ARTICLES

Dynamics of Intermolecular Hydrogen Bonds in the Excited States of 4′-Dialkylamino-3-hydroxyflavones. On the Pathway to an Ideal Fluorescent Hydrogen Bonding Sensor

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The spectroscopic behavior of the 4′-dialkylamino-3-hydroxyflavones in protic environments is very unusual. Previous studies showed that in contrast to other solvatochromic dyes containing carbonyl group (coumarins, Nile Red, PRODAN, etc.), their Stokes shift does not increase on the formation of intermolecular H-bonds with protic solvents. The present steady-state and time-resolved studies show that the ground-state equilibrium between the H-bonded and non-H-bonded forms of this derivative in mixed solvents is not changed significantly when the dye is excited to the normal (N*) excited state. New H-bonds do not form, but those already existing in the ground state can disrupt on a slow time scale. This last process is probably coupled with the slow excited-state intramolecular proton transfer (ESIPT) reaction of the H-bonded form of the dye. Therefore, the fluorescence spectra of the dye provide a measure of the ground state distribution between its H-bonded and non-H-bonded forms, which in turn reflects the H-bonding potential of the environment. Due to this feature, this dye can serve not only as a calibrator of solvent properties but also as a unique sensor of H-bonding potential in unknown media. This sensing can be provided by the relative intensities of the two separated emission bands in the fluorescence spectra.

Introduction

Molecular hydrogen-bonding sensors are strongly requested by modern science and technology. Among the systems that need to be characterized for their ability of forming intermolecular H-bonds are the interfaces[1,2] and the surfaces of natural[3,4] and synthetic[5,6] macromolecules. It is also important to characterize hydration in micelles,[7] polymer composites,[6] phospholipid bilayers, and biomembranes.[8,9] Moreover, to understand catalysis[2,10,11] the presence of H-bond donor and acceptor groups in catalytic sites needs to be identified. Of practical importance are also the partition of different solutes in solvent mixtures[12] and the determinations of protic impurities in apolar liquids,[13] and oils in particular.[14] Fast and very sensitive detection of intermolecular H-bonding can be based on fluorescence quenching, which occurs on formation or disruption of these bonds with fluorescence probe molecules.[15,16] However, this quenching is frequently difficult to quantify and distinguish from other quenching effects. Another possibility is to apply different solvatochromic dyes.[17,18] In these dyes, a redistribution of the electronic density occurs in the excited state between two groups, the electron donor and electron acceptor, and if the electron acceptor (commonly the carbonyl group) can form intermolecular H-bonds, then the fluorescence spectra exhibit a strong shift to the red.

The typical representative of these dyes is PRODAN (6-propionyl-2-dimethylaminonaphthalene)[19] (Scheme 1).

The carbonyl group in this dye contains a free electron pair capable of interacting with H-bond donor (protic) solvents. This intermolecular bond becomes stronger in the excited state, since the excited-state charge transfer (ESCT) increases the electronic charge on the carbonyl group. This leads to red shifts in absorption and fluorescence spectra.[20] Moreover, due to ESCT, the dipole moment also increases on electronic excitation, leading to shifts of absorption and fluorescence spectra with the increase of solvent polarity.[19,20] The effects of polarity are thus similar to that of H-bonding both in the direction and magnitude of the spectral shifts.[20] A very similar behavior was reported for 7-(dialkylamino)coumarins,[21,22] aminonaphthylimides,[23] fluorenones,[24] etc. Consequently, the selectivity of these dyes as H-bonding sensors is low, and it is almost impossible to distinguish between the polarity and H-bonding

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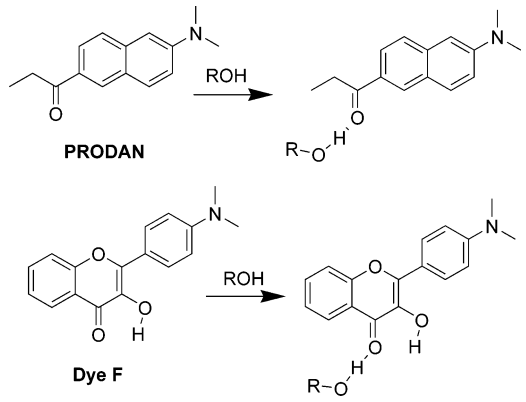
SCHEME 1: Formation of H-bonding Complex in the Ground State of PRODAN and Dye F



effects in unknown media. In model conditions the presence of H-bonding effects in spectral shifts can usually be distinguished only as a deviation from regularity described by common solvent polarity effects. This regularity can be constructed based on the spectroscopic data obtained in a sequence of different solvents, and the deviations described by solvent H-bonding basicity and acidity functions.[25−27] This approach finds only a limited application for sensing H-bonding interactions in systems of unknown polarity.

The other important problem in sensing unknown media is connected with the strongly increased basicity of the acceptor group forming the intermolecular H-bond in the excited state. Because of that, the excited state H-bonding equilibrium may shift strongly to the formation of new H-bonding complexes of the dye with the medium.[21,23] In the presence of protic solvents, this formation can be observed as the time-dependent red shift of the fluorescence spectrum and as biexponential fluorescence decay[23,28] with the presence of a component with a negative amplitude at the long-wavelength part of the emission spectra.[29] This process depends strongly on the proton donor concentration and its diffusion rate, and occurs in a very broad time range (0.1−5 ns and longer).[21,29] As a result, the information on the ground-state H-bonding equilibrium is lost in the fluorescence spectrum, preventing its quantitative analysis. Thus, an ideal fluorescent molecular H-bonding sensor should conform to two essential requirements: its fluorescence signal on H-bond formation should be easily distinguishable from that produced by a change of polarity and its electronic excitation should not modify the distribution of H-bonding species that exist in the ground state.

