Steady-State and Time-Resolved Spectroscopy of 2,2′-Bipyridine-3,3′-diol in Solvents and Cyclodextrins: Polarity and Nanoconfinement Effects on Tautomerization

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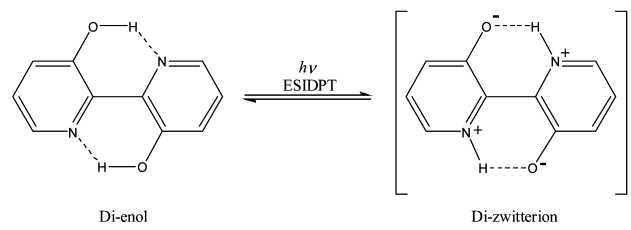
The ground- and excited-state tautomerization of the 2,2′-bipyridine-3,3′-diol molecule ($BP(OH)_2$) was studied in different solvents and in confined nanocavities of cyclodextrins (CDs) using steady-state and lifetime spectroscopic measurements. In all solvents, a dizwitterion (DZ) tautomer is produced in the excited state after intramolecular double-proton transfer. This tautomer is stabilized in the ground state in water only and produces two unique absorption peaks in the region of 400−450 nm. The DZ tautomer fluoresces in the green and as the solvent polarity increases, the fluorescence peak is blue-shifted (498 nm in cyclohexane versus 462 nm in water), and the fluorescence lifetime gets shorter (3.10 ns in cyclohexane versus 0.65 ns in water). The results indicate the sensitivity of this tautomer to solvent polarity, particularly the solvent's hydrogen-bonding capability. In water, another photoinduced tautomerization mechanism takes place via a water network solvating each of the two hydrogen-bonding centers of the molecule. The second tautomer is detected as a small shoulder in the blue side of the fluorescence peak and has a lifetime of 5.40 ns. Using $BP(OH)_2$ to probe the nanocavities of aqueous CDs reveals the degree of hydrophobicity of the cavities and the different mechanisms of probe encapsulation. As the cavity size decreases in the order γ-CD to β-CD to α-CD, the cavity is more hydrophobic, which is reflected in an intensity decrease of the absorbance of the DZ tautomer and a red shift in its fluorescence peak. The measured lifetimes show the same trend and reveal how the probe interacts with the CD moiety. In γ-CD, the probe is located near the secondary rim of the CD annulus, whereas in α-CD, the probe is completely sequestered between two CDs, and the hydrophobicity is close to that observed in cyclohexane. In β-CD and its derivatives, the spectral changes and the measured lifetimes indicate that the CD cavity gets more hydrophobic as a result of methyl substitution of the primary and secondary hydroxyls of the β-CD rims. In the fully methylated 2,3,6-tri-*O*-methyl-β-CD, the probe is exposed to water near the secondary rim due to the steric effect at the entrance rim that prevents the probe from full encapsulation.

Introduction

There has been an increased interest recently in studying the photophysics of molecules which possess one or more hydrogen bonds in their structure. These molecules can be photoinduced to tautomerize in the excited state, and upon returning to the ground state, reverse tautomerization brings the molecule to its original structure. Due to their extreme sensitivity to solvent polarity and hydrogen bonding with protic solvents, some of these molecules have been suggested as probes for the study of protein conformation and binding sites.[1–4] We proposed one such molecule, which is 2,2′-bipyridine-3,3′-diol ($BP(OH)_2$), as a sensitive probe to report on its local environment change by examining its steady-state spectra in different environments.[5–7]

The photoinduced excited-state intramolecular double proton transfer (ESIDPT) in $BP(OH)_2$, shown in Scheme 1, has been extensively studied both experimentally[8–19] and theoretically.[20–24] Electro-optical measurements and calculated excited-state dipole moments show that the dipole moments of the dienol (DE) and the dizwitterion (DZ) tautomers are negligible due to their symmetric structures.[13,14] At room temperature, $BP(OH)_2$ absorbs in the region of 330−360 nm yet fluoresces strongly in the green. Quantum yields of fluorescence on the order of 0.2−0.4

SCHEME 1: Phototautomerization of $BP(OH)_2$



were observed in different solvents at room temperature with lifetimes of a few nanoseconds.[8–12] Comparing the absorption and emission properties of $BP(OH)_2$ with related systems possessing only one hydrogen bond reveals that the second hydroxyl group is essential to the observation of the strong green emission. This emission is due to fluorescence from the DZ tautomer after an efficient ESIDPT process. Both concerted and stepwise mechanisms were proposed for the double proton transfer in $BP(OH)_2$.[15–24] The $BP(OH)_2$ molecule is also planar in the crystalline form[25] and is expected to retain its planarity in solutions of noninteracting solvents because of the two strong intramolecular hydrogen bonds.

We have recently reported the steady-state spectra of $BP(OH)_2$ in solvents of varying polarity and hydrogen-bonding capability and in binary mixtures of 1,4-dioxane/water.[5] Unique absorption

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due to water solvation was observed in the region of 400−450 nm. Compared to fluorescence in nonpolar solvents, a blue shift in the fluorescence band was observed in polar, protic solvents which is attributed to the formation of intermolecular hydrogen bonds. The shift increases with increasing solvent polarity and hydrogen-bonding capability. We also studied $BP(OH)_2$ in several cyclodextrins (CDs) in order to examine the effect of hydrophobic environments on its absorption and fluorescence spectra.[6,7] The results indicated that decreasing the cavity size and increasing its hydrophobicity cause a large reduction in the absorption features in the region of 400−450 nm and a red shift in the fluorescence peak of $BP(OH)_2$. We interpreted the results to be due to the decrease in water accessibility inside of the CD cavities. The results led us to propose $BP(OH)_2$ as a possible water sensor to study biological systems and inclusion in macromolecules.

