Electron Transfer Quenching and Photoinduced EPR of Hypericin and the Ciliate Photoreceptor Stentorin†

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Time-correlated single photon counting was used to observe dynamic quenching of the hypericin and stentorin excited singlet states. The fluorescence quenching data for hypericin and stentorin were interpreted in terms of electron transfer. The observed correlation between free energy change of electron transfer and quenching rate constant suggests that quenching proceeds via electron transfer from hypericin and stentorin to the quenchers. EPR spectra for hypericin, stentorin, and stentorin chromoprotein demonstrated that free radical formation was initiated or enhanced by visible light and that similar radical species were produced in each sample. Furthermore, the EPR signal for stentorin was significantly enhanced by 1,4-benzoquinone, but the overall shape and *g*-value was unchanged. We suggest that electron transfer in the excited state of these chromophores results in the formation of a cation radical. This electron transfer is a rapid and efficient pathway for deactivation of hypericin and stentorin excited singlet states and should be considered when discussing the photoreactivity of hypericin as a photodynamic agent and of stentorin as the *Stentor coeruleus* photoreceptor.

Introduction

Hypericin is a pigment of the naphthodianthrone family (Figure 1), found in plants of the genus *Hypericum*.[1] The ability of this quinoid molecule to produce hypericism, a condition of severe sensitivity to light, has been known for some time.[2,3] The photosensory ciliates, *Stentor coeruleus* and *Blepharisma japonicum*, use the hypericin-derived pigments stentorin and blepharismin, respectively, as the photoreceptor chromophores.[2] Recently, this photosensitizing pigment has been studied for its multitude of pharmacological activities and has been employed as an antidepressive agent, as an antitumoral agent, and as an antiviral agent.[4] Probably the most notable of these activities is hypericin's ability to destroy the virus that causes equine infectious anemia[5] and its relative, the human immunodeficiency virus, HIV.[6] The exact mechanism of hypericin's therapeutic activity is not clear but the role of light initiation or enhancement has been established.

Hypericin has been shown to produce singlet oxygen,[2,7,8] and much of the experimental data points to $^1O_2$ as the source of the photodynamic activity. Superoxide ($O_2^{\bullet-}$) has been detected in DMSO solutions of hypericin, in aqueous suspensions of the hypericin lysine salt,[8,9] and in hypericin bound to artificial membranes.[10] Thus hypericin's photobiological activity may also be superoxide radical mediated. Both type I and II quenchers suppressed photokilling of a human fibroblast cell line.[10,11] However, studies of ciliate photoreceptors, stentorins and blepharismins, and their primary photoprocesses have raised some doubt about the role of singlet oxygen and superoxide in hypericin-induced cellular and viral death.

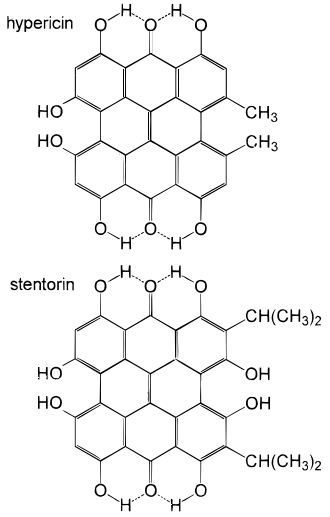

Figure 1. Structures of hypericin and stentorin.

The ciliated protozoa *S. coeruleus* and *B. japonicum* contain hypericin-like pigments[3,12−17] sequestered in subpellicular granules.[18,19] The recently elucidated structure of the stentorin chromophore[13] is given in Figure 1. Action spectra indicate that stentorin and blepharismin are responsible for the photoinduced motile responses of *Stentor*[20] and *Blepharisma*,[21] respectively. Although photokilling of both *Stentor* and *Blepharisma* under high light fluence is related to the formation of singlet oxygen,[7,22,23] it is unlikely that $^1O_2$ initiates the signal cascade resulting in their photophobic and phototactic responses. On the other hand, the primary photoprocesses may originate from proton transfer. Indirect evidence for a light-driven pH decrease across the cell membrane has been found for *Stentor*,[14,20,24,25] and a light-induced acidification has recently been observed for hypericin inserted in phosphatidyl vesicles.[26] An intramolecular proton transfer to an appropriate amino acid has been proposed for the stentorin protein to explain an ultrafast bleaching process observed at 565−630 nm,[27] and proton transfer can be efficiently coupled to electron transfer.[28]

† Dedicated to Professor Saburo Nagakura on his 75th birthday.

