Intramolecular Proton Transfer in Inclusion Complexes of Cyclodextrins: Role of Water and Highly Polar Nonaqueous Media

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The molecule 4-methyl-2,6-dicarbomethoxyphenol (CMOH) which undergoes ultrafast excited-state intramolecular proton transfer (ESIPT) has been employed as a guest to probe ESIPT within the interior of the host α- and β-cyclodextrins (CDx) in water and highly polar nonaqueous solvents. The ESIPT reaction of CMOH is favored in its microencapsulated form in both aqueous and nonaqueous media. As a consequence, resultant enhancement of tautomer emission, following ESIPT, relative to that in homogeneous bulk media is observed. CMOH forms 1:2 and 1:1 complexes with α- and β- CDx's (host), respectively, in both water and highly polar nonaqueous media. However, tautomer emission due to ESIPT is significantly enhanced in water compared to nonaqueous solvents, despite formation of stronger guest−host (inclusion) complexes with much higher binding constant in the nonaqueous media. The results have been interpreted as due to the formation of ternary complexes comprising CMOH, second guest, and cyclodextrin in nonaqueous media where the nonaqueous solvent acts as the second guest molecule.

Introduction

The increasing research activity dealing with inclusion compounds shows that the general interest in their physical and chemical properties has grown considerably over the past decade. This is due, on one hand, to the fact that the study of inclusion compounds in fundamental research furnishes information about noncovalent, intermolecular forces. On the other hand, they also serve as models for studying topochemical problems and the mode of action of enzymes.[1] The inclusion compounds are also utilized in laboratories, industry, and the home as ion exchangers, as catalysts in chemical reactions, or for the microencapsulation of sensitive, active, and aromatic substances.

The most important property of an inclusion complex is that a "host" component can admit a "guest component" into its cavity without any covalent bonds being formed. This offers a means of studying intracomplex reactions; e.g., quite a few studies have been made for twisted internal charge transfer (TICT)[2−4] and intermolecular excited-state proton transfer (ESPT)[5,6] reactions in inclusion complexes of cyclodextrins.

Research activity involving ESIPT in inclusion complexes of cyclodextrins in water is rare.[7] But so far, no studies have been done regarding ESIPT in inclusion complexes of cyclodextrins in highly polar nonaqueous solvents although it is known that substrate−cyclodextrin complexation can occur also in nonaqueous polar media.[8] Recently, Warner et al. studied 2-(2-hydroxyphenyl)benzimidazole (HBI) in cyclodextrins and in different aqueous solvent mixtures.[9] They observed that intermolecular interactions of HBI with cyclodextrins and various solvents appear to weaken intramolecular hydrogen bonding in HBI and facilitate the formation of strong intermolecular hydrogen bonds with the various cyclodextrins and solvent molecules.

Experimental Section

The molecule CMOH has been synthesized and purified as described earlier.[10] Triply distilled, deionized water was used throughout the study. All other solvents were of spectroscopic grade and used without further purification. Concentrations of CMOH solutions were maintained at $(1.0-1.2) \times 10^{-5}$ dm^3 mol^{-1}. Steady-state absorption and emission spectra were recorded in JASCO UV−vis absorption and Perkin-Elmer MPF 44B fluorescence spectrophotometers, respectively.

Results and Discussion

A. Steady-State Spectra in the Absence of Cyclodextrins. The molecule of interest, CMOH, exhibits a single absorption band in the 322 nm region in both aqueous and highly polar nonaqueous media. This absorption band is attributable to the intramolecularly hydrogen-bonded closed conformer (**I**) of CMOH (Scheme 1).

On photoexcitation, this closed conformer undergoes rapid ESIPT, the signature of it being a largely Stokes shifted emission in the 453−459 nm region attributable to the ESIPT phototautomer (**II**) of **I**. The fluorescence excitation spectra of this largely Stokes shifted emission agree reasonably well with the corresponding absorption spectra. We observe similar observations for CMOH in dry hydrocarbon (*n*-hexane, 3-methylpentane) solvents which support the ESIPT model (Scheme 1) for CMOH in water and highly polar nonaqueous solvents such as dimethyl sulfoxide (DMSO), *N*,*N*-dimethylformamide (DMF), and formamide.

