results, however, no lifetime component is observed that can be attributed to this complex. This is possibly because of the nonemissive nature of the OX1 dimers.[32−35] To ascertain this further, fluorescence decays were measured with very high (52.8 μM) and very low (∼1 μM) dye concentrations in the presence of 30 mM γCD and exciting the samples with 594 nm pulsed LED, where dimeric OX1 would be excited preferentially. Observed decays are shown in Figure S3 of the Supporting Information. As is seen from this figure, there is effectively no difference between the decays measured at the two extreme dye concentrations used. From Figures 2C and 1B, it was evident that dimeric dye−γCD complex formation is very large at higher dye concentration but it is extremely less at low dye concentration. Therefore, the results in Figure S3 in the Supporting Information undoubtedly suggest that the dimeric dye−γCD complexes are nonemissive in nature.

3.5. Time-Resolved Fluorescence Anisotropy Studies on OX1−CD Systems. Time-resolved fluorescence anisotropy measurements were also carried out for the present systems to get more insight into the host−guest complexes formed.[14,19] The observed anisotropy decays for the free dye as well as OX1−βCD and OX1−γCD systems in the presence of the highest CD concentrations are shown in Figure 7. In all the

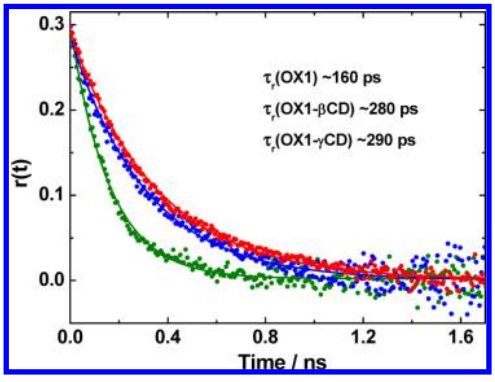

Figure 7. Fluorescence anisotropy decays of OX1 dye in aqueous solution in the absence and in the presence of 18 mM βCD and 30 mM γCD hosts. Dye concentrations in all the cases were ∼1 μM. Excitation and emission wavelengths were 636 and 672 nm, respectively.

cases, the decays fit reasonably well with a single-exponential function, giving the rotational correlation times (τ_r) as 0.16 ± 0.03, 0.28 ± 0.03, and 0.29 ± 0.03 ns, respectively. Since τ_r is directly proportional to the hydrodynamic volume of the fluorophore,[36,37] the increase in the τ_r value for the dye in the presence of CD hosts suggests the inclusion complex formation in the present systems.[14,19] Similar τ_r values for both OX1−βCD and OX1−γCD systems suggest that the 1:1 complexes are mainly responsible for the observed anisotropy decays in both systems. It should be mentioned that, for the OX1−γCD system, even though about 10% fluorescence was indicated due to the 1:2 (dye−host) complex at the highest γCD concentration (cf. section 3.4), its effect on anisotropy decay could not be resolved with confidence, because a biexponential analysis of the decay resulted in the physically unreasonable values for the fitting parameters.

3.6. Quantum Chemical Calculations on the OX1−CD Systems. Quantum chemical calculations on the present systems were carried out to get support for the inferences drawn from the photophysical studies. In the present calculations, we initially investigated the geometry-optimized structures of OX1 using both PM6-DH2 and DFT-D methods.[38,39]

The geometry-optimized *cis* and *trans* conformations of the dye were thus realized, as expected from the rotations of the two amino ethyl groups (cf. Figure 8a,b), with only a marginally

