

Quenching of Excited Triplet State Hypericin with Energy Acceptors and Donors and Acceptors of Electrons

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The quenching of the triplet state of hypericin by acceptors of electronic energy and donors and acceptors of electrons was studied by nanosecond laser photolysis. The energy of triplet hypericin is $E_T = 13\,350\text{ cm}^{-1}$, and for acceptors with $E_T < 13\,300\text{ cm}^{-1}$ the observed energy transfer rate constant declines with increasing exothermicity. Quenching of triplet hypericin by amines in acetonitrile and electron acceptors in DMSO occurs via electron transfer. Spectroscopic and kinetic properties and the quantum yield of hypericin radical ion formation were established. The rate constant profiles for electron transfer (in both directions) are consistent with an excited-state reaction occurring before electron transfer. The rate constant of the interaction of reduced hypericin with O_2 was estimated to be $3.8 \times 10^8\text{ M}^{-1}\text{ s}^{-1}$ in acetonitrile.

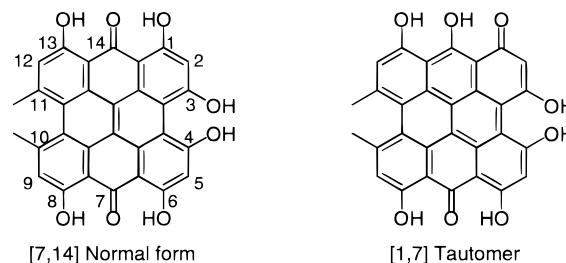
Introduction

Hypericin is a polycyclic quinone and a naturally occurring pigment in plants of the genus *Hypericum* that has long been used as a folk medicine. More recently, it has been found to possess strong photodynamic activity.^{1,2} Hypericin inactivates the human immunodeficiency virus (HIV) in a manner that requires light.^{3–5} Singlet oxygen, superoxide, and proton release have all been observed as a result of its irradiation. This has led to the proposal of several mechanisms for biological action, including type I and type II oxygenation reactions and photo-induced proton release. This question has not been fully resolved, but undoubtedly the primary photoprocesses from the excited singlet and/or triplet states of hypericin have fundamental importance.

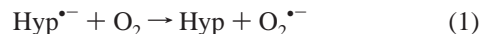
NMR and X-ray crystallographic studies point out that among five energetically reasonable tautomers⁶ the natural and most stable form of hypericin in the solid state and in aprotic solvents is the [7,14] tautomer (Chart 1).^{7,8} Petrich et al. established that in the excited singlet state hypericin undergoes tautomerization on the picosecond time scale after laser excitation in both protic and aprotic solvents.^{9–12} They have, in fact, argued that the biological activity may result from acidification of the surroundings. Evidence suggests that the triplet is the proton donor but does not yet exclude the possibility that the singlet can also donate its proton.¹³ Uncertainty remains regarding which tautomer or tautomers is or are formed;^{9–12,14} the [1,7] tautomer, a reasonable possibility, is also illustrated.

Reactive intermediates have been observed in various photophysical studies of hypericin. Hypericin has been shown to have a high quantum yield of triplet formation (0.5–0.7),^{14,15} and it efficiently generates singlet oxygen,¹⁶ though this may not be the mechanism for its photodynamic action. Superoxide ($\text{O}_2^{\bullet-}$) formation upon irradiation of hypericin has been detected in aqueous dispersions, in solution in dimethyl sulfoxide (DMSO), in aqueous liposomal media, and for hypericin bound to liposome membranes.^{17–19} Superoxide and the radical anion

CHART 1. Tautomeric Forms of Hypericin



($\text{Hyp}^{\bullet-}$) and/or radical cation ($\text{Hyp}^{\bullet+}$) may be directly or indirectly responsible for the photobiological activity of hypericin, and electron-transfer quenching of the fluorescent singlet state has been observed.^{20–22} The literature lacks data about the photophysical parameters of $\text{Hyp}^{\bullet-}$, and the rate constant of the reduction reaction 1 is unknown, though the reaction of anionic aggregates of hypericin with O_2 has been reported.²³



The quenching of hypericin fluorescence by electron acceptors such as quinones, nitro- and azobenzene in DMSO occurs via electron transfer, as established by EPR.²² The quenching of triplet hypericin by duroquinone in ethanol also goes by electron transfer with a rate constant close to the diffusion-controlled limit,¹⁵ and electron transfer to methyl viologen has been observed.²⁰ However, despite knowledge that hypericin is easily reduced, the quenching of the excited singlet and triplet states of hypericin by electron donors is not reported in the literature to the best of our knowledge.

The energy of triplet state of hypericin (^3Hyp) has been estimated in rigid media from the position of the 0–0 vibronic band of the phosphorescence spectrum in ethanol as $13\,190\text{ cm}^{-1}$ at 1.2 K ²⁴ and $13\,250\text{ cm}^{-1}$ at 77 K .²⁵ There is very little Stokes shift in the room-temperature fluorescence of hypericin, but because of the possibility of significant energy relaxation

due to conformational change or tautomerization in less rigid media and on a longer time scale, it is worthwhile to have a reasonable estimate of the triplet energy in solution at room temperature. For example, the triplet state energies of normal and tautomeric forms of the natural antioxidant quercetin (3,5,7,3',4'-pentahydroxyflavone) were estimated using energy transfer in 2-propanol at $\sim 20\,000$ and 7000 cm^{-1} , respectively.²⁶

Here we report a study of the quenching of triplet hypericin (^3Hyp) by different amines in acetonitrile and by electron acceptors in dimethyl sulfoxide. We refine a previous rough estimate of the solution phase triplet energy ($\sim 13\,700\text{ cm}^{-1}$ in ethanol)¹⁴ by using an appropriately chosen set of energy transfer acceptors. Photophysical properties and formation efficiency of $\text{Hyp}^{\bullet-}$ and $\text{Hyp}^{\bullet+}$ radicals are reported, along with rate constants for a series of quenchers that give rise to these species. From these data, it is proposed that it is not the "normal" form of hypericin proper which is being reduced, i.e., that there is a fast process that produces a triplet species which in turn is quenched by electron transfer. Also given is an estimate of the rate constant for the reaction of $\text{Hyp}^{\bullet-}$ with O_2 to produce superoxide. It is found that the radical ions of hypericin are themselves reactive with various electron donors and acceptors, and these data are presented. Finally, it is shown that the rate constant for triplet energy transfer to various acceptors gradually falls off as the acceptor triplet energy falls noticeably below that of hypericin, and it is suggested that this is a rare example of bimolecular energy transfer in the inverted region.

Experimental Section

Hypericin was synthesized and purified as described in the literature.^{27,28} It should be noted that a newer synthesis has appeared.²⁹ Other compounds were obtained commercially and purified by recrystallization or sublimation as necessary. Spectrometric grade acetonitrile and tetrahydrofuran (THF) were used as received. No special precautions were taken to rigorously exclude water. Experiments were carried at $23\text{ }^\circ\text{C}$, and solutions were deoxygenated by Ar bubbling for 15 min.

Absorption spectra were recorded on a UV-2101 PC Shimadzu spectrophotometer. The formation of ground-state charge-transfer complexes between hypericin and any of the quenchers was not observed in acetonitrile or DMSO, as determined by UV/vis absorption spectroscopy in the concentration ranges investigated here.

All flash experiments were carried out using a nanosecond laser photolysis apparatus described elsewhere.³⁰ The solutions of hypericin in acetonitrile, $\text{OD}(532\text{ nm}) \approx 0.1$, $[\text{Hyp}] \approx 1.0 \times 10^{-5}\text{ M}$, and $\epsilon_{\text{max}}(595\text{ nm}) = 4.1 \times 10^4\text{ M}^{-1}\text{ cm}^{-1}$, were irradiated with the second harmonic of a Continuum Surelite Nd:YAG laser (532 nm, 5 ns, 10 mJ). The decay kinetics and transient absorption spectra were recorded with a help of 1P-28 and FEU-84 and -83 photomultipliers. The yield of transients was directly proportional to the energy of the laser pulse. The thermodynamic parameters of the energy transfer were determined in a thermostatic cell over a range of $5\text{--}60\text{ }^\circ\text{C}$. The accuracy of quantum yields, molar extinction coefficients, and various rate constants is estimated to be $\pm 15\%$.