B. Steady-State Spectra in the Presence of Cyclodextrins. *Absorption Spectra*. The optical density of the absorption band increases (Figure 1) in the presence of α- and β-CDx, the increment being most prominent in an aqueous medium relative

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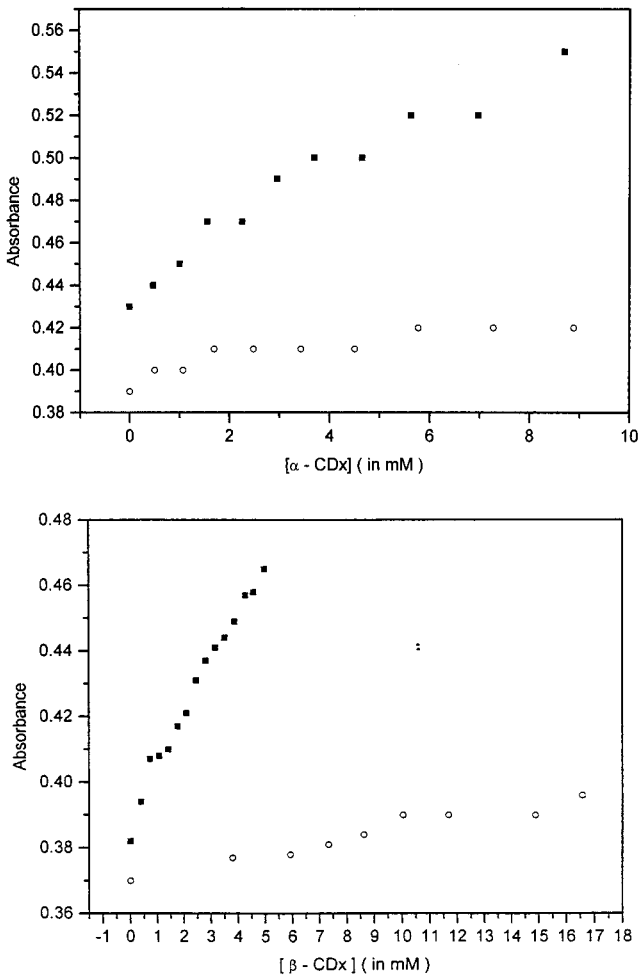
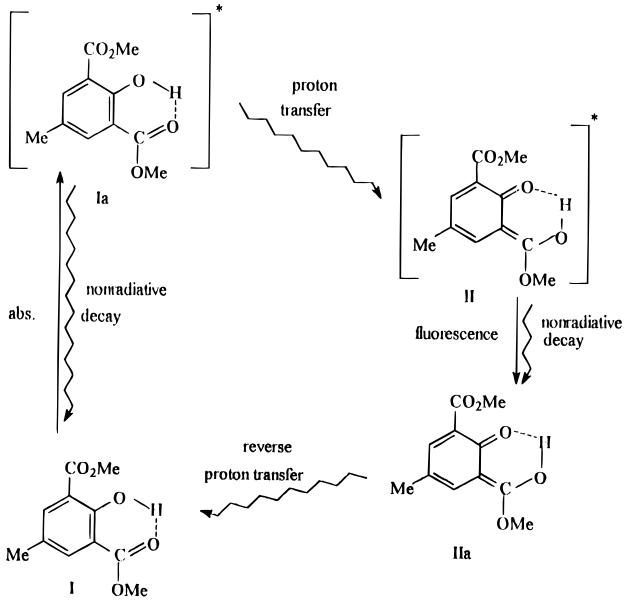


Figure 1. Variation of absorbance of CMOH with increasing concentration of cyclodextrins (CDx) in different homogeneous media: (a, top) α-CDx in water (■) and DMF (○) and (b, bottom) *β*-CDx in water (■) and DMSO (○).

