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Caging anionic structure of a proton transfer dye in a hydrophobic nanocavity with a cooperative H-bonding

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Abstract

We report on UV–vis absorption and emission studies of 3-hydroxyflavone (3HF) complexed to cyclodextrins (CD). The results show a stabilization of a caged anion through a proton-transfer reaction and inclusion of 3HF into CD. The cooperative H-bonding network of CD plays a central role in the deprotonation of the guest. Picosecond fluorescence measurements of the confined structure suggest no zwitterionic species formation of 3HF within the nanocage. Time-resolved emission anisotropy experiment on the complexes shows a large degree of confinement of the nanostructure. The specific and no specific interactions between the guest and the host observed here are reminiscent to many phenomena found in enzymatic reactions where hydrophobic as well as hydrophilic effects play a key role in biochemistry.

Keywords: Proton transfer; Cyclodextrin nanocavity; Cooperativity; Emission; Fast dynamics

1. Introduction

The ability of cyclodextrins (CD) to encapsulate organic and inorganic molecules has led to intensive studies of their inclusion complexes [1–4]. Recently, we have used CD to study proton-transfer reactions and twisting motion in confined systems, and shown the role of twisting on the photodynamics of these systems in solution and in chemical and biological nanocages [5–13].

Here, we report on studies of 3-hydroxyflavone (3HF) complexed to β- and γ-CD in *N*,*N*-dimethylformamide (DMF) (Scheme 1). Several studies have been done on 3HF and its derivatives reporting on the intermolecular H-bond with the solvent, the formation of its ionic (A) structure, the rate of proton transfers, and the involvement of a charge-transfer reaction modulating the proton-transfer one to form the zwitterionic (Z) structure [14–42]. In no H-bonding solvent, the dye shows an ultrafast excited state intramolecular proton-transfer reaction to produce in less than 50 fs a Z structure emitting at around 530 nm [26,27,29,39,40]. In hydrogen bonding solvent, H-bonded structures to the solvent molecules give normal emission at the blue side (around 400 nm), and detection of anionic species at both ground and excited state has been reported [14–20]. Recently, fs-observation gave a time constant of 0.5 ps to produce anionic structures from 3HF H-bonded to *N*,*N*-dimethylformamide (DMF), while the zwitterionic phototautomer is photoproduced from other H-bonded complexes in 5 ps [40]. The result was explained in terms of the difference between the energy barrier of intermolecular and intramolecular proton motions in these complexes. In other solvents like acetonitrile and alcohols, time constant of 5–10 ps has been observed in the transient absorption spectra, and it was assigned to several phenomena: the contribution of the normal forms, the presence of dissolvated intermolecular H-bonded complexes in alcohols, intramolecular vibrational redistribution, or to a fast equilibrium between two different excited Z structures [22,26,27,29]. On the other hand, 3HF trapped in different micellar environments has been studied using steady-state UV–vis absorption and emission spectra together with ns resolved-emission decays [25]. The result shows an enhancement of Z emission intensity inside the micelles, and it was explained in terms of a decrease of

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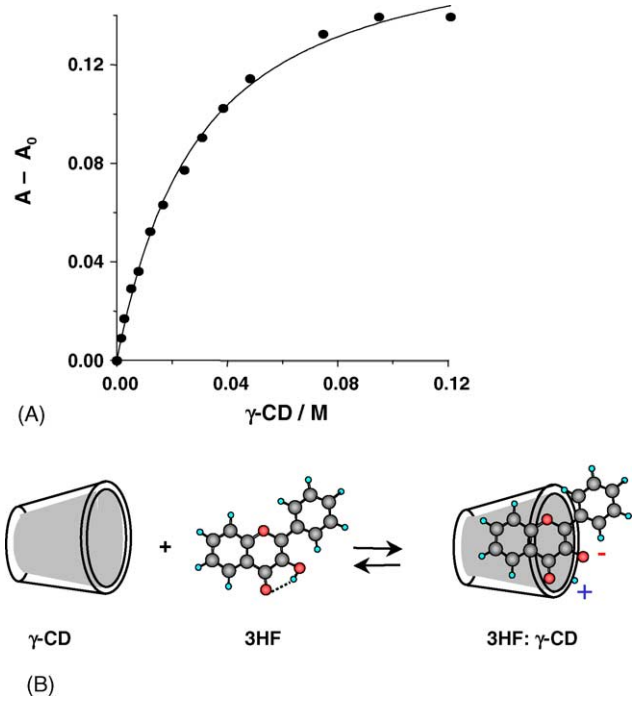

