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Excited-State Intramolecular Proton Transfer of 2-(2′-Hydroxyphenyl)benzimidazole in Cyclodextrins and Binary Solvent Mixtures

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The excited-state intramolecular proton transfer (ESIPT) of 2-(2′-hydroxyphenyl)benzimidazole (HBI) has been studied using absorbance, steady-state, and time-resolved emission spectroscopies in cyclodextrins (β-, γ-, and 2,6-di-*O*-methyl-β-cyclodextrins) and in binary aqueous solvent mixtures. The spectral characteristics of HBI in cyclodextrins and binary solvent mixtures (dioxane:water, MeOH:water, and solutions of cyclohexane containing MeOH and trifluoroethanol) are compared to further study the effects of various microenvironments on the ground and excited-state properties of the molecule. The 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. The acquired data are used to suggest the formation of a zwitterionic structure of HBI in cyclodextrins.

Introduction

Hydroxyphenylbenzazoles (HBAs) have been studied extensively[1−7] because of their ability to undergo very fast ($\sim 10^{12}$ s^{-1}) excited-state intramolecular proton transfer (ESIPT) reactions to form phototautomers. The ESIPT molecules often exhibit dual emission: a normal emission at lower wavelengths and a Stokes-shifted tautomer emission. These emissions have been shown to depend heavily on solvent polarity, pH, hydrogen-bonding character of solvent, and structure of the molecules in both the ground and excited states.[8,9] Molecules that give rise to fluorescent tautomers through ESIPT are useful in lasing systems as laser dyes,[10−11] in high-energy radiation detectors,[12−13] molecular energy storage systems,[14] and as fluorescent probes.[15] For example, the molecule 2-(2′-hydroxyphenyl)benzimidazole (HBI) has been used in the development of pulsed liquid lasers from photoinduced intramolecular proton transfer.[6] Tunable laser pulses with 10% efficiency have been obtained using HBI as a source of spontaneous emission. An important objective in enhancing the performance of proton-transfer lasers is the extension of applicability of the systems to aqueous media. The advantage of using water as a solvent in such systems is the minimization of artifacts that result from thermal gradients produced in organic solvents.[16]

HBI is believed to exhibit two different intramolecularly hydrogen-bonded isomers in the ground electronic state (S_0). Excitation of the first isomer (**I**) leads to formation of the keto-tautomer (**III**, Figure 1), while excitation of the second isomer (**II**) yields the normal emission.[17−19] The appearance of both the normal and tautomer emissions of HBI seems to be dictated by competition between intra- and intermolecular hydrogen bonding with solvent molecules. Such observations, combined with the potential utility of the molecule, have prompted a more detailed investigation of the microenvironment experienced by HBI molecules in various media.

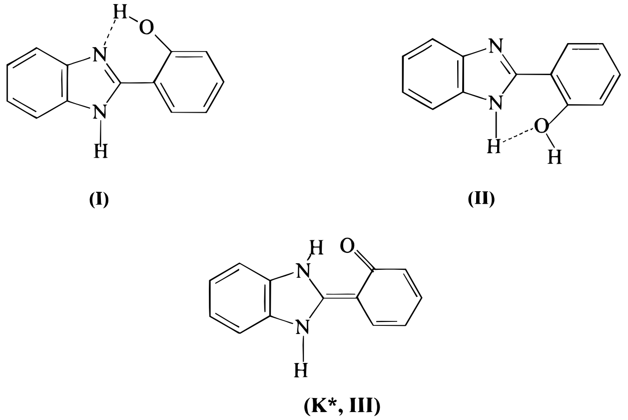

Figure 1. Structures of the ground-state isomers (**I**, **II**) and the excited-state keto-tautomer (K*, **III**) of HBI.

Cyclodextrins (CDs) have been used to perturb the physical and chemical properties of hydrophobic organic molecules in aqueous solutions. The CDs are capable of incorporating a wide range of guest molecules based on hydrophobic and geometrical considerations.[20] Upon inclusion or partial inclusion of molecules within their hydrophobic interior, CDs can effectively shield the excited singlet state of molecules from nonradiative processes and enhance their fluorescence intensity.[21] The aqueous solubility of complexed molecules has also been observed to be increased upon formation of inclusion complexes with CDs.[22,23] In a previous report,[24] we investigated the influence of cyclodextrins upon the spectral properties of 2-(2′-hydroxyphenyl)benzazoles. These studies provided more evidence to support the notion that HBAs possess different ground- and excited-state structural orientations. Furthermore, these studies seem to indicate differences in the extent of inter- and intramolecular proton transfer for the molecules in aqueous solutions of cyclodextrins. The results of recent studies of other ESIPT molecules in the presence of cyclodextrins[25,26] and micelles[27,28] have been used to suggest similar phenomena. In this study, we extend our examination of ESIPT in organized media to include the effects of cyclodextrins and binary aqueous solvents (methanol:water and dioxane:water) on the intramolecular proton transfer of HBI. The focus of this work is to characterize and compare the effects of microheterogeneous environments on the ESIPT process.