We continue the above study by examining in this paper the dynamics of $BP(OH)_2$ in different solvents and CDs. We focus here on the molecular relaxation after excited-state proton transfer by monitoring the time-resolved fluorescence decay. We revisit the steady-state spectra of $BP(OH)_2$ in different solvents and CDs in order to integrate both steady-state and dynamics results. The results obtained in different solvents shed light on the nature of water solvation of the hydrogen-bonding centers of $BP(OH)_2$, whereas the results in different CDs show that the $BP(OH)_2$ molecule is indeed a sensitive probe useful in exploring the hydrophobicity of nanocavities and the different mechanisms of encapsulation.

Experimental Section

$BP(OH)_2$ (98%) was obtained from Aldrich and was used without further purification. Acetonitrile (spectroscopic grade), anhydrous 1,4-dioxane, and methanol were obtained from Sigma−Aldrich Chemical Co. Anhydrous ethanol was received from Acros Organics. Spectroscopic-grade cyclohexane was purchased from BDH Chemicals. α-CD (≥98%), *β*-CD (≥99%), methyl-*β*-CD (M*β*-CD) (≥97%), 2,6-di-*O*-methyl-*β*-CD (DM*β*-CD) (≥98%), 2,3,6-tri-*O*-methyl-*β*-CD (TM*β*-CD) (≥98%), and *γ*-CD (≥98%) were all purchased from Fluka and used as received. Deionized water (Millipore) was used. The concentration of $BP(OH)_2$ in all solvents, including the CDs, was ∼0.01 mM. Complexes of $BP(OH)_2$ with CDs were prepared and allowed to equilibrate for 2 h before taking the measurements. Measurements were repeated after 12 h, and no significant differences were detected.

Absorption spectra were obtained with a HP 845x diode array spectrophotometer. Fluorescence spectra were recorded on a Shimadzu RF-5301 PC spectrofluorophotometer. Lifetime measurements were performed using a TimeMaster fluorescence lifetime spectrometer obtained from Photon Technology International. Excitation was at 340, 410, and 435 nm using LEDs. The system response time as measured from the scattered light was estimated to be approximately 1.5 ns (fwhm). The measured transients were fitted to multiexponential functions convoluted with the system response function. The goodness of the fits was judged by the value of the reduced chi-squared (χ^2). The experimental time resolution (after deconvolution) was approximately 100 ps. In all of the experiments, samples were contained in a 1 cm path length quartz cell, and the measurements were conducted at 23 ± 1 °C.

Results and Discussion

$BP(OH)_2$ in Different Solvents. The absorption spectra of $BP(OH)_2$ in different solvents are shown in Figure 1 for the spectral region from 280 to 480 nm. The broad peak in the region of 330−360 nm represents the transition to the lowest $^1(\pi,\pi^*)$ state of the DE tautomer.[8] Our previous study of $BP(OH)_2$ in different solvents shows that the absorbance intensity in the 400−450 nm region appears in water only and gains its strength at the expense of that in the region of 330−360 nm.[5–7] In all solvents, the position of the absorption maximum of the DE tautomer is only slightly changed, which is attributed to the negligible dipole moment of this tautomer.[13,14]

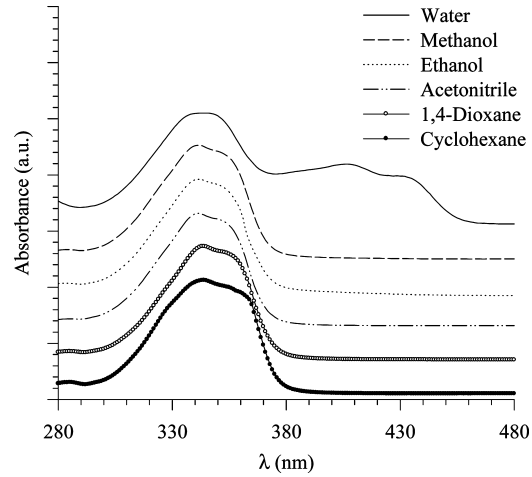


Figure 1. Absorption spectra of $BP(OH)_2$ in different solvents. The spectra are vertically spaced for clarity.

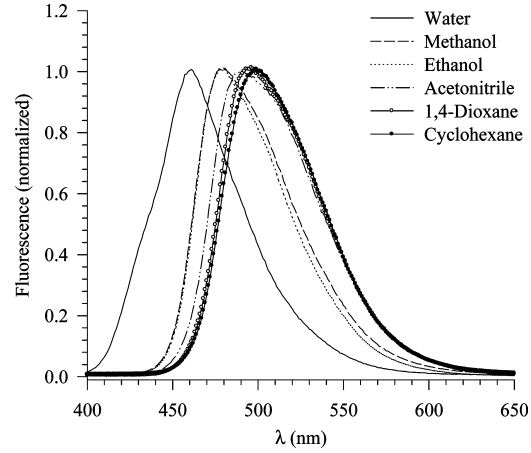


Figure 2. Normalized fluorescence emission spectra of $BP(OH)_2$ in different solvents; λ_{ex} = 340 nm.