Scheme 1. (A) Variation of the absorption intensity difference ($A - A_0$) at 420 nm of 3HF upon addition of γ-CD. A_0 is the absorption intensity at 420 nm in absence of γ-CD. The solid line is the result of a fitting model which assumes an 1:1 stoichiometry complex giving an apparent equilibrium constant $K = 48 \pm 10\,M^{-1}$ at 293 K. (B) Schematic representation of the equilibrium inclusion complex between free 3HF, γ-CD and the 3HF:γ-CD confined structure where the O–H proton of the guest (3HF) has shifted to the H-bonding network of the host (γ-CD).

intermolecular H-bonding interactions of the dye with environment. In addition to that, in SDS micelles, an emission at around 450 nm was observed and suggested to be due to the mission of complexes between the guest and the charged and polar heads groups of SDS micelles [25]. Other studies have reported on the use of 3HF and its derivatives as a probe for biological media [31]. However, the complex emission behavior of these dyes in polar and H-bonding media strongly limits their use in the field of fluorescent molecular probes. Here, we show that specific and hydrophobic interactions of the guest with the CD cavity play a key role in the ground state formation and stability of the caged ion of 3HF. At S_1, intermolecular proton transfer between the caged enol forms is fast (less than 10 ps) suggesting a small energy barrier for the reaction. At the best of our knowledge, this is the first report showing a clear ground-state proton-transfer reaction between a guest and CD leading to the formation of a caged anion. As said above other studies have reported on 3HF in neat solvents or on its derivatives in biological environments, where ground state anion formation has been observed.

2. Experimental

3HF and CD's (from Sigma–Aldrich) were used as received. Anhydrous DMF (Sigma–Aldrich, 99.8%) was used after drying and purification by standards methods. Steady-state absorption and emission spectra were recorded on a Varian (Cary E1) and Perkin-Elmer LS 50B spectrophotometers, respectively. Excited-state emission lifetimes were measured by using a time-correlated single-photon counting picosecond spectrophotometer (FluoTime 200, PicoQuant). The sample was excited by a 40 ps pulsed (20 MHz) laser centered at 393 or at 371 nm (PicoQuant) and the emission signal was collected at the magic angle. The pulse width measured by the apparatus was typically 65 ps. The emission decay data were convoluted with the cross-correlation signal and fitted to a multi-exponential function using the Fluofit package (Picoquant). The time-dependent anisotropy was constructed using the expression $r(t) = (I// - GI())/(I// + 2GI()$, where G is the ratio between the fluorescence intensity at parallel ($I//$) and perpendicular ($I()$ polarizations of the emission with respect to the excitation beam. The value of G was measured at a gating time of 2 to 3 ns, at which the fluorescence is almost completely depolarized (tail matching technique). The quality of the fits was characterized in terms of residual distribution and reduced χ^2 value. The magic-angle time-resolved emission spectra were recorded at different wavelengths (of 7 nm step) and exciting at 371 nm (or at 393 nm) and were constructed using the Fluofit package. Details on the apparatus and procedure of data analysis were described [12]. All the measurements were done at 293 ± 1 K.

