Ground- and Excited-State Structural Orientation of 2-(2′-Hydroxyphenyl)benzazoles in Cyclodextrins

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The effects of α-, β- , γ-, and 2,6-di-*O*-methyl-β-cyclodextrins (CDs) on the ground- and excited-state properties of 2-(2′-hydroxyphenyl)benzoxazole, 2-(2′-hydroxyphenyl)benzothiazole, and 2-(2′-hydroxyphenyl)benzimidazole in aqueous media are investigated. Steady-state fluorescence measurements are used to characterize the interaction of CDs with these azoles. Absorbance measurements indicate increased solubility of the azoles in aqueous solutions of CDs. Measurements of acidity constants (pK_a) and data from induced circular dichroism indicate increased ground- and excited-state acidities of the phenolic protons of the molecules in the presence of CDs and axial orientation of the molecules within the CD cavity, respectively. The data further suggest a planar stucture for HBO and a twisted confirmation for both HBT and HBI. The association constants of the inclusion complexes have also been estimated. These studies are further supplemented by comparative spectroscopic studies of 2-(2′-methoxyphenyl)benzothiazole in aqueous solutions of CDs. On the basis of the spectral data acquired, it is believed that the HBA molecules exist as zwitterionic tautomers in the presence of CDs.

Introduction

(Hydroxyphenyl)benzazoles (HBAs, Figure 1) are a class of compounds that are known to exhibit complex photochemical and photophysical properties and have thus been studied extensively.[1−3] Specifically, these molecules undergo very fast ($\sim 10^{12}$ s^{-1}) excited-state intramolecular proton transfer (ESIPT) reactions to form phototautomers.[4,5] The photophysical behavior of the HBAs is shown to be different in polar, protic solvents as compared to that observed in aprotic, nonpolar media. For example, the phototautomers of the molecules have been demonstrated to be more efficiently produced in 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.[6,7] As a result, in solvents of different polarity and pH, different emitting forms (e.g., neutral, anion, cation, and tautomers) of the HBAs are observed , suggesting complex excited-state proton transfer (ESPT) equilibria.[3,4,8] The formation of different rotameric (i.e., cis and trans) keto as well as zwitterionic tautomers upon electronic excitation has also been proposed in the literature.[3−5,8] In addition, recent studies have indicated a unique relaxation process[9] which involves torsional motion about the $C_1-C'_1$ bond in these molecules. The (hydroxyphenyl)benzothiazole analog is believed to possess a lower rotational energy barrier and is thus considered to rapidly undergo ESPT to produce cis and trans phototautomers. Similarly, (hydroxyphenyl)benzimidazole (HBI) is believed to exhibit two different intramolecularly hydrogen-bonded isomers in the ground electronic state that lead to distinctly different excited-state phenomena (S_0).[5]

Recently, the influence of cyclodextrins upon the photochemical processes of organic molecules has received much attention in the literature.[10−13] Cyclodextrins (CDs) are cyclic oligosaccharides composed of 6, 7, or 8 glucopyranose units and are named respectively as α-, β-, and γ-cyclodextrins. The CDs are capable of incorporating a wide range of guest molecules depending upon their size, shape and hydrophobicity.[14,15] The ability of CDs to include molecules within their hydrophobic interior has led to observations of increased solubilization[16] and enhanced fluorescence intensity for complexed guest molecules.[17] Upon inclusion into the CD cavity, the photophysical properties of the guest molecules are often changed. This has been utilized to shed light on the structures of molecules in solution. For example, the influence of CDs upon the photoactivity of anils has recently been investigated.[18] The anils *N*-5-chlorosalicylideneaniline and *N*-salicylidene-2-aminopyridine are shown to display photochroism upon interaction with β- and γ-cyclodextrin. We have recently reported a dual fluorescence of *trans*-stilbene in ternary aqueous solutions of γ-cyclodextrin.[19] The ternary component, either cyclohexane or toluene, is shown to play an active role in increasing the fluorescence intensity of *trans*-stilbene at 420 nm through the formation of excimers, thus restricting the cis−trans photoisomerization of stilbene in solution. More recently, we have studied the photochemistry of 10-hydroxybenzo[*h*]quinoline (HBQ)[20] and the influence of CDs and micelles upon its electronic spectral properties. The tautomer emission intensity of HBQ is significantly enhanced through inclusion of the molecule within the hydrophobic β- and γ-cyclodextrin cavities.