A prospective approach for more sensitive and selective detection of H-bonding is the application of fluorescent dyes undergoing excited-state intramolecular proton transfer (ESIPT) reaction, which is known to be highly sensitive to intermolecular H-bonding effects.[30,31] However, a close inspection of many dyes exhibiting ESIPT[31] shows that either they do not simultaneously display the signatures of the two forms (H-bonded and non-H-bonded) in emission, or the presence of these forms is connected with other coupled reactions (e.g., isomerizations).[32,33] In this respect, the most promising candidates for H-bonding sensors are 3-hydroxyflavones (3HFs).[34,35] In their structure, an intramolecular hydrogen bond connects the proximal 3-hydroxy group (H-bond donor) to the 4-carbonyl (H-bond acceptor) group belonging to the same rigid heterocycle. Upon electronic excitation to the N* state, a fast ESIPT reaction occurs in which the proton is transferred along this bond. The resultant tautomer T* exhibits a distinct emission band that is strongly shifted toward longer wavelengths with respect to the N* band.[34] Interestingly, any perturbation of the ESIPT reaction changes the relative intensities of the two emission bands. The extreme sensitivity of this reaction to intermolecular H-bonds has been shown in a number of experiments.[36−39] However, attempts to apply the parent 3-hydroxyflavone as a fluorescence probe[40−42] revealed a number of limitations. This dye is excited in the near UV (340−360 nm) and exhibits strong solvent-dependent variation of its quantum yield upon H-bonding. Furthermore, in most solvents, the relative intensity of the N* band of 3HF is very low, limiting its application as a sensor only for highly polar environments.

A significant improvement in the fluorescence properties was achieved by introducing 4′-dialkylamino-substituted 3HFs (the simplest compound being the 4′-(dimethylamino)-3-hydroxyflavone dye F, see Scheme 1).[43−45] Due to the strong electronic charge transfer in their N* state,[46] these dyes became strongly solvatochromic, in contrast to the parent 3HF.[47] 4′-Dialkylamino-substituted 3HFs have found much broader applications than the parent 3HF as fluorescence lipid membrane probes,[48−53] water sensors in reverse micelles,[54] protein ligands,[55] electrochromic probes,[52,56] and polarity sensors.[57] However, the role of H-bonding perturbations in fluorescence response of the dialkylamino-substituted 3HFs was not clearly established.

Recently, we performed a detailed analysis of the solvatochromic behavior of 4′-diethylamino-3-hydroxyflavone (FE) in a set of 21 representative solvents that include donors and acceptors of H-bonds.[57] It was shown that the relative intensity of the two emission bands, I_{N^*}/I_{T^*}, correlates strongly with solvent polarity. Furthermore, the emission spectra demonstrate a dramatic sensitivity to intermolecular H-bonding. Indeed, the I_{N^*}/I_{T^*} ratio of FE are up to 10 times higher in protic solvents than in aprotic solvents of the same polarity. This high sensitivity is clearly related to the H-bonding of solvent proton donors with the 4-carbonyl group of FE acting as H-bond proton acceptor (Scheme 1). In addition, using an analogue of FE with a 4-carbonyl group sterically protected from intermolecular H-bonding, we demonstrated that only this type of H-bonding affects the dual emission of dialkylamino substituted 3HFs, while the H-bonding interactions of the 3-hydroxy group with solvent proton acceptors are not detected in the fluorescence spectra.[58]

According to recent time-resolved data in aprotic solvents,[59,60] ESIPT in dye F is a fast reversible process, so that the intensity ratio of its two emission bands is determined by the ESIPT equilibrium in the excited state. Thus, with increase in solvent polarity this equilibrium shifts toward the N* state possessing a higher dipole moment with respect to the T* state, which results in the increase of the I_{N^*}/I_{T^*} ratio.[58,59] Meanwhile, the time-resolved data on 4′-dialkylamino-3-hydroxyflavones in the presence of protic additives are not available, and thus, the mechanism of their strong fluorescence response to H-bonding interactions remains unclear.

In the present work, to further investigate the mechanism of H-bonding of dye F, we performed steady-state and time-resolved fluorescence studies of dye F in protic and aprotic solvents as well as in their mixtures. The results demonstrate that the excited state intermolecular H-bond free species cannot form new H-bonds, but the intermolecular H-bonds of the excited state H-bonded species can break during the excited state lifetime. The latter process is rather slow and is probably controlled by the ESIPT reaction of the H-bonded species. The obtained data provide new insights for more efficient application of 3HF dyes as H-bond sensors.

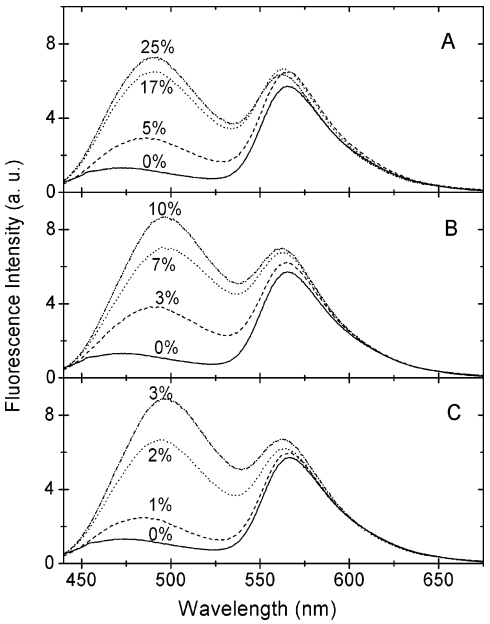


Figure 1. Steady-state fluorescence spectra of dye F in ethyl acetate with differing content of 2-methyl-2-butanol (A), ethanol (B), and water (C). Excitation wavelength was 430 nm. Dye concentration was 2 μM.

Materials and Methods

4′-(Dimethylamino)-3-hydroxyflavone (F) was synthesized and purified as described elsewhere.[45] The solvents of spectroscopic grade were purchased from Aldrich Chemical Co. Absorption and emission spectra were recorded on a Cary 400 spectrophotometer (Varian) and a FluoroMax 3.0 (Jobin Yvon, Horiba) spectrofluorometer, respectively. All fluorescence measurements were corrected for the instrumental response.