Experimental Section

Materials. HBI was synthesized and purified as described in previous reports.[24,29] The β-, γ-, and 2,6-di-*O*-methyl-β-cyclodextrins (β-CD$_x$, γ-CD$_x$, and β-CD$_m$, respectively) were obtained from American Maize Products (Hammond, IN) and Fluka Chemical (Ronkonkoma, NY) and were used as received.

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Anhydrous dioxane was obtained from Fluka Chemical. Anhydrous methanol (MeOH) was obtained from Sigma (St. Louis, MO). Spectrophotometric grade cyclohexane, methanol, and 2,2,2-trifluoroethanol were obtained from Sigma, Aldrich (Milwaukee, WI) and Fluka Chemical, respectively. All other solvents and reagents were of spectrophotometric grade and were used as received.

Methods. Crystals of purified 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 HBI solution was then filtered using 0.02 μm membrane syringe filters (Nalgene) to obtain saturated solutions of the compound in aqueous media. Equivolume amounts of the saturated solution of HBI were pipetted into test tubes containing appropriately weighed amounts of solid cyclodextrins to yield the desired concentration in solution. These samples were diluted with phosphate buffer (pH 7.0), filtered using syringe membrane filters, and allowed to equilibrate for 10−12 h. For the studies in binary aqueous mixtures, stock solutions of HBI in dioxane and methanol (10^{-3} M) were prepared. Equivolume amounts (50.0 μL) of HBI in dioxane and methanol were pipetted into separate test tubes and diluted with the appropriate amounts of dioxane/water and MeOH/water to make volume:volume (v/v) mixtures. Similar methods were used to prepare samples of HBI in cyclohexane:trifluoroethanol (TFE) and cyclohexane:MeOH mixtures.

Apparatus. Absorbance measurements were performed on a Shimadzu UV-3101PC UV−vis−NIR scanning spectrophotometer. Steady-state fluorescence spectra were acquired by use of a Spex Model P2T 211 spectrofluorometer 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. Fluorescence lifetime measurements were performed using a Photon Technology International (PTI) LS-100 luminescence spectrometer. A dilute starch solution was used as a scatterer to determine the lamp flash profile of the excitation source. The lamp was filled with a N_2/He gas mixture and operated at a pressure of 16.5 mmHg. The 337 nm emission of N_2 was used for sample excitation. The decay curves were obtained using a time-correlated single photon counting (TC-SPC) technique. The lifetime data were analyzed using a multiexponential decay analysis program, with curve fitting evaluated by reduced χ^2 and Durbin−Watson parameters. All measurements were performed at 25 °C.

Results

Fluorescence Spectra of HBI in Binary Solvents and Cyclodextrins. The fluorescence spectra of HBI have been studied in selected pure solvents and binary solvent mixtures. Figures 2 and 3 show the spectra in cyclohexane containing, respectively, various concentrations (*M*) of methanol and TFE. In pure cyclohexane, the molecule exhibits only a large Stokes-shifted emission band, the intensity of which increases in the presence of methanol. However, in the case of TFE the intensity of the long wavelength (LW) emission band decreases with the simultaneous appearance of a broad emission band at short wavelengths (SW) with the emission maximum between 370 and 400 nm. In both the cases of methanol and TFE, the LW emission shifts to shorter wavelengths with an increase in concentration of the solvent.

The fluorescence spectra in binary aqueous solvent mixtures of dioxane and water, and methanol and water (0−95%) are displayed in Figure 4. In both dioxane and methanol, two emission bands can be observed in the spectral range studied (330−580 nm). In dioxane, the intensity of the SW band is much weaker compared to that in methanol. However, in methanol, the intensity of the LW band is weaker than that in dioxane. In the presence of low concentrations of water of about <35% (v/v) the intensity of the LW as well as the SW band increases (not shown in the figure). However, at higher concentrations of water (>35%), the intensity of the band decreases along with a shift of the spectrum toward shorter wavelengths (i.e., from 442 to 430 nm). The intensity of the SW band continued to increase with increasing percentage of water up to 90%. Above 90%, the intensity of the SW band is observed to decrease.

As reported earlier,[24] the emission spectrum of HBI in the absence of cyclodextrins (pH 7.0) consists of a strong long

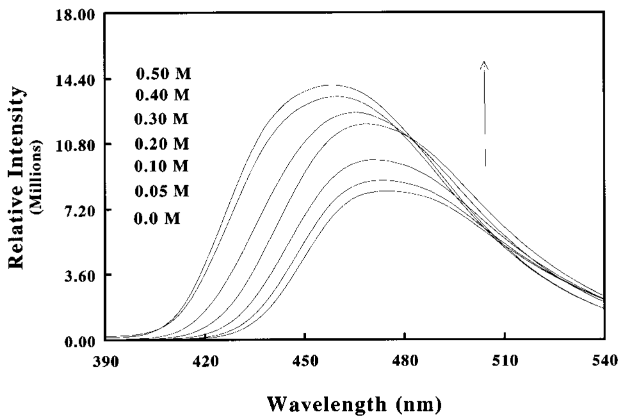

Figure 2. Influence of increasing concentrations of methanol (0.0−0.5 M) on the tautomer emission of HBI (5.0 × 10^{-5} M) in cyclohexane; λ_{ex} = 320 nm.