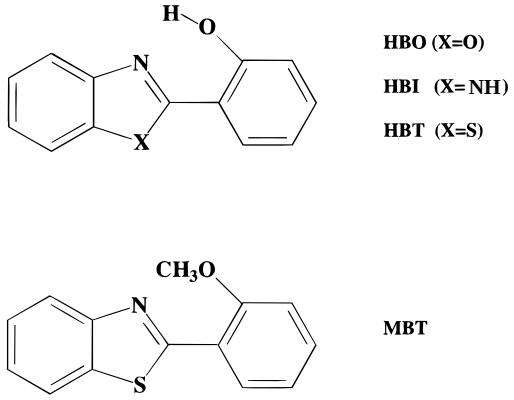

Figure 1. Structures of 2-(hydroxyphenyl)benzazoles (HBO, HBT, and HBI) and 2-(2′-methoxyphenyl)benzothiazole (MBT).

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Another interesting property of CDs is their ability to induce chirality into an achiral molecule when included into its cavity. This phenomenon is unique for molecules containing chromophoric groups (e.g., aromatic compounds) because, upon complexation with CDs, circular dichroism is induced. The induced circular dichroism (ICD) of many aromatic compounds have been reported[21−23] in the literature. This ICD signal is specific for a particular guest molecule, and only those molecules which are significantly included into the CD cavity exhibit ICD spectra. Furthermore, the ICD spectrum of a complexed molecule is often similar to its electronic absorption spectrum. Since an electronic spectrum of a molecule gives an idea of its ground-state structure, the ICD spectrum can be used to depict a molecule's orientation within the CD cavity. This can be used further to indirectly provide information concerning the geometry of the uncomplexed form of the molecule.

Molecules such as (hydroxyphenyl)benzazoles may, through the formation of inclusion complexes with the CDs, be afforded some protection from external quenchers in the bulk aqueous phase. Encapsulation of these molecules may also minimize other competing deactivation routes (e.g., cis, trans isomerization, etc.) from the excited state. In this report, we examine the influence of α-, *β*-, and *γ*-cyclodextrin upon the spectroscopic (i.e., absorbance and fluorescence) properties of HBI, HBO, and HBT. To compare the spectral characteristics and hence structural orientation, we have also included spectroscopic and induced circular dichroism studies of the HBAs and of 2-(2′-methoxyphenyl)benzothiazole (MBT) . The focus of this study is to investigate the possible existence of different rotamers of the HBAs in the ground (S_0) and excited (S_1) electronic states, as well as the effects of the CDs on the ground- and excited-state acidities of the molecules.

Experimental Section

Materials. HBO was obtained from Sigma Chemical (St. Louis, MO). HBT, HBI, and 2-(2′-methoxyphenyl)benzothiazole (MBT) were synthesized and purified using procedures reported in the literature.[4,24] The α-, *β*-, and *γ*-cyclodextrins (α-CD_x, *β*-CD_x, and *γ*-CD_x) were obtained from Sigma and American Maize Products Co. (Hammond, IN). The 2,6-di-*O*-methyl-*β*-cyclodextrin (*β*-CD_m) was obtained from Fluka Chemical (Ronkonkoma, NY). All other chemicals were obtained from Sigma and were used as received. Anhydrous cyclohexane and ethanol were obtained from Aldrich (Milwaukee, WI). All other solvents were of HPLC and/or spectroquality grade (Mallinckrodt/EM Science) and were used without further purification.

Method. *Preparation of Samples in Cyclodextrins for Spectroscopic Studies.* Due to the low solubility of the (hydroxyphenyl)benzazole compounds in water (∼10^{-7} M), crystals of purified HBO/HBT/HBI were added to 50.0 mL volumes of phosphate buffer (pH 7.0) prepared in distilled, deionized water (PureLab). These solutions were sonicated for 15−20 min and allowed to equilibrate for 30−40 min. The separate solutions of azoles were then filtered using 0.02 *μ*m membrane syringe filters (Nalgene) to obtain saturated solutions of the compounds in aqueous media . Equivolume amounts of the saturated solutions of azoles were pipetted into flasks containing appropriately weighed amounts of solid CDs to yield the desired concentrations in solution. These solutions were diluted with phosphate buffer (pH 7.0) and allowed to equilibrate for 10−12 h. The reference solutions for the absorbance studies contained the same concentrations of α-, *β*-, *γ*-, and 2,6-di-*O*-methyl-*β*-cyclodextrins and were diluted to the mark with deionized water.

