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Abstract
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Chemistry

Role of Solvent in Excited-State Proton Transfer in Hypericin

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Received: January 6, 1994; In Final Form: May 13, 1994®

The excited-state proton transfer of hypericin is monitored by the rise time (~6–12 ps in the solvents investigated) of the component of stimulated emission corresponding to the formation of the long-lived (~5 ns) fluorescent tautomer. The assignment of this excited-state process to proton transfer has been verified by noting that a hypericin analog (mesonaphthobianthrone) lacking labile protons is not fluorescent unless its carbonyl groups are protonated. Recent experimental studies on other systems have suggested that three solvent properties play important roles in excited-state proton transfer: viscosity, hydrogen-bonding character, and dynamic solvation. We find that for hypericin, in a range of protic, aprotic, hydrogen-bonding, and non-hydrogen-bonding solvents in which the viscosity changes by a factor of 60 and the average solvation time changes by a factor of 100, the excited-state proton-transfer rate of hypericin is uncorrelated with these properties and varies not more than a factor of 2 (~6–12 ps) at room temperature. The relative contribution of the bulk solvent polarity is considered, and the role of intramolecular vibrations of hypericin on the proton-transfer rate is discussed.

Introduction

Hypericin (Figure 1) is a naturally occurring polycyclic quinone that has received recent notoriety for its antiviral capacity, in particular its ability to deactivate the human immunodeficiency virus (HIV).[1] The antiviral activity of hypericin requires light.[2] Hypericin is also very closely related, both structurally and spectrally, to the chromophore of the photoreceptor complexes of the protozoan ciliates *Stentor coerulus*[3] and *Blepharisma japonicum*.[4] The hypericin-like chromophore is responsible for the photophobic and phototactic responses of the microorganism.[3,4] Optical excitation of hypericin-like chromophores produces both singlet oxygen[5] and a pH decrease.[6]

Figure 2 gives the steady-state absorption and fluorescence spectra of hypericin in octanol. The spectra of hypericin are very similar in all solvents in which it is soluble with the exception of shifts in absorbance and emission maxima. In previous work we have shown that in hypericin the ground state, and consequently the excited state, is inhomogeneous. Figure 3 presents a kinetic scheme based upon our current knowledge of the hypericin photophysics, which is discussed in detail elsewhere.[7,8] The salient observations consistent with this scheme are the following:

1. Immediately upon optical excitation a transient species is produced whose absorption lies between 580 and 650 nm, depending upon the solvent. In methanol measurement of the decay of this absorbance or of the rise time for the bleaching of the ground-state absorption, which overlaps the spectrum of the newly formed excited state, yields a lifetime of ~5–6 ps.

2. The hypericin analog with no hydroxyl groups and hence no intramolecular proton source, mesonaphthobianthrone, provides no absorption or emission in the visible region of the spectrum in aprotic solvents such as DMSO, unlike hypericin. In concentrated sulfuric acid, however, the absorption and emission spectra of the analog are very similar to those of hypericin. We thus interpreted these data in terms of the necessity of protonated carbonyl groups for the production of fluorescence at wavelengths longer than 580 nm. This result indicates that in the ground state at least one of the carbonyls of hypericin is protonated. This result also suggests the likelihood of excited-state tautomerization in hypericin.

3. The occurrence of excited-state tautomerization in hypericin is verified by the rise time of the stimulated emission signal,

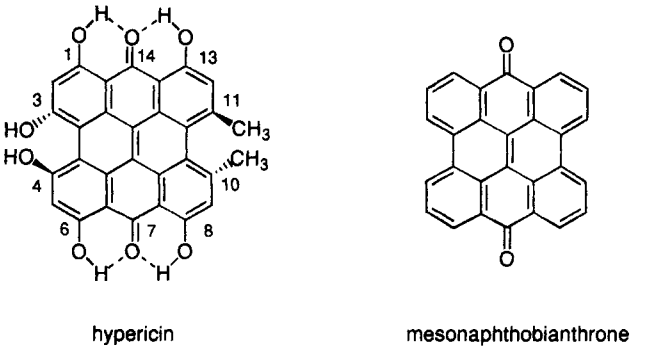

Figure 1. Structures of hypericin and mesonaphthobianthrone.

which measures the excited-state population. In methanol, the rise time of the stimulated emission is within experimental error identical to the decay of the absorption transient produced upon optical excitation. Another confirmation of the excited-state proton-transfer process is that in H_2SO_4 there is no transient with time constant > 1 ps.[8] This indicates that in the ground state the entire population of hypericin is already protonated and does not require an excited-state reaction to produce the long-lived fluorescent species (Figure 4).

We have suggested that upon light absorption hypericin undergoes excited-state proton transfer in ~5 ps in MeOH and ~9 ps in DMSO.[7,8] This small variation in rate has led us to inquire in more detail about the role of the solvent on the excited-state proton transfer.

The scheme presented in Figure 3 is the simplest that is consistent with the experimental data (see Conclusions).[7,8] Two species are believed to be in equilibrium in the ground state, N and MT: normal, or untautomerized, and monotautomerized forms of hypericin. Evidence for this is the appearance in the stimulated emission of an instantaneous component (N*) decaying with one lifetime and the appearance in transient absorption and bleaching measurements of another component decaying with a different lifetime (MT*). We propose that negligible amounts of the ditautomerized form exist in the ground state because in the absence of strong acid no long-lived (nanosecond duration) fluorescence appears instantaneously. Measurements of the hypericin analog lacking hydroxyl groups, mesonaphthobianthrone, suggest that protonation of both carbonyl groups of hypericin is a prerequisite for long-lived fluorescence. The decay of MT* matches the rise of DT*, demonstrating their kinetic

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® Abstract published in *Advance ACS Abstracts*, July 15, 1994.