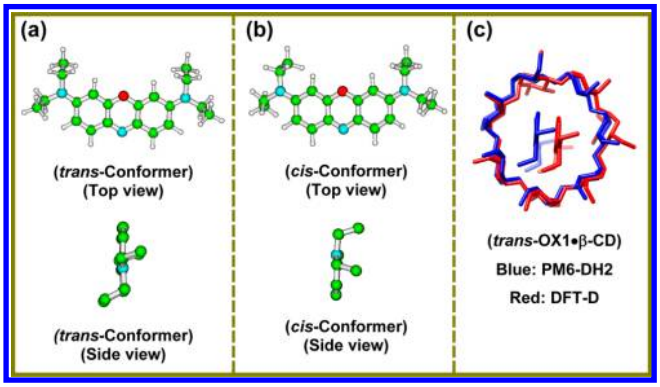

Figure 8. PM6-DH2-optimized structures of OX1 with (a) *trans* and (b) *cis* rotameric conformations. In the side view, the *cis* (panel a) and *trans* (panel b) arrangements of the two ethyl groups at one of the terminal amino nitrogens of the dye are shown. The arrangements of the two ethyl groups at the other terminal amino nitrogen are just behind these projections. Panel (c) shows the superimposed geometry-optimized structures of the 1:1 *trans*-OX1·β-CD complex obtained by using PM6-DH2 (blue) and DFT-D (red) methods. The structures derived from two methods are quite comparable.

higher stability for the *trans* form (by 1 kcal mol^{-1}). The highest occupied and the lowest unoccupied molecular orbitals (HOMO and LUMO) of the *cis* and *trans* conformers of OX1 are seen to be very similar (π and π* MOs). Representative HOMO and LUMO of the *trans* conformer of OX1 are shown in Figure 9. Present results suggest that the electronic structure of the chromophore does not alter appreciably in the two conformers of the OX1 dye.

Following geometry optimization of OX1, we computed the structures and binding energies of different host−guest complexes with varying stoichiometries. In these calculations, the starting structures for βCD and γCD were their crystallographic geometries and that of OX1 were its geometry-optimized *cis* or *trans* structures. As mentioned in

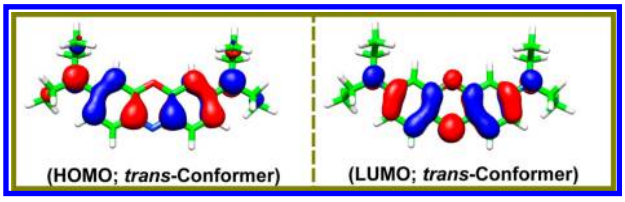

Figure 9. Calculated HOMO and LUMO of the OX1 molecule in its *trans* conformer are shown. The HOMO and LUMO of its *cis* conformer are very similar to the ones shown for its *trans* conformer.

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section 2, most of these calculations were carried out using the semiempirical PM6-DH2 method. Only for the OX1−βCD system, a calculation was also carried out using the more accurate DFT-D method, for a comparison with the results obtained from the PM6-DH2 method. Figure 8c shows the superimposed geometry-optimized structures for the 1:1 *trans*-OX1·βCD complex obtained from the two levels of theories, indicating that the structure derived from the semiempirical method is quite comparable to that obtained from the DFT-D method. Using the PM6-DH2 method, the calculated binding energy for the 1:1 *cis*-OX1·βCD complex is predicted as about −15.0 kcal mol^{-1}, a value somewhat less negative than that predicted by the DFT-D method (−20.5 kcal mol^{-1}). A small extent of underestimation in the binding energy was also observed for the *trans*-OX1 binding to the βCD host following the PM6-DH2 method in comparison to the DFT-D method. From the comparison of these results, we arrive at the conclusion that the semiempirical PM6-DH2 method can satisfactorily be applied to the present systems to predict the structures of the host−guest complexes, though, in relation to the binding energies, there could be some underestimation from the semiempirical method. However, considering the complexity of the systems studied and expecting that the quantum chemical results would mainly provide a qualitative support to the inferences drawn from the photophysical studies, the use of the PM6-DH2 method seemed to be quite reasonable for the present systems. On the basis of this idea, we thus calculated the structures and energies of the host−guest complexes using the semiempirical method, incorporating the solvent dielectric correction, as mentioned in section 2. The results thus obtained for different stoichiometric complexes for the present systems are presented in Figure 10 and Table 1, respectively. Comparisons of the results from gas-phase calculations and those incorporating solvent corrections are given in the Supporting Information (cf. Figure S4).

As indicated from Table 1, for the 1:1 complex, the calculated binding energy (B3LYP/def2-SV(P)/PM6-DH2) for the OX1−βCD system is more negative by about 3−6 kcal mol^{-1} than that for the OX1−γCD system, consistent with the photophysical results, indicating that the binding constant K_1 for the OX1·βCD complex is about 1 order of magnitude higher than that for the OX1·γCD complex (cf. section 3.3). Nevertheless, the negative values of the binding energies estimated for both OX1−βCD and OX1−γCD systems suggest that the host−guest complexes in these systems are easily formed with a 1:1 stoichiometry.