Time-resolved fluorescence measurements were performed with the time-correlated single-photon counting technique using the frequency-doubled output of a Ti−Sapphire laser (Tsunami, Spectra Physics), pumped by a Millenia X laser (Tsunami, Spectra Physics).[59] The excitation wavelength was set at 430 nm. The single-photon events were detected with a microchannel plate Hamamatsu R3809U photomultiplier coupled to a Philips 6954 pulse preamplifier and recorded on a multichannel analyzer (Ortec 7100) calibrated at 25.5 ps/channel. The instrumental response function was recorded with a polished aluminum reflector, and its full-width at half-maximum was 40 ps. Time-resolved data were analyzed by the Maximum Entropy method (MEM) using the Pulse 5.0 software.[61] The goodness of the fit was evaluated from the χ^2 values, the plots of the residuals, and the autocorrelation function.

Results

Intermolecular H-Bond Effect on the Fluorescence Spectra of F in Solvent Mixtures. Flavone F was studied in mixtures of aprotic ethyl acetate with three different protic solvents: 2-methyl-2-butanol, ethanol, and water. This allows one to compare the effects of H-bond formation in solvents of different properties. In neat ethyl acetate, the dye shows a classical two-band spectrum in which the short-wavelength and long-wavelength bands correspond to the emission of the normal (N*) and the tautomer (T*) excited states, respectively.[34,43−45] In all three mixtures, an increase of the concentration of the protic component results in a strong increase of the relative intensity of the short-wavelength N* band accompanied by its shift to longer wavelengths (Figure 1). In addition, the increase in the concentration of protic solvent increases the overall fluorescence intensity and thus, the quantum yield.

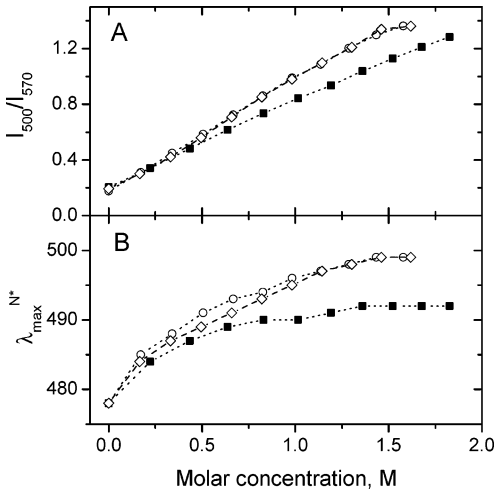


Figure 2. Fluorescence intensity ratio I_{500}/I_{570} (A) and position of the N* band maximum (B) of the dye F as a function of the molar concentration of the protic additives, 2-methyl-2-butanol (■), ethanol (○), and water (◇), in ethyl acetate. Experimental conditions are as in Figure 1.

The dependence of the intensity ratio of the short-wavelength to the long-wavelength band, I_{N^*}/I_{T^*}, on the molar concentration of the protic component is nearly linear (Figure 2A). Furthermore, it is very similar for all the three protic cosolvents and nearly identical for ethanol and water. Indeed, for the same molar concentration of water and ethanol in ethyl acetate (namely 1.7 M, which is 2% water and 7% ethanol), the two-band emission spectra (shown in Figure 1B and C) superimpose completely.

In contrast to the I_{N^*}/I_{T^*} ratio, the dependence of the N* band position on the cosolvent concentration is nonlinear, suggesting specific effects of small concentrations of protic cosolvent (Figure 2B). Noticeably, the dependence of the short-wavelength band on cosolvent concentration is nearly identical for ethanol and water. In the case of 2-methyl-2-butanol, the red shifts are less pronounced and reach a plateau at much lower concentrations. This may be explained by the low polarity of this alcohol.

Several important conclusions can be immediately drawn from these data.

(1) Similar effects are obtained with the three protic solvents (two alcohols of different polarities and water), suggesting that it is not the "dielectric enrichment" due to preferential solvation but the intermolecular H-bonding that is responsible for the observed changes in the fluorescence spectra. This is illustrated by 2-methyl-2-butanol, since its effect on the I_{N^*}/I_{T^*} ratio and the shift of the N* band (Figure 2) does not differ substantially from that of ethanol or water, though its polarity is similar to that of ethyl acetate ($\epsilon = 6.02$ for ethyl acetate and $\epsilon = 5.8$ for 2-methyl-2-butanol).

(2) The effects of the different cosolvents were obtained on the scale of molar concentrations. Since the I_{N^*}/I_{T^*} ratios exhibit a linear dependence with the concentration of cosolvents (Figure 2A), it can be derived that only binary intermolecular H-bonding complexes of the dye with the three protic solvent molecules are formed. Consequently, these results do not support the formation of H-bonds between both the OH− and carbonyl groups of F with solvent molecules.

(3) The oxygen in 2-methyl-2-butanol is less accessible to H-bonding with the H-bond donor solute, as compared to that of ethanol or water. Meanwhile, all three solvents produce very

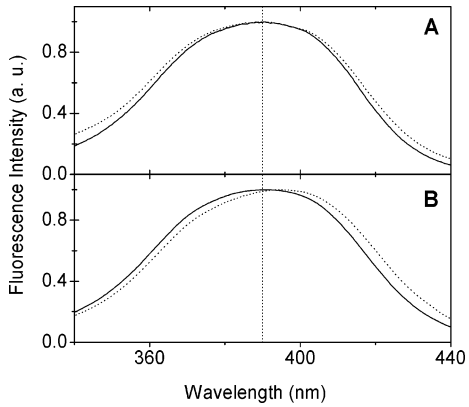


Figure 3. Excitation spectra of probe F in neat ethyl acetate (A) and in a mixture of ethyl acetate with 10% of ethanol (B) at different emission wavelengths: 580 nm (solid line) and 500 nm (dotted line). Vertical dots correspond to the excitation maximum in neat ethyl acetate, to underline the absence of spectral shift in (A) and its presence in (B). Dye concentration was 2 μM.