Results

The solvents used in the current study were tetrahydrofuran (THF), acetonitrile, and dimethyl sulfoxide (DMSO). Because the solvent can have an effect on the aggregation and protonation state of hypericin and this should be reflected in the absorption spectrum,^{17,21,23,31,32} it is worthwhile to note that the absorption spectra in these three solvents, shown in Figure 1, are virtually

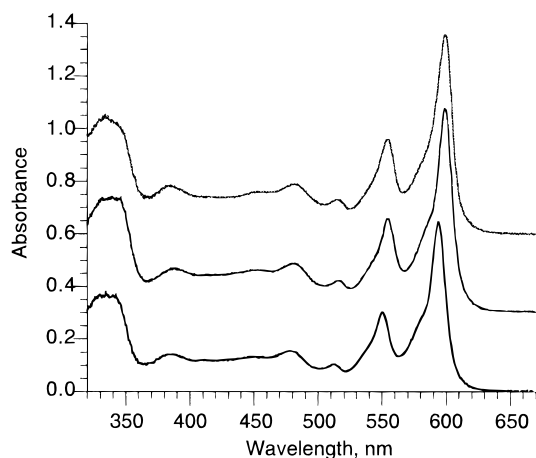


Figure 1. Absorption spectra of hypericin in THF, DMSO, and acetonitrile, from top to bottom. The top two spectra have been offset by 0.6 and 0.3 for clarity. The λ_{max} of the redmost bands are 599, 599, and 595 nm, respectively.

identical and are clearly those of monomeric hypericin. As a result, we will work under the presumption that the monomeric "normal form" of hypericin^{6,33} predominates in all experiments.

The protonation state of hypericin is also an issue, even in organic solvents. A very brief review of the literature is in order. Jardon has measured the pK_a s of hypericin in Brij 35 micelles.³⁴ Two deprotonations are observed, with $\text{pK}_a = 7$ and 11. The difference between the spectra of the neutral and the monoanion is subtle. A deprotonation in a similar pH range near 7 was observed in related electrochemical regions.¹⁷ Yamazaki and Song report a pK_a of Hyp in Triton micelles as 11.7.²¹ The spectral shifts and pK_a value are quite similar to those reported for the second pK_a of Hyp by Jardon.³⁴ Jardon also observes a protic equilibrium at approximately $\text{pH} = 1$ that he attributes to $\text{Hyp}^{\text{H}^+}/\text{Hyp}$.³⁴

In contrast, Falk reports the Hyp/Hyp^- pK_a (for a water-solubilized derivative) at approximately 2.³⁵ The assignment is based on an electrophoresis experiment on a cellulose support, in which the spot assigned to Hyp^- migrates over a wide pH range. Recent experiments using specifically protected hypericin derivatives³⁶ and atmospheric pressure chemical ionization mass spectrometry³⁷ strengthen this conclusion. On the basis of electrochemical data, Gerson has argued that hypericin is singly deprotonated in the solvents we used unless water is rigorously excluded.³² The spectra reported in rigorously dry solvents are apparently the same as those observed by Jardon at low pH. We were unable to reproduce this effect of rigorous dryness of solvent on absorption or emission spectra.

The spectra shown in Figure 1 are consistent with those Gerson and Falk attribute to the monoanion and others attribute to the neutral. For clarity of reading, because of the ambiguity in the literature about the aqueous pK_a and because of the somewhat unpredictable effect of solvent on pK_a , we have written using an arbitrary convention that hypericin is found in its neutral form (Hyp) in organic solvents. Therefore, for instance, we will refer to the one electron reduced compound as $\text{Hyp}^{\bullet-}$. At the same time, we must recognize that the predominant ground-state species under our conditions may very well be the monoanion, Hyp^- . Under such circumstances, the electron-transfer products are Hyp^{\bullet} and $\text{Hyp}^{\bullet 2-}$ for oxidation and reduction, respectively. Fortunately, this has very little impact on the interpretation of the work, and note will be made where it is important. Our experiments do not address this question directly.

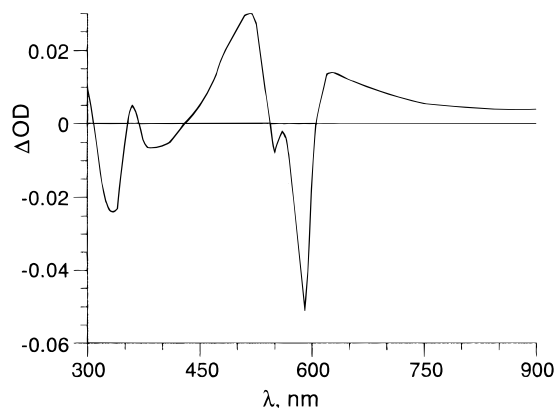


Figure 2. Differential T–T absorption spectrum of hypericin in acetonitrile.

The differential triplet–triplet absorption spectrum of hypericin in acetonitrile is shown in Figure 2. An identical T–T absorption spectrum was recorded using triplet–triplet energy transfer from anthracene with excitation by the laser pulse at 355 nm. The grow-in of ^3Hyp matched the decay of triplet anthracene. The extinction coefficient for ^3Hyp at $\lambda_{\text{max}} = 630$ nm was estimated by comparison of the bleaching of the ground state at 590 nm with the triplet absorption at 630 nm. A value of $\epsilon(630 \text{ nm}) = 9.0 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ was determined; the spectrum and extinction coefficient are in good agreement with previous reports.^{14,15} The lifetime of the triplet in deoxygenated solvent was about 50 μs , whereas in air-saturated solution it was 370 ns. Given that $[\text{O}_2]$ in air-saturated acetonitrile is 1.9 mM,³⁸ a crude estimate of the rate constant for the quenching of triplet hypericin by O_2 can be made: $1.4 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$. Quenching by O_2 does not lead to formation of radical ions detectable by our method.

Electron Transfer. The bimolecular rate constants (k_q) of triplet hypericin (^3Hyp) quenching by different amines in acetonitrile are collected in Table 1. Triplet energy transfer is energetically inaccessible,^{38,39} and quenching occurs via electron transfer. A new long-lived transient with an absorption maximum at 735 nm was observed in all cases; it is attributed to the radical anion of hypericin, $\text{Hyp}^{\bullet-}$. This absorption is not due to solvated electrons formed by photoionization of Hyp, as it would have also been observed in the absence of amines. The plots of τ^{-1} vs [amine] were strictly linear; there was no indication of acid–base or other interactions that might distort the plots used to determine the rate constants.⁴⁰ Additionally observed were transients attributable to the amine radical cations.⁴¹

The free energy of the electron transfer was estimated using the Rehm–Weller equation⁴⁵

$$\Delta G_{\text{et}}^{\circ} = E_{1/2}^{\text{ox}}(\text{D}/\text{D}^{\bullet+}) - E_{1/2}^{\text{red}}(\text{Hyp}/\text{Hyp}^{\bullet-}) - E_{\text{T}} - \frac{e^2}{\epsilon r_{\text{HA}}} \quad (2)$$

where $E_{1/2}^{\text{ox}}(\text{D}/\text{D}^{\bullet+})$ is the half-wave oxidation potential of amine vs SCE in acetonitrile. The first reduction potential of Hyp (or Hyp^-) is only slightly dependent on solvent (or counterion) in polar aprotic solvent.^{17,32,46} We adopt the value of -1.15 V vs SCE, which is arrived by near consensus among several measurements in polar aprotic solvents^{17,32,46} after adjustment to compensate for differences in reference electrode.⁴³ (Gerson attributes this reduction to the monoanion being reduced to the radical dianion. Regardless, it is the species in our solutions.)

The energy of the triplet state of hypericin acetonitrile is ca. $13\,350 \text{ cm}^{-1}$ or 38 kcal/mol (vide infra). The last term in eq 2

is the energy of Coulombic interaction between the two ions in the CRIP using $r_{\text{HA}} = r_{\text{H}} + r_{\text{A}}$. Using a spherical approximation for the size of hypericin from its crystallographic structure⁷ to give $r_{\text{H}} = 7 \text{ \AA}$ and taking r_{A} to be 5 \AA , we obtain $e^2/r_{\text{HA}}\epsilon = 0.03 \text{ eV}$. Although this value is obviously a rough approximation, the difference in Coulomb energies is small when using different values of r_{HA} . (The Coulomb term is doubled if the initial species is Hyp^- .) The estimated values of $\Delta G_{\text{et}}^{\circ}$ are given in Table 1.

The absorption spectrum of radical cation of N,N,N',N' -tetramethylbenzidine ($\text{TMB}^{\bullet+}$) and the T–T absorption of hypericin overlap strongly (Figures 2 and 3a). Thus, the k_q value was estimated at the isosbestic point of the T–T spectrum at 430 nm from the dependence of the buildup time of $\text{TMB}^{\bullet+}$ radical on the concentration of amine. The same method was used for N,N -diphenyl- p -phenylenediamine and 4-aminobiphenyl at the isosbestic point at 370 nm. In the case of N,N,N',N' -tetramethylphenylenediamine (TMPD), the k_q value was estimated from the dependence of the triplet hypericin lifetime on the concentration of amine by monitoring at 465 nm, the isosbestic point of the differential absorption spectrum of the radical ions (Figure 3a). The other radical cations of amines absorb light insignificantly at 630 nm,⁴¹ and k_q values were measured at this wavelength.