3. Results and discussion

Fig. 1A shows UV–vis absorption spectra of 3HF in DMF upon addition of γ-CD. In absence of CD, the blue absorption band (425 nm) is due to the formation of anionic form of 3HF (A) in DMF, in agreement with previous reports on 3HF in H-bonding solvents [14–42]. The increase of γ-CD concentration in DMF produces an increase of the absorption band intensity of A, and a decrease of that of enol (E) forms absorbing around 340 nm. The inset of Fig. 1A shows that addition of 1.5 M of water to 3HF in DMF in presence or in absence of 120 mM of γ-CD only provokes a small decrease (about 10%) of the absorption intensity at 420 nm. Therefore, the increase of absorption at 420 nm upon addition of γ-CD is not a result of a release of water due to the hydration of the host. The phenomenon is explained by formation of an inclusion complex between 3HF and γ-CD (Scheme 1). Analysis [12] of the absorption intensity upon addition of CD suggests an 1:1 stoichiometry of the complex in which the O–H proton of caged 3HF has shifted to the H-bonding network formed by the 16 OH groups of CD (Scheme 1). This is due to an increase of ground state 3HF acidity upon encapsulation. The apparent inclusion equilibrium constant is $48 \pm 10\,M^{-1}$ at 293 K. Using, 120 mM β-CD (a smaller nanocavity, interior diameter of ∼8.5 Å), and under similar experimental conditions, the increase of the 420 nm absorption intensity was relatively weak (5–10%) when compared to that observed using γ-CD and the same dried and purified