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Electron transfer may also be one of the primary photoprocesses in hypericin and hypericin-like pigments. Hypericin and its salts can be efficient acceptors of more than one electron.[29] Furthermore, the uptake of an electron would be enhanced by illumination because of the extra free energy of the excited state.[30] It is also possible for hypericin to function as both an electron acceptor and a donor. From evidence derived using the cytochrome *c* reduction method, it has been proposed that superoxide formation may be a result of electron transfer between the triplet state of hypericin and its ground state.[31] The formal potentials for hypericin as measured in DMSO, −0.87 and −1.18 V for the first two reductions and +0.9 V for the oxidation, also suggest hypericin can act as both a reductant and an oxidant.[28] Nonetheless, reducing agents such as cysteine and glutathione cannot reduce hypericin to a radical anion,[32] and neither dithionite nor ascorbate was effective at reducing stentorin.[12] Likewise, quenching of the excited state decay of hypericin was not observed in the presence of ferrous ions, dithionite, or ascorbate.[33]

The photoreduction of ferric to ferrous ions by hypericin has been observed in DMSO,[34] and mercaptoethanol was generated from the photoreduction of hypericin in the presence of dithiodiethanol.[35] Furthermore, we showed that electron acceptors such as *p*-benzoquinone are efficient quenchers of hypericin's excited singlet state.[33] Using hypericin as a model for stentorin, it was proposed that it is the excited singlet state (^{1}Hyp) from which the primary photoprocess originates. The present study was undertaken to further evaluate the ability of ^{1}Hyp and stentorin to donate an electron to suitable acceptors, thus implicating photoinduced electron transfer from the excited state as both the signal-initiating event in the ciliated protozoa and a possible photosensitizing mechanism of hypericin.

Materials and Methods

Hypericin (1,3,4,6,8,13-hexahydroxy-10,11-dimethylphenanthro[1,10,9,8-*opqra*]perylene-7,14-dione) was obtained from Carl Roth GmbH & Co., Karlsruhe, Germany. Stentorin was isolated by the method of Tao et al.[13] The 50 kDa stentorin chromoprotein, stentorin-2B was obtained as described recently.[35,36] 1,1-Diacetylferrocene, acetylferrocene, ferrocene, 1,4-benzoquinone, 1,4-naphthoquinone, duroquinone, anthraquinone 2-sulfonate, 9,10-anthraquinone, 2-methylanthraquinone, 2,3-dimethylanthraquinone, nitrobenzene, azobenzene, and benzophenone were purchased from Aldrich Chemical Co. (Milwaukee, WI). All measurements were performed in spectroscopic grade dimethyl sulfoxide (DMSO, Fisher Scientific, Pittsburgh, PA) or DMSO that was purified by fractional distillation under reduced pressure after drying over molecular sieves for a few days.

A single-compartment cell housing three electrodes (a 1 mm diameter platinum disk working electrode form Bioanalytical Systems, a platinum wire spiral counter electrode, and a saturated Ag/AgCl reference electrode) was used for measurements of the quencher potentials. The Ag/AgCl (in saturated KCl) electrode measures +0.197 V with respect to the standard hydrogen electrode. An EG&G Princeton Applied Research Model 273 potentiostat−galvanostat was used for recording cyclic voltammograms (CV). The standard electrode potentials were obtained by averaging the cathodic and anodic CV peak potentials. For 1,1-diacetylferrocene, whose oxidation wave was not reversible, it was estimated by subtracting about 50 mV from the oxidation CV peak potential. Reduction potentials were measured in DMSO solutions containing millimolar concentrations of a given quencher and 0.1 M tetra-*n*-butylammonium perchlorate as a supporting electrolyte.

Absorbance spectra were taken using a Hewlett-Packard 8452A diode array spectrophotometer. Steady state fluorescence measurements were performed employing a Shimadzu RF-540 spectrofluorophotometer.

Hypericin fluorescence decay curves were obtained using an Edinburgh 299T time-resolved fluorescence spectrometer, equipped with a H_2-filled nanosecond spark source. The coaxial flash lamp conditions were as follows: (a) 0.5 atm, (b) 1 mm electrode separation, (c) 40 kHz repetition rate, and (d) 7 kV switching voltage. The stability of the spark was monitored by correlation of the Thyraton gating pulses seen by a Thorn EMI 9661B side-window photomultiplier. The excitation wavelength and the emission wavelength were selected using a 560 ± 5 nm interference filter and a 610 nm cutoff filter, respectively. Instrument profiles were determined by scattering the 560 nm excitation pulse through Ludox HS-30 colloidal silica (DuPont). A full-width half-maximum (fwhm) of 1.0 ± 0.1 ns was maintained for all measurements. Reconvolution analyses were performed using the Edinburgh Analytical Instruments' FLA-900 Fluorescence Lifetime Analysis software.