The extinction coefficient of $\text{Hyp}^{\bullet-}$ at 735 nm was estimated by comparison of the absorption at 735 nm to that of the $\text{TMB}^{\bullet+}$ radical at 470 nm ($\epsilon = 4.1 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$)⁴⁷ and the absorption of $\text{TMPD}^{\bullet+}$ at 570 and 620 nm ($\epsilon = 1.3 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$)⁴⁸ as shown in Figure 3a,b. In both cases, $\epsilon_{\text{max}}(\text{Hyp}^{\bullet-}) = 2.0 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ was obtained and also when using bleaching of Hyp ground state at 590 nm as a standard. The differential absorption spectrum at $\lambda > 570 \text{ nm}$ in Figure 3c is related exclusively to $\text{Hyp}^{\bullet-}$. In Figure 3a at $\lambda > 750 \text{ nm}$, an additional absorption band was recorded that is due to an absorption of $\text{TMB}^{\bullet+}$.⁴⁹

The decay kinetics of $\text{Hyp}^{\bullet-}$ are first order in deoxygenated acetonitrile with a lifetime of 25 μs . In air-saturated solution, the lifetime is 1.3 μs . The rate constant for quenching of $\text{Hyp}^{\bullet-}$ by O_2 can be roughly estimated as $3.8 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$. It is assumed that this results in the formation of superoxide as shown in eq 1.

The lifetime of $\text{Hyp}^{\bullet-}$, formed by electron transfer from TMPD, decreases linearly with increasing [TMPD] in solution. At [TMPD] = 3 mM, the lifetime of $\text{Hyp}^{\bullet-}$ is 10 μs , and a rate constant for quenching of $\text{Hyp}^{\bullet-}$ by TMPD was estimated at $1.7 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$. It is tempting to ascribe this to a second reduction by TMPD to make another 1 equiv of Würster's Blue ($\text{TMPD}^{\bullet+}$), but this is not thermodynamically reasonable. For the more weakly electron donating amine TMB, the analogous rate constant is less than $1 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$. The exact nature of this process remains unclear.

As a result of electron donation by amine in the ^3Hyp quenching event, a triplet contact radical ion pair (CRIP) is born. The fraction of CRIPs that yield free ion pairs, $\Phi_{\text{Hyp}^{\bullet-}}$, was estimated by comparison of the triplet absorption of ^3Hyp at 630 nm to the $\text{Hyp}^{\bullet-}$ absorption at 735 nm using eq 3, where

$$\Phi_{\text{Hyp}^{\bullet-}} = \frac{\Delta\text{OD}(\text{Hyp}^{\bullet-})[\epsilon_{\text{T}}(630 \text{ nm})] k_0^{\text{T}} + k_q[\text{A}]}{\epsilon(\text{Hyp}^{\bullet-}, 735 \text{ nm})(\Delta\text{OD}_{\text{T}}) k_q[\text{A}]} \quad (3)$$

$\Delta\text{OD}(\text{Hyp}^{\bullet-})$ and $\Delta\text{OD}_{\text{T}}$ are the optical densities of $\text{Hyp}^{\bullet-}$ and T–T absorption, respectively, and k_0^{T} is τ^{-1} at the limit of $[\text{A}] = 0$. Optical densities for the triplet and for the radical anions were obtained from separate samples that were optically

TABLE 1: Quenching of Triplet Hypericin by Amines

quencher	$E_{1/2}^{\text{ox}}$, ^a V vs SCE	$\Delta G_{\text{et}}^{\circ}$, eV	k_q , $\text{M}^{-1} \text{s}^{-1}$	$\Phi_{\text{Hyp}}^{\bullet-}$	k_b , s^{-1}	$-\Delta G_b$, eV
TMPD	0.16 ^b	-0.38	1.4×10^{10}	0.43	6.6×10^8	1.48
<i>p</i> -phenylenediamine	0.18	-0.36	6.6×10^9	0.43	6.6×10^8	1.50
4-aminodiphenylamine	0.27	-0.27	8.5×10^9	0.48	5.4×10^8	1.59
TMB ^c	0.32	-0.22	1.5×10^{10}	0.32	1.1×10^9	1.64
<i>N,N</i> -diphenyl- <i>p</i> -phenylenediamine	0.335	-0.20	1.3×10^{10}	<0.34	<i>c</i>	1.56
<i>o</i> -phenylenediamine	0.40 ^b	-0.14	9.0×10^8	0.08	5.8×10^9	1.72
2-aminoanthracene	0.44	-0.10	1.1×10^8	0.094	4.8×10^9	1.76
2-aminofluorene	0.53	-0.01	1.8×10^8	0.04	1.2×10^{10}	1.85
1-aminonaphthalene	0.54	-0.00	8.0×10^6	0.045	1.1×10^{10}	1.86
DABCO ^d	0.57	0.03	1.1×10^6	>0.08		
<i>N,N</i> -dimethyl- <i>p</i> -toluidine	0.65	0.11	5.9×10^5	>0.10		
<i>N,N</i> -dimethylaniline	0.71	0.17	1.1×10^5			

^a In CH_3CN vs SCE.⁴² Corrections for reference electrode, when necessary, were as per ref 43. ^b Reference 44. ^c *N,N,N',N'*-tetramethylbenzidine. Value is not estimated due to a strong overlapping of absorption of $\text{Hyp}^{\bullet-}$ and radical cation of amine.⁴¹ ^d 1,4-diazabicyclo[2.2.2]octane.

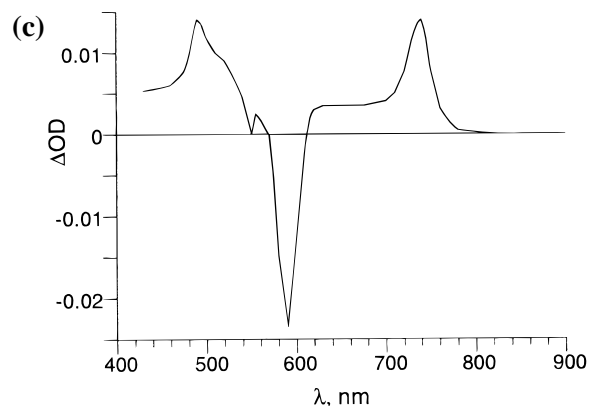
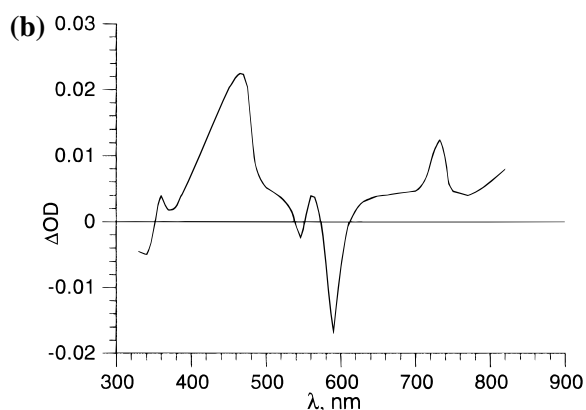
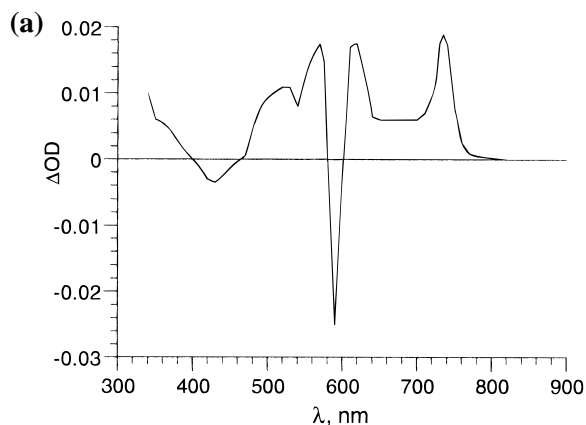


Figure 3. Differential absorption spectra of ion radicals recorded 5 μs after the laser pulse in quenching of triplet hypericin by amines: (a) $[\text{TMPD}] = 5 \times 10^{-5} \text{ M}$; (b) $[\text{TMB}] = 5 \times 10^{-5} \text{ M}$; (c) $[\text{p-phenylenediamine}] = 1 \times 10^{-4} \text{ M}$.

matched. Addition of amines at concentrations greater than 10 mM leads additionally to quenching of fluorescence, and the

