9-27-2005

Interaction of Glutathione S-Transferase with Hypericin: A Photophysical Study

Mintu Halder
Iowa State University

Pramit Kumar Chowdhury
Iowa State University

R. Das
Iowa State University

Prasun Mukherjee
Iowa State University

W. M. Atkins
University of Washington, Seattle

See next page for additional authors

Interaction of Glutathione S-Transferase with Hypericin: A Photophysical Study

Abstract
The photophysics of hypericin have been studied in its complex with two different isoforms, A1-1 and P1-1, of the protein glutathione S-transferase (GST). One molecule of hypericin binds to each of the two GST subunits. Comparisons are made with our previous results for the hypericin/human serum albumin complex (*Photochem. Photobiol.* **1999,** *69*, 633−645). Hypericin binds with high affinity to the GSTs: 0.65 μM for the A1-1 isoform and 0.51 μM for the P1-1 isoform (*Biochemistry* **2004**, *43*, 12761−12769). The photophysics and activity of hypericin are strongly modulated by the binding protein. Intramolecular hydrogen-atom transfer is suppressed in both cases. Most importantly, while there is significant singlet oxygen generation from hypericin bound to GST A1-1, binding to GST P1-1 suppresses singlet oxygen generation to almost negligible levels. The data are rationalized in terms of a simple model in which the hypericin photophysics depends entirely upon the decay of the triplet state by two competing processes, quenching by oxygen to yield singlet oxygen and ionization, the latter of these two are proposed to be modulated by A1-1 and P1-1.

Keywords
Hypericin, Isoforms, Photophysics, Ionization, Mathematical models, Molecular dynamics, proteins, dimethyl sulfoxide, glutathione transferase, perylene, chemistry, photochemistry, photolysis, physical chemistry, ultraviolet spectrophotometry

Disciplines
Chemistry | Physical Chemistry

Comments
Reprinted (adatped) with permission from *Journal of Physical Chemistry B* 109 (2005): 19484, doi: 10.1021/jp051645u.

Authors
Mintu Halder, Pramit Kumar Chowdhury, R. Das, Prasun Mukherjee, W. M. Atkins, and Jacob W. Petrich

Interaction of Glutathione S-Transferase with Hypericin: A Photophysical Study

M. Halder,[†] P. K. Chowdhury,[†] R. Das,[†] P. Mukherjee,[†] W. M. Atkins,[‡] and J. W. Petrich*,[†]

Department of Chemistry, Iowa State University, Ames, Iowa 50011, and Departments of Chemistry and Medicinal Chemistry, University of Washington, Seattle, Washington 98185

Received: April 1, 2005; In Final Form: June 27, 2005

The photophysics of hypericin have been studied in its complex with two different isoforms, A1-1 and P1-1, of the protein glutathione S-transferase (GST). One molecule of hypericin binds to each of the two GST subunits. Comparisons are made with our previous results for the hypericin/human serum albumin complex (*Photochem. Photobiol.* **1999,** *69*, 633−645). Hypericin binds with high affinity to the GSTs: 0.65 μM for the A1-1 isoform and 0.51 μM for the P1-1 isoform (*Biochemistry* **2004**, *43*, 12761−12769). The photophysics and activity of hypericin are strongly modulated by the binding protein. Intramolecular hydrogen-atom transfer is suppressed in both cases. Most importantly, while there is significant singlet oxygen generation from hypericin bound to GST A1-1, binding to GST P1-1 suppresses singlet oxygen generation to almost negligible levels. The data are rationalized in terms of a simple model in which the hypericin photophysics depends entirely upon the decay of the triplet state by two competing processes, quenching by oxygen to yield singlet oxygen and ionization, the latter of these two are proposed to be modulated by A1-1 and P1-1.

Introduction

Hypericin (Figure 1) is a major component of the botanical dietary supplement, St. John's Wort. It is a widely spread, naturally occurring perylene quinone pigment that is of great interest because of its broad spectrum of light-induced biological activities.[1−5] Here, we investigate the photophysics of hypericin in complex with a biologically important protein to which it binds with high affinity, glutathione S-transferase (GST).

GSTs (Figure 2) are biologically significant because they catalyze the conjugation of reduced glutathione (GSH) to a variety of electrophiles and thereby detoxify carcinogens, pesticides, and reactive products generated under oxidative stress. The conjugation of GSH with a foreign compound generally results in the formation of a nontoxic product that can be readily eliminated.[6] In addition to their catalytic function, GSTs also serve as nonenzymatic binding proteins, known as ligandins, that interact with various lipophilic compounds that include steroid and thyroid hormones. Finally, reactive oxygen species can trigger apoptosis, programmed cell death, in cells. The production of GSTs, by helping to protect the cell from oxidative damage caused by reactive oxygen species, assists in obviating the induction of apoptosis.[6] Cytochrome P450, especially the form CYP3A4 which predominates in hepatic tissue, has functions similar to that of GST. It metabolizes endogenous compounds and xenobiotics. It is believed to be capable of binding multiple ligands, of the size of pyrene, and we have shown that it can bind hypericin with high affinity.[7] Human serum albumin (HSA) is the archetypal drug transport protein, and we have studied hypericin bound to HSA extensively in the past.[8] Our hypericin/HSA results serve as a baseline reference.