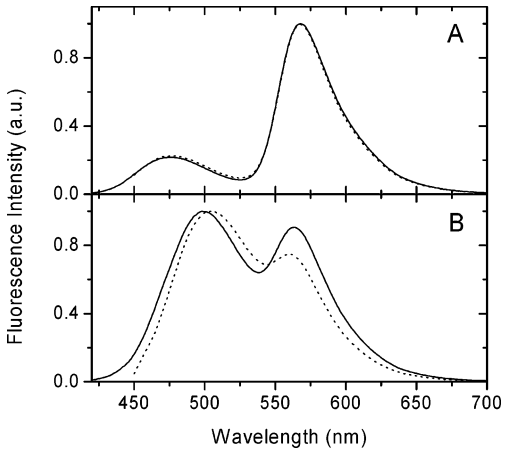


Figure 4. Emission spectra of probe F in ethyl acetate (A) and in a mixture of ethyl acetate with 10% of ethanol (B) as a function of the excitation wavelength: 390 nm (solid line) and 430 nm (dotted line). Dye concentration was 2 μM.

similar spectroscopic effects, signifying that it is not the oxygen but the hydrogen atom of the alcohol OH groups that forms an H-bond with the dye and produces the observed spectral changes. This result is in line with our previous data[58] demonstrating that only one type of H-bond, namely between the proton of the alcohol OH-group with the 4-carbonyl group of the dye, is responsible for the specific effects of protic solvents on the fluorescence of 4-dialkylamino-3-hydroxyflavones. Our results do not exclude the formation of other types of intermolecular H-bonding complexes, but they suggest that these putative complexes are not emissive.

(4) The similar effects of ethanol and water suggest that the structures of their H-bonding complexes with the flavone dye are the same. This indicates that only one proton per water molecule participates in intermolecular H-bonding with the dye. As a solvent, water has many special properties, such as its high polarity, its ability to serve as both donor and acceptor in intermolecular H-bond formation, its high capacity of solvating ions including proton, etc. Therefore, it is surprising that its behavior is similar to that of ethanol or 2-methyl-2-butanol. In fact, water probably does not behave in this case as a bulk solvent; rather, it behaves as a system of individual molecules forming H-bonding complexes with the dye.

(5) The larger red shifts of the N* band with the addition of water and ethanol as compared to 2-methyl-2-butanol (Figure 2B) could be explained by the significantly higher polarity of the former cosolvents. Additionally, the smaller shifts in 2-methyl-2-butanol can be also explained by steric factors that diminish the interaction of this bulky alcohol with the dye.

Taken together, our results show the essential role of intermolecular H-bonding in modulating the solvent-dependent variations of the fluorescence spectra of highly solvatochromic 3HF derivatives. These bonds are responsible for the formation of molecular complexes with a 1:1 stoichiometry.

Heterogeneity of Emissions in Steady-State Spectra. The excitation spectra of dye F were recorded at three different emission wavelengths: at 460 and 580 nm, corresponding to the N* and T* band maxima in ethyl acetate, and at 500 nm, corresponding to the H−N* band maximum (emission of the H-bonded N* form) in different alcohols.[57] In neat ethyl acetate, the position of the excitation spectrum is practically independent of the emission wavelength, demonstrating the presence of only one ground-state species (Figure 3A).

In the presence of a protic solvent, the excitation spectrum recorded at the T* band maximum (580 nm) occupies nearly the same position as that in neat ethyl acetate, while the spectrum recorded at 500 nm (on the long wavelength slope of the N* band) is significantly shifted to the red (by ca. 4 nm) (Figure 3B). This suggests that the T* emission in the presence of the protic component originates from the same ground state as in neat ethyl acetate, while the emission at 500 nm may originate from a different ground-state species. Moreover, the excitation spectrum recorded at 460 nm almost superimposes that recorded at 580 nm, demonstrating that the emission of the T* band and that of the short-wavelength part of the N* band originate from the same ground state. In previous studies on a series of neat solvents, we demonstrated that a red shift in the absorption spectrum can be a result of H-bonding with protic solvents.[57,58] Furthermore, data in protic solvents show that the H-bonded species of the F analogue emit commonly around 500−520 nm.[57] The result is that in solvent mixtures, the photoselection of the H-bonded species at 500 nm may cause the red shift of the excitation spectra with respect to those recorded at the emission regions of the H-bond free species (460 and 580 nm). Moreover, since the excitation spectrum recorded at the emission of the H-bonded form is shifted with respect to that of the H-bond free form, this suggests that the H-bonded and H-bond free forms do not undergo significant interconversion during the excited-state lifetime, so that the emission spectrum reflects the ground-state equilibrium of the H-bonding of the dye with protic media.

A similar conclusion can be drawn from the excitation wavelength dependence of the emission spectra (Figure 4). In the case of neat ethyl acetate, the emission spectra excited at 390 and 430 nm are identical, while these spectra are significantly different for the mixture of ethyl acetate with 10% of ethanol. Moreover, in the presence of protic solvent, excitation at the red-edge shifts the short-wavelength band to the red and increases the I_{N^*}/I_{T^*} ratio. These effects can be explained by photoselection of the H-bonded form of the dye, which is characterized by a red-shifted N* emission band with its increased relative intensity.