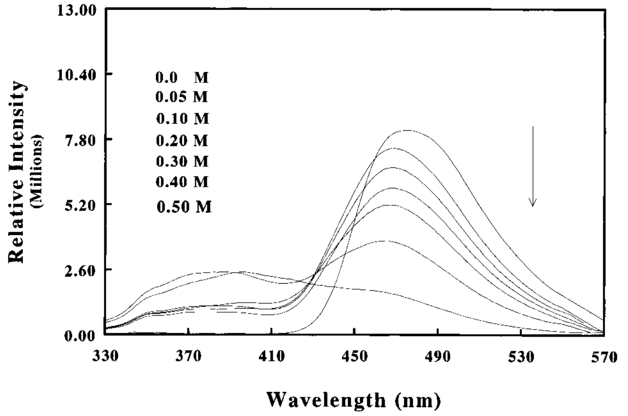

Figure 3. Influence of increasing concentrations of 2,2,2-trifluoroethanol (0.0−0.5 M) on the tautomer emission of HBI (5.0 × 10^{-5} M) in cyclohexane; λ_{ex} = 320 nm.

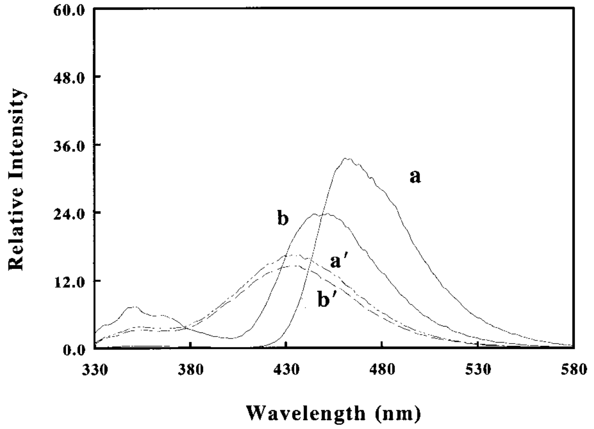

Figure 4. Emission spectra of HBI (5.0 × 10^{-5} M) in (a) 1,4-dioxane, (b) MeOH, (a′) (v/v) 95% water:dioxane, and (b′) 95% (v/v) water:MeOH mixtures; λ_{ex} = 320 nm.

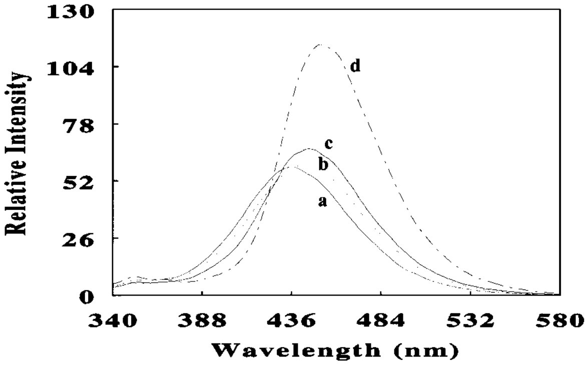

Figure 5. Emission spectra of HBI in (a) water and in the presence of (b) 8.0 mM γ-CD$_x$, (c) 8.0 mM β-CD$_x$, and (d) 8.0 mM β-CD$_m$ at pH 7.0; $\lambda_{ex} = 320$ nm.

TABLE 1: Lifetime Values of 2-(2′-Hydroxyphenyl)benzimidazole in Binary Aqueous Solvents of 1,4-Dioxane and Methanol

solvent	% water	$E_T(30)$ value[a]	lifetimes (ns) τ_1	τ_2	a_1	a_2	χ^2
MeOH	0.0	55.5	3.7	0.7	0.83	0.17	1.1
	10.0	56.1	3.9	0.6	0.82	0.18	1.2
	30.0	57.2	3.9	0.6	0.94	0.06	1.2
	50.0	58.3	3.5	0.7	0.83	0.17	1.1
	60.0	59.2	3.1	0.7	0.90	0.10	1.0
	70.0	60.0	2.7	0.7	0.91	0.09	1.2
	80.0	61.0	2.8	0.8	0.94	0.06	1.1
	90.0	62.2	2.5	0.7	0.77	0.23	1.1
pH 7	100.0	63.1	2.2	0.7	0.58	0.42	1.1
pH 11			2.7		1.00		1.0
basic MeOH			2.9		1.00		1.0
dioxane	0.0	36.0	4.1	0.8	0.78	0.22	1.1
	10.0	46.7	4.3	0.9	0.82	0.18	1.0
	30.0	50.9	4.5	1.0	0.68	0.32	1.1
	50.0	53.6	4.4	1.0	0.74	0.26	1.2
	60.0	55.6	4.1	0.9	0.75	0.25	1.1
	70.0	57.2	3.9	0.8	0.83	0.17	1.1
	80.0	58.6	3.8	0.8	0.84	0.16	1.0
	90.0	61.1	2.7		1.00		1.2

