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Unprecedented high fluorescence anisotropy in protic solvents: Hydrogen bond induced solvent caging?

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ABSTRACT

Atypical high fluorescence anisotropy has been revealed in protic solvents for excited state intramolecular proton transfer (ESIPT) prone 3-hydroxyflavone and some of its hydroxy derivatives. In non-protic solvents, however, the anisotropy values are low. The low and alike anisotropy values of 3-methoxyflavone in all the studied solvents and other experimental evidences ascribe the formation of hydrogen bond induced cage-like structures in case of the hydroxy compounds involving the probe and the solvent molecules to be responsible for the high values of fluorescence anisotropy in protic media.

1. Introduction

Because of their dual emissions, molecular systems exhibiting ESIPT cover a wide spectrum from blue to red in the visible region and hence serve as viable organic light sources [1,2]. ESIPT prone hydroxyflavone systems belong to this class. The flavones are abundant in the vegetal territory and act as anti-oxidants in the membrane environments [3,4]. Being the simplest molecular system in the flavonoid family 3-hydroxyflavone (3HF) is perhaps the most investigated ESIPT probe [1–4]. Along with 3HF, some other flavonoid systems studied here with a similar skeleton as that of 3HF but possessing additional hydroxyl groups and exhibiting dual emissions are 3,7-dihydroxyflavone (DHF), fisetin (F) and quercetin (Q) (Scheme 1) [5–7].

Fluorescence anisotropy is an angular correlation function that demonstrates the mutual orientation of the excitation and emission dipoles of a fluorophore eventually unraveling the intricacies of the rotational motion of the probe [8]. In most of the pure solvents, the photoexcited fluorophore rotates very fast within its fluorescence lifetime, giving rise to very low (close to zero) fluorescence anisotropy. However, enhanced rigidity of the environment due to increased viscosity or some specific interaction with the medium leads to an increase in the fluorescence anisotropy [8–11]. Also, due to the binding with the macromolecular systems in bio- and biomimetic microheterogeneous media like proteins, lipids, DNA, micelles, cyclodextrins, etc. the effective size of the fluorophore increases remarkably, making the rotational motion slow resulting in a higher anisotropy value. Thus, fluorescence anisotropy is often exploited to explore the rotational motion and thereby location of the fluorophore in the microheterogeneous environments [8–12].

Contrary to the general observation that the anisotropy values of probes are very low in pure solvents, in this letter we have reported, for the first time, unusually high fluorescence anisotropy values of 3HF and some other flavones, namely, DHF, F and Q (Scheme 1) in protic solvents. However, 3-methoxyflavone (3MF), the methoxy counterpart of 3HF, shows low values of fluorescence anisotropy in all the solvents studied. Based on the various experimental results, the surprisingly high values of fluorescence anisotropy in protic media are rationalized by proposing the formation of hydrogen bond induced solvated structures involving the probes and the solvent molecules.

2. Experimental

3-hydroxyflavone (Fluka, USA), 3-methoxyflavone (TCI Fine Chemicals, India), 3,7-dihydroxyflavone, fisetin (Aldrich, USA) and quercetin (Merck, Germany) were used as received. Spectroscopic grade solvents and de-ionized water from a Milli-Q water purification system (Millipore) were used.

Steady state fluorescence and fluorescence anisotropy measurements were carried out in a Horiba Jobin Yvon Fluoromax-4 spectrofluorometer. Fluorescence anisotropy (r) is defined as

$$r = (I_{VV} - G \cdot I_{VH})/(I_{VV} + 2G \cdot I_{VH}) \quad (1)$$

where I_{VV} and I_{VH} are the emission intensities obtained with the excitation polarizer oriented vertically and emission polarizer

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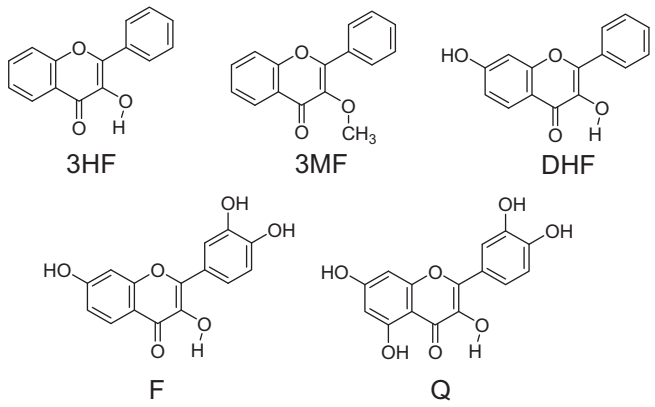

Scheme 1. Structures of 3-hydroxyflavone (3HF); 3-methoxyflavone (3MF); 3,7-dihydroxyflavone (DHF); fisetin (F); and quercetin (Q).

oriented vertically and horizontally, respectively. The G factor is defined as [8]

$$G = I_{HV}/I_{HH} \quad (2)$$

where the intensities I_{HV} and I_{HH} refer to the vertical and horizontal positions of the emission polarizer, with the excitation polarizer being horizontal.