Dynamics of Intermolecular H-Bonding in Neat Solvents. According to our previous data,[59] the dye F in ethyl acetate shows two lifetimes at all studied emission wavelengths: a short-lived component with a 30−40 ps lifetime assigned to the ESIPT reaction and a long-lived component of ca. 300 ps corresponding to the emission decay of both N* and T* forms. The positive preexponential factors at the wavelengths of the N* band emission together with the appearance of a negative

TABLE 1: Lifetime Data on Probe F in Different Solvents and Solvent Mixtures[a]

	λ, nm	τ_1, ns	α_1	τ_2, ns	α_2	τ_3, ns	α_3
ethyl acetate[b]	460	0.036	0.89	0.315	0.11	-	-
	500	0.040	0.88	0.272	0.12	-	-
	580	0.042	−0.47	0.340	0.53	-	-
2-methyl-2-butanol	470	0.085	0.60	0.256	0.08	0.655	0.32
	500	-	-	0.268	0.15	0.649	0.85
	580	0.082	−0.25	0.246	−0.23	0.641	0.52
ethanol	520	1.900	1.00	-	-	-	-
17% 2-methyl-2-butanol	460	0.063	0.68	0.291	0.32	-	-
	500	0.063	0.36	0.292	0.64	-	-
	580	0.063	−0.57	0.293	0.43	-	-
7% ethanol	460	0.048	0.66	0.351	0.34	-	-
	500	0.050	0.22	0.343	0.67	0.566	0.11
	580	0.052	−0.47	0.347	0.53	-	-
2% water	460	0.054	0.66	0.324	0.31	0.535	0.03
	500	0.059	0.45	0.331	0.48	0.525	0.07
	580	0.056	−0.54	0.324	0.46	-	-

[a] λ: emission wavelength at which decay curve was registered; τ_1, τ_2, and τ_3: emission lifetime components; α_1, α_2, and α_3: preexponential coefficients. [b] Data of Shynkar et al.[59] Excitation wavelength was 430 nm.

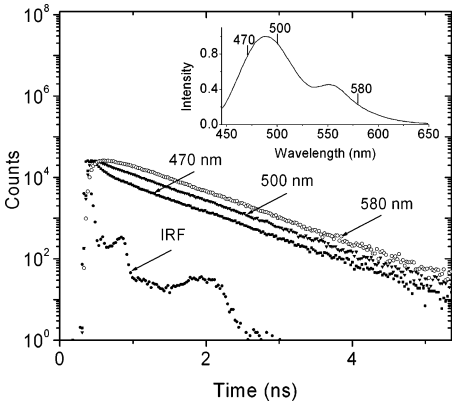


Figure 5. Emission decays of the dye F in neat 2-methyl-2-butanol at 470 nm (■), 500 nm (▼), 580 nm (○), and instrumental response function (●, IRF). Excitation wavelength was 430 nm. The insert shows the fluorescence emission spectrum of the dye F in 2-methyl-2-butanol, with indicated wavelengths for time-resolved studies. Dye concentration was 2 μM.

preexponential factor for the T* band emission are consistent with a fast reversible two-state ESIPT reaction, in which the equilibrium is reached during the excited-state lifetime.[43,59] The present results show that in neat protic solvents, the situation is different (Table 1). In ethanol, where the dye F demonstrates only one band, a monoexponential decay is observed with a much longer lifetime (1.9 ns). This suggests that no ESIPT occurs, and thus the single lifetime describes the decay of the H-bonded N* form of the dye (H−N* form). Moreover, since no short lifetime is observed at any emission wavelength, the H−N* form does not derive from the N* excited state, but must have a ground-state precursor (H−N state) that is excited directly.

A more complicated situation is observed in neat 2-methyl-2-butanol (Figure 5, Table 1), since the decay curves recorded at 470, 500, and 580 nm are significantly different. Three decay components can be detected in the emission (Table 1). Since 2-methyl-2-butanol is capable of forming H-bonds with the dye, and in line with the higher lifetime values in protic solvents, the longest lifetime component may be assigned to the emission decay of the H−N* state. If this form is transformed into an H−T* (or T*) form by an ESIPT reaction, a negative amplitude should appear for the H−T* band decay.

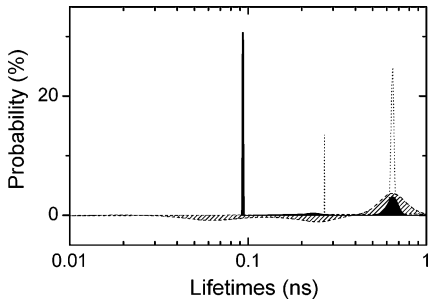


Figure 6. MEM recovered lifetime distributions for dye F in 2-methyl-2-butanol. The analyzed decay curves correspond to emission wavelengths of 470 nm (solid curve), 500 nm (dotted curve), and 580 nm (dashed curve).

In fact, two distinct components with negative amplitudes are observed at 580 nm (Figure 6). The same two short components with positive amplitudes are observed at 470 nm, suggesting that the excited state reaction involves two populations of excited molecules for which the ESIPT reaction rates are different. In fact, steric effects in the formation of H-bonding complexes of 2-methyl-2-butanol with the dye may result in H-bond-free species. We may consequently assign the 0.25-ns lifetime to the ESIPT occurring in the H-bonded complexes and the 0.08-ns lifetime to ESIPT occurring in a smaller subpopulation without intermolecular H-bonding. In line with this assignment, the fastest component is not observed at 500 nm (the maximum of the H−N* band).

Since the H-bonded and non-H-bonded forms of the dye are thought to not interconvert during the emission, the fluorescence decay should be composed of four lifetimes corresponding to the decay of the H−N* form, the decay of the N* form, and the components due to the two ESIPT reactions, respectively. Since only three lifetimes were observed at 470 and 580 nm, we hypothesized that the 0.26-ns lifetime may correspond to both the emission decay of the H-bond free N* and T* forms (which is close to the corresponding lifetime in ethyl acetate, Table 1) and to the ESIPT of the H−N* form. Moreover, the 0.65-ns lifetime component that shows the largest amplitude at 500 nm corresponds to the emission decay of the H−N* and H−T* species. Since no negative amplitude is observed at 500 nm (Table 1), this suggests that N* and H−N* species do not interconvert in the excited state. Moreover, at this wavelength the H−N* form probably dominates to such an extent that the emission of the N* form is negligible (the shortest lifetime component is not observed). It is worth noting that the longer-lifetime values observed in ethanol and 2-methyl-2-butanol as compared to ethyl acetate are consistent with the higher quantum yields of dye F and its analogue FE in protic solvents.[45,57] Thus, it is probably a general trend that the H-bonded form of 4′-dialkylamino-3-hydroxyflavones is characterized by a longer-lifetime and a higher quantum yield than the H-bond-free form. Correlation of the quantum yield and the lifetime indicates that H-bonding does not considerably change the radiative emission rate constants; instead, it significantly decreases the nonradiative deactivation processes.