[a] $E_T(30)$ values reported in kcal/mol. Emission monitored at 470 nm.

wavelength (LW) emission at ~430 nm and a weak short wavelength (SW) band at 350 nm (Figure 5a). The LW emission band is red-shifted compared to that of the anionic form (410 nm). In the presence of β-CD$_x$, the emission spectrum of HBI remains almost unchanged except for a red-shift (Figure 5c) of the LW emission to 438 nm. The fluorescence spectrum of HBI in γ-CD$_x$ is similar to that observed in β-CD$_x$, although the relative emission intensity for the former is slightly lower with a maximum at 434 nm (Figure 5b). In the presence of β-CD$_m$, HBI exhibits a strong LW emission as well as a slightly enhanced SW emission band. The LW emission band is also red-shifted (452 nm, Figure 5d) with respect to that in the presence of β-CD$_x$. This is consistent with the estimated binding constants of HBI in these media (i.e., $K = 256$, 131, and 45 M^{-1} in β-CD$_m$, β-CD$_x$, and γ-CD$_x$, respectively).[24]

Time-resolved Fluorescence Studies of HBI in Binary Solvents and Cyclodextrins. Time-resolved fluorescence measurements were performed to estimate the fluorescence lifetimes of HBI. The lifetime data obtained for HBI in binary aqueous solvents of 1,4-dioxane and methanol (MeOH) appear in Table 1. In methanol, the fluorescent decay of HBI obtained from monitoring the emission at 470 nm is biexponential, with lifetime values of 3.7 (τ_1) and 0.7 ns (τ_2). The lifetime (τ_1) remains relatively unchanged up to 50% water in MeOH (3.9 ns). At volumes of 50% water and greater, τ_1 is decreased while τ_2 is generally unchanged. The corresponding amplitudes of these lifetime values show very little variation with increasing percentage of water. In pure dioxane, the lifetimes of HBI are estimated to be 4.1 and 0.8 ns, corresponding to τ_1 and τ_2, respectively. The τ_1 and τ_2 values are slightly increased in dioxane:water mixtures containing up to 50% water (Table 1). Above 50% water (i.e., 50−90% water), the lifetime of the long-lived decay is decreased sharply from 4.4 to 2.7 ns (30−90% water). The value of the short-lived decay (τ_2) is also decreased with increasing percentage of water in dioxane.

TABLE 2: Lifetime Values of 2-(2′-Hydroxyphenyl)benzimidazole in Cyclodextrins

cyclodextrin	concn (mM)	lifetimes (ns) τ_1	τ_2	a_1	a_2	χ^2
β-CD$_x$	0.0	2.2	0.7	0.58	0.42	1.1
	1.0	3.1	1.6	0.15	0.85	1.1
	2.0	3.4	1.6	0.17	0.83	1.2
	4.0	3.4	1.5	0.33	0.67	1.1
	6.0	3.3	1.5	0.34	0.66	1.2
	8.0	3.4	1.5	0.36	0.64	1.1
	10.0	3.5	1.5	0.41	0.59	1.0
β-CD$_m$	1.0	3.7	1.8	0.46	0.54	1.1
	2.0	4.0	2.0	0.51	0.49	1.1
	4.0	4.3	2.1	0.52	0.48	1.2
	6.0	4.6	2.1	0.55	0.45	1.2
	8.0	4.8	2.0	0.57	0.43	1.1
	10.0	4.9	2.1	0.66	0.34	1.1

In aqueous solutions (pH 7), the decay corresponding to the long-wavelength emission of HBI ($\lambda_{em} \approx 450$ nm) is also observed to be a double exponential with lifetimes of 2.2 and 0.7 ns. When the concentration of β-CD$_x$ is increased, the lifetime of the long-lived species (τ_1) and that of the short-lived species (τ_2) are increased (Table 2). The amplitude of the component corresponding to τ_1 is also observed to steadily increase with increasing [β-CD$_x$]. In 2,6-di-*O*-methyl-β-cyclodextrin (β-CD$_m$), the τ_1 value of HBI increases sharply from 2.2 ns in water (pH 7) to 4.9 ns in the presence of 10.0 mM β-CD$_m$. The lifetime of the short-lived component (τ_2) is slightly increased in β-CD$_m$. Similar to that in β-CD$_x$, the amplitude corresponding to τ_1 is also increased with increasing [β-CD$_m$]. In the presence of γ-CD$_x$ (not shown), the lifetimes of HBI are relatively unaffected (τ_1 and τ_2 = 2.3 and 0.7 ns, respectively, in 10.0 mM γ-CD$_x$).