The corresponding fluorescence spectra of $BP(OH)_2$ in different solvents are shown in Figure 2 after excitation in the DE region (λ_{ex} = 340 nm). ESIDPT results in a highly fluorescent DZ tautomer, with a large Stokes shift (∼9000 cm^{-1} in nonpolar, aprotic solvents). The fluorescence peak maxima are shown in Table 1 for different solvents, along with solvent dielectric constants (ε) and empirical parameters of solvent polarity (π^* and E_T^N).[26] A summary of the fluorescence lifetime data is also included in the table. The lifetime results agree with the previously reported data in some of the solvents used here.[27]

The largest red shift in the fluorescence peak position was observed in aprotic solvents such as cyclohexane. A solvent such as 1,4-dioxane, which appears to be nonpolar according to its static dielectric constant (ε = 2.21), has a high solvent polarity parameter (π^* = 0.49) and an E_T^N value of 0.16.[26] 1,4-Dioxane has two CH_2-O-CH_2 groups opposite to each other, which results in a net zero dipole moment. Hence, it is considered a nondipolar solvent. However, 1,4-dioxane exhibits

TABLE 1: Fluorescence Spectral Peak Positions and Lifetimes of $BP(OH)_2$ in Different Solvents

solvent	ε^a 25 °C	π^{*a}	$E_T^{N a}$ 25 °C	fluorescence peak maximum (nm)[b]	λ_{ex} (nm)	τ_1 (ns)[c]	α_1	τ_2 (ns)[d]	α_2	χ^2
cyclohexane	2.02	0.00	0.00	498	340	3.10				1.15
1,4-dioxane	2.21	0.49	0.16	497	340	2.08				0.91
acetonitrile	35.94	0.66	0.46	493	340	1.00				0.98
ethanol	24.55	0.54	0.65	476	340	1.97				1.16
methanol	32.66	0.60	0.76	476	340	1.24				1.11
water	78.30	1.09	1.00	462	340	0.65	0.34	5.40	0.66	0.93
					410	0.65	0.78	5.40	0.22	2.10
					435	0.65				1.50

[a] Obtained from ref 26. [b] $\lambda_{ex} = 340$ nm. [c] Uncertainty in measurements is ±0.02 ns. [d] Uncertainty in measurements is ±0.31 ns.

a large quadrupole moment[28,29] which is reflected in its π^* parameter that mainly takes into consideration the polarizability and the dipolarity of the solvent.[30] The corresponding E_T^N value indicates that 1,4-dioxane exhibits only 16% of the solvent polarity of water, which classifies 1,4-dioxane as an apolar, non-hydrogen-bond donor solvent.[26] Comparing the steady-state fluorescence peak positions of $BP(OH)_2$ in cyclohexane and 1,4-dioxane provides evidence of weak solute−solvent interactions in both solvents. On the other hand, the measured fluorescence lifetime of $BP(OH)_2$ in 1,4-dioxane ($\tau = 2.08$ ns) is shorter than that in cyclohexane ($\tau = 3.10$ ns). The latter results point to the presence of some interaction between the polar groups of 1,4-dioxane with the $-N^+H$ groups of the DZ tautomer in the excited state. This interaction may tend to break the hydrogen bonds in the $BP(OH)_2$ molecule, which results in a possible distortion of the molecular backbone from planarity, leading to an increased nonradiative decay rate and a subsequent reduction in the fluorescence lifetime.

In a dipolar, aprotic solvent such as acetonitrile, the fluorescence peak maximum of $BP(OH)_2$ is slightly blue-shifted with respect to that in cyclohexane (only blue-shifted by ~200 cm^{-1}). This small shift indicates that the excited state of the DZ tautomer is slightly sensitive to the solvent polarity. This is in agreement with the nature of this tautomer, which has a negligible dipole moment due to its symmetric structure.[13,14] However, the measured fluorescence lifetime of $BP(OH)_2$ in acetonitrile yields a single exponential decay component of 1.00 ns. The reduction in the fluorescence lifetime is consistent with a structure which is more prone to nonplanarity due to strong interaction between the $-N^+H$ groups of $BP(OH)_2$ and acetonitrile in the excited state. This interaction is enhanced through the lone electron pairs of acetonitrile, which tend to solvate cations.[26]

In polar, protic solvents, the behavior of $BP(OH)_2$ in the excited state is different, and the blue shift in the fluorescence peak is much more pronounced. In ethanol, for example, the fluorescence peak is blue-shifted by ~725 cm^{-1} from that in acetonitrile. According to the reported values of ε and π^*,[31,32] ethanol is less polar than acetonitrile.[26] The blue shift in protic solvents must then derive from an intermolecular hydrogen-bonding interaction between $BP(OH)_2$ and the protic solvent in the excited-state potential energy surface, as previously proposed.[18] The blue shift is similar in methanol but increases in water (~1361 cm^{-1} from that in acetonitrile). The measured fluorescence lifetime of $BP(OH)_2$ in ethanol ($\tau = 1.97$ ns) is much longer than that measured in acetonitrile. This longer lifetime confirms the different mechanism of solvation of the polar centers in $BP(OH)_2$ by a protic solvent such as ethanol.