SCHEME 1: Diagram Showing the ESIPT Process in CMOH



to that in nonaqueous media. This enhancement in absorbance indicates that CDx promotes formation of inclusion complexes (guest−host complex).

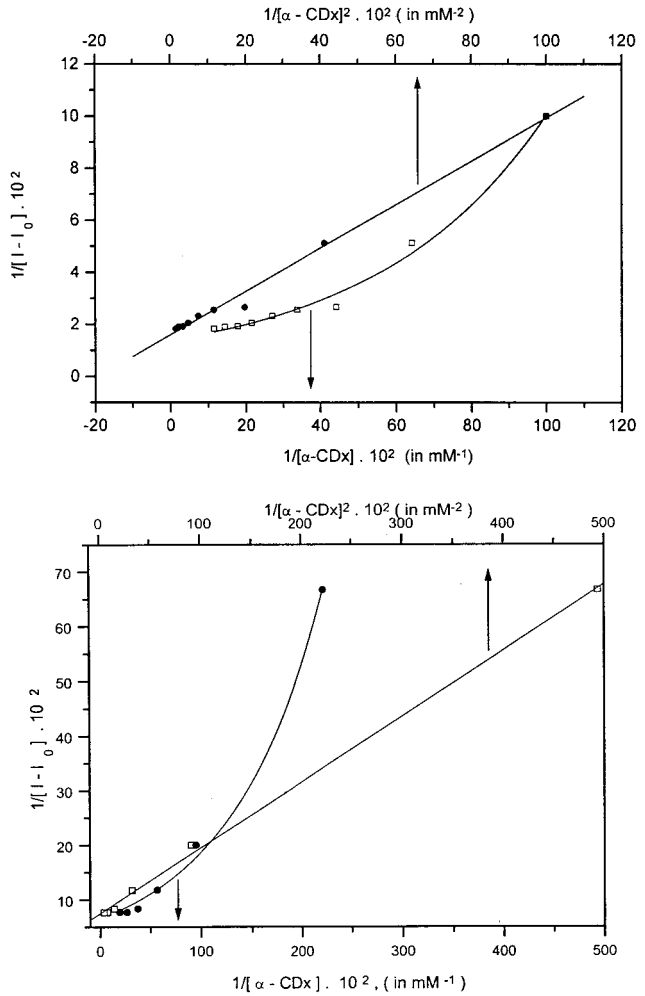


Figure 2. Benesi−Hildebrand plots using fluorescence data for the complexation of CMOH with α-CDx: (a, top) In water; solid line (●) is for 1:2 complexation. (b, bottom) In DMSO; solid line (□) is for for 1:2 complexation. *I* and I_0 are the fluorescence intensity of CMOH in the presence and absence of α-cyclodextrin, respectively.

Emission Spectra. The tautomer emission of CMOH due to ESIPT becomes enhanced on binding to cyclodextrins in both aqueous and nonaqueous media.The apparent binding constants and stoichiometric ratios of the inclusion complexes of CMOH with CDx's were estimated from the Benesi−Hildebrand (BH)[11] plots (Figures 2 and 3) using fluorescence data. The following equation[12] was used for 1:1 and 2:1 CDx:CMOH association.

$$\frac{I}{I_0} = \frac{1 + aK_i[\mathrm{CDx}]^n}{1 + K_i[\mathrm{CDx}]^n} \qquad (1)$$

where [CDx] represents the analytical concentration of the CDx's, I_0 and *I* are the fluorescence intensity of CMOH in the absence and presence of CDx, respectively, K_i's represent the equilibrium constants of CDx:CMOH association, and *a* is the ratio of the fluorescence intensities of the complexed and uncomplexed chromophore.