EPR experiments were conducted with a Bruker ECS-106 X-band spectrometer. Cryogenic temperatures were controlled by an Oxford liquid helium cryostat and an ITC4 temperature controller. A calibrated thermocouple, situated beneath the 3 mm i.d. quartz sample tube, was used to monitor sample temperatures. The microwave frequency was sampled during the measurements with a Hewlett-Packard 5340A frequency counter. Sample solutions of hypericin and free stentorin were photolyzed in an EPR cell with a 400 W tungsten lamp and a 520 nm cutoff filter. During the photolysis process the samples, initially at room temperature, were lowered into liquid N_2 to prevent the decay of the radical species and were then placed in the microwave cavity for recording their EPR spectra at 15 K. Light from a 1 kW xenon arc lamp (Oriel Optics, Stratford, CT) equipped with a 520 nm cutoff filter was directed into the EPR cavity during the measurements. The signal/noise ratio for the method described above was better than that obtained by using only direct irradiation in the EPR cavity at 15 K. The conditions for EPR measurements for the whole cells and the 50 kDa stentorin-2B are given in the figure captions.

All samples for the EPR measurements were initially prepared in an anaerobic chamber using DMSO that was degassed under vacuum, saturated with high purity N_2, and then stored in the anaerobic chamber for several days. The hypericin/stentorin solutions were not removed from the anaerobic chamber until after they were loaded into EPR tubes and sealed. The same samples were bubbled with air for no less than 15 min for EPR measurements under aerobic conditions.

Results

Fluorescence quenching data were analyzed according to Stern−Volmer kinetics:[37]

$$\frac{\tau_0}{\tau} = 1 + K_{SV}[Q] = 1 + k_q\tau_0[Q] \qquad (1)$$

where the upper limit of k_q, the bimolecular quenching constant, is given, in the case of a noninteracting compound, by the diffusion constant, k_D, in a given solvent. In DMSO at 298 K, $k_D = 4.8 \times 10^9\ M^{-1}\ s^{-1}$. The quenching and diffusion constants are related by the relation

$$k_q = \gamma k_D \qquad (2)$$

where γ is the quenching efficiency, ranging from 0 to 1.