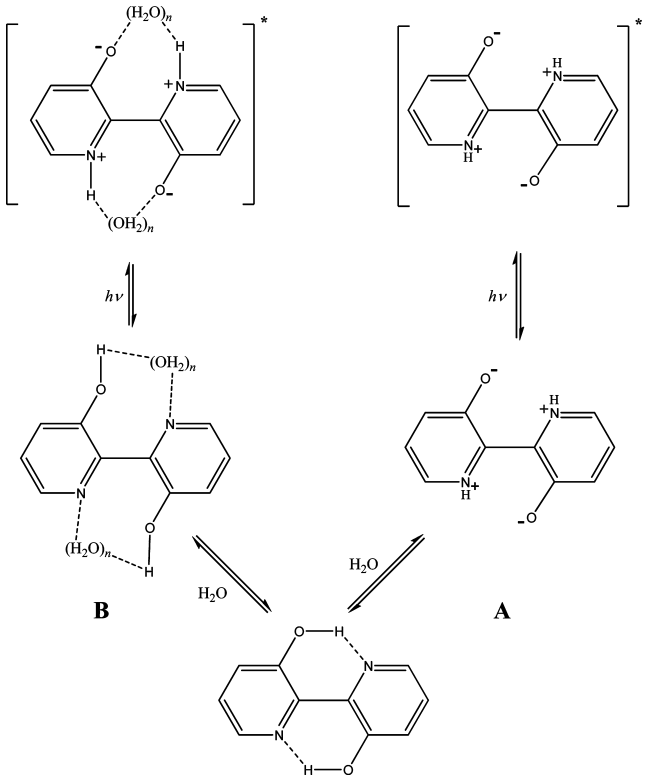
Both methanol and ethanol are considered as hydrogen-bond donors/acceptors.[26] The ability of such solvents to form hydrogen bonds with the cation part ($-N^+H$) and the anion part ($-O^-$) of the DZ tautomer may explain the stability of this tautomer after the rupture of its intramolecular hydrogen bonds. A stronger interaction between $BP(OH)_2$ and methanol may explain the shorter fluorescence lifetime of $BP(OH)_2$ in methanol ($\tau = 1.24$ ns) compared with that in ethanol, which can be attributed to the larger polarity and smaller size of the former solvent. Formation of a solvent network or a solvent wire that is capable of solvating both the cation and the anion parts of the DZ tautomer has a lower probability in the cases of methanol and ethanol due to their weak association properties. The mechanism of solvation is more likely to involve local solvation of each polar part by one or more solvent molecules through which tautomerization takes place.

In water, the steady-state spectra and fluorescence lifetimes of $BP(OH)_2$ show additional components that merit discussion. The absorption spectrum shows two additional red-shifted peaks (Figure 1), whereas the fluorescence spectrum shows one peak (the most blue-shifted in all solvents, Figure 2) with a small shoulder on the blue side. Two lifetime components were measured, as shown in Table 1, after excitation at 340 nm.

Solute−solvent interactions not only determine the relative stability of the tautomeric forms but can also influence the interconversion mechanism. A protic solvent like water, a strong hydrogen-bond donor/acceptor, can accept a proton from the donor site of the solute molecule and transfer a different proton to the acceptor site of the solute. Water-assisted proton-transfer mechanism studies have shown that the assistance of a water molecule significantly lowers the free-energy barriers in proton-transfer-related reactions.[33,34] The dynamics in such proton-transfer reactions can be greatly influenced by the presence of water molecules through short-range hydrogen-bonding interactions. In this case, an explicit interaction with a limited number of water molecules could influence the whole reaction path by lowering the energy barrier due to the direct participation of water molecules in the proton-transfer process. The two absorption peaks in the region of 400−450 nm are thus due to the DZ tautomer of $BP(OH)_2$ in the ground state, which is stabilized in water, as has been suggested theoretically.[22,35] It was found that the DZ tautomer is stabilized in aqueous solution and becomes just 3 kcal/mol less stable than its DE counterpart.[22] The absence of the DZ absorption in other protic solvents such as methanol and ethanol may indicate that, unlike water, short-range interaction between solvent molecules and the polar centers in $BP(OH)_2$ is not enough to stabilize the DZ tautomer in the ground state.

The tendency of water molecules to strongly associate with each other through intermolecular hydrogen bonds allows more than one molecule of water to form a solvent network or a solvent wire along which proton transfers can take place to and from the solute. Such proton-transfer mechanisms through a water bridge have been formulated theoretically.[22,34–37] We have recently estimated the number of water molecules solvating the

SCHEME 2: Proposed Mechanisms for the Effect of Water Solvation on the Ground- and Excited-State Tautomerization of $BP(OH)_2$



hydrogen-bonding centers of several systems experimentally in binary mixtures of 1,4-dioxane/water[5,38,39] and theoretically using ab initio methods.[38,39] For $BP(OH)_2$, we estimated that number to be three water molecules for each of the two hydrogen-bonding centers.[5]

On the basis of the above discussion, the results in water can be explained in light of Scheme 2. Mechanism A involves water interaction with each polar part in $BP(OH)_2$, leading to enol deprotonation and imine protonation to produce the DZ tautomer. This tautomer is stabilized in the ground state and absorbs in the spectral region of 400−450 nm. After excitation, the DZ tautomer is still stabilized in the excited state by water interaction with each polar part in a similar mechanism as in alcohol solvents. The lifetime of this "open structure" tautomer shows behavior similar to that in alcohols with a shorter lifetime (0.65 ns) due to the smaller size and more polar character of water. Mechanism B shows a different solvation mechanism of the two hydrogen-bonding centers of the DE tautomer in the ground state through a water network. This solvation pattern produces a DZ tautomer in the excited state through the transfer of protons to and from the water wires. Due to the strong solvation of the polar groups by a water wire at each hydrogen-bonding center, the DZ tautomer has a restricted rotation around the central bond between the two aromatic rings. The lifetime of this tautomer is then the longer component measured in the biexponential fluorescence decay curve (5.40 ns in Table 1).

