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Modulation of Excited-State Intramolecular Proton Transfer by Viscosity in Protic Media

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3-Hydroxyquinolones undergo excited-state intramolecular proton transfer (ESIPT), resulting in a dual emission highly sensitive to H-bonding perturbations. Here, we report on the strong effect of viscosity on the dual emission of 2-(2-thienyl)-3-hydroxyquinolone in protic solvents. An increase in viscosity significantly decreases the formation of the ESIPT product, thus changing dramatically the ratio of the two emission bands. Time-resolved studies suggest the presence of solvated species characterized by decay times close to the solvent relaxation times in viscous media. The intramolecular H bond in this species is probably disrupted by the solvent, and therefore, its ESIPT requires a reorganization of the solvation shell for restoring this intramolecular H bond. Thus, the ESIPT reaction of this dye and its dual emission depend on solvent relaxation rates and, therefore, on viscosity. The present results suggest a new physical principle for the fluorescence ratiometric measurement of local viscosity.

1. Introduction

Viscosity is a fundamental parameter in biological systems.[1−5] Since the viscosity distributes heterogeneously on the nanometer scale in living cells, molecular probes are efficient tools for its measurement. Specially designed fluorescent molecular rotors have notably been developed for this purpose.[6−11] These molecules are characterized by a high rotational mobility of their π-conjugated system so that their fluorescence quantum yield depends on this mobility and thus on the viscosity. In order to obtain a ratiometric fluorescent probe of viscosity, which is highly desirable for biological applications,[12,13] a combination of a molecular rotor fluorophore with a reference (environment-insensitive) fluorophore has been realized.[14] However, application of two fluorophores in one sensor complicates the data interpretation since factors like FRET, quenching, and photobleaching of individual fluorophores will interfere with the fluorescence response of this probe. A single fluorophore-based approach for ratiometric measurement of viscosity, which up to now has not been sufficiently explored, is to use the excited-state intramolecular proton-transfer (ESIPT) reaction. In protic environments, which are ubiquitous in biological systems, microviscosity can, in principle, be characterized through the dynamics of H bond exchange that may affect the proton-transfer rate. However, ESIPT is generally poorly dependent on viscosity. One exception is 7-azaindole,[15,16] which undergoes a "solvent assisted" proton transfer. This transfer requires appropriate solvent rearrangement so that the whole process depends on viscosity. Meanwhile, the application of this dye as a viscosity probe in protic media is limited because 7-azaindole exhibits a low fluorescence quantum yield (ca 1%) and a weak variation of the relative intensities of its two emission bands in response to viscosity. Most of other ESIPT dyes are not sensitive to solvent viscosity since their intramolecular H bond forms a stable six-membered ring. This stable intramolecular H bond cannot be readily disrupted by protic solvents, and therefore, no sensitivity to viscosity is observed.[17] In contrast, in dyes with a less stable five-membered H-bonded ring, such as 3-hydroxyflavones (3HF), the intramolecular H bond can be disrupted by protic solvents, leading to a decrease of the ESIPT rate. However, the dual emission of 3HF dyes is also not sensitive to viscosity,[18] probably because the dye forms with a disrupted intramolecular H bond are nonemissive at room temperature.[19] In this respect, the recently introduced 3-hydroxyquinolones (3HQs)[20] are highly promising since they exhibit high fluorescence quantum yields in protic solvents.[21] Moreover, the ESIPT in these dyes occurs (Figure 1) on a slower time scale (around 100 ps),[21,22] which is close to the time scale of solvent relaxation in viscous protic environments ($\geq$100 ps).[23] The ESIPT of 3HQs is highly sensitive to H-bonding perturbations since their intramolecular H bond forms a less stable five-membered ring.[20−22,24] An additional attractive feature of 3HQs is the independence of their fluorescence properties on pH in the biologically relevant range (pH 4−9).[21] However, nothing is reported so far on the effects of viscosity on the fluorescence of 3HQ dyes. Among the 3HQ dyes, *N*-methyl-substituted dyes show a significant sensitivity of their dual emission to solvent polarity, which is observed as an increase in the relative intensity of their normal (N*) emission band with respect to the long wavelength band, belonging to the ESIPT tautomer (T*)