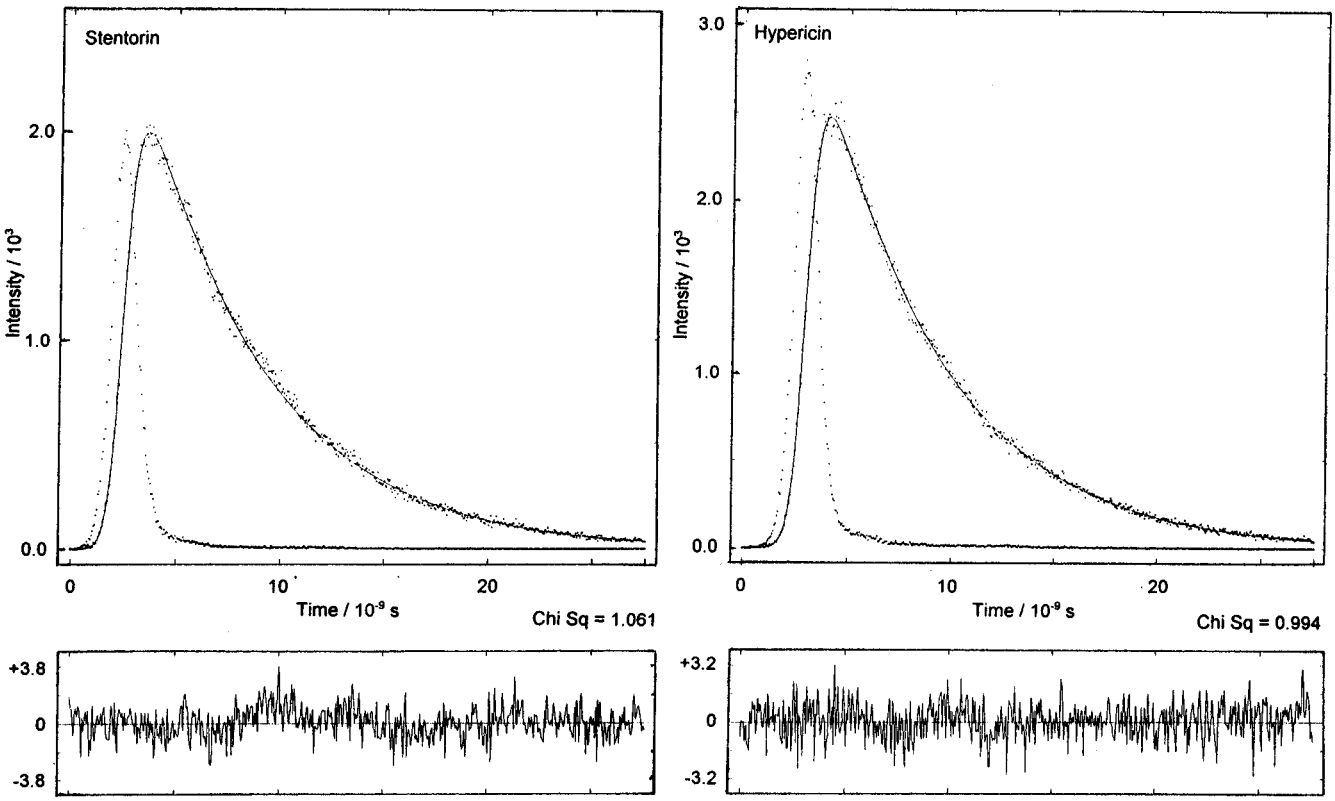

Figure 2. Time-resolved decays of hypericin and stentorin fluorescence. The solid line in each decay represented was obtained by nonlinear least squares analysis using a monoexponential model. The excitation and emission wavelengths were selected by using a 560 ± 5 nm interference filter and a 610 nm cutoff filter, respectively. All decays were collected at room temperature.

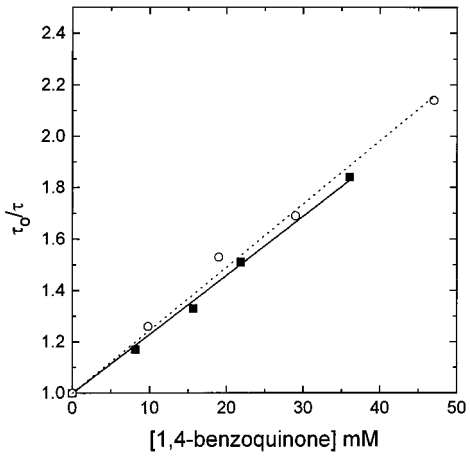

Figure 3. Stern−Volmer plots for fluorescence quenching of hypericin (■) and stentorin (○) by 1,4-benzoquinone. Regression lines are also reported. Conditions were the same as in Figure 1.

The time-correlated single photon counting data were analyzed by reconvolution fit analysis. Hypericin and stentorin decays were exponential in DMSO and were described well by lifetimes of 5.6 and 5.5 ns, respectively. These decay kinetics are in excellent agreement with our previously reported lifetime of ∼5.5 ns for hypericin in aprotic solvents.[33] Figure 2 shows representative hypericin and stentorin decays and illustrates the statistical significance of limiting the fits to a single-exponential term.

The redox potential of hypericin in the singlet excited state was determined to be ∼−1.2 V using the method of Bensasson et al.[38] Compounds such as quinones, which have reduction potentials less negative than this value, may be effective fluorescence quenchers through an electron transfer process. Stern−Volmer plots for 1,4-benzoquinone are shown in Figure 3. The fitted lines were determined by least squares analysis using eq 1. K_{SV}'s, the Stern−Volmer constants, were taken as the slope of each line. Using eq 1, the bimolecular quenching rate constants, k_q's, were determined to be 4.3×10^9 and 4.4×10^9 for stentorin. Table 1 gives the k_q's for a series of quinonic quenchers, many of which were found to be close to the diffusion constant in DMSO, as expected for a diffusion-controlled process. In agreement with the above redox potential for ^{1}Hyp, quenching rate constants approach zero as the quencher's reduction potential approaches −1.2 V (see Table 1).

TABLE 1: Reduction Potentials, E^0, of the Quenchers Employed, Free Energy Change, ΔG_{el}, of the Photoinduced Electron Transfer, and Quenching Rate Constants, k_q, for Hypericin Fluorescence Quenching[a]

quencher	E^0 (V)	ΔG_{el} (eV)	k_q (10^9 M^{-1} s^{-1})
1,4-benzoquinone	−0.134	−1.111	4.3
1,4-naphthoquinone	−0.309	−0.936	3.5
2-sulfonate-9,10-anthraquinone	−0.530	−0.715	1.7
9,10-anthraquinone	−0.546	−0.699	4.3
2-methylanthraquinone	−0.577	−0.668	2.5
2,3-dimethylanthraquinone	−0.607	−0.638	2.5
nitrobenzene	−0.8	−0.445	0.76
azobenzene	−1.022	−0.223	0.12
benzophenone	−1.448	0.203	0

[a] ΔG_{el}'s were calculated by eq 3, while k_q's were evaluated with eq 1.