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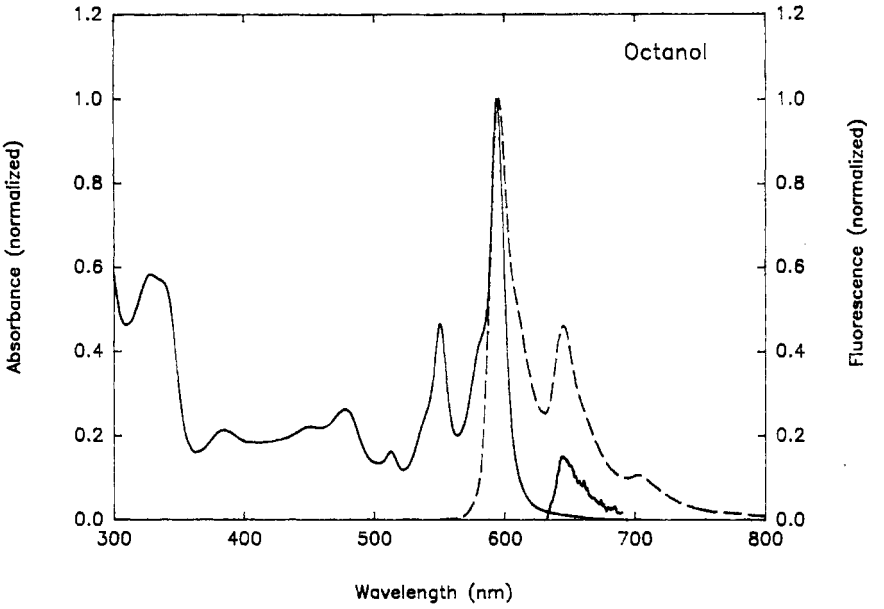

Figure 2. Normalized fluorescence spectrum (- - -) and absorption spectrum (—) of hypericin in octanol. The steady-state emission spectrum bears a "mirror symmetry" relationship to the visible portion of the absorption spectrum. We attribute this part of the absorption spectrum to the presence of ground-state MT (see Figure 3). The solid curve centered at ~650 nm is the spectrum of the stimulated emission that appears instantaneously and decays in ~12 ps (Figures 5 and 7). It is the "zero time" curve from Figure 4 scaled according to the relative amplitudes of the components of stimulated emission appearing instantaneously and with a finite rise time in the region from 640 to 645 nm (Figure 7 and Table 1). We propose that this emission arises from untautomerized hypericin that exists in equilibrium with a monotautomer in the ground state.

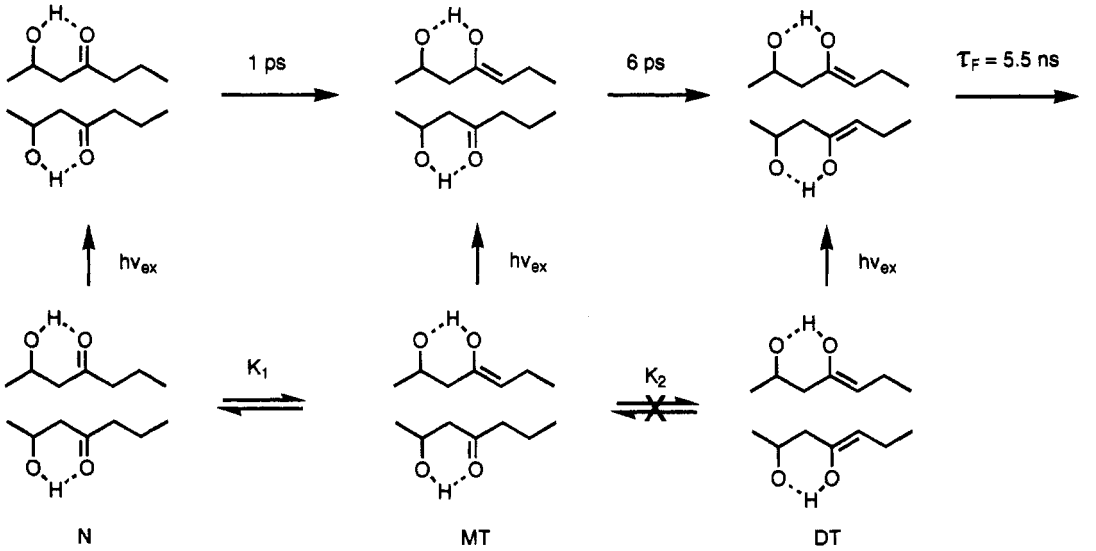

Figure 3. Proposed kinetic scheme for hypericin in the ground and excited states. The values quoted are for methanol. The time constant of 1 ps given for the conversion of N* to MT* is an upper limit determined by the duration of the laser pulse. In other solvents, for example octanol (Figure 7), this time constant is larger. Hypericin is represented schematically: only two of its six hydroxyl groups are pictured. We note that the tautomeric forms of DT can exist with the proton being donated from the "upper right" and the "lower left" hydroxyl groups as well as by the "upper left" and "lower left" hydroxyl groups, as indicated in the figure. The delocalization of the double bonds upon tautomerization may contribute significantly to the intramolecular component of the reorganization energy. We have tentatively assigned the rapid decay of N* to formation of MT*. Other nonradiative pathways such as internal conversion are also a possibility, as is demonstrated by the anthraquinones.[35] We note, however, that both the triplet yield and the fluorescence quantum yield of hypericin have been reported to be very high and that $\phi_F + \phi_{ISC} \sim 1$.[5] It thus seems unlikely that other nonradiative processes, besides proton transfer, play a significant role in the deactivation of N*. A problem for which at present we do not have a completely satisfactory response is why we observe no emission from MT*. It may be that there is not a large enough population of the species to be detected in the midst of all the other transients observed. This question requires further investigation.