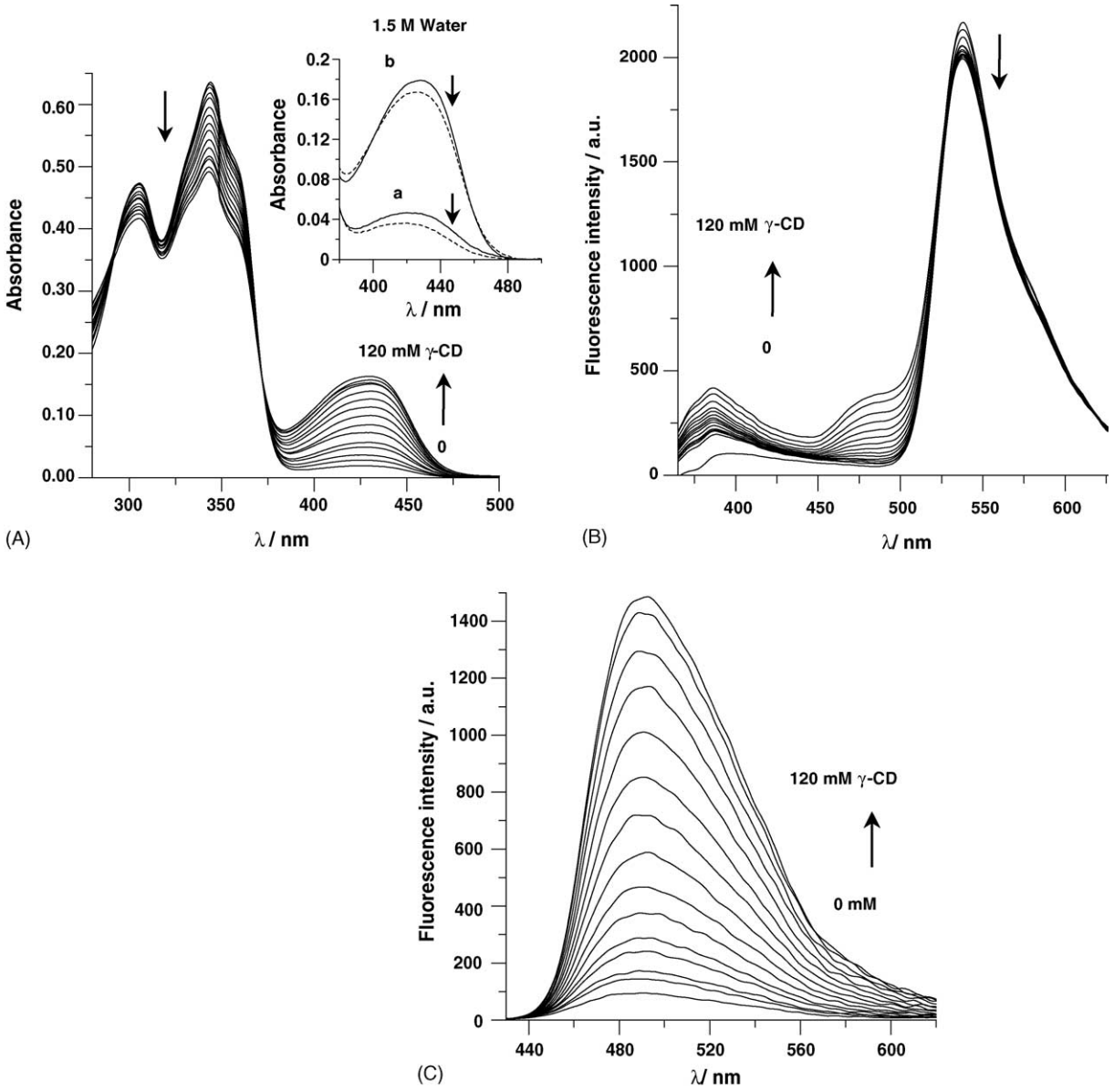

Fig. 1. UV–vis (A) absorption and (B, C) emission spectra of 3-hydroxygflavone (3HF) in *N*,*N*-dimethylformamide (DMF) in presence of γ-CD (see Scheme 1 for the values of $[CD]_0$). The inset of A shows the week effect of addition of 1.5 M of water on the 420 nm absorption band of 3HF in DMF in absence (a) and in presence (b) of 120 mM of γ-CD. For (B) and (C) the excitation wavelengths were 340 and 420 nm, respectively.

DMF. The equilibrium constant at 293 K for β-CD is 0.2 M^{-1}. Supposing that both CDs have comparable number of water molecules due crystallization, one can conclude that the observed deprotonation of 3HF in presence of CD is not due to the trace of amount of water content in these cavities, but rather it is rising from an interaction with the OH-groups of the host. On the other hand, adding maltose (two linked glucose units lacking a hydrophobic nanocavity but having eight OH groups) to the 3HF/DMF solution, we have observed a very weak (about 5%) increase of the absorption intensity of the anion. Thus, using γ-CD in DMF, 3HF is included into its cavity, and the deprotonation of its OH group by the H-bonding network (16 OH groups at the largest gate) of the host is enhanced and stabilized by its cooperativity and the hydrophobic effect of the nanocage. Hydrophobic interactions (between the aromatic part of the guest and the D-glucopyranose units of the host) can help in the stabilization process of the trapped anion, and thus of the formed confined nanostructure.

Fig. 1B and C show the emission spectra of 3HF in DMF solutions of γ-CD. In absence of CD, the 380 nm emission is due to that of 3HF molecules bonded to DMF, while that observed at 530 nm is from zwitterionic structures formed upon an intramolecular proton-transfer reaction in the enol (E) forms of 3HF. A weak emission appears around 480 nm and was assigned to that of anionic (A) species, in agreement with previous reports [14–20,37,40,41]. When exciting at 420 nm, where caged A absorbs, the emission intensity