Binding possibilities in OX1−CD complexes with other stoichiometric compositions were also investigated in the present study using quantum chemical calculations. Coinclusion of two OX1 into a smaller βCD cavity to give dimeric dye−βCD complex appeared to be not realizable due to the size incompatibility and the steric restrictions. For βCD, however, the formation of a 1:2 (dye−host) complex is found to be possible, as indicated from the geometry-optimized structure and the binding energy (−36.6 kcal mol^{-1}) shown in Figure 8 and Table 1, respectively, and accordingly supports the presence of the longer fluorescence lifetime component in the time-resolved fluorescence measurement, albeit with a very small contribution. Drawing an analogy with these results of the OX1−βCD system, a similar 1:2 complex is also suggested for the OX1−γCD system, as also indicated from time-resolved fluorescence, though we avoided the calculation for this system due to its larger size. Though coinclusion of two OX1 into a smaller βCD cavity is not feasible; such a situation is, however, found to be quite feasible involving a relatively larger γCD cavity. In this case, as the coinclusion of two *trans*-OX1 conformers into a single γCD cavity seemed to have a large steric hindrance, we investigated the binding possibility of two *cis*-OX1 conformers into a single γCD cavity using the PM6-DH2 method, and the result shows a favorable binding energy (−12.7 kcal mol^{-1}), quite comparable to that of the corresponding 1:1 complex, supporting the possible formation of some dimeric dye−γCD complexes in the system, as indicated from the ground-state absorption and ICD measurements.

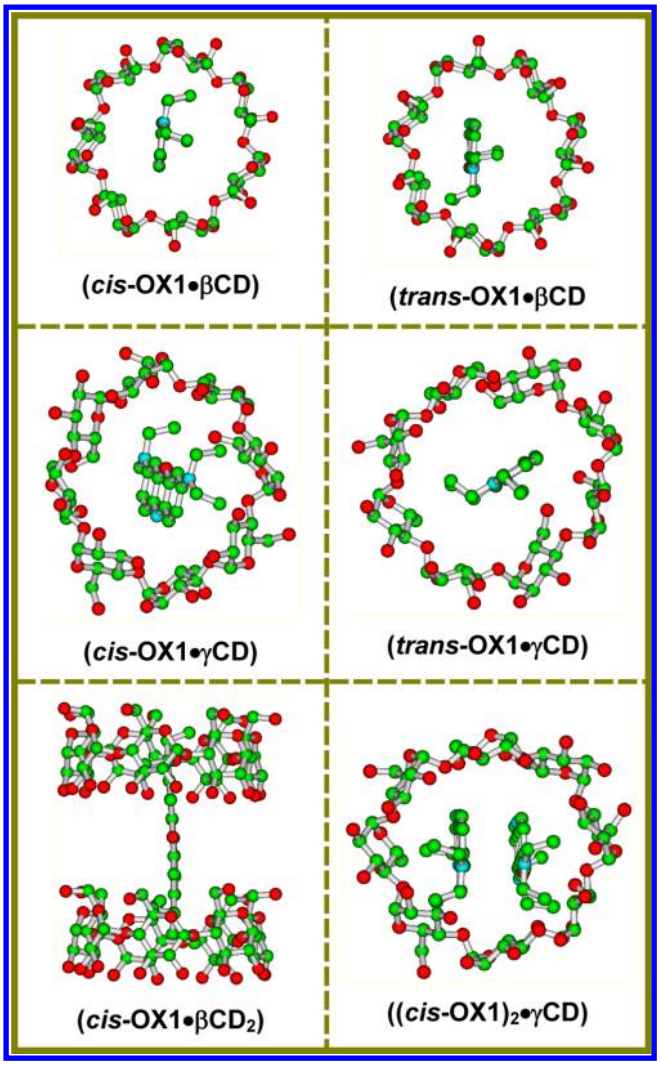

Figure 10. Geometry-optimized structures of host−guest complexes with different stoichiometries as obtained by using quantum chemical calculations of the OX1−βCD and OX1−γCD systems.