relatedness. It is likely that N* tautomerizes to form MT* and that this proton-transfer step represents the component of stimulated emission appearing instantaneously and decaying rapidly. For completeness, we note that it may be possible that N* undergoes a two-proton transfer reaction that converts it *directly* to DT*. Song, Yamazaki, and co-workers[9] have presented results on hypericin from which they conclude that excited-state intramolecular proton transfer does not occur. All of their conclusions, however, are based on observations of the long-lived fluorescence that is produced from the excited state whose duration is only several picoseconds. While their conclusions are thus not appropriate for the primary photoprocesses of hypericin, they may be relevant to the tautomer, which we refer to as DT*. The light-induced pH drop produced by hypericin may result from the intermolecular deprotonation of the tautomer by the solvent.

In this article the excited-state tautomerization of hypericin is studied in a range of solvents that vary greatly in their viscosity, their average solvation time, their ability to form hydrogen bonds with the solute, and their polarity. The choice of solvents and solvent properties studied was determined by reports in the literature suggesting they play an important role in other proton-transfer systems. Of all the solvent properties investigated, only

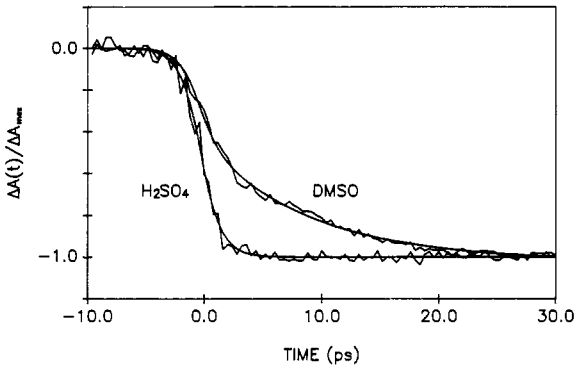

Figure 4. Comparison of the rise time for the formation of excited-state transients as measured by the delay time required to bleach maximally the ground-state absorption[7,8] (Table 1). This rise time is finite for hypericin in an aprotic solvent where a portion of the ground-state population is not tautomerized (DMSO). But in a solvent where the entire ground-state population is protonated (H_2SO_4), the rise time is instantaneous. The fits to the data are as follows: DMSO, λ_{probe} = 610 nm, $\Delta A(t) = 0.23 \exp(-t/9.6) - 0.41$; H_2SO_4, λ_{probe} = 630 nm; the bleaching of the ground-state absorption is complete within the time resolution afforded by the system, and the excited state formed is long-lived on the time scale of the measurement.

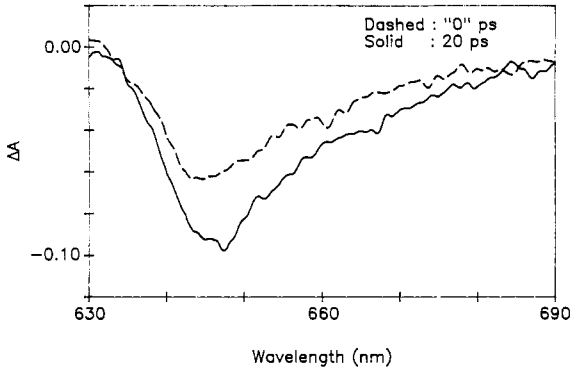

Figure 5. Time evolution of stimulated emission of hypericin in octanol.

polarity was well correlated with the proton-transfer time. We compare results obtained in these model system for excited-state proton transfer with those of the more complex system, hypericin, whose fascinating biochemical action and enormous medicinal potential have clearly been demonstrated to depend on light.

Materials and Methods

Hypericin was obtained from Carl Roth GmbH & Co. and used without further purification. The hypericin analog, mesonaphthobianthrone (Figure 1), was prepared as described by Koch et al.[10] Solvents were obtained from Aldrich. Fluorescence spectra were measured with a Spex Fluoromax. The time-resolved absorption (stimulated emission) experiments were performed with ≤1-ps resolution with the apparatus described elsewhere.[11-13] Transient absorption spectra were obtained with a liquid nitrogen cooled charge-coupled device (CCD) (Princeton Instruments LN/CCD-1152UV) mounted on an HR320 (Instruments SA, Inc.) monochromator with a grating (1200 grooves/mm) blazed at 5000 Å. The CCD pixels were binned such as to allow simultaneous collection of both the probe and the reference beams, I and I_0, respectively, of the transient absorption spectrometer. For the absorption and stimulated emission experiments, identical kinetics were observed whether the pump beam was rotated parallel, perpendicular, or at the magic angle (54.7°) to the probe beam. Unless otherwise indicated, experiments were performed at room temperature, 22 °C. Measuring excited-state kinetics by the increase in probe transmission owing to stimulated emission is a well-known technique. See ref 23 for an example of its application to a system executing excited-state proton transfer, 3-hydroxyflavone. Temperature-dependent measurements were performed with an Air Products system. A helium expander module (DE-202) was connected to a water-cooled compressor (HC-2) for helium exchange. The cryostat was evacuated by a Welch Duo Seal mechanical pump.