Discussion

HBI in Binary Solvents. The observed spectral characteristics of HBI in pure and binary mixtures can be explained in terms of the differences in hydrogen bond (HB) donor/acceptor properties of the solvent or solvent mixture. The increase of fluorescence intensity of HBI upon increasing the percentage of water (0−35%) in dioxane and methanol is indicative of the formation of the intermolecularly hydrogen-bonded tautomer **IV** (K$_2$*, Figure 6). The appearance of the normal emission band of HBI in dioxane and methanol (Figure 4) suggests that the intermolecular interaction also exists in the S$_0$ state, which results in an increase in the population of the intermolecularly hydrogen-bonded molecules **I** and **II** (Figure 6). Both dioxane and methanol have strong HB acceptor and donor properties[30,31] that facilitate intermolecular hydrogen-bonding with HBI in solution. This intermolecular interaction is further supported by the biexponential decay observed for HBI in the binary aqueous mixtures (Table 1), which are both slightly increased with increasing percentage of water (up to 35−40%). In the presence of higher percentages of water (i.e., >40%), species **II** (Figure 6) gives rise to the formation of the HBI anion. This is supported by the lifetime values of HBI in these solvents, which resemble those of the molecule in basic media ($\tau = 2.7$ and 2.9 ns at pH 11 in water and aqueous MeOH, respectively; see Table 1). Furthermore, based on the *Y* values determined

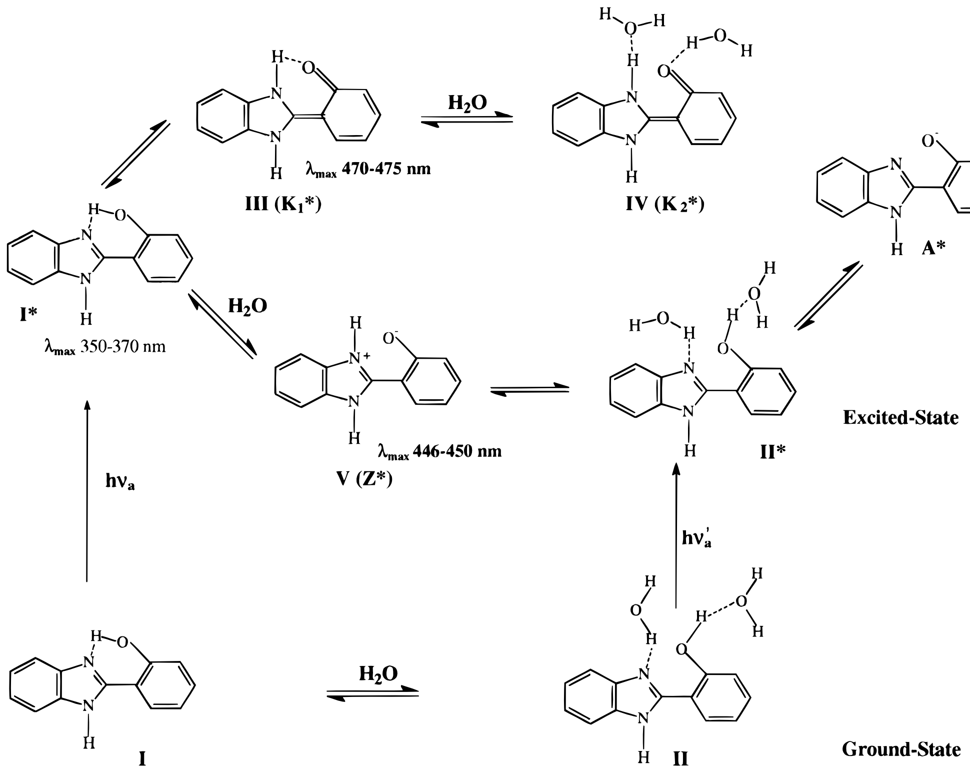

Figure 6. Ground- and excited-state PT reactions of HBI in various binary solvents. (The asterisks (*) are used to indicate the excited-state structures of the molecules.)

for different binary solvent mixtures by Fainberg and Winstein,[32] it is apparent that solutions of methanol and dioxane containing high percentages of water have higher ionizing strength than such solvents containing lower percentages of water. Thus, the increased percentage of water increases the population of the anion in the S_1 state (A*, Figure 6) and decreases the population of the tautomer **IV** (K_2*, Figure 6). These effects can also be discussed when considering the spectral behavior of HBI in other solvent systems.