TABLE 2: Quenching of Triplet Hypericin by Electron Acceptors

quencher	$E_{1/2}^{\text{red}}$, ^a V vs SCE	$\Delta G_{\text{et}}^{\circ}$, eV	k_q , $\text{M}^{-1} \text{s}^{-1}$	$\Phi_{\text{Hyp}}^{\bullet-}$
chloranil	+0.08	-1.16	2.2×10^9	0.54
fluoranil	-0.04 ^b	-1.04	2.9×10^9	0.50
1,4-benzoquinone	-0.40	-0.68	2.0×10^9	0.52
1,4-dihydroxyanthraquinone	-0.57 ^c	-0.51	1.8×10^9	0.53
1,4-naphthoquinone	-0.60 ^c	-0.48	9.7×10^8	0.50
duroquinone	-0.73	-0.35	3.5×10^7	0.57
4-nitrophthalimide	-0.75 ^c	-0.33	1.3×10^7	
3-nitrophthalimide	-0.84 ^c	-0.24	1.2×10^5	
9,10-anthraquinone	-0.86 ^{c,d}	-0.22	4.6×10^6	
maleic anhydride	-0.88 ^c	-0.20	3.0×10^4	

^a In DMSO vs SCE.⁴² ^b In CH_3CN . ^c DMF. ^d Reference 38.

yield of ^3Hyp decreases. The second term in eq 3 compensates when limited amine concentrations meant less than complete triplet quenching. For *N,N*-dimethyl-*p*-toluidine and *N,N*-dimethylaniline we could estimate only a lower limit for $\Phi_{\text{Hyp}}^{\bullet-}$ due to the low k_q values.

Also given in Table 1 are estimates of rate constants for first-order back electron transfer from the ion pairs, k_b . These are derived from $\Phi_{\text{Hyp}}^{\bullet-}$ by assuming that "escape" to form free $\text{Hyp}^{\bullet-}$ and A^{*+} and back electron transfer are the only available processes, i.e., $\Phi_{\text{Hyp}}^{\bullet-} = k_{\text{esc}}/(k_{\text{esc}} + k_b)$. For purposes of making this estimate, k_{esc} is taken to be $5 \times 10^8 \text{ s}^{-1}$, which has been determined by Weller for ion pairs from magnetic field measurements in acetonitrile.⁵⁰

The quenching of ^3Hyp by electron acceptors was studied in DMSO. In Ar-flushed solvent, the lifetime of ^3Hyp was 70 μs . Quenching with the series of compounds in Table 2 occurs through electron transfer with the formation of radical anions of the electron acceptors and a new long-lived transient ($\tau \sim 150 \mu\text{s}$) that has a broad differential absorption band with λ_{max} at 710 nm and is attributed to Hyp^{*+} .⁵¹ This transient was recorded with all quenchers in the series. Typical differential absorption spectra are shown in Figure 4. The quenching rate constants were determined by monitoring ^3Hyp at $\sim 500 \text{ nm}$ where the absorption of the radical ions are insignificant. Strong electron acceptors, such as chloranil, quenched the fastest among this set, very near the diffusion controlled limit³⁸ of $3 \times 10^9 \text{ M}^{-1} \text{s}^{-1}$.

The extinction coefficient of Hyp^{*+} at 710 nm was established by comparison of the absorption of this radical with the absorption of chloranil radical anion at 450 nm ($\epsilon = 9.0 \times 10^3 \text{ M}^{-1} \text{cm}^{-1}$ in acetonitrile⁵²) or with the ground-state bleaching of hypericin at 598 nm (Figure 4a). The estimates give $\epsilon_{\text{max}}^{\bullet-}(\text{Hyp}^{*+}, 710 \text{ nm}) = 2.0 \times 10^4 \text{ M}^{-1} \text{cm}^{-1}$. In the case of 1,4-naphthoquinone (NQ), the absorption of $\text{NQ}^{\bullet-}$ ($\lambda_{\text{max}} = 390 \text{ nm}$, $\epsilon = 1.25 \times 10^4 \text{ M}^{-1} \text{cm}^{-1}$)⁵³ and Hyp^{*+} overlap strongly (Figure

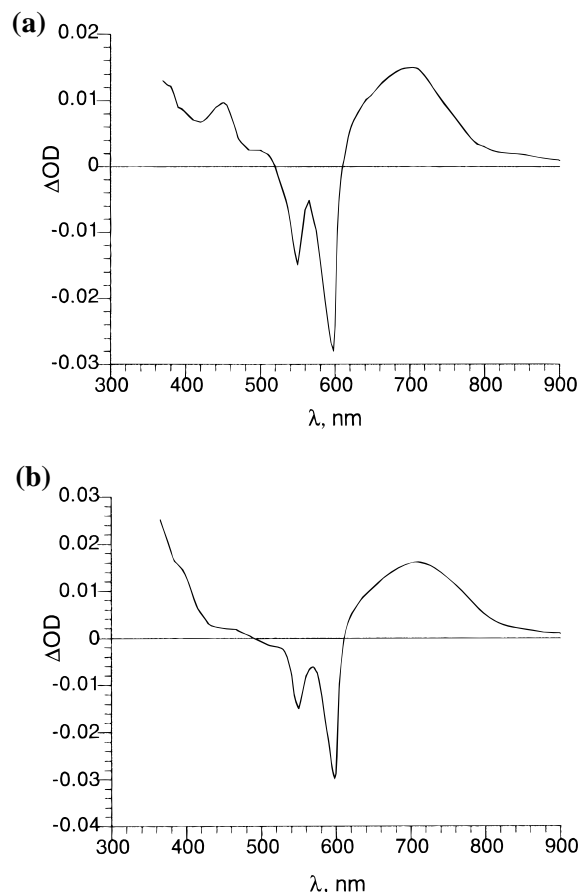


Figure 4. Differential absorption spectra of ion radicals recorded 15 μ s after the laser pulse in quenching of triplet hypericin by carbonyl compounds: (a) [chloranil] = 1.3×10^{-4} M; (b) [1,4-naphthoquinone] = 2.6×10^{-4} M.

TABLE 3: Interaction Rate Constants of $\text{Hyp}^{\bullet+}$ with Electron Donors in DMSO

reagent	$E_{1/2}^{\text{ox}, a}$ V vs SCE	k_r , $\text{M}^{-1} \text{s}^{-1}$
TMPD	0.16	1.0×10^9
TMB	0.32	1.6×10^8
2-aminoanthracene	0.44	9.2×10^7
triphenylamine	0.92	3.5×10^6
1,2,4-trimethoxybenzene	1.12 ^b	4.5×10^5

^a Reference 42. ^b Reference 55.

4b), but the same estimate is obtained using the bleaching band of hypericin ground state as a standard. In the region of $\lambda > 500$ nm the absorption spectrum is related exclusively to $\text{Hyp}^{\bullet+}$ (Figure 4). Spectra of other electron acceptors could not be recorded. The quantum yields of $\text{Hyp}^{\bullet+}$ were measured as above using eq 3 with the parameters of this radical and are collected in Table 2.

It is necessary to estimate the free energy for electron transfer in these systems as well and a properly adapted version of eq 2 is used. An irreversible oxidation wave for hypericin is observed in DMSO with a peak potential of 0.93 V vs NHE.^{17,46} Redpenning has shown that 0.85 V (vs NHE) is a reasonable estimate of the thermodynamic oxidation potential,⁴⁶ and thus we adopt a value of 0.61 V vs SCE.⁴³ The Coulomb term is 0.03 eV.⁵⁴ For some electron acceptors the reduction potentials are known only in DMF. For carbonyl compounds the reduction potentials in aprotic solvents DMSO and DMF practically coincide,⁴² so we used data in DMF in order to estimate the Δ

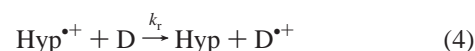
TABLE 4: Rate Constants of Energy Transfer from Triplet Hypericin to Various Acceptors

energy transfer acceptor	E_T , cm^{-1} (kcal/mol) ^a	$10^{-9}k_{\text{en}}$, $\text{M}^{-1} \text{s}^{-1}$		E_a , ^b kcal/mol
		CH ₃ CN	THF	THF
β -carotene	7 100 (20)	1.8	0.48	1.6
rubrene	9 300 ^c (26.3)	4.6	0.93	
tetracene	10 250 (29.3)	4.7	3.5	
1,3-diphenylisobenzofuran	11 900 (33.9)	7.0	3.2	
perylene	12 600 (36.1)	8.6	2.4	
ferrocene	13 300 (38.0)	11.5	3.5	
azulene	13 600 (38.9)	0.12	0.028	2.8
9,10-dibromoanthracene	14 000 (40.0)		0.037	

^a Triplet energies from ref 38. ^b ± 0.15 kcal/mol. ^c Reference 58.

G_{et}° values in DMSO without further correction when values in DMSO were unavailable (Table 2).

The interaction rate constants (k_r) of $\text{Hyp}^{\bullet+}$ with different electron donors were also determined (Table 3). The addition of quenchers leads to the decrease of $\text{Hyp}^{\bullet+}$ lifetime and the formation of radical cations of amines as shown in eq 4.