To prepare samples for absorbance and induced circular dichroism (ICD) studies, purified crystals of each of the various (hydroxyphenyl)benzazoles were added to separate vials. A buffered solution of monosodium/disodium phosphate (pH 7.0) was prepared and filtered using 0.45 *μ*m Nylon syringe membrane filters (Nalgene) to eliminate particulates. Stock solutions of α-CD_x, *β*-CD_x, *γ*-CD_x, and 2,6-di-*O*-methyl-*β*-CD_m (*β*-CD_m) were prepared by dissolving appropriate amounts of each cyclodextrin in the prepared phosphate buffer to make 10^{-2} M solutions. Similarly, these cyclodextrin stock solutions were filtered after preparation using 0.45 *μ*m membrane syringe filters. Solutions of the (hydroxyphenyl)benzazoles were prepared (10^{-2} M) in ethanol (EtOH). A 500 mL aliquot of each of these stock solutions was pipetted into separate vials, and the EtOH was completely evaporated under a stream of dry N_2. Appropriate volumes of cyclodextrin stock solutions were added to the vials and diluted to 5.0 mL with phosphate buffer (pH 7.0). The solutions were equilibrated with the (hydroxyphenyl)benzazole crystals deposited on the inner wall of the vials for 24 h at room temperature. After equilibration, the solutions were filtered using a 0.02 *μ*m pore size syringe filter (Nalgene) to remove the excess (hydroxyphenyl)benzazole crystals.

Determination of Binding Constants. The binding constants and stoichiometric ratios of the inclusion complexes of the HBAs with CDs were estimated from the Benesi−Hildebrand (BH)[25] plots using fluorescence as well as ICD data. The derivation of the BH relationships has been discussed elsewhere[20,26] and therefore will not be detailed here. The following equations were used for 1:1 and 2:1 CD:HBA association:

$$\frac{1}{F - F_0} = \frac{1}{F_1 - F_0} + \frac{1}{K_1[\mathrm{CD}]_0(F_1 - F_0)} \tag{1}$$

$$\frac{1}{F - F_0} = \frac{1}{F_2 - F_0} + \frac{1}{K_2[\mathrm{CD}]_0^{\,2}(F_2 - F_0)} \tag{2}$$

where $[CD]_0$ represents the analytical concentration of the cyclodextrin, F_0 and F_1 (and F_2) are the fluorescence intensities in the absence and presence of cyclodextrin, respectively, and K represents the equilibrium constant. Similar to eqs 1 and 2, the following equation was used to estimate apparent binding constants using ICD data:[27]

$$\frac{[\mathrm{CD}]}{\Delta\theta_s} = \frac{[\mathrm{HBA}] + [\mathrm{CD}] - [\mathrm{CD{:}HBA}]}{\Delta\theta_{\mathrm{CD:HBA}}} + \frac{1}{K\Delta\theta_{\mathrm{CD:HBA}}} \tag{3}$$

where [HBA], [CD], and [CD:HBA] represent the concentration of the guest, host, and the inclusion complex, respectively, $\Delta\theta_s$ is the molar ellipticity of the guest in solution, and $\Delta\theta_{\mathrm{CD:HBA}}$ is the molar ellipticity of the complex.

The apparent K values were obtained from the slope and intercept of the respective BH plots. The K value thus obtained was then used as an initial guess in an iterative nonlinear regression program (NLR)[28] which provides estimates for K for 1:1 and 2:1 complexes by fitting the data using the following equations:

$$F = \frac{F_0 + F_1K_1[\mathrm{CD}]_0}{1 + K_1[\mathrm{CD}]_0} \tag{4}$$

$$F = \frac{F_0 + F_2K_2[\mathrm{CD}]_0^{\,2}}{1 + K_2[\mathrm{CD}]_0^{\,2}} \tag{5}$$

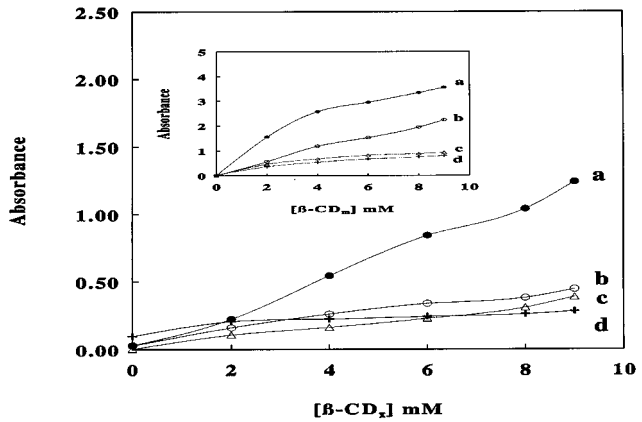

Figure 2. Influence of β-CD$_x$ (0.0−9.0 mM) on the absorbance of (a) MBT, (b) HBO, (c) HBT, and (d) HBI. (inset) Influence of β-CD$_m$ (0.0−9.0 mM) on the absorbance of the HBA molecules (b−d) and MBT (a).