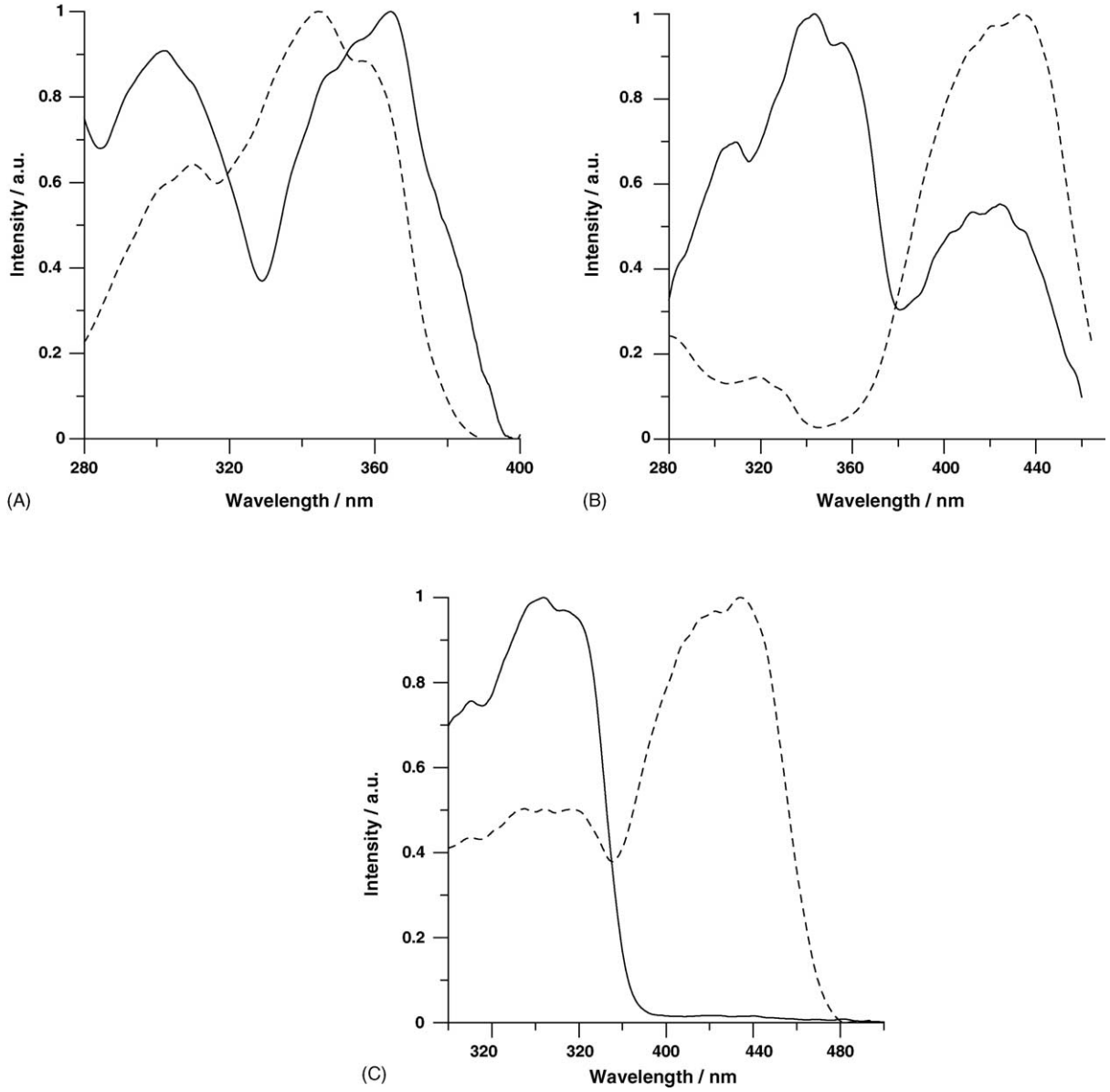

Fig. 2. Excitation spectra of 3HF in DMF (solid line) and in presence of 120 mM of γ-CD (dashed line) observed at (A) 420 nm, (B) 480 nm, and (C) 580 nm.