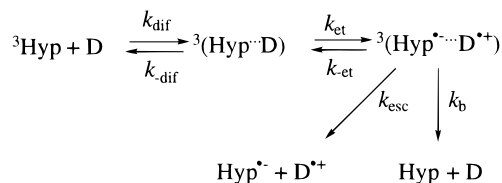
The rates of decay of $\text{Hyp}^{\bullet+}$ and grow-in of $\text{D}^{\bullet+}$ were equal. The rate constants of reaction 4 correlate well with the oxidation potential of the electron donors. $\text{Hyp}^{\bullet+}$ does not interact rapidly with molecular oxygen, and the rate constant may be estimated as $< 10^7 \text{ M}^{-1} \text{s}^{-1}$.

Energy Transfer. The energy transfer from ^3Hyp to the different triplet energy acceptors was recorded by monitoring the transient decay of hypericin at 520 or 630 nm, and the so-obtained rate constants (k_{en}) are shown in Table 4. Quenching occurs via energy transfer; the rise-times of the triplet acceptor absorptions⁵⁶ coincided with the lifetimes of triplet hypericin, and the formation of radicals was not observed. Except for ferrocene and β -carotene, the oxidation potentials of all quenchers in Table 4 are greater than 0.8 eV vs SCE.⁴² According to data in Table 1, reductive electron-transfer quenching of ^3Hyp is insignificant in this range. The oxidation potential for β -carotene vs SCE in THF is 0.74,⁵⁷ and triplet quenching is expected to strongly dominate. For ferrocene, the oxidation potential is low ($E_{1/2}^{\text{ox}} = 0.31$ V vs SCE in CH₃CN) and one might expect some contribution of electron transfer. However the data imply that even the ferrocene quenching occurs mainly via energy transfer, as the rate constants for quenching in THF are all smaller by a very comparable factor when compared to acetonitrile and no additional transients were observed.

Azulene,³⁸ 9,10-dibromoanthracene,³⁸ and ferrocene have short-lived triplet states, and the concentration of hypericin used in this work is very low (ca. 1×10^{-5} M). Therefore, reversible energy transfer from the acceptor triplet state to hypericin in secondary encounters may be neglected.

Discussion

Electron Transfer. A standard Rehm–Weller type kinetic scheme for the quenching of hypericin by amines as donors (D) is given in Scheme 1. Using a sequential steady-state approximation, the observed quenching rate constant k_q is given by eq 5 where the equilibrium constant for electron transfer is eq 6.⁴⁵ The form of the forward electron-transfer rate constant

SCHEME 1. Kinetic Scheme for Electron Transfer Quenching of Hypericin


is given by eq 7.

$$k_q = \frac{k_{\text{dif}}}{1 + \frac{k_{-\text{dif}}}{k_{\text{et}}} + \frac{k_{-\text{dif}}}{k_{\text{b}} + k_{\text{esc}}} \exp(\Delta G_{\text{et}}/RT)} \quad (5)$$

$$k_{\text{et}}/k_{-\text{et}} = \exp(-\Delta G_{\text{et}}/RT) \quad (6)$$

$$k_{\text{et}} = A \exp(-\Delta G_{\text{et}}^\ddagger/RT) \quad (7)$$

Modern electron transfer theory uses k_{et} based on Fermi's golden rule

$$k = \frac{2\pi}{\hbar} |V|^2 F \quad (8)$$

where V is an electronic coupling matrix element connecting initial and final states and F is the Franck–Condon weighted density of states. Using a classical expression for F , this gives

$$k = \frac{2\pi}{\hbar} |V|^2 (4\pi\lambda k_{\text{B}}T)^{-1/2} \exp[-(\Delta G_{\text{et}} + \lambda)^2/4\lambda k_{\text{B}}T] \quad (9)$$

where λ is the total reorganization energy, which can be broken into solvent (λ_{s}) and internal (λ_{v}) components. Using a single quantized mode approximation, eq 10^{59,60} has enjoyed considerable success in describing experimental results.

$$k = \frac{2\pi}{\hbar} |V|^2 (4\pi\lambda_{\text{s}} k_{\text{B}}T)^{-1/2} \sum_{n=0}^{\infty} \frac{e^{-S} S^n}{n!} \exp\left(-\frac{(\lambda_{\text{s}} + \Delta G_{\text{et}} + \hbar\nu)^2}{4\lambda_{\text{s}} k_{\text{B}}T}\right) \quad (10)$$

Here, $S = \lambda_{\text{v}}/\hbar\nu$, where $\hbar\nu$ is the energy of the representative molecular vibration.

While quantitatively different reorganization energies and coupling matrix elements are found using the Marcus (0-mode)⁶¹ and single-mode semiclassical expressions,^{62–64} the latter is affected by assumptions about quantities that cannot be easily tested with our data. It is clear from the literature that eq 10 is superior for describing electron transfer in the inverted region, but eq 9 is adequate for endothermic to moderately exothermic “normal region” electron transfer. It has the advantages of requiring only two parameters and easily reducing to the form of eq 7 and will be used here by treating the preexponential part as a single adjustable parameter, A . Other equations that try to explain why an inverted region is generally not observed for bimolecular charge separations, such as those based on asymptotic reorganization energies⁶⁵ or diffusion mediated rate constants,⁶⁶ are also unnecessary for the current analysis.

For purposes of evaluating eq 5, we take k_{dif} to be $1.9 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$.³⁸ For $k_{\text{dif}}/k_{-\text{dif}}$, we obtain 4.4 M^{-1} from the Eigen–Fuoss equation, eq 11. The values for k_{b} and k_{esc} are obtained as previously described.

The data in Table 1 and Figure 5 are not sufficient to supply a unique fit to eq 5. Using two adjustable parameters (A and λ), no satisfactory fit was obtained. Arbitrarily allowing $(k_{\text{b}} + k_{\text{esc}})$

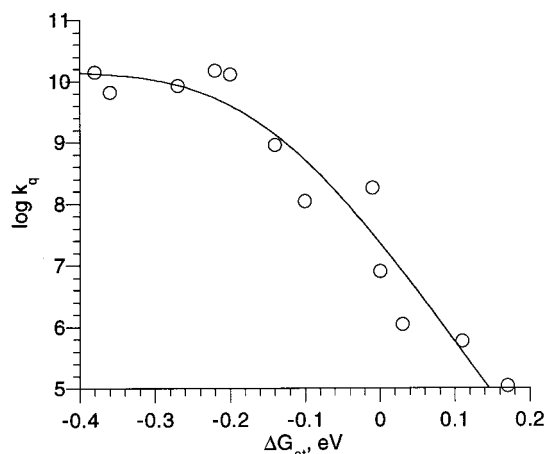


Figure 5. Dependence of the observed quenching rate of triplet hypericin by amines on the free energy of electron transfer. The solid line is the Rehm–Weller curve with $A = 10^{12} \text{ s}^{-1}$, $\lambda = 0.73 \text{ eV}$, and $\delta = 0.17 \text{ V}$. See text for details.

$$\frac{k_{\text{dif}}}{k_{-\text{dif}}} = \frac{4\pi N r_{\text{HA}}^3}{3000} \quad (11)$$

also to independently vary could produce reasonable fits, but they gave physically unreasonable values of A and $(k_{\text{b}} + k_{\text{esc}})$.

If, instead of $(k_{\text{b}} + k_{\text{esc}})$, a third parameter δ was allowed to float as $\Delta G_{\text{et}} = \Delta G_{\text{et}}^\circ + \delta$, reasonable values of A and λ could be obtained. Experimenting showed that the best values of δ were 0.15–0.21, regardless of the other parameters. If δ was fixed at 0.17 eV, the problem was again reduced to two parameters, A and λ . At this point, a range of physically reasonable values of A and λ gave good fits because there were not enough data to uniquely determine the curvature in the region of $\Delta G_{\text{et}}^\circ \approx -0.3$ to 0.1. For example, $A = 10^{12} \text{ s}^{-1}$ and $\lambda = 0.7 \text{ eV}$ gave a similarly good fit as $A = 10^{14} \text{ s}^{-1}$ and $\lambda = 1.2 \text{ eV}$.

The solvent reorganization energy for the charge separation reaction can be estimated from the usual equation on the basis of the dielectric continuum model⁶¹

$$\lambda_{\text{s}} = e^2 \left(\frac{1}{2r_{\text{H}}} + \frac{1}{2r_{\text{A}}} - \frac{1}{r_{\text{HA}}} \right) (n^{-2} - \epsilon^{-1}) \quad (12)$$

where n is the refractive index of acetonitrile and ϵ is the dielectric constant. Substituting in eq 12 the values of r_{H} , r_{A} and r_{HA} defined above, we obtain $\lambda_{\text{s}} = 0.67 \text{ eV}$, which is consistent with the range of λ values that fit the data in Figure 5.