In all cases the pump–probe data include a contribution from stimulated emission that grows in with a finite rise time and a contribution from stimulated emission that appears instantaneously. The component with the finite rise time is represented by a rising exponential with a positive prefactor $a_1[\exp(-t/\tau_1) - 1]$. For large values of t, the amplitude of this term is determined by the stimulated emission corresponding to the long-lived—several nanoseconds—fluorescent state that does not decay on the time scale of the experiment. The instantaneous component of stimulated emission is represented in the data-fitting analysis by an exponential with a negative prefactor, $-a_2$. In addition, long-lived absorption owing to the presence of the solvated electron[8] may in some cases need to be taken into account by a constant, c. There are thus three possible factors to be considered in the pump–probe data: (1) stimulated emission with a finite rise time that arises from a long-lived fluorescent state; (2) instantaneous components of stimulated emission; and (3) long-lived transient absorption owing to the presence of the solvated electron. The pump–probe data are thus fit to the following form, which takes into account the above contributions in the order in which they have been discussed:

$$\Delta A(t) = a_1[\exp(-t/\tau_1) - 1] - a_2 \exp(-t/\tau_2) + c \quad (1)$$

The construction of the spectrum (Figure 2) of the species, N*, giving rise to the instantaneous component of stimulated emission that decays rapidly is performed as follows. First, the spectrum of the stimulated emission at "time zero" is obtained (Figure 5). Because this spectrum is obtained at zero time, it does not include contributions from the state that grows in with a finite time constant. Second, the amplitude of this spectrum at a given wavelength, with respect to that of the steady-state spectrum, is determined from the ratio of the amplitude of the component of stimulated emission appearing instantaneously, [N*], to that of the component appearing with a finite time constant from [MT*] (Figures 3 and 7 and Table 1). It might be objected that the shape of this spectrum does not accurately represent that of the transient in question because of the presence of other species absorbing at the probe wavelength. A transient species with significant oscillator strength in this spectral region is the solvated electron.[8] We note that the absorption spectra of the solvated electrons in methanol, ethanol, and 2-propanol are all very broad and that the maximum of the spectrum shifts to *longer* wavelength with increasing size of the alkyl chain.[14] For 2-propanol, for example, the maximum is at ~800 nm and the absorbance tails off slowly toward shorter wavelengths. The spectrum of the solvated electron in octanol would be expected to be shifted even farther to the red. We thus conclude that the distortion of the emission spectrum that we attribute to N* from the solvated electron is negligible and that if any distortion were to be expected, it would appear on the red, not the blue, edge of the spectrum. On the other hand, we cannot yet exclude the possibility that excited-state absorption from MT* distorts the observed transient spectrum.

Results

Figure 5 presents the negative-going transient absorbance signal of hypericin in octanol. This signal is attributed to stimulated emission from excited-state hypericin because it is observed in a region where there is no ground-state absorbance. The signal thus cannot be assigned to ground-state bleaching. The salient feature of Figure 5 is that *a finite time is required for the stimulated emission to be fully developed.* We have thus used

TABLE 1: Dependence of Proton-Transfer Times in Hypericin in Selected Solvents[a]

solvent	η (cP)[b]	$\langle\tau_s\rangle$[c] (ps)	$E_T(30)$[33]	decay of time[d,f] (ps)	rise time[e,f] (ps)	I_F/I_S[f,g]
MeCN	0.37	0.9	45.6	10.8 (600 nm)	11.6 (645 nm)	1.0 (645 nm)
BuCN	0.57	3.6	43.1	11.7 (600 nm)	10.4 (645 nm)	1.2 (645 nm)
CCl_4/BuCN[h]					12.8 (645 (nm)	
MeOH	0.57	3.3, 6.2	55.4	6.4 (600 nm)	6.7 (645 nm)	0.84 (645 nm)
DMSO	1.99[i]	3.1, 1.2	45.1	9.6 (610 nm)	9.2 (658 nm)	0.43 (658 nm)
BuOH	2.75	61	05.2	7.5 (600 nm)	11.0 (645 nm)	0.75 (645 nm)
OcOH	7.36[i]		48.3	10.3 (610 nm)	12.6 (645 nm)	0.49 (640 nm)
						0.51 (645 (nm)
EgOH	18.25	100	56.3	5.8 (600 nm)	6.4 (645 nm)	1.2 (645 nm)
						0.99 (650 nm)

[a] All experiments were performed at room temperature, 22 °C. [b] Except where otherwise noted, the solvent viscosity at 22 °C.[32] [c] Average solvation time as determined from measurements of time-resolved Stokes shifts. The cited solvation times are obtained from the tabulation in ref 15. [d] Decay of the excited-state absorption as measured by the rise time for the ground-state bleaching of hypericin. The absence of a value indicates that the measurement was not performed. [e] Time constant for the "long" rising component of stimulated emission, which is attributed to the intramolecular proton transfer in hypericin. [f] The value in parentheses is the probe wavelength. [g] Ratio of the component of stimulated emission appearing instantaneously, I_F, to that appearing with a finite rise time, I_S. The dependence of this ratio on solvent can be interpreted in terms of a ground-state equilibrium between N and MT (Figure 3) insofar as the emission spectra of N* and MT* do not change greatly with respect to solvent at the probe wavelength. [h] The ratio of solvents in the mixture is 1/4 and is based on volume. This corresponds to a solution of 0.18 mole fraction in CCl_4. The viscosity of the mixture was not determined. [i] Viscosity at 25 °C.[34]