increases. However addition of CD and excitation of E (absorbing at 330 nm), shift the normal emission (380 nm) to shorter wavelengths and increase its intensity, contrary to the absorption one at this excitation wavelength. This indicates that a population of caged 3HF is H-bonded to CD but does not undergo a proton-transfer reaction. The emission quantum yield of these nanostructures is higher than that of 3HF bonded to DMF. Further more, Fig. 1B shows an increase of A emission intensity (480 nm) and a decrease of the zwitterionic (Z) tautomer one (530 nm) upon addition of CD. The excitation spectrum of A emission (Fig. 2B) shows a ∼15 nm red-shift in γ-CD solution (435 nm) when compared to that recorded in DMF (420 nm). This suggests a larger relaxation of A inside the hydrophobic nanocavity, and accounts for its stability. We notice also that the excitation spectrum of the normal emission at 420 nm (Fig. 2A) in presence of γ-CD is different from that observed in neat DMF, indicating caged E forms of 3HF have different electronic distribution from that of H-bonded E to DMF, and most probably hydrophobic interactions of the cage play a role in this difference. Finally, in presence of CD the excitation spectrum recorded at 580 nm is a combination of those of caged A and free E forms (Fig. 2).

Femtosecond (fs) experiments on 3HF in DMF have shown that proton motion in H-bonded enol forms of the dye to DMF occurs in ∼500 fs to give excited A and 5 ps to give excited Z [40]. The lifetime of A and Z are 1.8 ns and 270 ps, respectively. While that of weakly H-bonded forms is about 5 ps. Fig. 3 shows 480 nm emission decays in absence and presence of γ-CD. Exciting at 371 nm and in absence of CD (Fig. 3A), the decay fits to a three-exponential function with

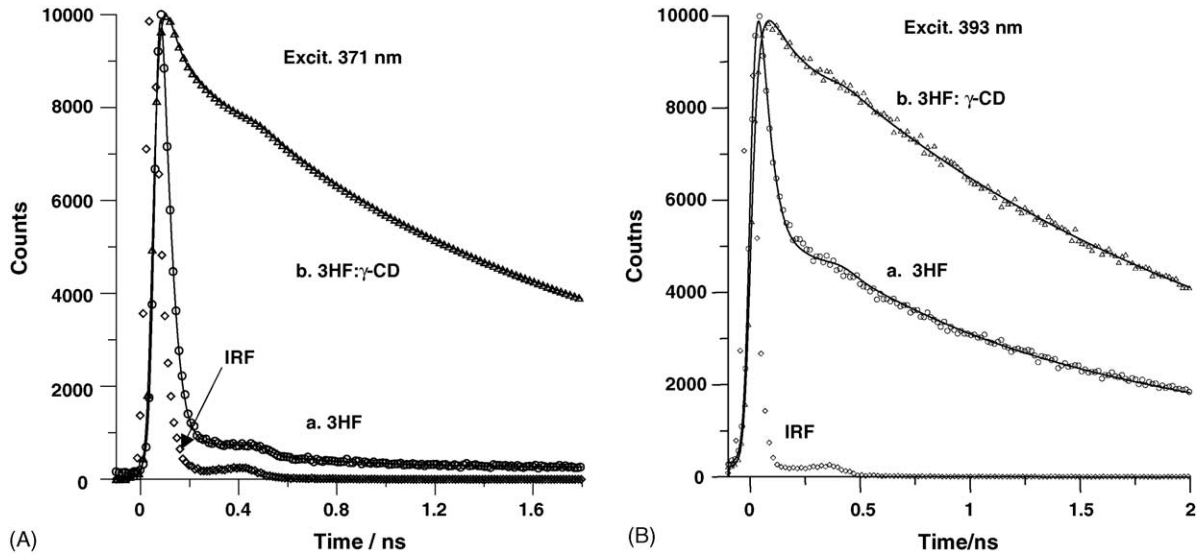

Fig. 3. Emission decays (magic angle) of 3HF in DMF at 480 nm (a) without and with (b) 120 mM of γ-CD upon ps-excitation at (A) 371 nm and (B) 393 nm. See text for the values of lifetimes and pre-exponential factors.