Measurement of Acidity Constants (pK_as). The acidity constants of the azoles in β-CD$_x$ were determined spectrophotometrically. Saturated solutions of the HBAs in β-CD$_x$ (i.e., 5.0 mM) were prepared as described previously. The following buffers were prepared and used for this study: 0.1 M $Na(CH_3)_2AsO_2 \cdot 3H_2O$/0.1 M HCl (pH 2.0−7.0); 0.1 M NaH_2PO_4/Na_2HPO_4 (pH 7.0−8.7); 0.1 M Na_2CO_3/0.1 M $NaHCO_3$ (pH 9.0−10.70); and 0.1 M Na_2HPO_4/ 0.1 M NaOH (pH 11.0−12.50). The pK_a values were obtained from Henderson−Hasselbalch plots as well as from absorbance titration curves.

Apparatus. Absorbance measurements were performed on a Shimadzu UV-3101PC UV−vis−near-IR scanning spectrometer. Steady-state fluorescence spectra were acquired by use of a SPEX Model P2T 211 spectrofluorimeter and a Perkin-Elmer LS-50 luminescence spectrometer. Samples were measured in a 1 cm^2 quartz cell using excitation and emission bandwidths of 2−5 nm. Circular dichroism studies were performed using a Jasco J-710 spectropolarimeter. All spectra were recorded at room temperature. ICD spectra were corrected by subtraction of the corresponding reference ICD spectrum.

Results and Discussion

Absorbance Studies. Absorbance measurements were performed to qualitatively assess the solubility of HBAs in the presence of cyclodextrins. Figure 2 depicts the absorbance of MBT (a), HBO (b), HBT (c), and HBI (d) at various concentrations of β-CD$_x$. Since the absorbance change of the HBAs is very small in α-CD$_x$ and γ-CD$_x$, these values are not included in Figure 2. It is evident that the solubility of the HBAs increases with increasing β-CD$_x$ concentration. As can be seen from the plots in Figure 2 (inset), the solubilities of the HBAs are much higher in β-CD$_m$ compared to those in β-CD$_x$. Both plots show that the solubilities of the HBAs are on the order MBT > HBO > HBT > HBI.

It has been well-established that the enhanced solubilities of organic compounds in water in the presence of CDs is due to formation of inclusion complexes. Molecules can be included into the CD cavity depending upon their size, geometry, and hydrophobicity. Of the CDs used in this study, α-CD$_x$, has the smallest (∼5.7 Å) cavity diameter and the γ-CD$_x$ has the largest diameter (∼9.5 Å), while β-CD$_x$ has a cavity diameter of ∼7.8 Å. However, all of the CDs have similar height (∼7.0 Å). The HBA molecules have an average length of ∼10 Å and width of ∼5.0 Å (including C−H bonds). Thus, the probability of equatorial inclusion of the HBAs in the CDs is very low. The low solubility of the HBAs in α-CD$_x$ is probably due to smaller cavity size and, therefore, lack of complexation. On the other hand, the cavity size of γ-CD$_x$ appears to be too wide to form a stable complex with the compounds when oriented axially in the CD cavity. Also, the γ-CD$_x$ cavity being wider, water molecules can freely enter into the cavity, making the cavity less hydrophobic than that of β-CD$_x$. This is indicated by the low values of the association constants (Table 1) of the HBAs with γ-CD$_x$. The higher solubilities of the HBAs in β-CD$_m$ as compared to β-CD$_x$ may be due to the strong hydrogen-bonding ability of the alcoholic OH and the methoxy groups on the CD ring with the phenolic OH group of the HBAs. This will be discussed later in detail. Among the HBAs, HBO has the highest solubility as well as largest association constants with the CDs (i.e., β-CD$_x$ and β-CD$_m$). Since all of the HBAs are similar in size, this difference in the binding constant values and hence the difference in solubilities may be attributed to the differences in their molecular structure. It seems that the HBO molecule is more hydrophobic, as compared to HBI and HBT. The most likely orientation of HBO which can explain its hydrophobicity is one in which the phenyl ring is coplanar with the benzoxazole ring through strong intramolecular hydrogen bonding. This is confirmed by the vibronic fine structure of the long-wavelength absorption bands in its electronic spectrum (Figure 3, inset) in β-CD$_m$. Similar spectral characteristics of HBO in nonpolar solvents have also been reported in the literature.[8] In contrast, the absorption bands of HBI and HBT are broad, thus suggesting that the phenyl ring is not in the plane of the benzazole ring. This suggests that the dipole moments of HBI and HBT are higher than that of HBO and therefore are more polar compared to HBO. Consequently, the inclusion of HBI and HBT into the β-CD$_x$ cavity is weaker. Further evidence for the structural differences among the HBAs lies in the differences in their acid dissociation constants for the phenolic proton and has been discussed below in a separate section.

TABLE 1: Estimated Binding Constants (*K*) for HBAs in Various CDs

	K (M^{-1})[a]				
	fluorescence data			ICD data	
compd	β-CD$_x$	β-CD$_m$	γ-CD$_x$	β-CD$_x$	β-CD$_m$
HBI	131	256	45	53	208
HBO	340	462	140	228	274
HBT	220	253	161	95	124
MBT	385	530	252	307	528

[a] Values represent average of four trials.