It is somewhat unorthodox to allow ΔG_{et} to be an adjustable parameter in fits such as these. The implication of the result is that the reduction potential of the species being reduced is actually -1.32 V vs SCE rather than the -1.15 V previously adopted for Hyp. It is possible that this simply reflects an error in the assumption of the reduction potential of Hyp, though this “correction” seems a little large for that. (This conclusion applies whether the hypericin is deprotonated, since the potentials were measured under conditions similar to those used here.)

We offer a speculative interpretation here. There is ample evidence^{9–12} for very rapid tautomerization of hypericin in its singlet chemistry. If either (a) tautomerization occurs in the excited singlet manifold and intersystem crossing occurs after the tautomerization or (b) tautomerization is very rapid and occurs in the triplet manifold as well, then the species that is being reduced is actually the tautomer. As a result, the reduction

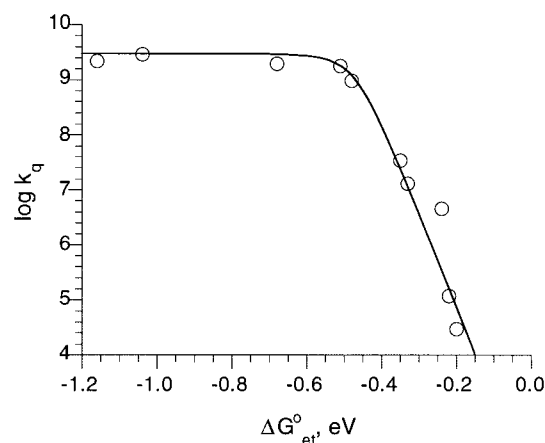


Figure 6. Dependence of the observed quenching rate of triplet hypericin by electron acceptors on the free energy of electron transfer. The solid line is the Rehm-Weller curve with $A = 10^{12} \text{ s}^{-1}$, $\lambda = 0.72 \text{ eV}$, and $\delta = 0.48 \text{ V}$. See text for details.

potential of the normal [7,14] form of hypericin is not actually the appropriate one to be used in calculating ΔG_{et}^o in eq 2. *The correct reduction potential is that of the tautomer.* Given the optimal value of δ , it may be suggested that the tautomer (Taut) has a reduction potential in the range of -1.32 V vs SCE. Support for this suggestion will come from the oxidative quenching of Hyp as well.

The free energy for back electron transfer from $\text{Hyp}^{\bullet-}$ (or $\text{Taut}^{\bullet-}$) to the amine radical cation is given by

$$\Delta G_b = -E_{1/2}^{\text{ox}}(\text{A}/\text{A}^{\bullet+}) + E_{1/2}^{\text{red}}(\text{H}/\text{H}^{\bullet-}) \quad (13)$$

where $E_{1/2}^{\text{red}}(\text{H}/\text{H}^{\bullet-})$ is the reduction potential of hypericin or its tautomer under our proposed interpretation. These values are given in Table 1, using $E_{1/2}^{\text{red}}(\text{H}/\text{H}^{\bullet-}) = -1.32 \text{ V}$. The largest values of k_b are at the most exothermic rates; i.e., these are still in the normal region for electron transfer. It is fairly typical for such charge recombination reactions to have their maximum rates at $-\Delta G_b = 1.5\text{--}2.0 \text{ eV}$,^{67–73} as appears would be the case here if this were a longer series.

The charge-transfer quenching of ^3Hyp by electron acceptors in DMSO can be analyzed in much the same way as was done for the amine donors in CH_3CN . Since $\Phi_{\text{Hyp}^{\bullet-}}$ is close to 0.5 for the whole series, $k_b + k_e$ was taken to be $1 \times 10^9 \text{ s}^{-1}$ for all quenchers. A value of $3.0 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ was used for k_{dif} .³⁸ Again, no reasonable fit could be obtained without using an additional parameter δ such that $\Delta G_{\text{et}} = \Delta G_{\text{et}}^o + \delta$. A range of $\delta = 0.45\text{--}0.51 \text{ V}$ gave reasonable fits, with the best at $\delta = 0.48 \text{ V}$. Again, a range of A values and correlated λ values gave reasonable fits. With A held at 10^{12} s^{-1} , λ was 0.72 eV , as shown in Figure 7. The value for λ was 1.2 eV if A was as large as 10^{14} .

The shift of nearly 0.5 V would appear to be much more than is reasonable to attribute to error in the thermodynamic oxidation potential. Thus, the oxidative electron-transfer quenching data for ^3Hyp also support the notion that it is inadequate to describe the electron-transfer energetics with the reduction potential of hypericin in its ordinary [7,14] form. The fits again suggest that it is a tautomeric form of Hyp that is oxidized. The tautomer is more difficult to oxidize than Hyp by about 0.48 V , giving it an oxidation potential of about 1.09 V vs SCE.⁷⁴

Energy Transfer. The energy of the triplet state of hypericin may be estimated from the dependence of the energy transfer rate constant on the energy of triplet acceptors.⁷⁵ The triplet

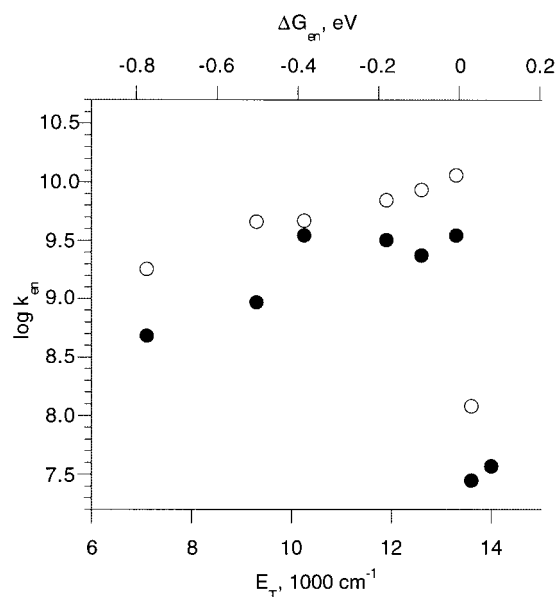


Figure 7. Dependence of the energy transfer rate constant from triplet hypericin to energy acceptors on the triplet energy of sensitizers. Open circles are data in acetonitrile, and filled circles are data in THF.

energy is taken at the energy where the observed energy transfer rate constant is half of the maximum limiting value (usually near the diffusion-controlled limit). If one takes the datum for ferrocene to represent $k_{\text{et}, \text{max}}$ and draws a line through the point for azulene (Figure 7), an estimate of $13\,350 \text{ cm}^{-1}$ is obtained for $E_T(\text{Hyp})$. Taking the point along that line that corresponds to $0.5k_{\text{dif}}$ from the reference³⁸ value of k_{dif} yields a nearly equivalent triplet energy of $13\,300 \text{ cm}^{-1}$. A very similar estimate is obtained in THF using the data for ferrocene, azulene, and dibromoanthracene. This triplet energy is in good agreement with previous spectroscopic determination in rigid media at very low temperatures and indicates that there are not gross structural changes in the triplet state that lead to energy relaxation.^{24,25} However, it is also consistent with an excited-state rearrangement with only minor geometrical changes and the very small Stokes shift observed in the fluorescence.

A modest but real decrease in energy transfer rate constants is observed with decreasing E_T of the acceptor below $13\,000 \text{ cm}^{-1}$ (Figure 7). This decrease does not appear attributable to steric factors. Energy transfer from anthracene (a flat molecule like hypericin) to β -carotene, for instance, occurs with a rate constant of $1.4 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ in toluene.⁷⁶ This datum also argues against the current series being limited by diffusion.

Despite the unusual appearance of the rate constant profile, it seems reasonable to suggest that the rate constant falloff is an example of the Marcus inverted region for energy transfer. This was not a result that was easily anticipated since most analogous triplet-triplet energy transfer series simply achieve the diffusion-controlled limit when exothermic. The essential requirement to observe the inverted region for cases like this is a small matrix element V that leads to maximum rate constants below diffusion control. Though comparatively rare in the literature, both unimolecular and bimolecular examples of the inverted region in energy transfer have been reported.^{77–83}

There have been theoretical and experimental efforts to link triplet energy transfer and electron transfer.^{77,83–86} In the weak coupling limit, both are nonadiabatic processes that should follow the golden rule and rates of energy transfer are usually predicted by some variation of eq 11. We adopt eq 14, used previously by Meyer and Schanze, that takes into account a single quantized mode for both the donor and acceptor.^{80,82} All

$$k = \frac{2\pi}{\hbar} (4\pi\lambda_s k_B T)^{-1/2} |V|^2 \sum_{n_i} \sum_{n_j} e^{-(S_D + S_A)} \frac{(S_D)^{n_i}}{n_i!} \frac{(S_A)^{n_j}}{n_j!} \exp \left(\frac{-[\lambda_s + \Delta E_T + h\nu_D + h\nu_A]^2}{4\lambda_s k_B T} \right) \quad (14)$$

of the terms are the same as above, save that both donor and acceptor modes are considered.