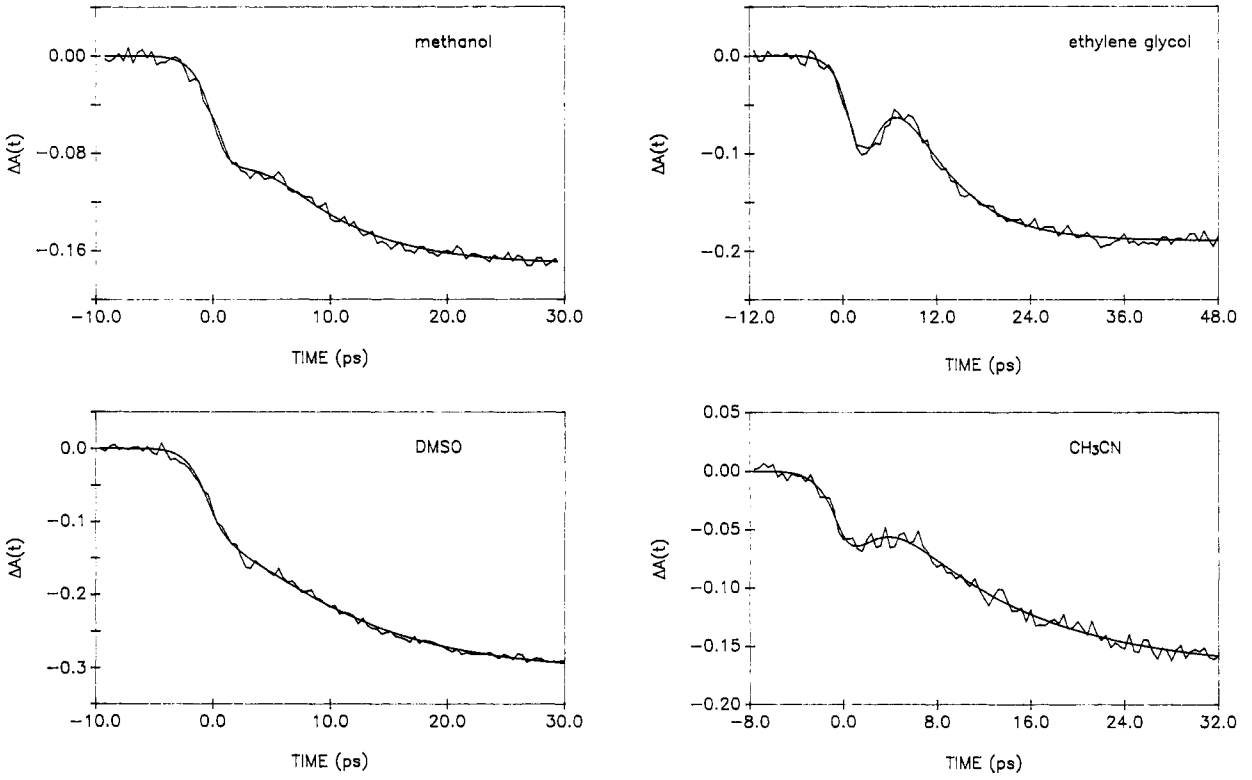

Figure 6. Time-resolved stimulated emission profiles of hypericin in methanol, DMSO, ethylene glycol, and acetonitrile; λ_{ex} = 588 nm; see Table 1 for further details: methanol, $\Delta A(t) = 0.17[\exp(-t/6.7\text{ ps}) - 1] - 0.14\exp(-t/1.9\text{ ps})$; DMSO, $\Delta A(t) = 0.30[\exp(-t/9.2\text{ ps}) - 1] - 0.13\exp(-t/1.9\text{ ps})$; ethylene glycol, $\Delta A(t) = 0.45[\exp(-t/6.4\text{ ps}) - 1] - 0.53\exp(-t/2.4\text{ ps}) + 0.26$; acetonitrile, $\Delta A(t) = 0.19[\exp(-t/11.2\text{ ps}) - 1] - 0.19\exp(-t/1.4\text{ ps}) + 0.025$.

the rise time of stimulated emission as a measure of the time required to produce the long-lived excited-state species. We have argued[8] that measurable fluorescence (by steady-state or conventional photon-counting techniques) in hypericin is obtained only from the species with both carbonyl groups protonated.

In all the solvents in which we have investigated hypericin, except strong acids such as sulfuric and triflic acid where it is expected to have both carbonyl groups protonated, we observe *a finite "rise time" for the ground-state bleaching.* Such a phenomenon is most easily rationalized by the presence of an excited-state species, produced within the excitation pulse, that has oscillator strength in the same region as the ground-state molecules. We have directly observed such an excited-state species in transient absorption measurements.[7,8] As indicated by the summary presented in Table 1, the agreement between the lifetime of this excited state and the rise time of the "slow" component of the stimulated emission is excellent. We have discovered that measurement of the rise time for ground-state bleaching provides a more accurate determination of the lifetime of the short-lived excited state that is a precursor, MT*, to the long-lived fluorescent species of hypericin, DT*. Measurement of the decay of the transient absorbance of MT* can be obfuscated by the presence of absorption from the biphotonically produced solvated electron[8] as well as from stimulated emission, or transient absorption from the species producing the stimulated emission.

Figures 6 and 7 also indicate that upon optical excitation a species is created that produces stimulated emission immediately and that subsequently decays. This prompt stimulated emission is consistent with the suggestion made above (and in the caption to Figure 3) that at least two ground-state species are optically excited. Figure 6 and Table 1 suggests that the solvent seems to affect the ground-state equilibrium between N and MT, which