Time resolved fluorescence decay measurements were performed by the time-correlated single photon counting (TCSPC) technique in Horiba–Jobin–Yvon FluoroCube system using NanoLED at 370 nm (IBH, UK) as the excitation source and TBX photon detection module as the detector. The decays were analyzed using IBH DAS-6 decay analysis software. The lamp profile was collected by placing a scatterer (dilute micellar solution of sodium dodecyl sulfate in water) in place of the sample. Goodness of fits was evaluated from χ^2 criterion and visual inspection of the residuals of the fitted functions to the data. Average fluorescence lifetimes (τ) for multiexponential iterative fittings were calculated from the decay times (τ_i) and the normalized pre-exponential factors (a_i) using the following relation:

$$\langle\tau\rangle = \sum_i a_i\tau_i \quad (3)$$

For time-resolved fluorescence anisotropy decay measurements, the polarized fluorescence decays for the parallel [I_{VV}] and perpendicular [I_{VH}] emission polarizations with respect to the vertical excitation polarization were collected at the respective emission maxima of the probes. The anisotropy decay function $r(t)$ was constructed from these I_{VV} and I_{VH} decays following the standard procedure [11]. A Laser-Diode having an output of 375 nm was used as the excitation source.

Dynamic light scattering (DLS) measurements were carried out on a Malvern Nano-ZS90 instrument equipped with a He–Ne laser (λ = 632.8 nm). The operating procedure is programmed with DTS software. Each measurement was averaged over 15 runs and each run was averaged for 20 s.

3. Results and discussion

Upon photoexcitation at 345 nm, 3HF exhibits dual emissions with band maxima at around 410 nm and 510 nm (peak positions depend on the solvent), corresponding to the normal and the photoproduced tautomer, respectively (Figure S1 of the supporting information) [3,13–17]. Monitoring at the two respective emission bands, the steady state fluorescence anisotropy values for both the species of 3HF in different solvents are measured (Table 1). Unprecedented high fluorescence anisotropies

Table 1
Fluorescence anisotropy values for the normal (r_N) species of 3HF, DHF, F, Q and 3MF in different solvents at 300 K. The same for the tautomer (r_T) species of 3HF is also recorded. Each anisotropy value is an average of 20 individual measurements.

Solvent	3HF[a]		DHF[a]	F[b]	Q[b]	3MF[c]
	r_N	r_T	r_N	r_N	r_N	r_N
Water	0.298	0.045	0.271	0.279	0.302	0.031
Ethanol	0.216	0.029	0.236	0.238	0.243	0.036
Methanol	0.192	0.024	0.228	0.195	0.228	0.042
Chloroform	0.045	0.031	0.056	0.048	0.040	0.037
Heptane	0.037	0.033	0.049	0.039	0.032	0.033

[a] λ_{ex} = 345 nm.
[b] λ_{ex} = 370 nm.
[c] λ_{ex} = 355 nm.

of the normal species ($\lambda_{em} \approx 410$ nm) of the fluorophore have been recorded in water and other protic solvents; while the tautomer emission ($\lambda_{em} \approx 520$ nm) shows low anisotropy values. Table 1 reflects that with an increase in the protic character of the solvents the fluorescence anisotropy of the normal form increases, although that of the tautomer species remains invariant. The low anisotropy values of the tautomer in the studied solvents may be justified by considering the change in the orientation of the dipole of 3HF on proton transfer as reported by Bellucci and Coker from their molecular dynamics study [18]. A detailed theoretical treatment is required to divulge the picture more explicitly. However, for the present purpose we focus on the normal species only. Nevertheless, this will not lessen the impact of the present work at all since this is the first systematic report of such high fluorescence anisotropy in conventional protic solvents. We have extended the study to some hydroxyl derivatives of 3HF, viz., DHF, F and Q (Scheme 1). The measured anisotropy values monitoring at the normal emission bands of these probes in different solvents are collected in Table 1. The values are found to go parallel to that of 3HF.

On photoexcitation at 355 nm, 3-methoxyflavone (3MF), lacking the 3-hydroxyl proton and hence incapable of undergoing ESIPT, yields a single fluorescence band ($\lambda_{em}^{max} \approx 440$ nm) corresponding to the normal species (Figure S2 of the supporting information). For this molecular system we notice low fluorescence anisotropy values (0.03–0.04) in all the solvents studied (Table 1).

The anomalous high values of fluorescence anisotropy of the normal forms of 3HF, DHF, F and Q in protic solvents, especially in water, establish the dominating role of hydrogen bonding of the solvent in this context. For 3MF, however, the anisotropy values are low in all the studied solvents as generally observed for the fluorophores in pure solvents. The observations thus, substantiate the commanding role of the 3-hydroxyl group in controlling the value of the fluorescence anisotropy. Again, parallel values of anisotropy for the normal forms of 3HF, DHF, F and Q in different solvents negate any perceptible role of the additional hydroxyl groups (apart from the 3-hydroxyl) in this respect. Low values of fluorescence anisotropy in solvents like heptane and chloroform enforce the impact of interaction of the solvents, particularly the protic ones, with the probe. In short, the results imply that the rotational motions of the probes are restricted in the protic environments. The solvated probes, thus, appear to possess a larger effective volume making their rotational motions slower leading to the higher values of fluorescence anisotropy in the protic solvents. Substitution of the hydroxyl group of 3HF by methoxy group in 3MF restricts the hydrogen bonding with the solvents and hence the anisotropies of 3MF are reduced in all the solvents - augmenting the role of solvation. The study with 3MF further points to the proposition that the effective size enhancement of the solvated probe involves principally the 3-hydroxyl group and the carbonyl group of the flavone systems, thereby designing the effective rotating