Electronic charge distribution in the OX1 molecule was also calculated, and the results indicate that the cationic charge of the dye is largely delocalized between the two terminal dialkyl amino nitrogens (cf. Figure S5, Supporting Information). Such a delocalization of the cationic charge suggests that the binding of the CD hosts would be equally likely at both ends of the dye and thus supports the 1:2 (dye−host) complex formation. To substantiate it further that the cationic charge of a quaternary ammonium moiety does not deter the CD binding significantly, we also carried out calculations on diethtylaniline−βCD and protonated diethtylaniline−βCD systems using the DFT-D

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Table 1. Calculated Binding Energies (kcal mol^{-1}) for OX-1 Dye with βCD and γCD Hosts

dye−host	stoichiometry	B3LYP-D//PM6-DH2[a]	B3LYP-D//BP86-D[a]
OX1·βCD	1:1	−15.0 (−19.8)	−20.5 (−20.4)
OX1·γCD	1:1	−12.5 (−13.0)	
OX1·βCD_2	1:2	−36.6	
$OX1_2$·γCD	2:1	−12.7	

[a]Values given outside the parentheses are for the complexes involving the *cis* conformer, and those inside the parentheses are for the complexes involving the *trans* conformer of the OX1 dye.

method. The results give quite comparable binding energies for both systems (cf. Figure S6, Supporting Information), suggesting that the quaternary ammonium moiety does not largely affect the CD binding.

Calculations using the DFT-D method were also carried out on the possibility of exclusion complex formation in the present systems. The results indicate almost similar binding energies for the 1:1 exclusion complexes with both side and top binding (−10 and −13 kcal mol^{-1}, respectively; cf. Figure S7, Supporting Information). However, these binding energies are almost half that of the 1:1 OX1-βCD inclusion complex estimated using the DFT-D method (BE = 20.4 kcal mol^{-1}; cf. Table 1). It is thus suggested that the exclusion complexes will be much weaker than the corresponding inclusion complexes and thus would hardly contribute in the present OX1−CD systems. In brief, the results from the quantum chemical calculations are qualitatively in accordance with the results obtained from photophysical studies on the OX1−CD systems and, accordingly, substantiate the inferences drawn on different host−guest complexes formed in the systems studied.

4. CONCLUSIONS

The results from ground-state absorption, steady-state and time-resolved fluorescence, and ICD studies indicate that the OX1 dye undergoes inclusion complex formation with βCD and γCD hosts. The dye binds relatively strongly with the βCD host than the γCD host, and the results are rationalized on the basis of the tighter binding of the dye with the smaller βCD cavity than the larger γCD cavity. That the binding constant (K_1) values for the present systems are only in the moderate range suggests that weaker hydrophobic interaction is mainly responsible for the formation of the inclusion complexes in the present systems. For both OX1−βCD and OX1−γCD systems, the monomeric dye−host complexes are mainly formed, especially when the dye concentration is kept significantly low, with major contributions from the 1:1 complexes and only a minor contribution from 1:2 (dye−host) complexes. For the OX1−γCD system, however, formation of some dimeric dye−γCD complexes is also indicated, whose contribution increases significantly on increasing the dye concentration in the solution. Interestingly, where γCD promotes aggregation of OX1, especially at higher dye concentration, βCD causes a complete deaggregation of the dye, a contrasting effect ascribed to the relative dimensions of the two host cavities. Formation of inclusion complexes with different stoichiometries in the present systems is nicely supported by the results from quantum chemical calculations using the semiempirical molecular orbital method incorporating both dispersion and solvation effects. It is understood that the synergy between experimental data and theoretical predictions gives a better understanding of the studied host−guest systems, which show complexity due to the formation of different stoichiometric complexes. Present results demonstrate that the differential cavity size of the CD hosts largely influences the strength of interactions as well as the stoichiometries of the complexes formed in these systems. Modulations in the photophysical properties of the OX1 dye, as observed in the present study, are believed to have relevance to host-assisted binding and stabilization of sensitive dyes and drugs and their possible uses in drug delivery, aqueous dye lasers, fluorescence sensors, and others.

■ ASSOCIATED CONTENT

Supporting Information

Additional figures, tables, and explanations related to time-resolved fluorescence, fluorescence titration, and quantum chemical calculations are given in the Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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