We clarified the above mechanisms by measuring the fluorescence decay curves after excitation in the region of the DZ tautomer (400−450 nm). As shown in Table 1, the contribution of the short-lifetime component increases after excitation at 410 nm, and the decay curve becomes a single exponential with only the short component when exciting at 435 nm. We also measured the steady-state fluorescence

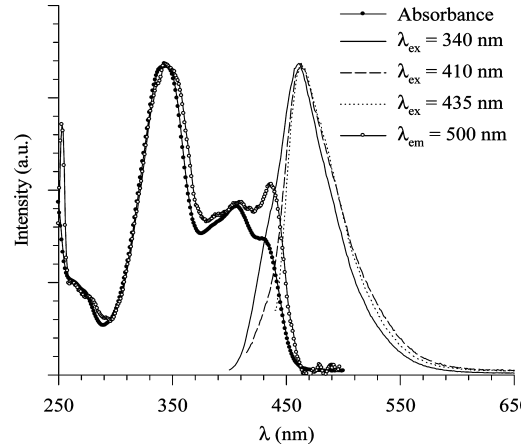


Figure 3. Normalized absorption, fluorescence emission, and fluorescence excitation spectra of $BP(OH)_2$ dissolved in water.

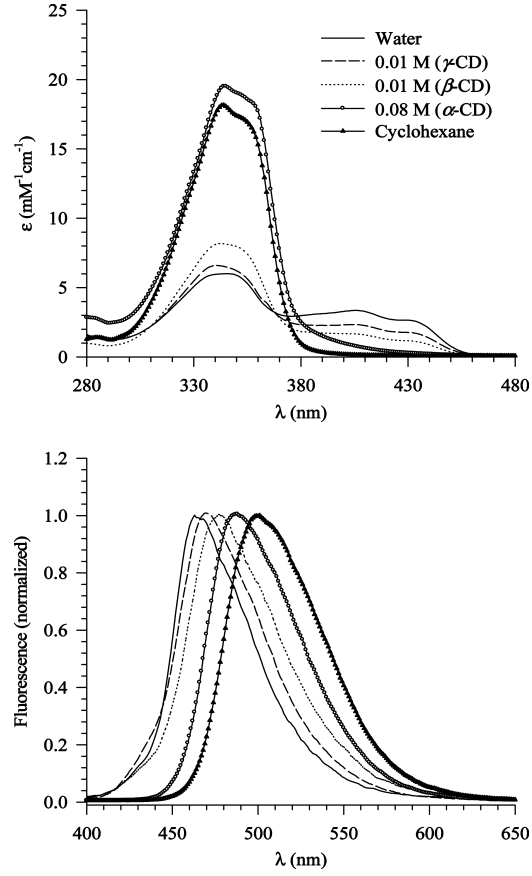


Figure 4. Absorption (upper) and normalized fluorescence emission (lower) spectra of $BP(OH)_2$ dissolved in water, cyclohexane, and aqueous γ-, β-, and α-CDs; $\lambda_{ex} = 340$ nm.

emission and fluorescence excitation spectra at different wavelengths, as shown in Figure 3. Excitation at 410 and 435 nm causes the blue shoulder in the fluorescence band to disappear. The results show that the major peak in the fluorescence spectrum is due to the DZ tautomer produced by mechanism A in Scheme 2. Measuring the fluorescence excitation spectrum for observation at $\lambda_{em} = 500$ nm causes an increase in the signal intensity in the region of the DZ tautomer in the ground state.

$BP(OH)_2$ in Aqueous CDs. The absorption and fluorescence spectra of $BP(OH)_2$ in aqueous γ-, β-, and α-CDs are depicted in Figure 4. The corresponding spectra of $BP(OH)_2$ in water and cyclohexane are included for comparison. Water and cyclohexane represent the two limits of highest polarity and

TABLE 2: Fluorescence Spectral Peak Positions and Lifetimes of $BP(OH)_2$ in Different CDs ($\lambda_{ex} = 340$ nm)

CD[a]	fluorescence peak maximum (nm)	τ_1 (ns)	α_1	τ_2 (ns)	α_2	χ^2
γ-CD	469	0.70 ± 0.07	0.47	3.72 ± 0.27	0.53	0.88
β-CD	477	1.00 ± 0.06	0.55	4.90 ± 0.35	0.45	0.94
α-CD	488			3.66 ± 0.03		1.29
Mβ-CD	481	1.23 ± 0.07	0.52	4.93 ± 0.43	0.48	0.96
DMβ-CD	484	1.48 ± 0.02				0.94
TMβ-CD	478	0.97 ± 0.10	0.58	2.97 ± 0.34	0.42	0.85

[a] The concentration of CDs was 10 mM, except for α-CD, which was 80 mM. The concentration of $BP(OH)_2$ was 0.01 mM.

highest nonpolarity conditions, respectively. As discussed above, the absorption in the region of 400−450 nm appears only in water and at the expense of that in the region of 330−360 nm. Fluorescence of $BP(OH)_2$ in water shows the smallest Stokes-shifted peak, while the largest Stokes-shifted peak is observed in cyclohexane. On the basis of our previous results on determining the binding constants for $BP(OH)_2$/CD complexes,[6,7] the concentrations used here for the different CDs are enough to cause maximum caging with no further changes in the absorption or fluorescence spectra for higher CD concentrations.