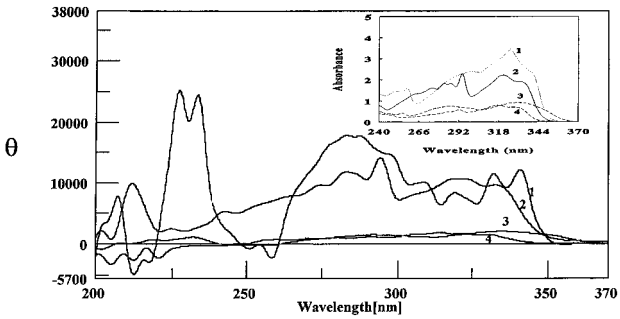

Figure 3. Induced circular dichroism (ICD) spectra of β-CD$_m$ (9.0 mM) with (1) MBT, (2) HBO, (3) HBT, and (4) HBI. (inset) Corresponding absorbance spectra of the molecules in 9.0 mM β-CD$_m$, from 240 to 370 nm.

Induced Circular Dichroism Studies. The circular dichroism spectra of the four HBAs in β-CD$_m$ are portrayed in Figure 3. Since the compounds are less soluble in α-CD$_x$ and γ-CD$_x$, the ICD spectra were measured only in β-CD$_x$ and β-CD$_m$. The

molar ellipticity (θ) of the HBAs is higher in the latter apparently because of their higher binding constant. The ICD spectra of the molecules are very similar in both β-CD_x and β-CD_m and also resemble their respective electronic absorption spectra (in β-CD_m, Figure 3 inset). Similar to the absorption spectrum, the long-wavelength bands of the ICD spectrum of HBO are also structured, indicating again that the molecule is rigid and is included into the hydrophobic environment of the CD cavity. All of the HBAs exhibit positive CD signals with maxima corresponding to long-wavelength electronic absorption bands. Molecular orbital calculations by Dey and Dogra[29] and also by Chou et al.[9] have shown that the lowest energy electronic transition of HBAs are polarized along the long axis of the molecule. According to the Kirkwood−Tinoco rules based on the coupled oscillator theory,[17] these data suggest that the molecules are oriented axially in the CD cavity; i.e., the long axis of the molecules is parallel to the symmetry axis of the CD cavity.

The association constants of the HBAs (Table 1) obtained by using the ICD data suggest that the binding of HBI and HBT is weaker in β-CD_x as compared to β-CD_m. The association constants obtained by ICD data are lower than those obtained from fluorescence data. Since these two methods measure ground- and excited-state phenomena (circular dichroism and fluorescence, respectively), the values obtained by the above-mentioned techniques may reflect structural differences in the HBA molecules. However, the trend is similar in both sets of data. The low binding constant of HBI with β-CD_x is indicative of the polar structure of the molecule. The higher binding constant in β-CD_m implies that the cavity of β-CD_m is less polar than that of β-CD_x. Because of higher polarity, the molecule is also less soluble in γ-CD_x. Similar conclusions can also be drawn about HBT on the basis of the estimated K values.

The ICD spectrum of MBT in β-CD_x or β-CD_m is stronger than that of HBT, suggesting that MBT forms a tighter complex with the cyclodextrins. This is manifested in the binding constant value which is higher than that for the other HBAs. This implies that the HBA molecules enter the CD cavity with the benzazole ring forward and phenyl group protruding into the bulk solvent. The OCH_3 group of MBT acts as a capping group, making the binding stronger. If the molecules entered into the cavity with the phenyl group first, the binding constant of MBT would be smaller compared to that of HBT and HBI. It has been reported that the binding efficiencies of disubstituted benzene derivatives are in the order ortho < meta < para.[30] In the case of HBI, HBO, and HBT, the benzazole ring lies inside the cavity and the phenyl ring is on the surface. It seems that, in the case of HBO, the inclusion is deeper compared to that of HBT and HBI because of its more rigid planar structure. This is indicated by the higher binding constant value compared to that of HBT and HBI. In the cases of HBI and HBT, probably the hydrogen-bonding interaction of the phenolic OH with the alcoholic OH groups of the CD ring prevents deeper inclusion of the molecules. This further weakens the intramolecular hydrogen bond and thus enhances the noncoplanarity of the phenyl group. This will be discussed in detail in the following section. The above observations thus suggest that the molecular structure of the HBAs are different. Of these, HBO has a planar structure, and both HBI and HBT as well as MBT have nonplanar structures. The MO calculations by Chou et al. have also suggested similar results.[9]