The time-resolved data in neat protic solvents suggest that the ground-state H−N complex is directly excited to H−N* and then exhibits ESIPT with a time constant of 0.25−0.26 ns. This time is probably sufficient to achieve equilibrium during the ESIPT reaction, so that the H−N* and H−T* forms decay with the same time constant of 0.65−66 ns. This conclusion is in line with the fact that the longest-lifetime component is the same for the three studied emission wavelengths (Table 1).

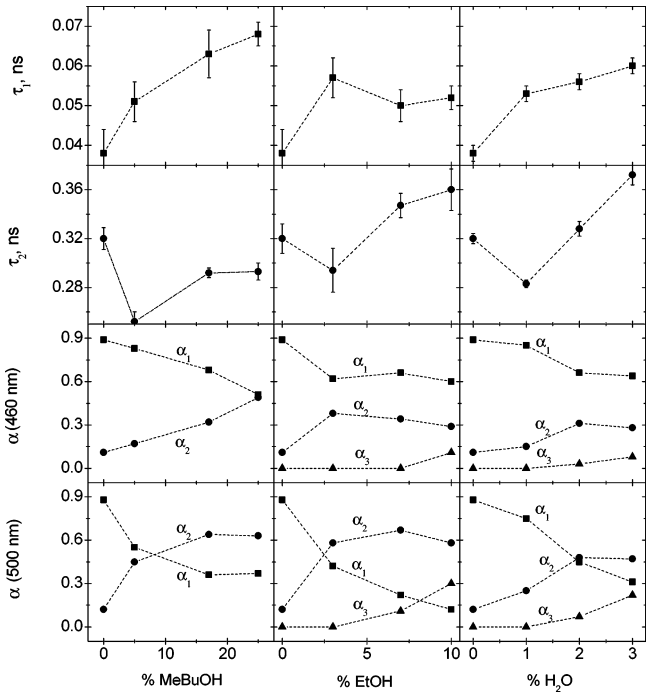


Figure 7. Dependence of the lifetimes (τ_1 and τ_2) and amplitudes (α_1, α_2, and α_3) of the fluorescence decays for dye F on the concentration of 2-methyl-2-butanol (MeBuOH), ethanol (EtOH), and water in ethyl acetate. Lifetimes τ_1 and τ_2 are presented by their mean value with standard deviation. The experimental conditions are the same as in Figure 5.

Dynamics of Intermolecular H-Bonding in Solvent Mixtures. The fluorescence decay kinetics in mixtures of ethyl acetate with 2-methyl-2-butanol offer a good opportunity to study the excited state dynamics of intermolecular H-bonds. Due to the similar dielectric constants of these solvents, the results of these studies should not be complicated by the temporal effects of dielectric enrichment. In fact, the emission decays of F in this system appear rather simple, with only two components over the whole range of studied concentrations. The short-lived component (50−70 ps), having a positive amplitude at short wavelengths (460 nm), shows a negative amplitude at 580 nm (Table 1). Due to its similarity to the short-lived component of F in neat ethyl acetate, this component may be assigned to the ESIPT reaction of the H-bond-free form. An increase in the alcohol concentration gradually increases the short lifetime and decreases its amplitude, suggesting a decreased ESIPT rate at higher concentrations of alcohol. The long-lived component at about 0.29 ns is observed over all of the emission spectrum and its value is almost independent of the concentration of alcohol. In contrast, the amplitude of this component increases with the alcohol concentration (Figure 7). Moreover, it depends on the emission wavelength, with the maximal values corresponding to the maximum of the H−N* form. The same two lifetime components are observed for dye F in a mixture of ethyl acetate with ethanol or water (Table 1). For all of the studied solvent mixtures, the ratio of the amplitudes of these two components at the short-wavelength band, α_1/α_2, which to some approximation describes the transformation of the N* to T* form,[56] strongly decreases with the increase in alcohol concentration (Figure 7). Furthermore, while in neat ethyl acetate the α_1/α_2 ratio is constant over the whole N* band spectrum,[59] in the presence of alcohol it is strongly wavelength-dependent with its smallest value at 500 nm (Table 1).

Thus, in the presence of alcohol, the short-wavelength band is heterogeneous, indicating at least two N* emissive species. At 460 nm, we observe the emission of the H-bond-free N* species that undergoes fast ESIPT as in neat ethyl acetate, while at 500 nm, the emission is mainly due to the H−N* species, for which ESIPT is probably much slower and cannot be detected. The fact that the N* and H−N* species are characterized by a similar long lifetime demonstrates that the interconversion of these two forms in the excited state occurs on a similar or faster time scale. Moreover, the fact that we can distinguish these two forms by a fast ESIPT component suggests that their interconversion is much slower than the ESIPT reaction.

In the case of ethanol and water used as protic additives to ethyl acetate, biexponential decays are only observed at low concentrations of these additives (1% water and 3% ethanol). Higher concentrations of these cosolvents allowed the detection of a third long-lived component (0.53−0.56 ns; see Table 1). This component is most pronounced at 500 nm and its amplitude grows with the cosolvent concentration (Table 1). These data allow us to assign this new component to the emission decay of the H-bonded (H−N*) form. This assignment is additionally supported by the fact that the value of this component is close to that observed in neat 2-methyl-2-butanol. The fact that the long lifetime of the H−N* component becomes resolvable shows that in the case of ethanol and water at high concentrations (or in neat 2-methyl-2-butanol) the H-bonding complex of the dye with solvent is stable and persists longer than the emission lifetime. This conclusion is in full agreement with our steady-state data showing the dependence of the excitation spectra on the emission wavelengths (Figure 3) and the emission spectra on the excitation wavelength (Figure 4).