The results for quenchers reported in Table 2 are given for both time-resolved and steady state measurements. Comparison of the two k_q's reported for duroquinone, ferrocene, acetylferrocene, and 1,1-diacetylferrocene suggests possible ground state interactions between these compounds and hypericin. Furthermore, the absorption spectra of hypericin in the presence of these quenchers was strongly affected (for hypericin−duro-

TABLE 2: Quenchers Strongly Affecting the Absorption Spectrum of Hypericin[a]

quencher	E^0 (V)	k_q (10^9 M^{-1} s^{-1})	k_q (10^{10} M^{-1} s^{-1})
duroquinone	−0.457	8.7	28
ferrocene	+0.701	3.6	0.7
acetylferrocene	+0.921	4.2	7
1,1-diacetylferrocene	+1.119	10	15.6

[a] Symbols are defined in Table 1. [b] Fluorescence quenching constants calculated from static fluorescence measurements are also presented[a].

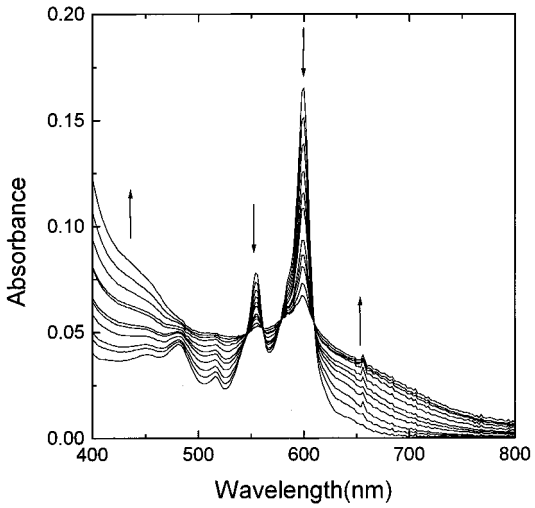

Figure 4. Absorption spectra of hypericin in the presence of duroquinone. Arrows indicate the directions of peak changes upon increasing duroquinone concentration, ranging from 0 to 0.95 mM.

quinone complexation, see Figure 4), indicating that irreversible reactions were occurring. In this case, the simple diffusion−reaction model for noninteracting compounds is not satisfactory[39] and the quenchers were not used for subsequent analyses.

Excited states have an extra free energy content that is approximately equal to the energy difference between the excited D* and ground state. The free energy change for electron transfer from the excited state of hypericin was calculated by[38]

$$\Delta G_{el} = F[E(\mathrm{Hyp}^{\bullet+}|\mathrm{Hyp}) - E(\mathrm{Q}/\mathrm{Q}^{\bullet-})] - \Delta E^{00} - \frac{e^2}{4\pi\epsilon_0\epsilon_s r} \quad (3)$$

where F is the Faraday constant, $E(\mathrm{Hyp}^{\bullet+}/Hyp)$ and $E(\mathrm{Q}/Q^{\bullet-})$ are the oxidation potential of hypericin and the reduction potential of the quencher, ΔE^{00} is the 0−0 electronic excitation energy, and the last term represents the free energy gained by bringing together the two radical ions at the encounter distance r (an average value assumed to be 7 Å for a series of quinones[39]), in a solvent with a dielectric constant ϵ_s.[40] With the assumed encounter distance, the last free energy term in eq 3 was taken to be 0.045 eV in DMSO for an approximate correlation between the free energy change and electron transfer. The free energy change values for electron transfer from hypericin to the various quenchers used are given in Table 1. The ΔG_{el}'s for the compounds in Table 1 correlate well with the results of dynamic fluorescence quenching measurements. A free energy change less than zero represents a thermodynamically favorable processes, and the lack of exothermicity for benzophenone further explains why it was not an effective quencher of hypericin fluorescence in the same concentration range as the other compounds.