A BH plot assuming a 2:1 association between α-CDx and CMOH reveals a good linear fit. Conversely, a plot assuming a 1:1 association reveals a nonlinear regression having a curvature (Figure 2), suggesting that the stoichiometry of the complex is 2:1. On the other hand, the BH plot that assumes a 2:1 stoichiometry between *β*-CDx and CMOH exhibits nonlinear regression with a curvature (Figure 3). But the BH

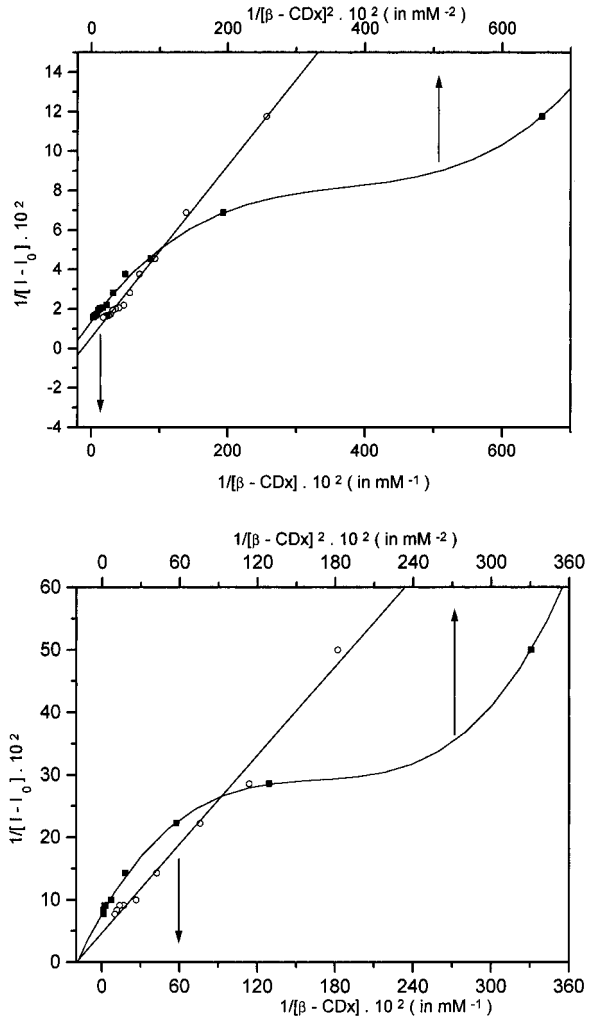


Figure 3. Benesi−Hildebrand plots using fluorescence data for the complexation of CMOH with β-CDx: (a, top) in water and (b, bottom) in DMSO. Solid line (○) is for 1:1 complexation. I and I_0 are fluorescence intensity of CMOH in the presence and absence of β-cyclodextrin, respectively.

plot for an assumed 1:1 inclusion complex shows an excellent linear fit, affirming a 1:1 stoichiometry of the β-CDx−CMOH complex.

The apparent K_i values, obtained from the slope and intercept of the respective BH plots, were then used as an initial guess in an iterative nonlinear regression program (NLR) based on the Levenberg−Marquardt algorithm[13] for the nonlinear curve fitting of the fluorescence data with varying CDx concentration (Figure 4), which provides estimates for K_i for 1:1 and 2:1 complexes using the following equations:

$$I = \frac{I_0 + I_1K_1[\mathrm{CDx}]}{1 + K_1[\mathrm{CDx}]} \tag{2}$$

$$I = \frac{I_0 + I_2K_2[\mathrm{CDx}]}{1 + K_2[\mathrm{CDx}]} \tag{3}$$

where $I_1 = aI_0$ for the 1:1 complex and $I_2 = aI_0$ for the 2:1 complex.

The equilibrium constants for binding (Table 1) reflect that CMOH is bound to cyclodextrins (CDx) much more strongly in nonaqueous media than in aqueous media. Why is there such a variation in equilibrium constant values? We will look out for its answer in the next section.