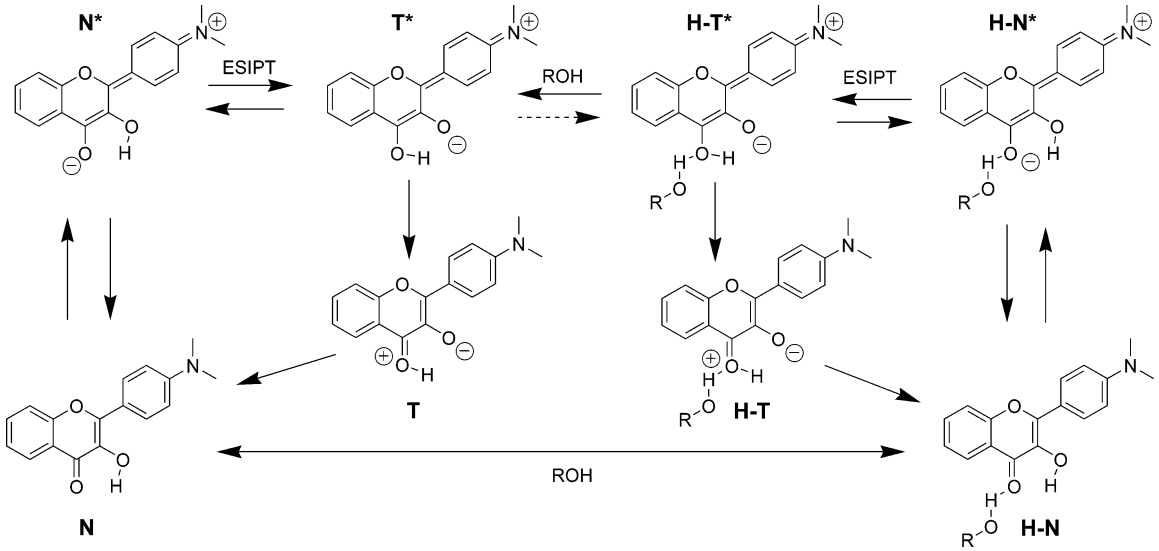
Discussion

For most H-bond-forming dyes, one can derive the general rule that if the dye contains carbonyl as the π-electron acceptor group, the basicity of this group in the excited state always increases, so that the intermolecular H-bonds with protic solvent molecules become stronger, and new H-bonds are easily formed in mixed fluid solvents. In contrast, the 3-hydroxyflavone dye F exhibits a quite different behavior since no signature for formation of new intermolecular H-bonds was detected. In addition, the slow exchange between H-bonded and non-H-bonded species enables their selective excitation.

To emphasize this unique behavior of 3-hydroxyflavone dye F, a comparison with typical solvatochromic dyes PRODAN,[19,20] 7-(dialkylamino)coumarins,[21,22] and aminonaphthylimides[23] can be made. For these dyes the formation of an H-bond with the protic partner is favored by electronic excitation. It is commonly faster than the emission decay and is controlled by the diffusion rates and concentrations of protic cosolvent.[21,29] The driving force for the formation of this H-bond is the highly increased basicity of the acceptor carbonyl group in the excited state.[22,23] The formation of a new H-bond in the excited state of PRODAN can be derived from the steady-state spectra by observing in protic solvents significantly larger Stokes shifts than those expected from the Lippert equation calibrated with aprotic solvents.[20] This is definitely not the case for 4-diethylamino-3-hydroxyflavones, since the Stokes shifts for a homologue of F in both protic and aprotic solvents have been recently shown to fit the same linear Lippert function.[57] Together with the data of the present study, this observation provides evidence that in the excited state the relaxation processes that could strengthen intermolecular H-bonds or form new H-bonds do not occur.

What is the origin of the uniqueness of 3-hydroxyflavones as carbonyl-containing dyes? Why do intermolecular H-bonds

SCHEME 2: Ground and Excited State Transformations of Dye F in Protic Environments



Ground-state N and H−N forms are in equilibrium, and on electronic excitation they generate two unconnected N* and H−N* states, which can be photoselected by choosing appropriate excitation and emission wavelengths. Both of these forms undergo ESIPT reaction, but for H−N* it proceeds in a much slower time scale. H−T* can convert into the T* form.

not form or strengthen in the excited state, so that these dyes basically retain the distribution of H-bonded and non-H-bonded species existing in the ground state? As a rule, the formation of new intermolecular H-bonds is not always favorable since it decreases the entropy of the system. However, in the cases when basicity of a carbonyl (or other H-bond acceptor) group of a dye increases strongly on electronic excitation, the gain in enthalpy is high enough to shift the equilibrium toward the formation of H-bonds. Why does this not happen in the case of 4′-dialkylamino-3-hydroxyflavones? We hypothesize that this may be due to the already-present *intramolecular* H-bond, which is involved in the ESIPT reaction. An intramolecular H-bond may be more favorable than an intermolecular H-bond, since it is not associated with entropy loss. The increase of the carbonyl electron density (basicity) that occurs in the excited state may strengthen this intramolecular H-bond. This may prevent in turn the formation of intermolecular bonds, so that the distribution between H-bonded and non-H-bonded species in the excited state does not differ substantially from that in the ground state.