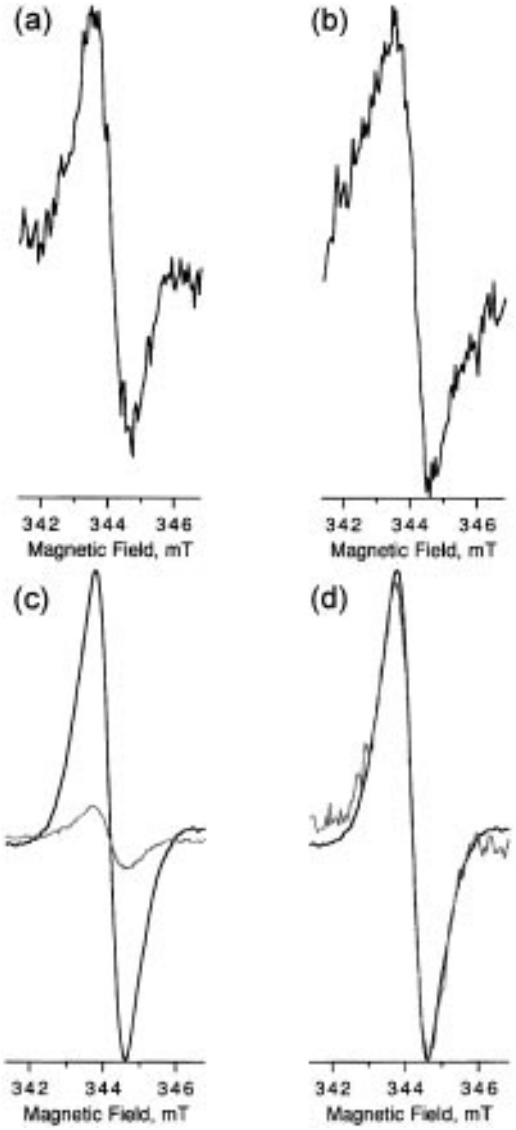

Figure 5. EPR spectra of (a) hypericin under anaerobic conditions; (b) hypericin in the presence of oxygen; (c) stentorin under anaerobic conditions, the light gray line, and stentorin + 10 mM 1,4-benzoquinone, the solid black line; (d) same as part c except the stentorin spectrum was adjusted to a similar amplitude to stentorin + 10 mM 1,4-benzoquinone (multiplication factor was 7.7). Spectra shown are light-on minus light-off produced at room temperature and were recorded at 15 K, as described in the Materials and Methods section.

Photolysis of hypericin in DMSO gave EPR singlet spectra under both anaerobic and aerobic conditions (Figure 5a,b). These two spectra have similar intensity, but the one taken in the presence of O_2 is slightly broader on the low-field side. EPR spectra for stentorin under identical conditions produced results that can be superimposed with those for hypericin. The singlet spectra shown are light minus dark, but this was done only as a background correction since an unpaired electron was not detected in the dark controls for the free chromophores. Figure 5 (c and d) shows photoinduced EPR spectra of stentorin under anaerobic conditions in the absence and presence of 10 mM 1,4-benzoquinone. The EPR signal was approximately 8-fold higher when photolyzed in the presence of the electron acceptor (quinone). No EPR signal was observed for stentorin in the presence of benzoquinone in the dark.

Figure 6 shows the light-induced EPR spectra of a *Stentor* cell suspension, a crude stentorin chromoprotein solution, and stentorin-2B. In all of these samples, the EPR spectra of the bound chromophore are almost indistinguishable from those of

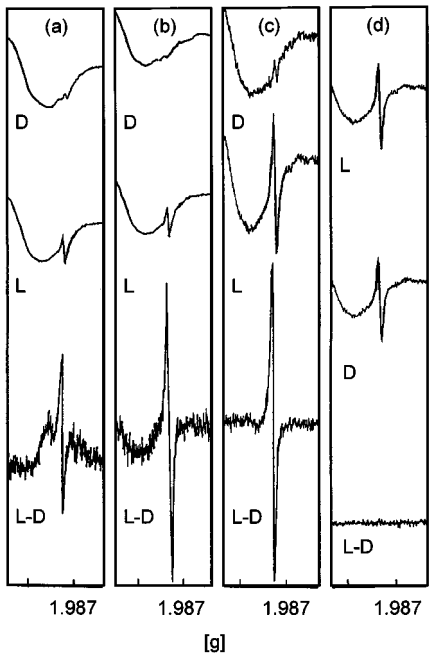

Figure 6. D: in the dark measurement. L: light on measurement, irradiated at 15 K. L−D: the difference between L and D. (a) EPR spectra of *Stentor* cell suspension recorded at 15 K. Healthy cells were collected and immediately subjected to EPR measurements. (b) EPR spectra of a crude stentorin chromoprotein solution recorded at 15 K. The concentration of chromoprotein was adjusted to give a red absorption peak of about 3 in 20 mM Tris buffer, pH 7.8, containing 2% CHAPS (3-[(3-cholamidopropyl)dimethylammonium]-1-propanesulfonate). (c) EPR spectra of purified stentorin-2B recorded at 15 K. The stentorin-2B concentration was adjusted to give a red absorption peak of about 3 in 20 mM Tris buffer, pH 7.8, containing 0.5% CHAPS. (d) EPR spectra of stentorin-2B recorded at 15 K; L, light on; D, same sample kept in the dark for 10 min after light-on measurement. The third spectrum is light minus dark (L−D). Conditions were the same as part c. The *g*-value for hypericin and all radical species shown was 2.000.

hypericin and free stentorin. Unlike hypericin and stentorin, the bound chromophore did have an EPR signal in the dark control. This signal is greatly enhanced upon irradiation with visible light. The photoinduced EPR signal for stentorin-2B was observed even after 10 min in the dark at 15 K (Figure 6d).