C. Solvent Participation in Guest−Host Complexation: Formation of "Ternary Complex". Table 1 reveals that the association constants for the formation of inclusion complexes of CDx's are distinctly higher in nonaqueous solvents such as DMSO and DMF than in water. Since same guest (CMOH) and host (CDx) partners are involved in the formation of inclusion complexes in both media, it is presumed that solvent molecules might play a significant role in determining the complexation efficiency. The formation of a host−guest inclusion complex by cyclodextrins is primarily determined by the tightness of fit, i.e., by the size and shape matching between the guest and cyclodextrin cavity. An imperfect fit of the guest in the CDx interior leads to decreased equilibrium constant of binding.[14,15] If the guest does not occupy the whole internal space of the cavity, the void space will be filled with solvent molecules. In the case of water, its incorporation is thermodynamically unfavorable. The efficiency of CDx complexation can be altered by addition of ternary complexation agents such as alcohols or alkyl sulfate, leading to either an increase or a decrease of the CDx−guest association constants. Enhancement of association efficiency in the presence of ternary complexation agents has been ascribed to the capacity of these molecules to fill the "void space" within the CDx cavity.[16,17]

We will now verify the possibility of formation of ternary complexes in nonaqueous media.

D. Stoichiometry of Ternary Complex. The "continuous variation method" has been used to determine the stoichiometry of the ternary systems.[18] Although this method is applicable to binary systems, it can also be applied to this three-component system to determine the stoichiometry between the CDx and the nonaqueous solvents.[14]

In this case, the CMOH concentration was fixed at 1.0×10^{-5} M. Under the condition of [β-CDx] + [DMSO] = 10 mM, the observed fluorescence intensity at 453 nm was measured as a function of DMSO mole fraction. Figure 5 shows a plot for the β-CDx/CMOH/DMSO system. The difference in the fluorescence intensity goes through a maximum value at a DMSO mole fraction of 0.3. This result provides clear evidence of a 1:2 stoichiometry between β-CDx and DMSO. Now, as the stoichiometry of the binary β-CDx/CMOH complex is 1:1, a ternary complex of stoichiometry of 1:1:2 β-CDx/CMOH/DMSO is proposed (Figure 6). Similarly, we have determined a 1:1:1 stoichiometry of the ternary complex involving α-CDx, CMOH, and DMSO.

Therefore, the formation of ternary complexes in case of α- and β-CDx's enhances CMOH−CDx complexation efficiency in nonaqueous media and gives rise to enhanced equilibrium constants for binding (compared to water) where these nonaqueous solvent molecules fill the "void space" within the CDx interior. But in water only binary complexes involving CDx's and CMOH are formed. The water enclosed within the "empty" CDx cavity is in an unfavorable, hydrophobic environment and cannot satiate its (tetrahedral) hydrogen bond potential and is thus activated.[19,20] On its expulsion, inclusion complex formation is favored by a gain in potential energy.[21,22] In aqueous medium, therefore, the water molecules are excluded from the CDx interior during the formation of an inclusion complex, and the complexation efficiency is much less than in nonaqueous media due to the absence of water molecules which could fill the "void space" within the CDx cavity.

The tautomer emission at 453−459 nm due to ESIPT in CMOH has been found to enhance on binding to cyclodextrins (CDx's) in both aqueous and nonaqueous media. What is the