In all of the CDs, the absorbance intensity in the region of 330−360 nm is higher than that in water, and the intensity in the region of 400−450 nm is lower than that in water. The fluorescence spectra (normalized for clarity) show red shifts in CDs compared to fluorescence in water. The caging effect on the absorption and fluorescence spectra increases as the cavity size of the CD decreases (going from γ-CD to β-CD to α-CD).[40,41] As the cavity size decreases, the guest molecule is buried inside a more hydrophobic environment where water is expelled from the cavity.[42]

The largest caging effect on the $BP(OH)_2$ molecule was observed in the complex between $BP(OH)_2$ and α-CD. We previously showed that the guest ($BP(OH)_2$)-to-host (CD) ratio is 1:1 for all CDs except in α-CD, where this ratio is 1:2.[7] Two α-CD molecules capping the $BP(OH)_2$ molecule are necessary to stabilize the complex due to the small cavity size of α-CD (an inner diameter of 0.57 nm).[40,41] The two α-CD molecules will encapsulate the $BP(OH)_2$ molecule and shield it from water. This is manifested in the almost complete absence of absorption in the 400−450 nm region as shown in Figure 4. The position of the fluorescence peak of the $BP(OH)_2$:(α-CD)$_2$ complex also approaches that of $BP(OH)_2$ in cyclohexane.

The fluorescence peak maxima and fluorescence lifetimes of $BP(OH)_2$ in different CDs are shown in Table 2 for excitation at 340 nm. For $BP(OH)_2$ in γ-CD, two lifetimes were measured. The value of the short-lifetime component is similar to that measured in water, which is assigned to the DZ produced via mechanism A in Scheme 2. From our previous results, the least stable complex was that between $BP(OH)_2$ and γ-CD.[6,7] This can be explained by the large cavity size of γ-CD (inner diameter = 0.95 nm),[40,41] which may only sustain partial inclusion of the guest molecule. The decrease in the lifetime of the longer component (3.72 ns) compared to the corresponding lifetime in pure water (5.40 ns) may thus be explained in terms of a direct interaction between the DZ moieties (produced via mechanism B) with the secondary hydroxyls of the glycopyranose units of γ-CD. This interaction may result in a disruption of the water network, which will lead to a less rigid molecule.

Two lifetimes were measured for the $BP(OH)_2$:β-CD complex. The shorter component (1.00 ns) is slightly longer than the corresponding one in pure water (0.65 ns). This result is explained by the high degree of encapsulation of the $BP(OH)_2$ molecule by β-CD, which tends to isolate the DZ tautomer and hence increase its fluorescence lifetime. We calculated the structure of the $BP(OH)_2$:β-CD complex in the ground state, and the results showed that the $BP(OH)_2$ molecule is completely sequestered in the center of the β-CD cavity with nearly axial orientation.[6] The value of the short-component lifetime is close to those measured when $BP(OH)_2$ is dissolved in acetonitrile and methanol (see Table 1). Also, the fluorescence peak maximum of the $BP(OH)_2$:β-CD complex (Table 2) is similar to that measured when $BP(OH)_2$ is solvated in methanol. The results indicate a local environment around the guest molecule in the CD cavity which differs from that in bulk water. Nevertheless, water accessibility inside the β-CD cavity is not ruled out,[42] which is supported by the measured long-lifetime component and the existence of the blue shoulder in the fluorescence peak. The long-lifetime component is similar, within experimental uncertainty, to that measured in pure water.

In α-CD, only one lifetime was measured (3.66 ns). As mentioned above, the formation of a 1:2 complex between $BP(OH)_2$ and α-CD results in complete isolation of the guest within the cavities of the two α-CD molecules. We calculated the structure of the $BP(OH)_2$:(α-CD)$_2$ complex in the ground state and found that the inclusion of the $BP(OH)_2$ molecule is axial and centered between the two cavities of α-CDs with van der Waals and electrostatic interactions dominating the binding.[7] From the steady-state absorption and fluorescence results, and the fact that there is only one lifetime measured for the fluorescence decay of the $BP(OH)_2$:(α-CD)$_2$ complex, all results point to the absence of water around the DZ tautomer. The slight blue shift in the fluorescence peak of $BP(OH)_2$ in the $BP(OH)_2$:(α-CD)$_2$ complex compared to that in cyclohexane ($\sim$411 cm^{-1}) indicates the existence of a somewhat polar environment around the guest molecule. The calculated structure of the $BP(OH)_2$:(α-CD)$_2$ complex shows that the two hydrogen-bonding centers of $BP(OH)_2$ are located near the wider rims of the two α-CD molecules.[7] These rims include secondary hydroxyl groups which may explain the stability of the $BP(OH)_2$ molecule when its polar groups are close to the secondary OHs of the two α-CD molecules. The value of the lifetime (3.66 ns) is then a manifestation of a mixed environment around the $BP(OH)_2$ molecule, but the absence of the blue shoulder in the fluorescence peak indicates that solvation of the hydrogen-bonding centers of $BP(OH)_2$ by water networks is not possible in this complex.

The data presented so far show that γ-CD is too wide to enhance strong inclusion and α-CD is too small to sustain 1:1 complexes. On the other hand, β-CD seems to have an appropriate cavity size to stabilize strong complexation. It is worth noting here that the solubility of the CDs in aqueous media varies in an irregular manner, with a relatively low solubility of β-CD.[42] This fact was a reason for the modification of the β-CD structure in order to improve its solubility and increase the hydrophobicity of its cavity as its annulus is of a size particularly suitable for the complexation of drug complexes which are more soluble than the drugs in their free states.[42] We investigate next the effect of changing the cavity hydrophobicity of β-CD while maintaining the same cavity diameter. This can be achieved by substitution in the OHs of the primary and secondary rims. We study the simple cases of inclusion of $BP(OH)_2$ in methyl-substituted β-CD (Mβ-CD),[43] dimethyl-substituted β-CD (DMβ-CD), and the fully methylated trimethyl-substituted β-CD (TMβ-CD). Figure 5 displays the absorption