Unimolecular rate constants for the microscopic energy transfer step $k_{\text{en}}^{\text{uni}}$ were extracted from the observed bimolecular rate constants by adapting Scheme 1 and eq 5. Only the point for ferrocene in acetonitrile cannot be easily determined in this way, since it is so close to the diffusion-controlled limit. The resulting unimolecular rate constants $k_{\text{en}}^{\text{uni}}$ are lower than the observed bimolecular rate constants k_{en} , but the overall appearance of the rate constant profile is very similar to Figure 7.

Reasonable parameter values must be measured or assumed to try to fit eq 14 to the data. It is expected theoretically and apparent from the rapid falloff of the energy transfer rate constants in the endothermic region that λ_s is small. Meyer's value of $S_A = 0.9$, measured for anthracene and used with tetracene also,⁸⁰ was adopted for all acceptors. Well-precedented approximations were used for the acceptor vibrational spacing (i.e., ca. 1300 cm^{-1}), and a similar value was adopted for the vibrational spacing in ³Hyp, based on its phosphorescence spectrum.²⁵

Fits were attempted by fixing the S parameters and vibrational spacing, while allowing a preexponential term and λ_s to vary. Despite repeated attempts using a wide range of the fixed values, no reasonable fit of the data to eq 14 could be obtained. This might have been anticipated because of the asymmetry of Figure 7. Using a classical expression for the Franck–Condon factors gives a truly parabolic rate constant profile, but the use of quantized modes such as in eq 14 allows the rate constants to fall off more slowly in the inverted region than in the endothermic region. However, asymmetry as gross as observed here could not be accommodated. Whether our failure to fit the data to eq 14 results from approximations such as using the same values of V and S_A for all acceptors or from a more fundamental reason still remains to be determined.

The activation energies measured for β -carotene and azulene are both within reasonable expectations. The rate constants for both of them at room temperature are far enough below the diffusion-controlled limit that it can be reasonably expected that the activation energies reflect the inherent energy transfer process. That of azulene reflects the slightly endothermic energy transfer and, presumably, a small contribution from reorganization. That for β -carotene is more interesting, being within the apparent inverted region. Almost a decade ago, it was recognized that classical Marcus theory failed to predict activation energies in the inverted region of electron transfer with any accuracy.^{87,88} The apparent barrier is expected to be very small, as observed here. In fact, over wide enough temperature ranges, small negative activation barriers are observed, but these have been attributed to temperature dependence of the solvent reorganization energy.^{87–89} Ideally, the temperature dependence can be fit to eq 14, but this was not justified on the basis of the uncertainty of the other parameters and our inability to fit all the data at a single temperature.

Conclusions

Quenching of triplet hypericin by amines in acetonitrile occurs via electron transfer from the amine to the hypericin. The

opposite mode of electron transfer is observed when quenching the triplet state of hypericin in DMSO with electron acceptors. In both cases, the rate constant profile is inconsistent with the use of the appropriate redox potential for ground-state hypericin in the usual Rehm–Weller energy formulation. It is suggested that this is because the actual species being reduced or oxidized is not merely the triplet state of hypericin, but probably a tautomer formed in its triplet state before the electron transfer step. Within this model, the tautomer is somewhat more difficult to oxidize and to reduce than “normal” hypericin and is also likely the phosphorescent species at low temperature.

If one works under the assumption that hypericin is neutral under the current conditions, the species formed by electron donation is $\text{Hyp}^{\bullet-}$. This species is found to interact with oxygen with a rate constant greater than $10^8 \text{ M}^{-1} \text{ s}^{-1}$, which may account for the formation of superoxide in biologically relevant conditions. The one-electron oxidized form of hypericin does not react with O_2 at an appreciable rate.

The quenching of triplet hypericin by energy transfer with sensitizers whose triplet energy is less than $13\,300 \text{ cm}^{-1}$ apparently occurs in the inverted region. The apparent solvent reorganization energy is very low, but we were unable to achieve a reasonable fit of the data to the usual expressions for nonadiabatic energy transfer.

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References and Notes

- Giese, A. C. *Photochem. Photobiol. Rev.* **1980**, *5*, 229–245.
- Duran, N.; Song, P.-S. *Photochem. Photobiol.* **1986**, *43*, 677–680.
- Meruelo, D.; Lavie, G.; Lavie, D. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 5230–5234.
- Lavie, D.; Valentine, F.; Levin, B.; Mazur, Y.; Gallo, G.; Lavie, D.; Weiner, D.; Meruelo, D. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 5963–5967.
- Lenard, J.; Rabson, A.; Vanderof, R. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 158–162.
- Petrich, J. W.; Gordon, M. S.; Cagle, M. J. *Phys. Chem. A* **1998**, *102*, 1647–1651.
- Etzlstorfer, C.; Falk, H.; Müller, N.; Schmitzberger, W.; Wagner, U. G. *Monatsh. Chem.* **1993**, *124*, 751–761.
- Etzlstorfer, C.; Falk, H.; Oberreiter, M. *Monatsh. Chem.* **1993**, *124*, 3384–3385.
- Gai, F.; Fehr, M. J.; Petrich, J. W. *J. Am. Chem. Soc.* **1993**, *115*, 5795.
- Gai, F.; Fehr, M. J.; Petrich, J. W. *J. Phys. Chem.* **1994**, *98*, 5784–5795.
- Gai, F.; Fehr, M. J.; Petrich, J. W. *J. Phys. Chem.* **1994**, *98*, 8352–8358.
- English, D. S.; Das, K.; Zenner, J. M.; Zhang, W.; Kraus, G. A.; Larock, R. C.; Petrich, J. W. *J. Phys. Chem. A* **1997**, *101*, 3235–3240.
- Fehr, M. J.; McCloskey, M. A.; Petrich, J. W. *J. Am. Chem. Soc.* **1995**, *117*, 1833–1836.
- Jardon, P.; Lazorchak, N.; Gautron, R. *J. Chim. Phys.* **1986**, *83*, 311–315.
- Michaeli, A.; Regev, A.; Mazur, Y.; Feitelson, J.; Levanon, H. *J. Phys. Chem.* **1993**, *97*, 9154–9160.
- Darmanyan, A. P.; Burel, L.; Eloy, D.; Jardon, P. *J. Chim. Phys.* **1994**, *91*, 1774–1785.
- Burel, L.; Jardon, P.; Lepretre, J.-C. *New J. Chem.* **1997**, *21*, 399–403.
- Hadjur, C.; Jeunet, A.; Jardon, P. *J. Photochem. Photobiol. B* **1994**, *26*, 67–74.
- Weiner, L.; Mazur, Y. *J. Chem. Soc., Perkin Trans. 2* **1992**, 1439–1442.
- Xia, G.; He, X.; Zhou, Y.; Zhang, M.; Shen, T. *J. Photochem. Photobiol. A* **1998**, *114*, 31–35.
- Yamazaki, T.; Ohta, N.; Yamazaki, I.; Song, P.-S. *J. Phys. Chem.* **1993**, *97*, 7870–7875.
- Wells, T. A.; Losi, A.; Dai, R.; Scott, P.; Park, S.-M.; Golbeck, J.; Song, P.-S. *J. Phys. Chem. A* **1997**, *101*, 366–372.
- Malkin, J.; Mazur, Y. *Photochem. Photobiol.* **1993**, *57*, 929–933.