Acidity Constants. The acidity constants (pK_as) of the HBAs in the presence and absence of β-CD_x (5.0 mM) were determined spectrophotometrically. The estimated pK_a values for the dissociation of the phenolic proton in the various HBAs appear in Table 2. The pK_a values of the HBAs in aqueous solution (in absence of CDs) are in the order HBO > HBT > HBI. These values, within the experimental error, are in reasonably good agreement with the values reported in the literature.[31] In all cases, the pK_a was observed to decrease in the presence of β-CD_x, most dramatically for HBO. Similar negative ΔpK_a values have also been reported for other aromatic alcohols.[32] The ΔpK_a values for HBT and HBI are observed to show a similar trend. The estimated pK_a value for the conjugate acid of HBI formed by protonation at the pyridinic ring nitrogen also shows a slight decrease in the presence of β-CD_x.

TABLE 2: Estimated Acidity Constants (pK_a) of HBAs in Presence and Absence of β-CD_x

	pK_a		
compd	water (W)	β-CD_x	ΔpK_a[b]
HBO	10.48	9.70	−0.78
HBT	10.16	9.60	−0.56
HBI	9.98	9.50	−0.48
HBI	4.84[a]	4.60[a]	−0.24

[a] Acidity constants for protonation of pyridinic nitrogen of HBI. [b] $\Delta pK_a = pK_a(CD) - pK_a(W)$.

The difference in the ground-state pK_a values among the HBAs suggests differences in the strength of intramolecular hydrogen bonding. Thus, the strength of the intramolecular hydrogen bond in the HBAs is in the order HBO > HBT > HBI. This implies that HBO is more planar compared to HBT and HBI. It seems that the phenyl ring of HBI is almost out of plane of the benzimidazole ring since the pK_a value is equal to any non-hydrogen-bonded phenol, and consequently it is highly solvated in aqueous solution. The negative ΔpK_a value implies that the anionic form of the HBAs is more stable when complexed with the CDs. Since the CD molecule is uncharged, there must be some type of strong specific interaction(s) between the phenolate anion and the CD molecule. The decrease in the pK_a value of the conjugate acid formed by protonation at the pyridinic ring nitrogen of HBI in the presence of β-CD_x is because of the higher stability of the complex with the neutral base than that formed with the conjugate acid. This indicates that the formation of inclusion complexes with CDs occurs in such a manner that the benzazole moiety enters first into the cavity.

The increased acidity of the phenolic OH group of the HBAs seems likely due to formation of strong intermolecular hydrogen bonding between the phenolic -OH group and alcoholic OH groups of the cyclodextrin in which the latter acts as a hydrogen bond donor. The secondary OH groups of β-CD_x are reported to have $pK_a \sim 12$.[32] As already discussed above, the HBAs enter the CD cavity from the upper rim side of β-CD_x with the benzazole moiety forward, though a partial penetration is achieved. Thus, the phenyl group is solubilized at the surface of the upper rim of cyclodextrin. This allows hydrogen-bonding interactions between the HBA and the alcoholic OH groups of β-CD_x, which weakens the intramolecular hydrogen bonding in HBAs and thus facilitates formation of stronger intermolecular hydrogen bonds with H_2O molecules in which water acts as a proton acceptor. As a result, proton transfer to the solvent becomes simple. The large negative ΔpK_a value (−0.78) for HBO is indicative of the fact that the intramolecular hydrogen bonding is stronger in HBO compared to that in HBI and HBT. This is also indicated by the higher pK_a value of the molecule in water as compared to the other HBAs.

Fluorescence Spectra. The steady-state fluorescence spectra of the HBAs employed in this study were measured in aqueous solutions (pH 7.0) in the presence of 8 mM β-CD_x, β-CD_m, and

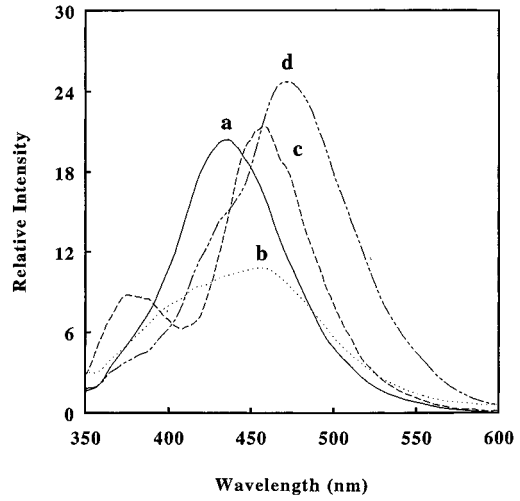

Figure 4. Emission of HBO in (a) water (pH 7.0), (b) 8.0 mM β-CD$_x$, (c) 8.0 mM β-CD$_m$, and (d) 8.0 mM γ-CD$_x$. $\lambda_{exc} = 320$ nm.