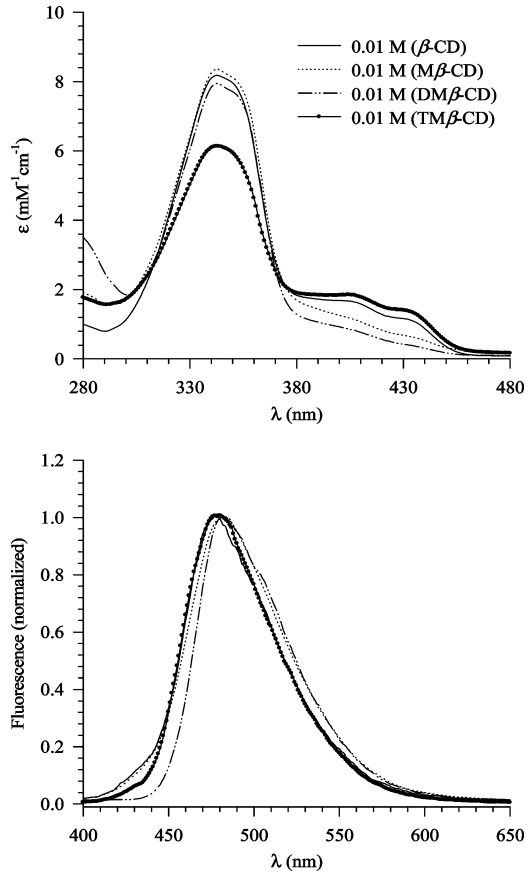


Figure 5. Absorption (upper) and normalized fluorescence emission (lower) spectra of $BP(OH)_2$ dissolved in β-CD and its derivatives; λ_{ex} = 340 nm.

and fluorescence spectra of $BP(OH)_2$ in β-CD and its three derivatives. The corresponding fluorescence lifetimes are included in Table 2.

β-CD and its three derivatives all have the same cavity diameter but varying solubility in water, with DMβ-CD as the most soluble among all of the derivatives. This is because the intramolecular hydrogen bonding between the secondary alcoholic −OH groups at the 2- and 3-positions of the adjacent glucopyranose rings is destroyed due to substitution of the alcoholic protons at the 2-position with methyl groups. As a consequence, the intermolecular hydrogen-bonding ability of the alcoholic −OH group at the 3-position with water is enhanced. In contrast, in β-CD, the secondary alcoholic −OH groups at the 2- and 3-positions are engaged in intramolecular hydrogen bonding with each other. As a result, β-CD has a relatively low solubility in water.

Substitution at the 2- and 6-positions in DMβ-CD also increases the hydrophobicity of the cavity.[42] This leads to a stronger association between $BP(OH)_2$ and DMβ-CD compared to that between $BP(OH)_2$ and β-CD and enhances more penetration of $BP(OH)_2$ inside of the CD cavity. This is reflected in the dramatic decrease in the absorbance intensity in the region of 400−450 nm for the $BP(OH)_2$:DMβ-CD complex and also the large red shift in its fluorescence peak as shown in Figure 5. The measured fluorescence lifetime of the $BP(OH)_2$:DMβ-CD complex indicates the presence of only one decay component of 1.48 ns. The longer value relative to that measured in the parent β-CD (1.00 ns) is explained by the increased stability of this tautomer inside the more hydrophobic cavity of the DMβ-CD molecule due to the caging effect. The absence of the long-lifetime component indicates that no DZ tautomer is produced through mechanism B in the case of the $BP(OH)_2$:DMβ-CD complex. This is in agreement with the steady-state results in which there is no blue shoulder in the fluorescence peak. This is a consequence of the increased hydrophobicity of the DMβ-CD cavity due to methyl substitution, which tends to expel water outside the cavity during the process of encapsulation.[42]

For Mβ-CD, the methyl substitution ratio is 1.7−1.9.[43] This indicates that Mβ-CD is not monomethylated and may carry up to two methyl groups in each sugar unit. Both absorption and fluorescence spectra of $BP(OH)_2$:Mβ-CD show a caging effect intermediate between the caging effects in $BP(OH)_2$:β-CD and $BP(OH)_2$:DMβ-CD. The measured lifetimes (two components) confirm this conclusion. The short component (1.23 ns) is in the middle between the corresponding values measured for $BP(OH)_2$:β-CD and $BP(OH)_2$:DMβ-CD. The long component (4.93 ns) is close in value to that measured for $BP(OH)_2$:β-CD.

For the $BP(OH)_2$:TMβ-CD complex, the absorption spectrum shows a reverse action where the absorption intensity in the region of 330−360 nm is less than that in the parent β-CD and the intensity in the region of 400−450 nm is slightly higher than that in β-CD. The fluorescence peak of $BP(OH)_2$:TMβ-CD has a similar Stokes shift to that measured in β-CD. Two lifetime components were measured for the fluorescence decay of the $BP(OH)_2$:TMβ-CD complex (Table 2). The short-lifetime component (0.97 ns) implies similar conditions for the DZ tautomer as that in the $BP(OH)_2$:β-CD complex. The second component (2.97 ns) is much shorter than the long-lifetime component measured in $BP(OH)_2$:β-CD (4.90 ns). The latter observation implies a different inclusion mechanism in $BP(OH)_2$:TMβ-CD for the DZ tautomer produced via mechanism B in Scheme 2. In addition, the slight reduction in the intensity of the blue shoulder in the fluorescence peak compared to that in $BP(OH)_2$:β-CD points to the less favored DZ formed via mechanism B.