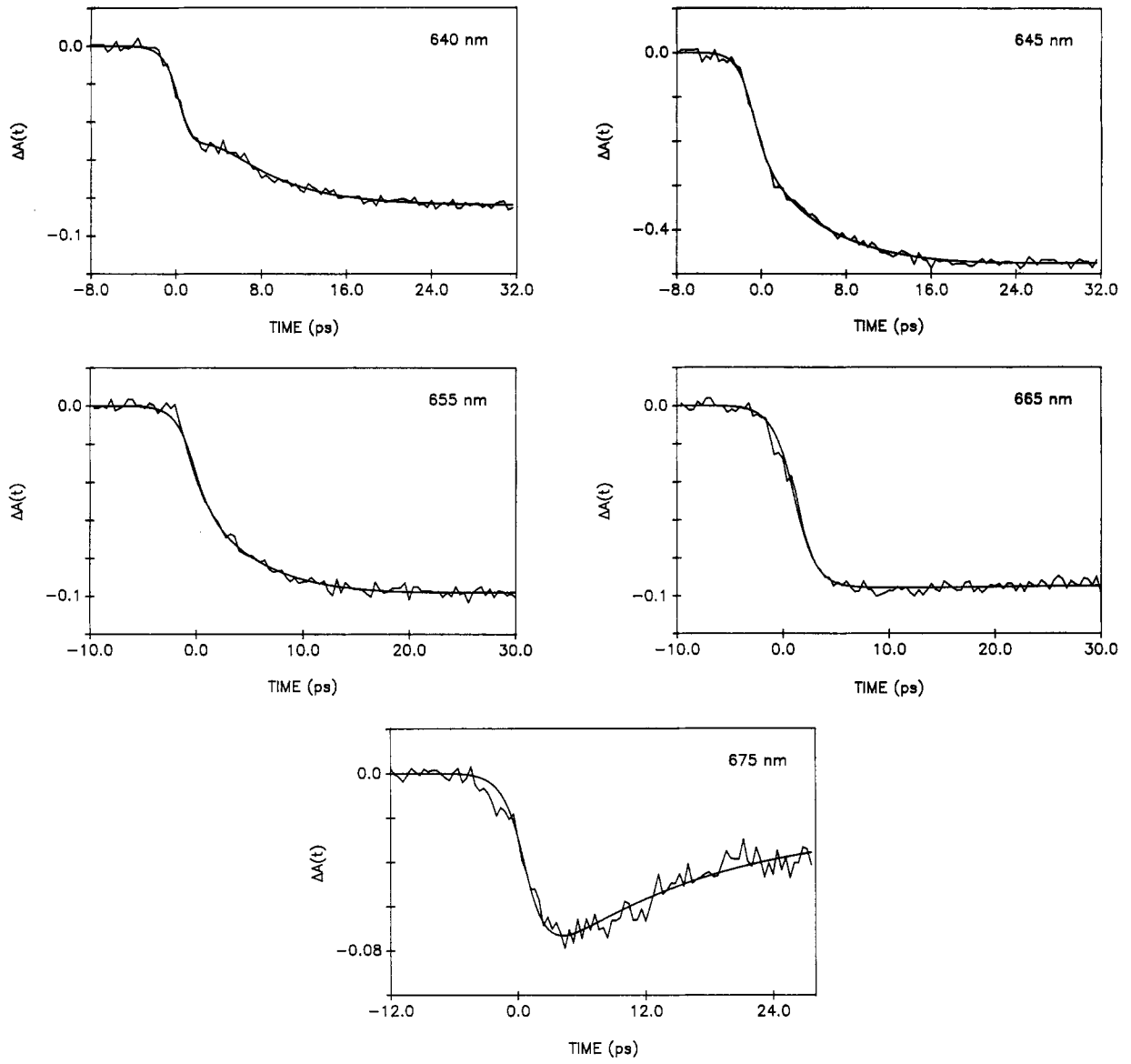

Figure 7. Stimulated emission profiles of hypericin in octanol at different probe wavelengths, λ_{ex} = 588 nm. Probe wavelengths are indicated in the panels: λ_{probe} = 640 nm; $\Delta A(t) = 0.086[\exp(-t/11.0\text{ ps}) - 1] - 0.042\exp(-t/13.4\text{ ps})$; λ_{probe} = 645 nm; $\Delta A(t) = 0.47[\exp(-t/12.6\text{ ps}) - 1] - 0.24\exp(-t/18.8\text{ ps})$; λ_{probe} = 655 nm, $\Delta A(t) = 0.097[\exp(-t/7.6\text{ ps}) - 1] - 0.054\exp(-t/11.0\text{ ps})$; λ_{probe} = 665 nm, $\Delta A(t) = -0.12$; λ_{probe} = 675 nm; $\Delta A(t) = -0.067\exp(-t/16.0\text{ ps}) - 0.022$.

is subsequently manifested in the ratio of the components of stimulated emission appearing with an instantaneous or a finite rise time, I_F/I_S. The component of the stimulated emission that appears instantaneously is not attributable to vibrational relaxation because its decay is the same whether the excitation is at 294 or 588 nm. Furthermore, it does not arise from dynamic solvation of the excited state[15] because it decays equally rapidly in acetonitrile and ethylene glycol, whose average solvation times differ by a factor of 100 (Table 1, Figure 6). We suggest that the rapid decay of N* represents a tautomerization step to MT*. This is discussed in more detail elsewhere.[8]

Figure 7 demonstrates the variation in the stimulated emission kinetics of hypericin in octanol as a function of probe wavelength. Tuning the probe from 640 to 675 nm reveals smoothly varying contributions of the components of stimulated emission that appear instantaneously and that appear with a finite rise time. The instantaneous component is most easily identified at 640 and 675 nm. The simplest interpretation of these data is that the normal form of hypericin, N*, which we suggest gives rise to the instantaneous component, has a fluorescence spectrum that in some regions is more intense than that of the fully tautomerized form, DT*. The solid curve centered at ~650 nm in Figure 2

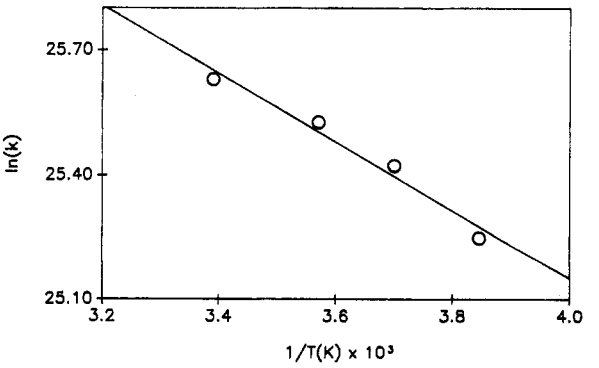

Figure 8. Arrhenius plot of the time constant of the longer-lived component of stimulated emission in hypericin in ethylene glycol.

represents the fluorescence spectrum of N* obtained from the ratio of the instantaneous to the rising components (Table 1).