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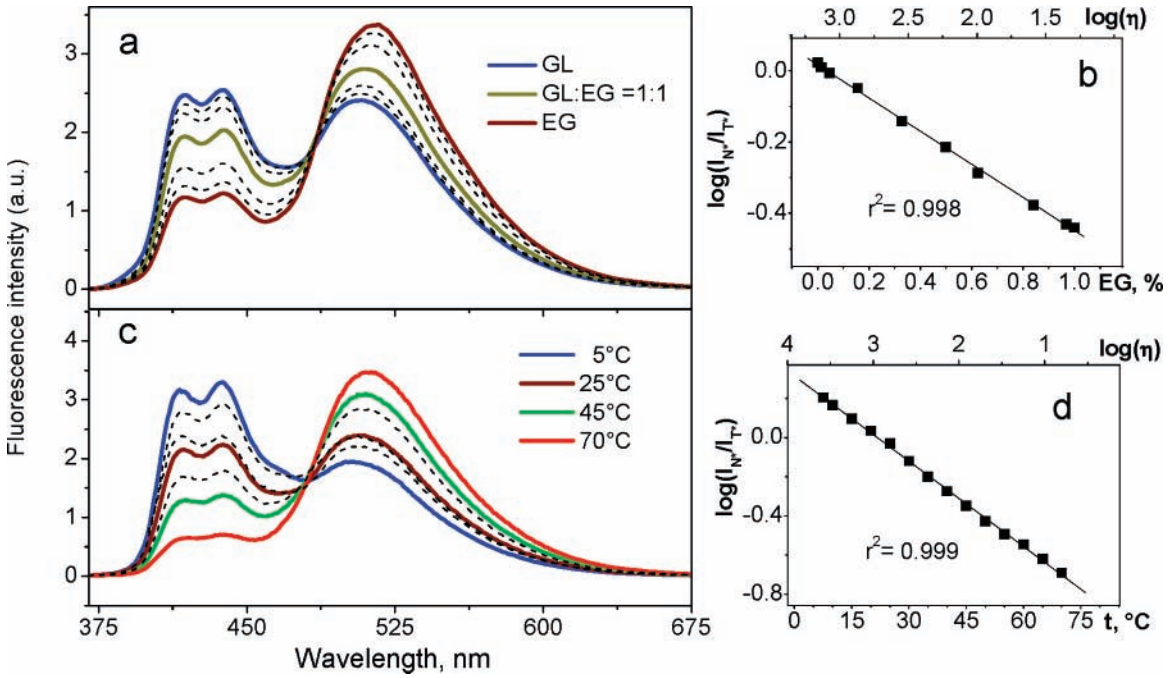
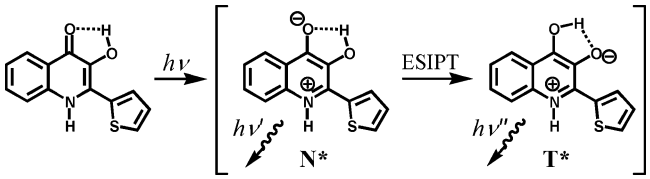


Figure 1. Viscosity dependence of 3HQT fluorescence. The spectra were performed (a and b) in mixtures of ethylene glycol (Eg) and glycerol (Gl) of various compositions at 20 °C and (c and d) in glycerol at various temperatures. The corresponding values of viscosity were taken from the literature.[14,25]

SCHEME 1: 3HQT Dye and Its Excited-State Transformation



product.[20−22] Meanwhile, N-nonsubstituted 3HQs are poorly sensitive to polarity and show a low relative intensity of their short-wavelength band in most nonviscous organic solvents.[21] Therefore, to study the effect of viscosity independently of polarity, we selected a N-nonsubstituted 3HQ, namely, 2-(2-thienyl)-3-hydroxy-4(1H)-quinolone (3HQT, Scheme 1). Due to the 2-thienyl substituent, this molecule is flat in the ground state and therefore, unlike other 3HQs, exhibits a limited rotational mobility of its 2-aryl group and a rather constant fluorescence quantum yield in a variety of solvents.[21] The dual emission of this dye was found to be highly sensitive to viscosity in protic media, showing a dramatic decrease of the relative intensity of its T* band with increasing viscosities. Time-resolved data suggest that this phenomenon is connected with the viscosity-dependent reorganization of the solvation shell of the dye, which is required for the ESIPT reaction. We expect that the observed phenomenon can be used for the development of new fluorescence ratiometric probes of viscosity.