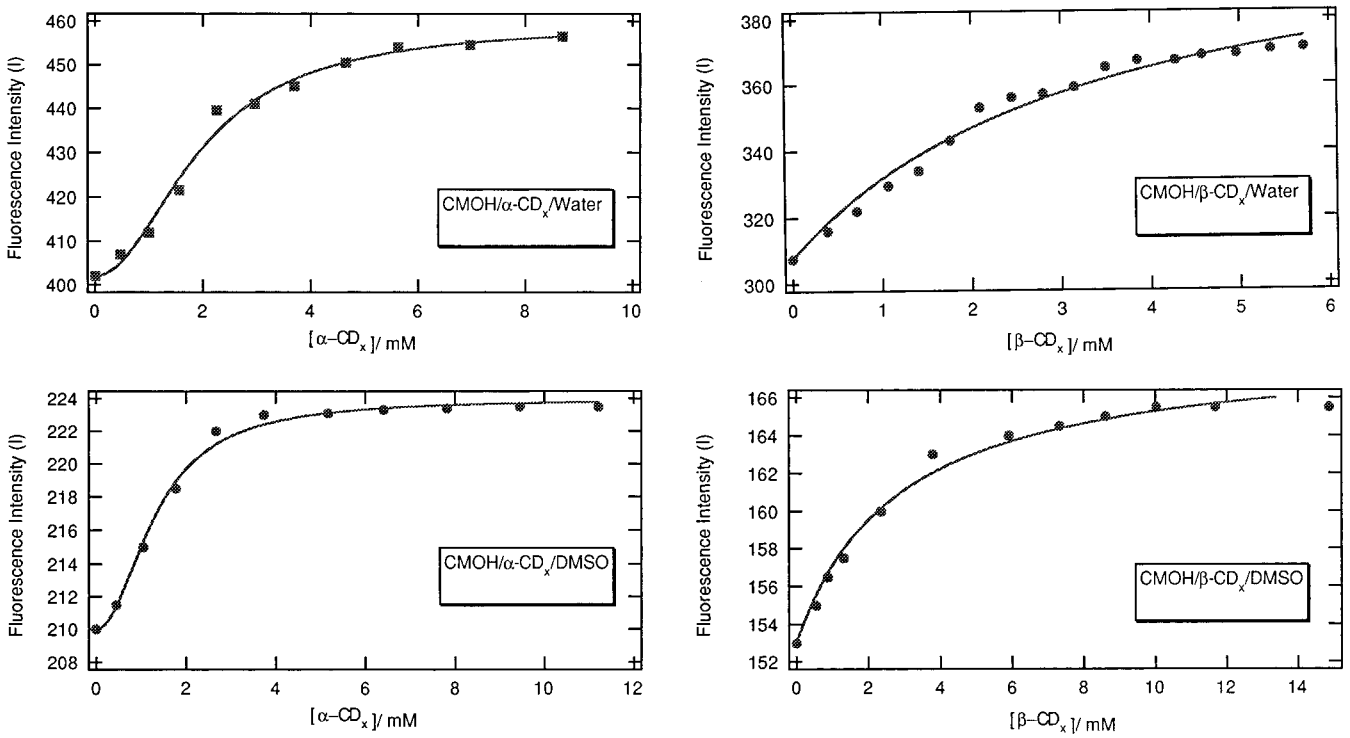


Figure 4. Variation of fluorescence intensity of tautomer emission of CMOH with increasing concentrations of cyclodextrins: (a, top) with α-CDx in water (■) and DMSO (●); (b, bottom) with β-CDx in water (●) and in DMSO (●).

TABLE 1: Association Constants (K_i) for the Formation of Inclusion Complexes of Cyclodextrins in Aqueous and Nonaqueous Media

host	guest	solvent	host:guest	K_i (M^{-1}/M^{-2})	a^a
β-CDx	CMOH	water	1:1	290.3	1.34
β-CDx	CMOH	DMSO	1:1	354.0	1.10
β-CDx	CMOH	formamide	1:1	663.4	1.09
α-CDx	CMOH	water	2:1	2.6×10^5	1.14
α-CDx	CMOH	DMSO	2:1	5.6×10^5	1.06
α-CDx	CMOH	DMF	2:1	36.7×10^5	1.05

[a] $a = I/I_0$, where I and I_0 are fluorescence intensity of tautomer emission of CMOH in the presence and absence of cyclodextrins.

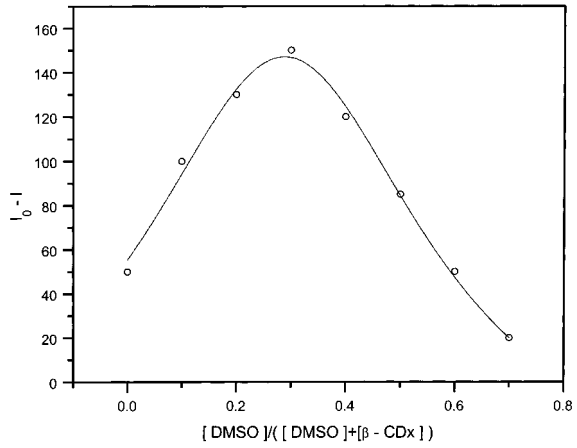


Figure 5. Continuous variation plot monitored at emission wavelength of 453 nm and excitation wavelength at 323 nm. The CMOH concentration is 1.0×10^{-5} M. [DMSO] + [β-CDx] = 10 mM.

origin of such an enhancement of tautomer emission in inclusion complexes of CDx's?