Measurements of the slower component of the stimulated emission in ethylene glycol over a rather limited temperature range were used to construct an Arrhenius plot (Figure 8). The barrier for this proton-transfer reaction is thus estimated to be 1.5 kcal/mol in ethylene glycol. Lastly, Figure 9 presents a plot

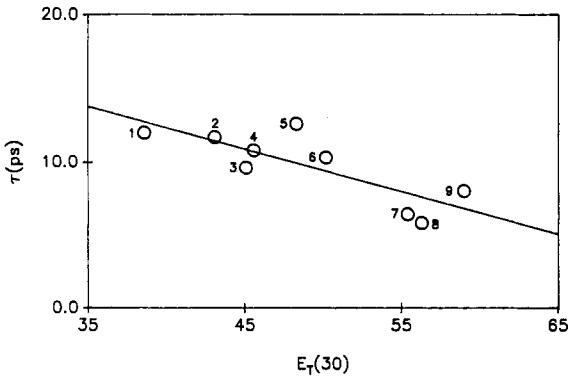

Figure 9. The time constant of the longer-lived component of stimulated emission in hypericin plotted as a function of polarity as measured by $E_T(30)$:[33] 1, diethylene glycol dimethyl ether; 2, butyronitrile; 3, DMSO; 4, acetonitrile; 5, 1-octanol; 6, 1-butanol; 7, methanol; 8, ethylene glycol; 9, 2,2,2-trifluoroethanol.

of the time constant for the slower component against solvent polarity as measured by $E_T(30)$.

Discussion

In a range of solvents, both hydrogen-bonding and non-hydrogen-bonding and protic and aprotic, over which the viscosity and the average solvation time change by factors of 60 and 100, respectively, the proton-transfer time as measured by the rise time of the longer-lived component of the stimulated emission is found to be uncorrelated with these properties and to change by at most a factor of 2. This result is very surprising when it is considered in the context of other examples of excited-state proton transfer.

Hochstrasser and co-workers[16] have observed proton-transfer rates for a 2-phenylbenzotriazole bearing an octyl group on the 5-position that agree very well with the longitudinal relaxation time of the solvent, τ_L. These data are particularly intriguing because despite this dependence on τ_L no time-dependent Stokes shift is observed in this system.

The time-dependent Stokes shift is related to τ_L and is characterized by an average solvation time $\langle\tau_S\rangle$.[15,17] When a solute is promoted to its excited state, its charge distribution is altered. The solvent is no longer in equilibrium and must relax to its new equilibrium structure, thus affording the temporally evolving Stokes shift. Charge-transfer reactions similarly alter the charge distribution of the solute, and in many cases the rates of such reactions have been shown to depend on the dynamic response of the solvent, characterized by τ_L or $\langle\tau_S\rangle$, to the charge-transferred species.[16,17]

In hydrogen-bonding solvents such as alcohols, on the other hand, the ability of the solvent to weaken or to break the *intramolecular* hydrogen bond in 3-hydroxyflavone is the rate-determining factor in the excited-state proton-transfer reaction.[18-23] If both the carbonyl and the alcohol groups of 3-hydroxyflavone are strongly coordinated to different solvent molecules, proton transfer occurs relatively slowly, on a time scale of $\geq$10 ps.[23] If, however, the intramolecular hydrogen bond of the solute is not perturbed (as occurs in hydrocarbon solvents such as methylcyclohexane), excited-state proton transfer is very rapid. Harris and co-workers have measured this time to be $\sim$240 fs.[23] These workers have also proposed that if a *single* alcoholic solvent molecule can form a cyclic hydrogen-bonding interaction with the carbonyl and the alcohol groups of the solute, an even more rapid transfer time of $\sim$80 fs results. Similarly rapid proton-transfer times have been observed in benzothiazole derivatives.[24,25] These results have been interpreted in terms of the wave packet prepared upon optical excitation. The evolution of this wave packet toward the tautomeric form on the excited-state potential surface will initially depend very strongly on the vibrations displaced upon light absorption.[23,24]

The proton-transfer time in hypericin ranges from about 6 to 12 ps in the solvents we have investigated and hence is similar to the proton-transfer time observed in 3-hydroxyflavone and attributed to solute–solvent structures in which two different alcohol molecules are coordinated to the carbonyl and alcohol groups of the solute.[23] Such a state of solvation cannot, however, explain the proton-transfer rates in hypericin because the same results are obtained in both hydrogen-bonding and non-hydrogen-bonding solvents. This suggests that the intramolecular interactions between the O—H···O group formed by the alcohol oxygen, the proton, and the carbonyl oxygen of hypericin are much stronger than any potential hydrogen-bonding interactions with the solvent.