2. Experimental Section

3HQT dye (Scheme 1) has been synthesized by condensation of the corresponding anthranilic acid ester (prepared from phenacyl bromide and anthranilic acid) in polyphosphoric acid.[21] All of the solvents and chemicals were purchased from Aldrich. The solvents for fluorescence measurements were of spectroscopic grade. The viscosity values for different viscous solvents and their mixtures were taken from the literature.[14,25,26] Absorption spectra (for fluorescence quantum yield calculations) were recorded on a Cary 4 spectrophotometer (Varian) and fluorescence spectra on a FluoroMax 3.0 (Jobin Yvon, Horiba) spectrofluorometer. For fluorescence spectroscopy measurements, the excitation wavelength was systematically 360 nm. For determination of fluorescence quantum yields, quinine sulfate in 0.5 M sulfuric acid ($Q = 0.577$) was used as a reference.[27] In all of the spectroscopic measurements, the concentration of the dye was $\sim 5 \times 10^{-6}$ M.

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).[28] The excitation wavelength was set at 320 nm. The fluorescence decays were collected at the magic angle (54.7°) of the emission polarizer. 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 50 ps. The time-resolved decays were analyzed by the iterative reconvolution method.[29] The goodness of the fit was evaluated from the χ^2 values, the plots of the residuals and the autocorrelation function.

3. Results and Discussion

Like other 3HQs, 3HQT shows two emission bands in organic solvents, and the excitation spectra recorded at these two bands are identical.[21,22] These data indicate that 3HQT also undergoes an ESIPT reaction, resulting in the emission of both a normal form (N*) and a phototautomer (T*) (Scheme 1).

To vary viscosity, we used two alternative approaches, (a) solvent mixtures of different compositions and (b) neat viscous solvents at different temperatures. The increase in the glycerol content of a glycerol−ethylene glycol mixture, which increases the solvent viscosity, resulted in a large increase of the intensity ratio of the two emission bands, I_{N^*}/I_{T^*} (Figure 1a). Moreover,

TABLE 1: Time-Resolved Data of 3HQT in Solvent Systems of Different Viscosities[a]

T,°C	Gl,%	τ_1^{N*}, ns	α_1^{N*}	τ_2^{N*}, ns	α_2^{N*}	τ_1^{T*}, ns	α_1^{T*}	τ_2^{T*}, ns	α_2^{T*}	*Q*
20	0	0.59 ± 0.06	1.00			0.51 ± 0.12	−0.40	8.02 ± 0.1	0.60	0.32
20	20	0.66 ± 0.06	0.81	0.69 ± 0.06	0.19	0.55 ± 0.13	−0.37	7.96 ± 0.1	0.63	0.32
20	40	0.59 ± 0.05	0.57	0.99 ± 0.07	0.43	0.55 ± 0.13	−0.32	7.84 ± 0.1	0.68	0.34
20	60	0.57 ± 0.05	0.50	1.22 ± 0.09	0.50					0.36
20	80	0.43 ± 0.05	0.38	1.29 ± 0.09	0.62	0.42 ± 0.11	−0.27	7.53 ± 0.1	0.73	0.36
20	100	0.38 ± 0.04	0.29	1.28 ± 0.09	0.71	0.40 ± 0.11	−0.27	7.53 ± 0.1	0.73	0.36
70	100	0.30 ± 0.04	1.00			0.30 ± 0.10	−0.37	6.38 ± 0.1	0.63	0.36
60	100	0.38 ± 0.04	0.95	0.67 ± 0.06	0.05	0.38 ± 0.11	−0.37	6.66 ± 0.1	0.63	0.39
50	100	0.33 ± 0.04	0.46	0.65 ± 0.06	0.54	0.43 ± 0.11	−0.34	6.91 ± 0.1	0.66	0.33
40	100	0.33 ± 0.04	0.38	0.80 ± 0.07	0.62	0.50 ± 0.12	−0.34	7.16 ± 0.1	0.66	0.37
30	100	0.40 ± 0.05	0.41	1.05 ± 0.08	0.59	0.50 ± 0.12	−0.30	7.35 ± 0.1	0.70	0.37
20	100	0.38 ± 0.04	0.29	1.28 ± 0.09	0.71	0.40 ± 0.11	−0.27	7.53 ± 0.1	0.73	0.36
methanol		0.25 ± 0.03	0.98	1.30 ± 0.09	0.02	0.24 ± 0.09	−0.43	6.73 ± 0.1	0.57	0.30

[a] *T*,°C temperature; Gl,% volume % of glycerol in the mixture with ethylene glycol; τ_1^{N*}, τ_2^{N*}, τ_1^{T*}, and τ_2^{T*} fluorescence decay times recorded at the N* (415 nm) and T* (520 nm) bands; α_1^{N*}, α_2^{N*}, α_1^{T*}, and α_2^{T*} amplitudes of the corresponding decay components; *Q* fluorescence quantum yield.