time constants of ∼10 ps (99%), 273 ps (0.7%) and 1.9 ns (0.3%). The contribution of the ns-component assigned to A emission is very small. Addition of 120 mM of γ-CD, gives time constants of ∼12 ps (68%), 270 ps (7%) and 2.14 ns (25%). A clear increase of the ns component is recorded, and therefore the 2.14 ns lifetime is assigned to caged A. We have not observed any significant risetime (within the 10 ps time resolution of the ps-apparatus) at this wavelength, and which could be assigned to the photoformation of caged A. In absence of CD, intermolecular proton-transfer reaction between 3HF and DMF happens in 0.5 ps to give excited A [40]. The short lifetime (about 12 ps (12%) at 400 nm) of caged structures emitting at the blue side suggests a fast intermolecular reaction with the host. Gating the decay at 560 nm or at longer wavelengths (Z emission) shows a 12 ps rising component (limited by the time resolution of the apparatus) comparable to that (5 ps) observed in neat DMF, and therefore assigned to the formation of Z from 3HF bonded to DMF [40]. When exciting at 393 nm (Fig. 3B), the contribution of A emission (1.8 ns) at 480 nm in neat DMF is 18%, and 60% in presence of 120 mM of γ-CD (2 ns). Fig. 4 shows time-resolved emission spectra of 3HF excited at 371 nm and in presence of 120 mM of γ-CD. The figure shows the absence of equilibrium between Z and caged A emitting respectively at the green and blue regions.

Comparing now the effect of nanocavity size, for β-CD (smaller cavity) and exciting at 371 nm, caged A emission lifetime becomes relatively longer (2.34 ns) than that found in γ-CD (2.14 ns), suggesting a stronger confinement of β-CD. As said in Section 1, 3HF has been studied in several media, and a relevant work on confined media to the present one was carried in micelles [25]. Using Triton X-100 micelles, the emission intensity of Z decreases and its decay at 530 nm shows a bi-exponential behavior: a time constant of less than 0.8 ns (limited by the time resolution of the apparatus) and 1.9 ns. [25]. The bi-exponential behavior was interpreted in terms of two different environments for Z tautomer in the micelles. However, the possibility of A emission contribution in the 530 nm signal was not considered. The longest time (1.9 ns) is similar to the one observed in CD, and which we assign to the emission lifetime of caged A. In presence of micelles like SDS or Triton X-100, the anionic structure of 3HF should contribute to the emission decay (as it does in the

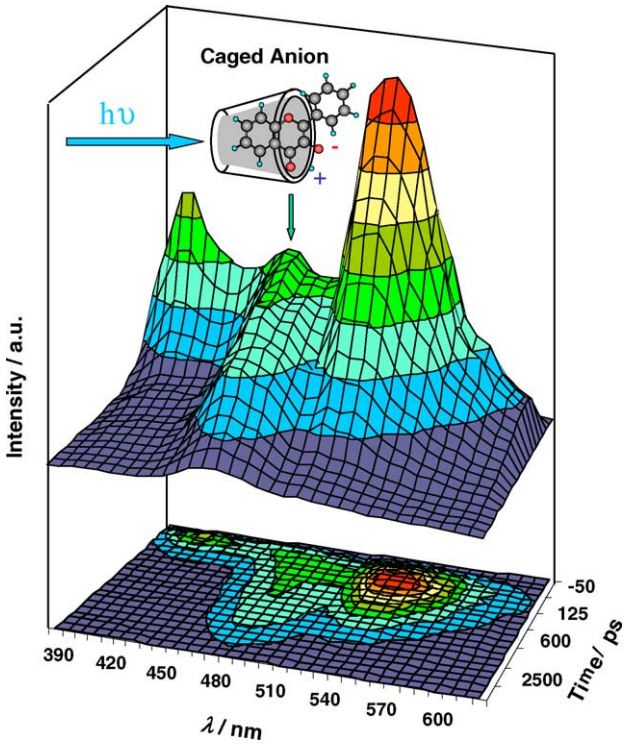

Fig. 4. Time-resolved emission spectra of 3HF in a DMF solution containing 120 mM of γ-CD. The excitation wavelength was 371 nm.