Discussion

The data in Table 1 and the Stern−Volmer plots clearly show that the formation of an encounter complex between hypericin and quencher during the lifetime of the excited state resulted in increased deactivation of ^{1}Hyp. We propose that the additional process that competed with fluorescence was electron transfer and can be described by the Rehm and Weller kinetic scheme.[41] When an electron donor, D, is excited to its lowest excited singlet state, D*, in a polar solvent, the fluorescence can be quenched by an electron acceptor, A:

$$D \xrightarrow{h\nu} D^* + A \underset{k_{-D}}{\overset{k_D}{\rightleftharpoons}} [D^*\bullet\bullet\bullet A] \underset{k_{-el}}{\overset{k_{el}}{\rightleftharpoons}} [D^{+}\bullet\bullet\bullet A^{-}] \xrightarrow{k_{bt}} D + A \qquad (4)$$

$$D^{+} + A^{\bullet -}$$

where k_{-D} is the dissociation rate constant for the encounter complex, k_{el} is the electron transfer rate constant, and k_{bt} is the rate constant for back electron transfer to form the reactants in their ground state.

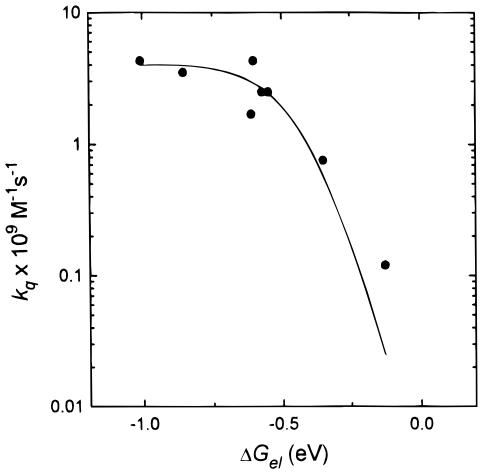

Figure 7. Logarithmic plot of the quenching rate constants as a function of ΔG_{el}. A fitted curve using eq 5 is also reported. The fitted curve was generated using nonlinear least squares analysis with k_{-D}/k_{el}^0 and λ as variable parameters and keeping k_{bt} equal to k_{-D}.

Plotted in Figure 7 is ΔG_{el} vs log k_q for the series of quenchers in Table 1. As ΔG_{el} becomes increasingly negative, k_q becomes larger, until the free energy change is approximately −0.70 eV at the point $k_q \approx k_D$ and the data level off. Using the steady state approximation, the bimolecular quenching rate constant, k_q, for the above reaction scheme is[38,41]

$$k_q = \frac{k_D}{1 + \frac{k_{-D}}{k_{el}^0}\exp\left(\frac{\Delta G_{el}^{\#}}{RT}\right) + \frac{k_{-D}}{k_{bt}^0}\exp\left(\frac{\Delta G_{el}}{RT}\right)} \qquad (5)$$

The term k_{el}^0 is the preexponential factor for the forward electron transfer rate constant, defined by $k_{el} = k_{el}^0 \exp(\Delta G^{\#}_{el}/RT)$.[38] Using the Eigen equation for reactants with no Coulombic interaction,[42] a value of 5.5×10^9 s^{-1} was obtained for k_{-D} in DMSO. The solid line in Figure 7 was calculated by assuming the quenchers are a homologous series and fitting the experimental data with eq 5 above.

Free energy of activation for electron transfer ($\Delta G^{\#}_{el}$) was related to the free energy change, ΔG_{el}, by the theoretical "quadratic" form of the Marcus theory:[43,44]

$$\Delta G_{el}^{\#} = \frac{(\Delta G_{el} + \lambda)^2}{4\lambda} \qquad (6)$$

where λ is the total reorganization energy. During the fitting k_{bt} was kept equal to k_{-D}. Previously it has been shown that the optimization is not sensitive to values of $k_{bt} > 10^8$ s^{-1}.[45,46] The variable parameters were k_{-D}/k_{el}^0 and λ. Nonlinear least squares analysis returned $\lambda = 1.05$ eV. To obtain a value of λ similar to this, one can adopt for the series of quenchers the inner sphere reorganization energy, λ_v, to be 0.25 eV[47] and calculate λ_s, the outer sphere reorganization energy, using an average molecular radius of 3.5 Å for the quinone quenchers[39] and an assumed molecular radius for hypericin (calculated to be 5 Å using the molecular axis for hypericin[26] and assuming spherical). Such calculations yielded $\lambda = \lambda_v + \lambda_s = 1.03$ eV. Clearly, with the crude assumptions and parameters used the agreement should be considered qualitative.