The assignment of the excited-state process to intramolecular proton transfer may be criticized because we do not observe an isotope effect.[8] There is precedent for proton-transfer processes that do not exhibit an isotope effect.[23,25] Whether an isotope effect is observed will also depend on such factors as the degree to which the reaction is nonadiabatic and characterized by tunneling through a potential barrier[26] or if the reaction occurs by means of a barrierless (or small barrier) process in which the role of vibrational motions other than the O—H stretch are important.[27]

Hynes, Borgis, and co-workers[26] have presented a theory of proton transfer in both adiabatic and nonadiabatic limits. Three coordinates play an important role: the coordinate for the proton itself; the intramolecular separation of the two atoms (in this case oxygens) between which the proton is transferred; and a collective solvent coordinate. In this treatment, the electrons are always treated adiabatically; but the proton-transfer process is considered to be in a nonadiabatic or an adiabatic limit depending on the separation of the oxygen atoms. For a large (>2.7 Å) separation, the wave function for the proton is localized about one of the oxygens and a sufficiently large barrier exists that proton transfer must be viewed as a nonadiabatic tunneling process. (The rate of this tunneling process is modulated by the oxygen–oxygen separation and the solvent fluctuations.) If, however, the separation is small (<2.7 Å), the barrier to proton transfer is greatly decreased and the extent to which tunneling contributes to the rate of proton transfer can be greatly reduced. Finally, in reactions that involve the generation of charged or partially charged species, the solvent polarity is expected to accelerate the rate. For example, in a reaction taking covalent reactants to ionic products, the products will be better solvated by a polar substance such as water than a nonpolar substance such a methylcyclohexane. Stabilization of the potential surface for the ionic species with respect to that for the covalent species will lower the point at which they cross and hence decrease the activation energy for the process.[28]

In the case of hypericin, the distances[29,30] between the keto and hydroxy oxygens between which the proton is transferred are all consistent with an adiabatic process: that is, they are all less than 2.5 Å. The solvent dependence of the time constant for the excited-state process is also consistent with its assignment to proton transfer. The time constant decreases with increasing solvent polarity, as measured by $E_T(30)$, and suggests a process that involves the transfer of a charged particle, molecular rearrangement, and charge reorganization.[26,28] Finally, temperature-dependent measurements in ethylene glycol (Figure 8) indicate that there is a small barrier ($\sim$1.5 kcal/mol) between MT* and DT*. This small barrier is in agreement with the short distances between oxygens in hypericin.

Construction of molecular models of hypericin and a recent X-ray structure[29] indicate that the aromatic polycycle is twisted. One might argue that the excited-state transients observed reflect transitions from one form of conformational isomer to another. Because such a process involves a large amplitude motion, it would be expected to be viscosity dependent. In solvents in which the visocisty changes by a factor of 60 we see, however, no more than

a change of a factor of 2 in the time constant of the longer-lived excited-state transient (~6-12 ps). Furthermore, the rate of the excited-state process is completely uncorrelated to viscosity: the small variation in rate cited can be effected just as easily when the viscosity is increased by less than a factor of 2,i.e. from methanol to acetonitrile (Table 1). This excludes the assignment to a conformational transition.

An obvious question that remains is whether intramolecular vibrational modes other than those modulating the oxygen–oxygen separation play a role in the reaction. Resonance Raman measurements will be indispensable in providing a response. Peteanu and Mathies[27] have shown that in the case of 2-hydroxyacetophenone, which is believed to execute a barrierless excited-state proton transfer, there is no displacement in the O—H stretching coordinate upon optical excitation. This result suggests that vibrations other than proton motion are responsible for the initial displacement of the wave packet away from the Franck–Condon region of the excited state. Consistent with these Raman measurements are the observations of Harris and co-workers[23] and of Elsaesser and co-workers[24,25] of proton-transfer rates that are independent of isotopic substitution. Cotton and co-workers[31] have measured the resonance Raman spectrum of hypericin under various conditions and observed bands in the region from ~1620 to 620 cm^{-1}, some of which were tentatively identified.

Conclusions

Measurements of the stimulated emission of hypericin with ≤1-ps resolution have been used to monitor the creation and decay of excited and hence fluorescent states. The excited-state characterized by nanosecond lifetimes and observed in steady-state measurements[8,9] appears in roughly 6–12 ps. The rise time for the appearance of this emission is attributed to an excited-state proton-transfer reaction. The similarity of the rates in both hydrogen-bonding and non-hydrogen-bonding solvents is the most surprising result given what is known about the behavior of 3-hydroxyflavone in these solvents. Polarity is the only solvent property that is well correlated with the proton-transfer time. In addition to the slower, ~6–12-ps rise time, a component of the stimulated emission is observed that appears instantaneously. This component is attributed to a ground-state untautomerized (normal, N) form of hypericin that decays rapidly, most probably by forming the excited-state tautomer MT*. The observation of this component demonstrates the inhomogeneous distribution of hypericin structures in the ground state and hence in the excited state. A proton-transfer reaction with a time constant of 6-12 ps is relatively slow.[19-25] Hypericin thus provides an extremely useful system with which to test current theories of proton transfer.[26]

A possible alternative to the excited-state reaction scheme presented in Figure 3 is that the slower component of stimulated emission arises from a *back-transfer* of a tautomerized species instead of from a *sequential* proton transfer. Other experiments are required to consider this possibility properly.

In conclusion, the primary photoprocess occurring in hypericin is intramolecular proton transfer, whose rate depends on solvent polarity. Understanding the light-induced activity in hypericin is of significance for appreciating its biochemical role in protozoa and exploiting its medicinal activity against viruses.

Acknowledgment. J.W.P. is an Office of Naval Research Young Investigator. M.J.F. is supported by an AASERT training grant from the ONR. F.G. is the recipient of a fellowship from Phillips Petroleum. Professor J. T. Hynes provided many very helpful comments. We thank Professor W. S. Struve for loaning us his low-temperature equipment. We are also grateful to Professor H. Falk for providing us the X-ray coordinates of hypericin.

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