Methylation at the O(3) position in TMβ-CD may restrict entry of the guest molecule through the secondary face (wider rim) of the annulus of the TMβ-CD molecule.[42] X-ray crystallographic results show distortion of the CD ring of TMβ-CD and steric crowding of the $-O(2)-CH_3$ and $-O(3)-CH_3$ groups.[44–47] Under such conditions, the DZ tautomer produced via mechanism B may not be fully encapsulated inside the CD cavity. This happens because the water networks associated with the DZ tautomer render it too large to fully enter the CD cavity from its wide rim. The tautomer is expected to be close to the surface of the secondary rim of TMβ-CD, where the polar conditions are different from those in bulk. It was also suggested that water molecules near the cavity rim of β-CD are more basic than those in the bulk.[48] The new conditions of different polarity and basicity may be the reason for the different lifetime measured in $BP(OH)_2$:TMβ-CD.

The above results in different CDs show that the $BP(OH)_2$ molecule represents a potentially useful new photophysical probe to study inclusion in supramolecular structures. The results may also suggest a method to quantify the polarity of a given CD cavity by comparing the position of the fluorescence peak with that measured in different solvents. However, such correlation is complicated for the present system by the fact that the fluorescence peak shift of $BP(OH)_2$ in different solvents proved to be a function of not only the polarity of the solvent but mostly of its hydrogen-bonding capability. Determination of the dielectric constant ε inside a CD nanocavity on the basis of the shift of the fluorescence peak of a guest molecule depends also

on the host−guest combination. Values of ε for different CDs were reported to range from 5 to 74.[49,50] For example, for β-CD, there has been a wide range of polarities reported, ranging from ($\varepsilon = 6$)[51] to ($\varepsilon = 48$).[52] However many studies put the polarity of β-CD in the range of some alcohol solvents ($\varepsilon = 20-24$).[53–56] This wide range in the reported polarities of CDs may be explained by the size difference between the guest molecule and the cavity diameter. If the guest molecule is much smaller than the CD cavity, the cavity may include water molecules together with the guest molecule. On the other hand, if the guest molecule is large, only part of the guest molecule will be incorporated into the cavity, and a substantial part of the guest may be exposed to the aqueous environment. In addition, a guest molecule in complexes with more than one CD molecules will experience more of the shielding effect from the aqueous medium than that in 1:1 complexes. The 1:2 complex of $BP(OH)_2$ with two α-CDs is a typical example when the spectral changes are compared with those in 1:1 complexes. Also, within the 1:1 complexes, different modes of inclusion strongly affect the absorption and fluorescence behavior of the encapsulated guest. This was observed in the current study for the complexes of $BP(OH)_2$ with β-CD and its derivatives.

Conclusions

The ground and excited states of the $BP(OH)_2$ molecule were investigated in different solvents and in confined nanocavities of cyclodextrins using steady-state and lifetime spectroscopic measurements. The absorption of the molecule free in solution shows unique absorption features in water in the region of 400−450 nm due to the stabilization of a DZ tautomer produced via proton transfer to and from water in the ground state. The measured fluorescence spectra in different solvents indicate the sensitivity of the tautomer's excited state to solvent polarity, particulary the solvent's hydrogen-bonding capability. Fluorescence lifetime measurements show only one component in each solvent, except in water, where two lifetime components were detected. One common lifetime component in all solvents is due to the excited-state dynamics of the DZ tautomer, which gets shorter as the solvent polarity increases, reflecting the sensitivity of this tautomer to its local environment. The other lifetime component in water is relatively long and is assigned to the photoinduced tautomerization through a water network solvating each of the two hydrogen-bonding centers of the $BP(OH)_2$ molecule.

Probing the nanocavities of several CDs in aqueous solution using the $BP(OH)_2$ molecule as a probe reflects the degree of hydrophobicity of the CD cavity and reveals the different mechanisms of probe encapsulation. As the cavity size decreases in the order γ-CD to β-CD to α-CD, the probe molecule enters a more hydrophobic environment, which is shown as an intensity decrease in the absorbance of the DZ tautomer and a red shift in its fluorescence peak. The measured lifetimes show the same trend and reveal how the probe interacts with the CD annulus. In γ-CD, the probe is located near the secondary rim of the CD annulus, whereas in α-CD, the probe is completely sequestered between two CDs, and the hydrophobicity is close to that observed in cyclohexane. In β-CD and its derivatives, the spectral changes and the measured lifetimes indicate that the CD cavity is more hydrophobic by methyl substitution of the primary and secondary hydroxyls of the CD rims. In the fully methylated TMβ-CD, the probe is exposed to water near the secondary rim due to steric effects, which prevent it from full encapsulation.

The present results show that the $BP(OH)_2$ molecule is a useful photophysical probe in inclusion studies, particularly those which probe the hydrophobicity of nanocavities in supramolecular systems.

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Supporting Information Available: Fluorescence decay transients of $BP(OH)_2$ in different solvents and CDs. This material is available free of charge via the Internet at http://pubs.acs.org.

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(32) The reported value of E_T^N for ethanol is larger than that for acetonitrile. This is because of the way the empirical parameter E_T^N was formulated, which is based on classifying solvents into protic hydrogen-bond donors ($E_T^N \approx 0.5. .1.0$), dipolar non-hydrogen-bond donors ($E_T^N \approx 0.3. .0.5$), and apolar non-hydrogen-bond donors ($E_T^N \approx 0.0. .0.3$). See ref 26 for more details.
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