the logarithm of the intensity ratio ($\log(I_{N*}/I_{T*})$), which is a linear function of different solvent parameters in 3HQ and 3HF derivatives,[21,22,30] exhibited a linear relationship with the glycerol content (Figure 1b). Since the logarithm of the viscosity ($\log \eta$) of this solvent mixture depends linearly on the glycerol content,[14,26] $\log(I_{N*}/I_{T*})$ depends linearly on $\log \eta$ (Figure 1b). Remarkably, a linear correlation between the logarithm of the fluorescence intensity and the logarithm of viscosity was also reported for molecular rotors.[14,31] Variation of the viscosity of glycerol at different temperatures also led to strong I_{N*}/I_{T*} changes (Figure 1c). In this case, the dependence of $\log(I_{N*}/I_{T*})$ on temperature was linear (Figure 1d); therefore, $\log(I_{N*}/I_{T*})$ was also a linear function of $\log \eta$ ($\log \eta$ being a linear function of temperature; see ref 25).

In glycerol−water mixtures as well as in 1,2-propylenglycol and 1,3-propylenglycol at different temperatures, we observed similar linear increases of $\log(I_{N*}/I_{T*})$ as a function of $\log \eta$. The slope of these linear relations varied from one system to another. As a general trend, we observed that, in experiments with temperature variation, the slope of the linear correlation was steeper than that in solvent mixtures. As a consequence, the temperature seems to additionally contribute to the increase in the relative emission of the tautomer (T*) form.

It is important to note that in aprotic solvents (such as toluene, tetrahydrofuran, and acetonitrile), the I_{N*}/I_{T*} ratio was very low; therefore, the emission of the ESIPT product T* dominated.[21] Moreover, a temperature-dependent variation of viscosity in a viscos aprotic solvent triacetin did not significantly modify the fluorescence spectrum of 3HQT (data not shown). Therefore, we can conclude that the viscosity dependence in the dual emission of 3HQT requires protic environments, and therefore, it is connected with the specific H-bonding interactions of the dye and solvent. In order to understand the mechanism of the viscosity-dependent changes of the intensity ratio, we performed time-resolved fluorescence measurements. At relatively low viscosities (neat ethylene glycol and glycerol at 70 °C or methanol), the decay of the N* band was monoexponential. At higher viscosities (with a decrease in the temperature of neat glycerol or an increase in the content of glycerol in mixtures with ethylene glycol), a longer decay component (τ_2^{N*}) appeared, and its relative contribution (α_2^{N*}) increased with the viscosity (Table 1). Moreover, an increase in viscosity resulted in a substantial increase in the decay time τ_2^{N*}, while the shorter component τ_1^{N*} exhibited only slight changes. For the T* emission, the fluorescence decay of 3HQT in glycerol was biexponential. The short decay time τ_1^{T*} exhibited a negative amplitude and corresponded well to the short decay time τ_1^{N*}

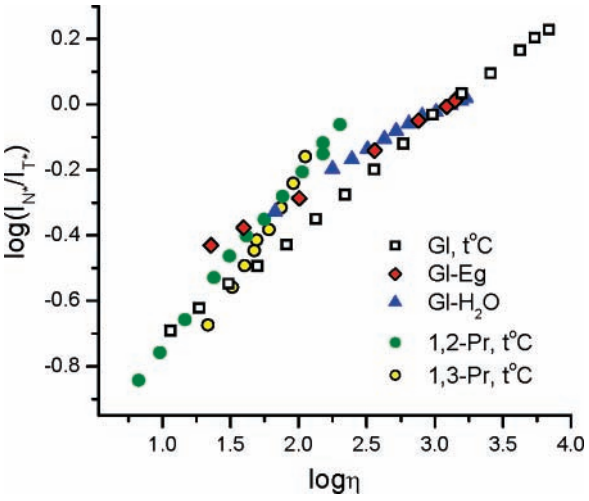
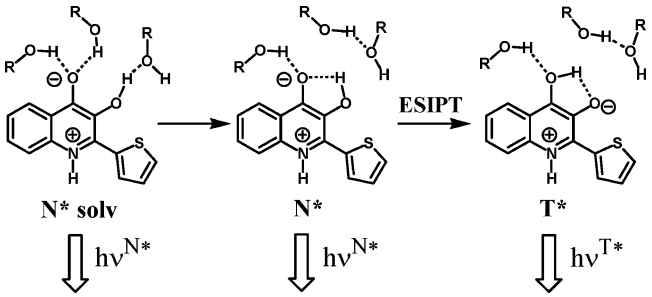