To further examine the role of intermolecular HB, we have measured fluorescence spectra of HBI in cyclohexane in the presence of MeOH and TFE. In the presence of MeOH, the emission maximum of the HBI tautomer (476 nm) is blue-shifted with increasing amounts of MeOH, to 456 nm ($\Delta E = 2.64$ kcal/mol, Figure 4). At higher concentrations of MeOH (i.e., 0.20−0.50 M in cyclohexane), an apparent isoemissive point for HBI appears at 478 nm, indicating the presence of equilibrium between two species in the excited state. In the presence of trifluoroethanol (CF_3CH_2OH), a similar trend is observed, i.e., the tautomer emission of HBI is blue-shifted with increasing TFE, from 476 to 460 nm ($\Delta E = 2.09$ kcal/mol). Furthermore, with increased concentrations of TFE (0.20−0.50 M), the emission of the tautomer is significantly decreased with the appearance of a slight emission between 350 and 400 nm (Figure 5). The addition of increased quantities of MeOH and TFE also results in an increase in the estimated bandwidths at half-maximum (bwhm) for HBI in cyclohexane (Table 3).

The fact that the long-wavelength (LW) emission band of HBI in cyclohexane is due to formation of the keto-tautomer (K_1*, Figure 6) has been well established in the literature.[2,17] In non-hydrogen-bonding solvents, the tautomer is expected to be present in the intramolecularly hydrogen-bonded form **III** (K_1*, Figure 6) in the excited-state. The dipole moment of the tautomer is expected to be low ($\mu = 4.03$ D),[17] since it is planar in structure. This intramolecular hydrogen bond provides an extra degree of stability to the structure of the HBI molecule. However, in the presence of hydrogen-bonding solvents, e.g., dioxane and methanol, this intramolecular HB is partially broken as the structure becomes destabilized. This is indicated by the blue-shift of the spectrum in dioxane compared to the spectrum of cyclohexane. Since methanol is a stronger HB acceptor compared to dioxane ($\beta = 0.62$ and 0.37 for methanol and dioxane, respectively),[30] the observed blue-shift is larger in the former solvent. Similarly, the blue-shift of the tautomer emission upon addition of MeOH and TFE is due to disruption of the intramolecular hydrogen bond of tautomer **III** (K_1*) to give tautomer **IV** (K_2*). The formation of the tautomer **IV** (K_2*) is further supported by the higher τ_1 value (4.4 ns) compared to that in cyclohexane (3.6 ns), as well as by the respective quantum yields ($\Phi_f = 0.46$ and 0.55, respectively).[33] These data support the fact that the existence of intramolecular HB usually increases the nonradiative decay rates in the excited state of the tautomer.[34] The decrease of fluorescence intensity of tautomer **III** (K_1*) upon addition of TFE is due to the decrease of the population of the hydrogen-bonded ground-state molecule (**I**), since TFE is a stronger HB-donating (HBD) solvent ($\alpha = 1.51$) compared to MeOH ($\alpha = 0.98$).[30,31] This interaction predominately accounts for the decrease of intensity as a result of HB breaking of the tautomer.

TABLE 3: Influence of Methanol and 2,2,2-Trifluoroethanol/Cyclohexane Mixtures on Emission Maxima and Bandwidths at Half-Maximum (bwhm) for the Tautomer Emission of HBI

[MeOH] or [TFE] (M)	λ_{max} (cm^{-1})		bwhm (cm^{-1})	
	MeOH	TFE	MeOH	TFE
0.0	21010	21010	2950	2950
0.025	21100	21190	3030	3050
0.050	21280	21280	3070	3140
0.10	21460	21370	3370	3180
0.15	21550	21550	3400	3270
0.30	21740		3400	
0.40	21830		3700	
0.50	21930		3790	

However, the large blue-shifts observed in these media cannot be due only to a disruption of the intramolecular HB because the energy change involved (e.g., $\Delta E = 2.64$ kcal/mol in MeOH:

cyclohexane) is too large too explain the N−H···O= hydrogen bond strength. For molecules such as HBI, the N−H···O bond is believed to be far weaker than the N···H−O bond.[35,36] Furthermore, the strength of the intramolecular hydrogen bond of HBI is reported to be weakest among the various (hydroxyphenyl)benzazoles (i.e., HBO > HBT > HBI).[35] Therefore, this large change in energy must involve formation of some other species that seem to fluorescece at longer wavelengths relative to that of the anionic species. The formation of a new species is also supported by the increased bandwidth at half-maximum (bwhm) of the emission spectra of HBI in binary solvent mixtures (Table 3).

We believe that this species is a dipolar zwitterion Z* (**V**, Figure 6) that is expected to be more stable in solvents of high dielectric constant. Since the tautomers **III** and **IV** are more rigid compared to the zwitterion (Z*), their emission maxima are red-shifted and the respective fluorescence quantum yields are higher relative to that of the latter. Moreover, since TFE is a stronger HBD solvent compared to water ($\alpha = 1.0$),[30] it can protonate the ring nitrogen of HBI to form the cationic species. This is indicated by the broad maximum (350−400 nm) of the short wavelength band, the intensity of which increases with increasing TFE concentration.