For 2-sulfonated anthraquinone the data point diverges from the fitted curve. This is related to the assumption that the series of quenchers were homologous and all parameters are constant in eq 5 except ΔG_{el} and $\Delta G^{\#}_{el}$. Furthermore, using the Eigen equation to determine k_{-D} is a good approximation in the

absence of Coulombic interactions but may not be valid for 2-sulfonated anthraquinone.

Figure 7 demonstrates a correlation between free energy change and the bimolecular quenching rate constant. This relationship is indicative of electron transfer mechanism and shows that the singlet excited state of hypericin and stentorin can be deactivated by donation of an electron to an appropriate acceptor molecule.

According to the above scheme, upon electron transfer from hypericin and stentorin to the quenchers, a cation radical could be formed under photolytic conditions. Radical ions can be easily formed by reduction of tetraketones or by oxidation of hydroxy quinones and detected by EPR.[48] The formation of EPR detectable radical ions from hypericin has also been reported.[29,32,49,50] The sodium and lysine salts of hypericin produced an EPR signal in ethanol and in water even in the dark.[32] This signal could be enhanced by treatment with $NaBH_4$ and by illumination with visible light.[32] It was suggested that upon irradiation a triplet state was formed and one electron transferred to a ground state hypericin, generating hypericin cation and anion radicals. The semiquinone radicals then reacted with O_2 to produce superoxide, which was EPR detected.[50] Here we show light-induced EPR spectra of hypericin, stentorin, and various stentorin chromoprotein preparations. However, our data shown in Figure 5 (a,b) show that O_2 is not required for the EPR spectra, but because of the broadening that occurs under aerobic conditions, O_2 may lead to production of additional species such as superoxide. Figures 5 and 6 together show that excitation of the quinoid molecules and chromoproteins resulted in generation of similar radical species.

We tentatively attribute the low-temperature EPR signals of stentorins and hypericin to their respective cation radicals. These arise from electron transfer from the excited chromophore to the solvent or to ground state hypericin/stentorin and to an electron acceptor residue (e.g., cystine) for the bound stentorin chromophore. Duroquinone can quench ^{3}Hyp decay, presumably by electron transfer, yielding a duroquinone anion radical when hypericin was irradiated in the presence of 1 mM duroquinone.[50] Presumably that would mean that a cation radical of hypericin was also formed. Our data on electron transfer quenching of hypericin and the related compound stentorin would also suggest that electron transfer from hypericin to various electron acceptors is possible, and the fluorescence data show that it can efficiently occur from the excited singlet state. For 1,4-benzoquinone the quenching efficiency as determined by eq 2 was 0.9. Because of its high quenching efficiency, benzoquinone was used to see if light-induced electron transfer from the chromophores to benzoquinone could result in an enhancement of the EPR signal. This would strengthen the case for electron transfer and subsequent formation of chromophore cation radicals. Oxidation of hydroxy quinones such as naphthacene dihydrotetrone has been shown to generate EPR detectable cation radicals.[48] The ~8-fold increase in EPR signal shown in Figure 5 (c,d) upon addition of quinone is indicative of an electron transfer process. Because the shapes of the two curves are virtually identical, it is likely that the signals originate from the same radical species in the presence and the absence of 1,4-benzoquinone. In light of the established electron transfer quenching data, it would then stand to reason that the radical being observed is a cation radical of stentorin.

We conclude that, upon photoexcitation of hypericin and stentorin, electron transfer occurs from the excited singlet state to an acceptor molecule (e.g. cystine for the stentorin chromoprotein).[35] Electron transfer possibly plays a major role in the fast deactivation of excited hypericin and stentorin, and electron transfer from the excited states of stentorin and hypericin should be considered a possible mechanism for the primary photoprocess of the former for the photophobic response in *S. coeruleus* and the photochemotherapeutic activity of the latter. Since the pK_a of an aromatic hydroxyl proton decreases dramatically in going from the neutral molecule to its cation radical,[51] the excited state electron transfer in stentorin could generate a transient pH change as a cellular signal in the ciliate photosensory transduction.

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