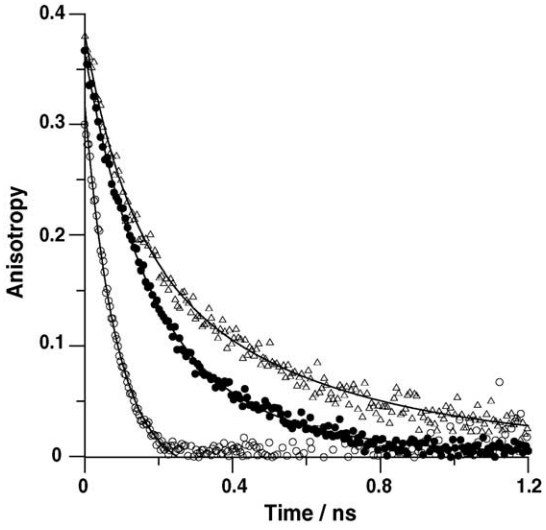

Fig. 5. Anisotropy ($r(t)$) decay of 3HF in DMF and in presence of 120 mM of γ-CD. In DMF: excitation at 371 nm and emission at 480 nm (and 560 nm) (◯). In presence of γ-CD: excitation at 371 nm and emission at 480 nm (△, and excitation at 393 nm and observation at 480 nm (●).

steady-state emission spectrum), as we show here in presence of CD in DMF.

To get information on the rotational times (ϕ) of the complex, we performed time-resolved anisotropy ($r(t)$) measurements exciting at 393 nm (Fig. 5). For 3HF in pure DMF, the decay of $r(t)$ at 480 nm (and at 560 nm) fits to a single exponential function giving a rotational time $\phi = 85 \pm 10$ ps (90 ps when exciting at 371 nm). Rotational times of 50 to 130 ps have been found for comparable (in size) aromatic molecules showing an ultrafast proton transfer [5]. Modeling 3HF as a prolate ellipsoid, non-hydrated rotor, and using the hydrodynamics theory, one gets orientation relaxation times of 90 and 30 ps under stick- and slip-boundary condition limits, respectively. The experimental value is close to the expected one under stick conditions suggesting that an attachment of the solvation shell to 3HF. This agrees with the existence of H-bonding interaction between the dye and the solvent molecules. For 3HF:γ-CD solution excited at 393 nm and observed at 480 nm, the fit of $r(t)$ decay gives two times: $\phi_1 \sim 169 \pm 20$ ps (80%) and $\phi_2 \sim 474 \pm 50$ ps (20%). Exciting at 371 nm and observing at 480 nm, the values of the times do not change (within the errors of experiment and fitting), while the contributions of ϕ_1 component is 40% and that of ϕ_2 one is 60%. Using β-CD these times are $\phi_1 \sim 150$ ps (50 %) and $\phi_2 \sim 438$ ps (50%). Based on these experiments, we attribute ϕ_1 to the rotational relaxation time of caged A. This time for both cavities of CD is twice that of free Z in DMF, indicating a docking of A in the cavities. The second relaxation time, ϕ_2, which is an overall rotational time of anion:CD complexes is longer for γ-CD complex reflecting the difference in complexes geometries and volumes. Modeling γ-CD a prolate ellipsoid, the stick condition limit gives 415 ps as a rotational time, comparable to the observed value. Finally, the initial value of $r(t)$ is ∼0.38, very close to the ideal one (0.4), indicating that the emission transient moment of caged A is almost parallel to that of its absorption.

4. Conclusion

The present results show a clear hydrophobic stabilization of caged anion through a proton transfer to a cooperative H-bonding network of CD, and suggest a fast intermolecular proton-transfer reaction between the caged enol and host. No Z formation rises from caged A or E. The unusual phenomenon reported here for guest:host CD complexes is relevant to those found in enzymatic reactions where hydrophobic as well as hydrophilic effects play a key role in the biochemical machinery.

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