Since the anion and cation of HBI fluorescence at ~410 and 386 nm, respectively, the λ_f(max) for Z* is expected at ~446 nm (i.e., assuming $\lambda_f(Z^*) = [\lambda_f(A^*) - \lambda_f(N)] + [\lambda_f(C^*) - \lambda_f(N)]$, where $\lambda_f(N)$ and $\lambda_f(C^*)$ represent the emission maximum for neutral and cationic HBI, respectively). The actual λ_f(max) for Z* would be slightly higher (~450−455 nm) because of solvation effects. Furthermore, the anion emission is not expected in the emission region in which the lifetime values were monitored ($\lambda_f \approx 470$ nm). The biexponential decay of HBI in the various binary solvents also supports the existence of a second species for HBI. The short-lived ($\tau_2 \approx 0.7$ ns) component of the fluorescence decay probably explains the existence of the zwitterionic (Z*) species in dioxane/water or methanol/water mixtures. The short lifetime of Z* compared to K_1* or K_2* is expected because of stronger solute−solvent interactions in solvents of high dieelectric constants, which increase the nonradiative decay rates. The observed effects will be further discussed below in relation to the spectral behavior of HBI in cyclodextrins.

HBI in Cyclodextrins. Similar to the data observed for HBI in the various binary solvent mixtures, the spectral characteristics of HBI in cyclodextrins can be explained in terms of hydrogen-bonding and hydrophobic interactions. HBI exhibits both inter- and intramolecular proton transfer (PT), but the rate of intermolecular PT in aqueous media is so fast that ionization is almost complete during the lifetime of the excited state.[24] Interaction of HBI with cyclodextrins stabilizes the nonionic form of HBI through intermolecular hydrogen-bonding, thus decreasing the emission intensity of the anionic form (A*, Figure 6) of HBI. The dramatic difference in the fluorescence of HBI in β-CD$_m$ compared to that in β-CD$_x$ and γ-CD$_x$ can be explained along similar lines. 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. As a result, β-CD$_x$ is less soluble in water than β-CD$_m$. In the case of β-CD$_m$, however, this intramolecular hydrogen bonding is partially destroyed because of substitution of one of the alcoholic protons by a $-CH_3$ group (at the 2- position). The hydrogen-bonding ability of β-CD$_m$ with HBI and water molecules is thus enhanced. This greater hydrogen-bonding ability and higher hydrophobicity increase the strength of association of HBI with β-CD$_m$ compared to that with β-CD$_x$ and γ-CD$_x$. The increased association of HBI with β-CD$_m$ is further supported by the enhancement of fluorescence intensities and measured lifetimes for the tautomer (K_2*) emission of HBI. With increasing [β-CD$_x$], the tautomer lifetime ($\tau_1(K_2^*)$) of HBI is increased from 2.2 ns in water to 3.5 ns in 10.0 mM β-CD$_x$. In β-CD$_m$, however, the tautomer emission lifetime of HBI is twice that in water (2.2 ns compared to 4.9 ns). It seems that β-CD$_m$ promotes formation of the tautomer **IV**(K_2*), while β-CD$_x$ promotes formation of species **III** (K_1*). Sarkar et al.[27] studied the inter- and intramolecular PT of HBI in nonionic and ionic micelles. Their results indicated a similar increase in the tautomer lifetime of HBI, suggesting a more pronounced effect of the micelles on the proton transfer of HBI than on the quantum yields of the molecule.[27] Rodriguez-Prieto et al.[37] noticed a similar difference in the tautomer lifetime values of HBI in water ($\tau_T \approx 1.86$) versus that in ethanol ($\tau_T \approx 4.10$). Similarly, in studying the ESPT of protonated 1-aminopyrene complexed with β-CD$_x$, Fleming et al.[25] indicated increased intermolecular PT rates of 1-aminopyrene in cyclodextrins compared to those in bulk water.

The microenvironment near the edge of the cyclodextrin cavity is often described as having the properties of a binary aqueous solvent (e.g., EtOH:water),[25,38] while the CD cavity has been estimated to be similar in polarity to oxygenated solvents such as EtOH, dioxane, isopropyl ether, and ethylene glycol.[39] Thus, the increased hydrophobicity of such an environment combined with other physicochemical properties (e.g., rigidity) often results in the protection of analyte excited states from nonradiative processes. This is observed by the relatively high lifetime values for the HBI keto-tautomer (K_2*) in β-CD$_m$ (4.9 ns) compared to apolar solvents such as cyclohexane (3.6 ns). It appears that the radiative deactivation rate constant for the excited-state tautomer (K*) is independent of the solvent while the nonradiative deactivation rate constant is greater in the absence of CDs.[33,37] Thus, it seems that, similar to micelles, complexation of HBI by cyclodextrins decreases nonradiative rates in the excited state of the HBI tautomer. The mode of interaction of HBI with cyclodextrins, however, appears to be more specific.