Figure 2. Dependences of $\log(I_{N*}/I_{T*})$ on $\log \eta$. Experiments were performed with glycerol at different temperatures and mixtures of glycerol (Gl) with ethylene glycol (Eg) at 20 °C. 1,2-Pr and 1,3-Pr correspond to 1,2- and 1,3-propylenglycol, respectively. The values of viscosity were taken from the literature.[14,25,26]

recorded at the N* band. This confirms that the T* form is generated from the N* form by an excited-state process (ESIPT).[32] Moreover, the second decay time τ_2^{T*} was more than 10-fold longer than τ_1^{T*} and was absent in the N* band. These results are in accordance with our previous measurements on 3HQs,[21,22] suggesting that the dye undergoes an irreversible ESIPT transformation N* → T* and the rate of this transformation is close to $1/\tau_1^{N*}$ (or $1/\tau_1^{T*}$).[32] Since τ_1^{N*} and τ_1^{T*} did not vary significantly with viscosity, we conclude that the ESIPT rate itself does not depend significantly on viscosity. The long decay time of the T* band τ_2^{T*} was also independent of viscosity, in line with the observed invariant quantum yields (Table 1). Thus, viscosity affects only the long-lived component of the N* emission. What is the origin of this decay time component and how it can be responsible for the strong dependence of the dual emission of the dye on viscosity? Since this component was observed only for the N* emission, we can attribute it to an additional N* form, which cannot undergo ESIPT. It is commonly accepted that the intramolecular H bond closing the five-membered ring in 3HQs and 3HFs can be easily disrupted by a protic solvent, resulting in an inhibition of the ESIPT reaction.[21,22,24] Therefore, the new lifetime observed in viscous solvents could be attributed to a solvated form of the dye (N*-solv) with a disrupted intramolecular H bond. In the excited state, the large increase of basicity of the 4-carbonyl

SCHEME 2: Model of Excited-State Transformations of 3HQT in Viscous Protic Solvents[a]



[a] In the solvated form (N*-solv), the intramolecular H bond of 3HQT is disrupted by the protic solvent. In the excited state, the increase of basicity of the 4-carbonyl group favors the intramolecular H bond and, thus, the N*-solv → N* reaction. In viscous solvents, the reorganization of the solvation shell is slowed down, preventing the formation of the N* form and its subsequent transformation into the T* form by ESIPT.

group should favor the intramolecular H bond of the dye and, thus, favor the transformation of the N*-solv form into the N* form. However, this transformation requires the reorganization of the H-bonding network of the solvation shell of the dye (Scheme 2). In relatively low viscosity environments, this process is much faster than ESIPT; therefore, the fluorescence decay of the N*-solv form cannot be detected, and only the decay of the N* form is observed (Table 1). However, with the increase in viscosity, the reorganization of H bonds slows down, and therefore, the decay of the N*-solv form can be resolved as an individual component. Importantly, the observed increase in the decay time of this new component (τ_2^{N*}) from 0.6 up to 1.5 ns upon increase in the solvent viscosity (Table 1) corresponds well to the viscosity-dependent increase in the solvent relaxation times reported in the literature for the relevant glycol systems.[23] Evidently, the decay time τ_2^{N*} and its amplitude α_2^{N*} are directly connected with the viscosity-dependent solvent reorganization rates. In this respect, the ESIPT transformation of 3HQT in viscous protic solvents may have an "activation" step which consists of the formation of the intramolecular H bond due to reorganization of the solvation shell (Scheme 2), which is a viscosity-dependent process. The increase in viscosity slows down this step, which was observed in the fluorescence spectra as an increase in the relative intensity of the short-wavelength emission (Figure 1). In the time-resolved measurements, this was observed as the increase of the τ_2^{N*} value and its amplitude α_2^{N*} (Table 1). A similar "solvent-assisted" two-step proton-transfer mechanism was previously reported for 7-azaindole, which also shows a clear correlation between the rates of proton transfer and the relaxation of the protic solvent.[15,16] Importantly, unlike 7-azaindole, the 3HQT dye shows a strong variation of its dual emission in response to viscosity as well as a high fluorescence quantum yield in protic media (Table 1). These properties make 3HQT and its derivatives attractive for development of fluorescence probes for ratiometric measurements of viscosity.

In conclusion, we report on the dramatic viscosity-dependent changes of the dual emission of a dye that undergoes ESIPT. Time-resolved data reveal an additional excited-state species of the dye in viscous protic solvents. This species is probably highly solvated and, thus, can undergo ESIPT only after an appropriate viscosity-dependent solvent rearrangement. This viscosity-dependent inhibition of the ESIPT reaction suggests a new principle in the design of ratiometric fluorescence probes of viscosity.

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