The relative enhancement of tautomer emission ($\Delta I/I_0$) of CMOH on binding to CDx's with respect to the homogeneous solvent media is explained as due to the following reasons: In aqueous medium, the water molecules are not completely

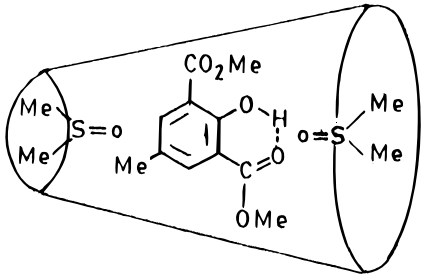


Figure 6. Ternary complex of 1:1:2 stoichiometry involving CMOH, β-CDx, and DMSO.

excluded from CDx interior during the formation of an inclusion complex. In pure aqueous solution water does enter the cyclodextrin cavity. In fact, the cyclodextrins (particularly β-CDx) are known to be in a strained configuration due to the presence of water under these conditions. In fact, this is one of the driving forces for complexation with hydrophobic CMOH, i.e., to release the strain by driving some of the water molecules out of the cyclodextrin cavity. As a result, the external hydrogen-bonding interaction of water molecules with CMOH in the CDx interior is minimized, and the intramolecular hydrogen bond in CMOH (**I**, Scheme 1) remains intact. Therefore, solvent perturbation of ESIPT through intermolecular hydrogen-bonding interaction[23,24] is minimized in the inclusion complexes of cyclodextrins and hence the resultant enhancement of tautomer emission. In case of nonaqueous highly polar media a similar situation prevails. The polar solvent molecules, incorporated within the solvophobic CDx interior, during dissolution of CDx in these nonaqueous media, became excluded from the interior, due to the formation of a guest−host inclusion complex. This leads to the encapsulation of CMOH in a less hydrogen bond interacting medium (CDx interior) than homogeneous solvent, favoring an increase in the ESIPT rate. However, in nonaqueous media, one or two solvent molecules remain in the CDx interior, due to the formation of a ternary

TABLE 2: Relative Enhancement ($\Delta I/I_0$)[a] of Tautomer Emission of the Inclusion Complexes of Cyclodextrins with Respect to Bulk Solvent Media

host	guest	solvent	host:guest	$\Delta I/I_0$ (%)
β-CDx	CMOH	water	1:1	34.0
β-CDx	CMOH	DMSO	1:1	10.0
α-CDx	CMOH	water	2:1	14.0
α-CDx	CMOH	DMSO	2:1	6.0

[a] $\Delta I = I - I_0$; I_0 is the intensity of tautomer emission of CMOH in bulk solvent in absence of cyclodextrin, and I is the intensity of tautomer emission of inclusion complexes of CMOH and cyclodextrin in the same bulk solvent.

complex. As a consequence, the extent of solvent perturbation to ESIPT through external hydrogen bonding interaction in inclusion complexes is much less than in a homogeneous solvent environment.

Also, the CDx interior offers a medium of less polar solvophobic environment. Although ESIPT is a very fast process and has no intrinsic barrier that could depend on the polarity of the solvent environment, the solvent may play a crucial role in terms of introducing a "solvent-induced barrier"[25,26] to ESIPT. The solvent-induced barrier to intramolecular proton transfer originates from the interaction of the normal and tautomer dipole with the polarization of the surrounding solvent and is determined by solvent dielectric properties.[25,26] In the solvophobic CDx interior, the "solvent-induced barrier" to ESIPT decreases, favoring enhancement of the rate of ESIPT, and enhanced tautomer emission is observed in inclusion complexes because rapid ESIPT outweighs the effect of competing nonradiative processes for the depopulation of photoexcited closed conformers (Scheme 1).