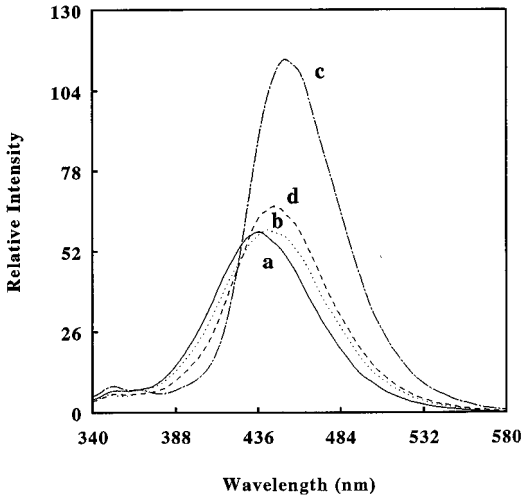

Figure 5. Emission of HBI in water (pH 7.0) and in the presence of (b) 8.0 mM β-CD$_x$, (c) 8.0 mM β-CD$_m$, and (d) 8.0 mM γ-CD$_x$. $\lambda_{exc} = 330$ nm.

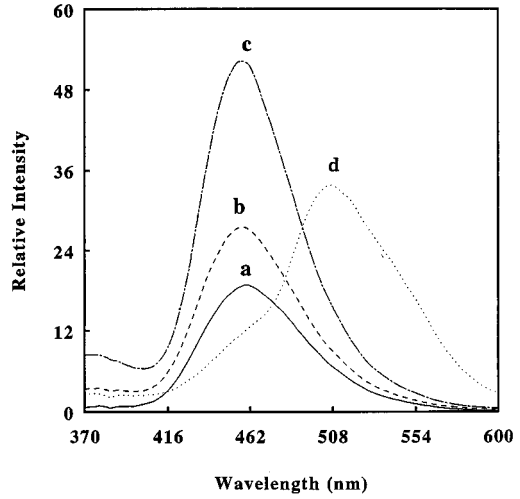

Figure 6. Emission of HBT in (a) water (pH 7.0), (b) 8.0 mM β-CD$_x$, (c) 8.0 mM β-CD$_m$, and (d) 8.0 mM γ-CD$_x$. $\lambda_{exc} = 340$ nm.

γ-CD$_x$. The spectra are displayed in Figures 4, 5, and 6. In aqueous solutions, in the absence of CDs, HBO exhibits a broad emission band (Figure 4a) with λ_{max} ~439 nm. On the basis of literature reports,[8] this emission can be attributed predominately to the phenolate anion with possible contribution from the undissociated neutral molecule. In the presence of β-CD$_x$ the overall intensity of the band decreased accompanied by a red shift ($\lambda_{max} \sim 463$ nm). The bandwidth at half-maximum (bwhm) also increased in comparison to pure aqueous solution. This suggests the existence of multiple fluorescent species. The emission spectrum in the presence of the same concentration (8 mM) of β-CD$_m$ is, however, clearly resolved into two bands. The short-wavelength band resembles the normal emission of the neutral molecule. The long-wavelength band, however, is displaced further to the red relative to that of the anion band and therefore can be associated with the tautomeric species. The fluorescence spectrum of HBO in γ-CD$_x$ is again broad. The maximum of the proton-transferred form ($\lambda_{max} \sim 478$ nm) is further red-shifted compared to that in β-CD$_m$. The spectrum also shows shoulders at ~370 and ~440 nm, indicating that the neutral molecule and the phenolate ion also contribute to the emission.

The fluorescence spectra of HBI in the absence and presence of cyclodextrins are shown in Figure 5. Similar to HBO (Figure 4), the emission spectrum of HBI in the absence of CDs consists of a strong emission at ~427 nm mainly due to the anionic species and a weak band at ~350 nm corresponding to the neutral molecule (Figure 5a). The long-wavelength emission band is red-shifted as compared to the anionic form (~410 nm)[33] and, therefore, likely to be due to both the tautomer and anion forms. In the presence of β-CD$_x$ the spectrum remains almost unchanged except for a small red shift of the long-wavelength emission. The small red shift is a result of the inclusion of the molecule into the hydrophobic cavity of the CD molecule which promotes the formation of the tautomer. This is consistent with the binding constant value (Table 1). However, the binding constant of HBI with β-CD$_m$ is higher, indicating a strong interaction. This is manifested in the appearance of a strong tautomer as well as the normal emission bands. The tautomer emission band is also red-shifted in comparison to β-CD$_x$. The fluorescence spectrum of HBI in γ-CD$_x$ is similar to that in β-CD$_x$.