Acidity constant determinations[24] of HBI in the presence of cyclodextrins indicate increased acidity of the phenolic −OH group of HBI. This increased acidity is believed to be due to strong intermolecular hydrogen bonding between the phenolic −OH of HBI and the alcoholic groups of the cyclodextrin. It has been further suggested that the water near the rim of the cavity of β-cyclodextrin is modified in such a way as to increase its basicity, with the pK_a of the CD hydroxyl groups estimated to be ~12.[18,25,37] Thus, we believe that the HBI molecule enters the CD cavity from the secondary rim side with the benzimidazole moiety forward, achieving only partial penetration. This arrangement allows for solubilization of the HBI phenyl group at the surface of the secondary rim of cyclodextrin and intermolecular hydrogen bonding in which cyclodextrin acts as a hydrogen bond donor. Such an arrangement is further supported by induced circular dichroism studies of HBI in β-CD$_x$ and β-CD$_m$, which suggests axial inclusion of the molecule in the CD cavity (~7.8 Å height) as opposed to equatorial orientation of the molecule in cyclodextrins.[24]

The spectral characteristics of HBI in CDs and binary solvent systems of TFE/MeOH in cyclohexane could further indicate the presence of an intermediate zwitterionic PT tautomer species of HBI whose formation is dictated by differences in inter- and intramolecular hydrogen bonding. The data obtained from absorbance, ICD, and pK_a studies, as well as from other spectroscopic studies,[17] further suggest that the normal form of

HBI has a higher dipole moment in the S_0 state. This means that the phenyl ring of HBI is twisted with respect to the benzimidazole ring and hence has a weaker intramolecular hydrogen bond than does the benzoxazole (HBO) derivative.[24] Furthermore, the HBI tautomer is suspected to be polar based upon the shifts in emission maximum for the molecule in CDs. In the presence of CDs, HBI has an emission maximum between that reported for the compound in polar (e.g., 430 nm in water) and apolar (e.g., 470 nm in cyclohexane) solvents.[2]

Thus, it is believed that these intermediate structures exist as zwitterionic species. Such emission forms have been suggested for other (hydroxyphenyl)benzazoles in the presence of hydrogen-bonding solvents. For example, Elsaesser and Schmetzer[8] demonstrated and observed complex equilibria for 2-(2-hydroxyphenyl)benzotriazole (HBT) in polar solvents. Similarly, Potter and Brown[3] studied the ESIPT of HBT in acidic and basic aqueous ethanolic solvents. These data were used to suggest complex equilibria of HBT involving formation of zwitterionic and quinoid forms of HBT ($\lambda_{em} \approx 500$ nm) produced as a result of the ESIPT process. Furthermore, these authors observed that 8% of the excited neutral HBT molecules deprotonate to form the excited anion in aqueous media. In a previously mentioned study, Sinha and Dogra[2] investigated the ground- and excited-state prototropism of HBI in various solvents. The authors suggest equilibria involving the formation of a zwitterionic species of HBI ($\lambda_{em} \approx 440$ nm) in water through electronic reorganization in the excited state (S_1). This reorganization facilitates the increase of acidity and basicity of the −OH group and tertiary nitrogen atom, respectively, in S_1 state. Using spectroscopic studies and AM1 calculations, Chou et al.[40] have provided more evidence for the formation of zwitterionic species for (hydroxyphenyl)benzazoles. Since the ESPT rate for HBAs is ultrafast ($>10^{12}$ s^{-1}), the authors suggest that the proton-transfer tautomer may be depicted by the zwitterionic or π-electron conjugated cis−keto -tautomer.[40] Intra- and intermolecular hydrogen bonding involving cyclodextrins (β-CD$_x$, β-CD$_m$) and HBI are believed to be similarly facilitated, with the CD molecules enhancing both the ground- and excited-state intermolecular PT of HBI through stabilization of the nonionic forms of HBI. Such interactions may, in the case of HBI, facilitate formation of a polar zwitterionic species in the presence of specific microenvironments.

Conclusions

It appears that the interaction of HBI with cyclodextrins is dictated by competing inter- and intramolecular processes. The molecule is oriented with respect to cyclodextrin such that intermolecular proton transfer is facilitated through interaction of the phenolic −OH of HBI with the alcoholic groups that reside on the periphery of the cyclodextrin cavity. The intermolecular proton transfer between β-CD$_x$ or β-CD$_m$ and HBI inhibits the intramolecular PT process, and hence, the formation of the keto tautomer (K_1* and K_2*). This inter- and intramolecular proton transfer is also observed in the presence of binary aqueous and binary organic solvents. In aqueous binary solvents of dioxane and MeOH, the intermolecular interactions disrupt the intramolecular hydrogen bond of HBI in the ground (S_0) and excited (S_1) state. Similarly, in the presence of stronger HBD solvents such as TFE, the population of the intramolecularly hydrogen-bonded ground-state molecule of HBI is decreased, thus decreasing the fluorescence intensity of the tautomer emission of HBI. Furthermore, the observed spectral behavior of HBI in significant concentrations of protic solvent suggests the formation of a new species that is stabilized in these polar microenvironments. On the basis of the fluorescence and fluorescence lifetime characteristics of HBI in cyclodextrins and binary solvents, we believe that the PT tautomer exists as a polar zwitterionic species.

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