- (24) Angerhofer, A.; Falk, H.; Meyer, J.; Schoppel, G. *J. Photochem. Photobiol., B* **1993**, *20*, 133–137.
- (25) Arabei, S. M.; Galaup, J. P.; Jardon, P. *Chem. Phys. Lett.* **1997**, *270*, 31–36.
- (26) Darmanyan, A. P.; Kasaikina, O. T.; Khrameeva, N. P. *Khim. Fiz.* **1987**, *6*, 1083–1092.
- (27) Brockmann, H.; Spitzner, D. *Tetrahedron Lett.* **1975**, 37–40.
- (28) Graver, G. D.; Adams, R. *J. Am. Chem. Soc.* **1923**, *45*, 2439–2455.
- (29) Falk, H.; Meyer, J.; Oberreiter, M. *Monatsh. Chem.* **1993**, *124*, 339–41.
- (30) Darmanyan, A. P.; Gregory, D. D.; Guo, Y.; Jenks, W. S. *J. Phys. Chem. A* **1997**, *101*, 6855–6863.
- (31) Wynn, J. L.; Cotton, T. M. *J. Phys. Chem.* **1995**, *99*, 4317–4323.
- (32) Gerson, F.; Gescheidt, G.; Häring, P.; Mazur, Y.; Freeman, D.; Spreizer, H.; Daub, J. *J. Am. Chem. Soc.* **1995**, *117*, 11861–11866.
- (33) Gutman, I.; Markovic, Z.; Solujic, S.; Sukdolak, S. *Monatsh. Chem.* **1998**, *129*, 481–486.
- (34) Eloy, D.; Le Pellec, A.; Jardon, P. *J. Chim. Phys.* **1996**, *93*, 442–457.
- (35) Altmann, R.; Falk, H. *Monatsh. Chem.* **1997**, *128*, 571–583.
- (36) Amer, A. M.; Falk, H.; Tran, H. T. N. *Monatsh. Chem.* **1988**, *129*, 1237–1244.
- (37) Ahrer, W.; Falk, H.; Tran, H. T. N. *Monatsh. Chem.* **1998**, *129*, 643–647.
- (38) Murov, S. L.; Carmichael, I.; Hug, G. L. *Handbook of Photochemistry*, 2nd ed.; Marcel Dekker: New York, 1993.
- (39) The triplet energies of most of the quenchers were established in the literature. Phosphorescence spectra at 77 K of the others showed that they all had substantially higher triplet energies than hypericin.
- (40) This is the strongest indication within our work that the predominant protonation state of hypericin may be monoanionic under neutral conditions.
- (41) Shida, T. *Absorption Spectra of Radical Ions*; Elsevier Science Publishing Co., Inc.: Amsterdam, 1988.
- (42) Mann, C. K.; Barnes, K. K. *Electrochemical Reactions in Non-aqueous Systems*; Marcel Dekker: New York, 1970.
- (43) Wayner, D. D. M. *Redox Properties*; Scaiano, J. C., Ed.; CRC Press: Boca Raton, FL, 1989; Vol. II, pp 363–368.
- (44) Chen, J.-M.; Ho, T.-I.; Mou, C.-Y. *J. Phys. Chem.* **1990**, *94*, 2889–2896.
- (45) Rehm, D.; Weller, A. *Isr. J. Chem.* **1970**, *8*, 259–271.
- (46) Redepenning, J.; Tao, N. *Photochem. Photobiol.* **1993**, *58*, 532–535.
- (47) Alkaitis, S. A.; Grätzel, M. *J. Am. Chem. Soc.* **1976**, *98*, 3549–3555.
- (48) Anderson, C. P.; Saimon, D. J.; Meyer, T. J.; Young, R. C. *J. Am. Chem. Soc.* **1977**, *99*, 1980–1982.
- (49) Shida, T.; Hamill, W. H. *J. Chem. Phys.* **1966**, *44*, 2369–2374.
- (50) Weller, A. Z. *Phys. Chem. (Munich)* **1982**, *130*, 129–138.
- (51) Should ground state be deprotonated, the assignment naturally switches to Hyp^{*}.
- (52) Andre, J. J.; Weill, G. *Mol. Phys.* **1968**, *15*, 97–99.
- (53) Patel, K. B.; Wilson, R. L. *J. Chem. Soc., Faraday Trans.* **1973**, *169*, 814–825.
- (54) The Coulomb term is formally zero if hypericin is deprotonated because this becomes a charge shift rather than charge recombination or separation reaction.
- (55) Zweig, A.; Hodgson, W. G.; Jura, W. H. *J. Am. Chem. Soc.* **1964**, *86*, 4124–4129.
- (56) Carmichael, I.; Hug, G. L. *J. Phys. Chem. Ref. Data* **1986**, *15*, 1–250.
- (57) Park, S.-M. *J. Electrochem. Soc.* **1978**, *125*, 216–222.
- (58) Darmanyan, A. P.; Kuz'min, V. A. *Dokl. Acad. Nauk SSSR* **1981**, *260*, 1167–1170.
- (59) Ulstrup, J.; Jortner, J. *J. Chem. Phys.* **1975**, *63*, 4358.
- (60) Jortner, J. *J. Chem. Phys.* **1976**, *64*, 4860–4867.
- (61) Marcus, R. A. *Discuss. Faraday Soc.* **1960**, *29*, 21–31.
- (62) Hug, G. L.; Marciniak, M. *J. Phys. Chem.* **1994**, *98*, 7523–7532.
- (63) Hug, G. L.; Barciniak, B. *J. Phys. Chem.* **1995**, *99*, 1478–1483.
- (64) Kumar, K.; Kurnikov, I. V.; Beratan, D. N.; Waldeck, D. H.; Zimmt, M. B. *J. Phys. Chem. A* **1998**, *102*, 5529–5541.
- (65) Agmon, N.; Levine, R. D. *Chem. Phys. Lett.* **1977**, *52*, 197–201.
- (66) Tachiya, M.; Murata, S. *J. Phys. Chem.* **1992**, *96*, 8441–8444.
- (67) See, for instance, refs 68–73.
- (68) Ohno, T.; Yoshimura, A.; Mataga, N. *J. Phys. Chem.* **1986**, *90*, 3295–3297.
- (69) Ohno, T.; Yoshimura, A. *J. Phys. Chem.* **1987**, *91*, 4365–4370.
- (70) Ohno, T.; Yoshimura, A.; Mataga, N. *J. Phys. Chem.* **1990**, *94*, 4871–4876.
- (71) Gould, I. R.; Ege, D.; Mattes, S. L.; Farid, S. *J. Am. Chem. Soc.* **1987**, *109*, 3794–3796.
- (72) Gould, I. R.; Farid, S. *J. Phys. Chem.* **1993**, *97*, 13067–13072.
- (73) Kikuchi, K.; Niwa, T.; Takahashi, Y.; Ikeda, H.; Miyashi, T. *J. Phys. Chem.* **1993**, *97*, 5070–5073.
- (74) It is interesting to note that Figure 7 of ref 22 might also benefit by a similar shift in the oxidation potential, though more data in the nearly thermoneutral region would be necessary to confirm this. The figure is comparable to our Figure 6, save that it is for quenching of a singlet state of hypericin by electron acceptors.
- (75) Sandros, K. *Acta Chem. Scand.* **1964**, *18*, 2355–2374.
- (76) Nielsen, R.; Jorgensen, K.; Skibsted, L. H. *J. Photochem. Photobiol. A. Chem.* **1998**, *127*–133.
- (77) Sigman, M. E.; Closs, G. L. *J. Phys. Chem.* **1991**, *95*, 5012–5017.
- (78) MacQueen, D. B.; Eyler, J. R.; Schanze, K. S. *J. Am. Chem. Soc.* **1992**, *114*, 1897–1898.
- (79) Murtaza, Z.; Zipp, A. P.; Worl, L. A.; Graff, D.; Jones, W. E., Jr.; Bates, W. D.; Meyer, T. J. *J. Am. Chem. Soc.* **1991**, *113*, 5113–5114.
- (80) Murtaza, Z.; Graff, D. K.; Zipp, A. R.; Worl, L. A.; Jones, W. E., Jr.; Bates, W. D.; Meyer, T. J. *J. Phys. Chem.* **1994**, *98*, 10504–10513.
- (81) Farrán, A.; Deshayes, K. D. *J. Phys. Chem.* **1996**, *100*, 3305–3307.
- (82) Schanze, K. S.; Lucia, L. A.; Cooper, M.; Walters, K. A.; Ji, H.-F.; Sabina, O. *J. Phys. Chem. A* **1998**, *102*, 5577–5584.
- (83) Parola, A. J.; Pina, F.; Ferreira, E.; Mauro, M.; Balzani, V. *J. Am. Chem. Soc.* **1996**, *118*, 11610–11616.
- (84) Closs, G.; Piotrowiak, P.; MacInnis, J. M.; Fleming, G. R. *J. Am. Chem. Soc.* **1988**, *110*, 2652–2653.
- (85) Closs, G. L.; Johnson, M. D.; Miller, J. R.; Piotrowiak, P. *J. Am. Chem. Soc.* **1989**, *111*, 3751–3753.
- (86) Balzani, V.; Bolletta, F.; Scandola, F. *J. Am. Chem. Soc.* **1980**, *102*, 2, 2152–2163.
- (87) Liang, N.; Miller, J. R.; Closs, G. L. *J. Am. Chem. Soc.* **1990**, *112*, 5353–5354.
- (88) Liang, N.; Miller, J. R.; Closs, G. L. *J. Am. Chem. Soc.* **1989**, *111*, 8740–8741.
- (89) Kroon, J.; Oevering, H.; Verhoeven, J. W.; Warman, J. M.; Oliver, A. M.; Paddon-Row, M. N. *J. Phys. Chem.* **1993**, *97*, 5065–5069.