The fluorescence spectral behavior of HBT in CDs reflects similarities and differences relative to that observed for HBI and HBO. Like HBO and HBI, the fluorescence spectrum of HBT (Figure 6) in the absence of CDs shows characteristic fluorescence from both the neutral molecule and the anion form. However, unlike the former molecules, the intensity of the short- and long-wavelength bands of HBT increased in the presence of both β-CD$_x$ and β-CD$_m$. The magnitude of the intensity change is much higher in the case of β-CD$_m$. In γ-CD$_x$, the fluorescence spectrum of HBT exhibits a decrease in intensity of both the normal and anion emission bands and the rise of a strong band with maximum at ~505 nm, which corresponds to the tautomer emission. The spectral behavior of HBT is thus different from that of either HBO or HBI.

The observed spectral characteristics of the HBAs in cyclodextrins can be explained in terms of hydrophobic and hydrogen-bonding interactions with the cyclodextrins. The HBAs exhibit ESPT (both inter- and intramolecular), but the rate of intermolecular proton transfer (PT) in aqueous solution is so fast that ionization is almost complete during the lifetime of the excited state. Consequently, in aqueous solution, even at neutral pH, nearly all of the emission occurs from the excited phenolate anion. Since the nonionic forms of the HBAs are stabilized by CDs through the formation of inclusion complexes, the intensities of the anionic forms of HBO and HBI are decreased. However, this is not true in the case of HBT, thus suggesting that the molecular structure of HBT is different from the other HBAs. This is also reflected in its association constants and

ground-state pK_a value and has already been discussed in the preceding section.

As mentioned earlier, the phenyl ring in HBT is noncoplanar with the benzazole ring, thus rupturing the intramolecular hydrogen bond. As a result, in the inclusion complex, the HBT molecule is oriented in such a way that the phenyl ring with the phenolic OH group is exposed to the bulk solvent. The strong hydrogen-bonding interaction between phenolic oxygen and the alcoholic hydrogens of the CD increases the acidity of the phenolic proton. Since this intermolecular hydrogen bond is in close proximity to the bulk solvent, the phenolate ion is more stable in CD. The hydrogen-bonding ability of the alcoholic hydrogens of β-CD$_x$ is low compared to that of β-CD$_m$ because in the former, the secondary alcoholic OH groups at the 2- and 3′-positions of the adjacent glucopyranose rings are engaged in hydrogen bonding themselves. This is the reason why the solubility of β-CD$_x$ is less than other CDs in water. In β-CD$_m$, however, this intramolecular hydrogen bonding is partially destroyed because of the substitution of one of the alcoholic protons by CH_3 group. This enhances the hydrogen-bonding ability of β-CD$_m$ with the HBAs as well as water molecules. As a result, β-CD$_m$ is more soluble in water. The greater hydrogen-bonding ability and higher hydrophobicity increases the association constant of the HBAs in β-CD$_m$. Thus, stronger intermolecular hydrogen bonding between HBT and β-CD$_m$ facilitates the ESPT, producing more phenolate anion. Moreover, the structure of the HBA tautomers is highly polar, which explains the shift in emission maxima of HBI, HBT, and HBO in cyclodextrins. In the presence of cyclodextrins, all of the molecules have emission maxima between those reported for the compounds in polar (e.g., alcoholic) and apolar (e.g., hydrocarbon) solvents. For example, HBT, in the presence of γ-CD$_x$, exhibits emission at ~505 nm, blue-shifted from the tautomer emission (520 nm). Potter and Brown[7] observed a similar emission for HBT at 500 nm in polar solvents and attributed it to the zwitterionic/quinoid form of HBT produced by ESPT. Both HBI and HBO exhibit similar intermediate emission maxima in CDs (450 and 478 nm, respectively) relative to those observed in apolar (470 and 502 nm, respectively) and polar (445 and 470 nm, respectively) solvents.[7,8,34,35] Therefore, we believe that the polar structure of the HBA tautomers exist as PT zwitterionic species.

Conclusion

The absorbance studies discussed in this paper indicate that the (hydroxyphenyl)benzazole molecules are more soluble in β-CD$_m$ than in β-CD$_x$. The induced circular dichroism spectra suggest that the HBA molecules are axially oriented in the CD cavity with the benzazole group forward. Cyclodextrins (β-CD$_x$, β-CD$_m$) increase the ground- and excited-state acidities of the phenolic protons for HBT and HBI, but in the case of HBO they increase the ground-state acidities but reduce the acidities in the excited state as manifested in the pK_a values. The respective pK_a values and association constants of the HBAs in CDs clearly suggest that HBO has a planar structure, whereas with HBI and HBT the phenyl group is twisted. On the basis of the fluorescence spectral characteristics of the HBAs in β- and γ-CD$_x$, we propose that the respective PT tautomers of the HBAs exist in the zwitterionic form.

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