This is not the only distinctive feature of 3-hydroxyflavone dyes. In the non-H-bonding species of dye F and its analogues, ESIPT occurs according to the classical scheme[34] as a fast (10^{-10}−10^{-11} s) reversible process.[43,59] The formation of an intermolecular H-bond with the 4-carbonyl group is inhibited directly by this fast ESIPT reaction, which transforms this group into a low basic hydroxyl group (Scheme 2). In the case of H-bonded species, we observe much slower ESIPT kinetics (0.25−0.35 ns). This can be explained both by the selective stabilization of the normal with respect to the tautomer excited states and by the weakening of the intramolecular H-bonding due to competition with the intermolecular H-bonding (Scheme 1). Therefore. in protic environments ESIPT may become so slow that the transformation of H−N* into H−T* species (Scheme 2) may occur on the same or longer time scale as compared with the fluorescence decay times. In this respect, the subsequent dissociation of H-bonds may not be revealed on the time scale of emission.

Our time-resolved data reveal essential differences in the excited state transformations in neat protic solvents and their mixtures with aprotic solvents. According to our time-resolved data, in neat 2-methyl-2-butanol, the longest lifetime component (0.65 ns) corresponding to the H-bonded species of the dye is the same at both emission maxima (Table 1). Therefore, the observed two bands in emission originate mainly from the H−N* and H−T* forms coupled by the reversible ESIPT reaction. Previously, we showed that emission of the long-wavelength band in protic solvents (H−T*) is significantly shifted to the blue with respect to the T* emission in aprotic solvents,[57] which suggests that the H−T* form of the dye is destabilized, being energetically less favorable than the T* form. The low stability of the H−T* form may cause its dissociation into the T* form (Scheme 2) at the lower concentrations of alcohols. The transformation of the H−T* into the T* form is probably much faster than the emission decay. This is supported by the fact that for solvent mixtures, we could not detect a lifetime component around 0.55−0.65 ns at 580 nm corresponding to the emission decay of the H−T* form (Table 1). Therefore, the dissociation of the H-bonding complex (i.e., H−N* → H−T* → T*; see Scheme 2) should be determined by the relatively slow ESIPT occurring in the 0.25−0.35 ns time scale. Noticeably, the third component (0.55−0.65 ns) corresponding to the emission decay of the H−N* form is observed in solvent mixtures containing ethanol and water, but not 2-methyl-2-butanol, which could be explained by higher stability of the H-bonding complex of the dye with the former solvents. Furthermore, the ESIPT transformation of the H-bonded species (H−N* → H−T*) with ethanol and water is probably slower compared to that of 2-methyl-2-butanol, since the second lifetime describing this process is longer for the former solvents (Table 1).

Based on the obtained results, we suggest the following scheme of possible transformations of dye F in the ground and excited states (Scheme 2). The ground and excited-state transformations are different for the N and H−N forms. Before excitation, these forms are in equilibrium. This equilibrium depends on the concentration and reactivity of the proton donors in the environment. They are excited independently and there are no direct transitions between the corresponding N* and H−N* forms. Some interchange (and possibly H-bond dissociation) may occur only in the H−T* and T* states.

The above-described properties of dye F that may be peculiar to a broader class of 3-hydroxychromones and 3-hyfroxyfla-

vones make this class very attractive for the application as H-bonding sensors. The ideal sensor should provide information about the ground-state distribution of H-bond proton donors in its surroundings rather than influencing actively this distribution by any excited state reaction. Since, in the excited state, the H-bond-free form of the dye does not tend to form H-bonds while the H-bonded form undergoes only a slow disruption of its H-bond, the excited state H-bonding equilibrium may confidently describe the ground state H-bonding equilibrium. This property is an important advantage of 3HFs as H-bonding sensors, since they allow a quantitative analysis of the ground state H-bonding by using fluorescence spectra. In contrast, for the common solvatochromic dyes such as 7-(dialkylamino)-coumarins, the formation of a new H-bond occurs in the excited state, is commonly faster than the emission decay, and is controlled by the diffusion rates and concentrations of the protic cosolvent.[21,23,29] Due to the formation of a new H-bond in the excited state, such dyes demonstrate strong effects of H-bond kinetics in fluorescence spectra without providing a quantitative description of the ground-state H-bonding equilibrium. In the case of dye F and its analogues, this formation of new H-bonds is prevented by the strengthening of the intramolecular H-bond and further ESIPT transformation that compensate for the increased basicity of the 4-carbonyl group.

An additional attractive feature of dye F and its analogues is the possibility to describe the ground-state H-bonding equilibrium (i.e., hydration, etc.) of the dye by using the ratio of the two emission bands, I_{N^*}/I_{T^*}, with all the advantages of fluorescence ratiometric measurements. Importantly, their two-band spectrum allows one to distinguish polarity and H-bonding effects,[57] a feature that is not possible with a single-band solvatochromic dye. These unique H-bond sensing properties of 3-hydroxyflavone dyes have already been applied for probing polarity (a function of nonspecific dielectric relaxation) and hydration (due to the H-bond donor ability of water) of lipid bilayers.[53] The results show that in lipid bilayers it is possible to identify the H-bonded and H-bond free forms of the dyes using fluorescence spectra. This allows characterizing their relative concentration (i.e., probe hydration) and the site polarity of the H-bond-free form of the dyes.

Conclusions

The present results demonstrate that dialkylamino 3HF forms with and without intermolecular H-bonds coexist in both the ground and excited states, and no significant shift of the equilibrium between these forms occurs in the excited state. For non-H-bonded species, the ESIPT reaction is fast and its equilibrium is established rapidly on the time scale of fluorescence decay. For H-bonded species, the situation is different. After electronic excitation, they exhibit a slow ESIPT transition to the H-bonded tautomer H−T* form, which is of low stability and tends to dissociate slowly with the formation of H-bond-free T* form. This H−N* → H−T* → T* transformation makes a rather small contribution to the formation of the two-band steady-state fluorescence spectra in protic media. Therefore, these spectra may describe the ground-state distributions of intermolecular H-bonds, which make 3-hydroxyflavone dyes promising sensors for H-bonding in unknown environments.

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