It is noteworthy here that the solvent polarity/dielectric properties which may play a role in controlling the dynamics of ESIPT is rarely visited. On the other hand, it is well established that the hydrogen-bonding property of solvent molecules perturbs ESIPT through external hydrogen-bonding interaction with the solute molecule and retards the ESIPT rate. For example, the phototautomers of (hydroxyphenyl)benzazoles due to ESIPT have been demonstrated to be more efficiently produced in dry hydrocarbon solvents as compared to alcohols or water due to less (or absence of) competition between intramolecular and intermolecular hydrogen bonding with the solvent molecules.[27,28] So, the most important and predominant factor that contributes to the enhancement of tautomer emission of CMOH in inclusion complexes of CDx's is the less hydrogen bond interacting environment of CDx interior rather than its less polar environment in comparison to homogeneous solvent media.

The binding constant values (Table 1) reveal stronger guest−host complexation in nonaqueous media compared to aqueous media. Now, stronger guest−host complexation should permit stronger intracomplex ESIPT reactions, and we should have obtained much stronger enhancement of tautomer emission in inclusion complexes in nonaqueous solvents than in water. However, quite interestingly, the experimental observations show much stronger enhancement in ESIPT tautomer emission in inclusion complexes in water than in nonaqueous solvents as evident from the *a* values (Table 1) and relative enhancement of tautomer emission ($\Delta I/I_0$) values displayed in Table 2. The relative enhancement of tautomer emission intensity ($\Delta I/I_0$) of CMOH in the inclusion complex of β-CDX in water with respect to bulk water is 34% while in dimethyl sulfoxide (DMSO) it is 10%, and in the case of the inclusion complex of α-CDX in water with respect to bulk water and in DMSO with respect to bulk DMSO, $\Delta I/I_0$ are 14% and 6%, respectively. Why is there such an anomaly in the enhancemnt of tautomer emission in inclusion complexes of CDx's between aqueous and nonaqueous media?

The formation of "ternary" and "binary" complexes in nonaqueous and aqueous media, respectively, successfully explains the lesser relative enhancement of tautomer emission ($\Delta I/I_0$) in inclusion complexes in nonaqueous media than in water. The solvent DMSO and DMF molecules which are strongly hydrogen bond interacting can disrupt the intramolecular hydrogen bond in CMOH through external hydrogen-bonding interaction and perturb ESIPT. Because it is well-known that external hydrogen-bonding interaction by solvent has a profound effect on ESIPT,[23,24] this effect of solvent perturbation to ESIPT of CMOH has become prominent in the case of DMSO and DMF. But in the case of inclusion complexes in water, the water molecules are excluded from CDx interior, and the type of complex formed is binary, involving only CMOH (as guest) and CDx (as host) partners. This leads to a medium in water that is less hydrogen bond interacting than the CDx interior in nonaqueous media, which favors an increase in the ESIPT rate. Therefore, although emission due to ESIPT of CMOH is enhanced in inclusion complexes of CDx's in nonaqueous media, its relative enhancement ($\Delta I/I_0$) is less (Table 2) than in inclusion complexes in water.

Conclusions

The study of excited-state intramolecular proton transfer (ESIPT) using CMOH as the probe molecule within cyclodextrin cavity in both aqueous and nonaqueous media shows that CMOH forms 1:1 and 1:2 complexes with α- and β-cyclodextrins, respectively. Although the equilibrium constants for binding to cyclodextrins are much higher in the case of highly polar nonaqueous solvents which should lead to stronger intracomplex ESIPT reactions, enhancement of tautomer emission is found to be much higher in water. This anomaly is due to the participation of nonaqueous solvent molecules in the formation of ternary complexes between CMOH, α-/β-CDx, and nonaqueous solvent molecules in the nonaqueous media.

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