The biological activity of hypericin and its analogues depends on light. Hypericin displays light-induced activity against several types of viruses, including the human immunodeficiency virus (HIV),[9−11] as well as antiproliferative and cytotoxic effects on tumor cells.[12−15] Hypericin is also a potent antidepressant,[16,17] exhibits light-dependent inhibition of protein kinase C (PKC), which has been suggested to be a locus of its antiviral activity,[18] and is reported to possess numerous other types of biological behavior.[19−21] Hypericin, like other anticancer drugs, also induces apoptosis.[22,23] Consequently, it is important to understand the primary photophysical processes occurring after photon absorption. Our work has produced several hypotheses concerning the excited-state H-atom transfer in these molecules, namely, that excited-state intramolecular hydrogen atom displacement ultimately triggers the ejection of a proton. We have argued that the antiviral activity of hypericin remains significant in the absence of oxygen.[24] The question then arises how hypericin interacts with a cell and by what means its biological effects

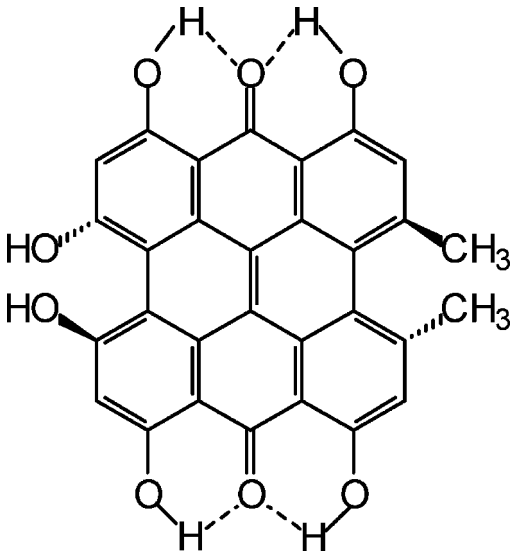

Figure 1. Hypericin (7,14 normal form).

* To whom correspondence should be addressed. E-mail: jwp@iastate.edu.
[†] Iowa State University.
[‡] University of Washington.

10.1021/jp051645u CCC: $30.25
Published on Web 09/27/2005

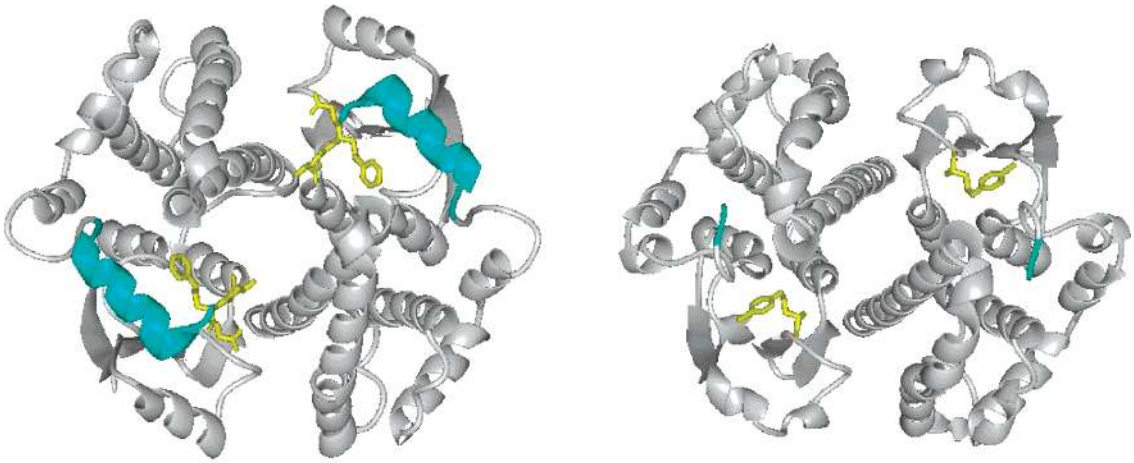

Figure 2. GST isoforms: A1-1 (left), P1-1 (right). Although the two proteins are structurally very homologous, they share only 29% sequence homology.[7]

are exerted. Photoactive molecules are traditionally depicted as either interacting directly with a substrate (type I mechanisms) or producing reactive oxygen species, especially singlet oxygen (type II mechanisms) that exhibit toxicity. Clearly, hypericin and its analogues produce parallel pathways, which work either independently of the type I and type II mechanisms or in such a way as to complement them. We wish to elucidate these parallel pathways.

Owing to this light-induced biological activity, which is diverse, widespread, and important, we have been studying the early time photophysics of hypericin and its analogues.[8,25−37] By means of H/D substitution, investigation of methoxy analogues, and complementary studies using both transient absorption and fluorescence upconversion spectroscopies, we have argued that the major primary photophysical process in hypericin in organic solvents is excited-state intramolecular hydrogen-atom transfer.

After the intramolecular H-atom transfer, there is an *intermolecular* proton transfer to the solvent.[38−40] This event is a reversible process in vitro, as demonstrated by the conservation of the sample optical density over the course of several cycles of pH measurement. We have suggested that these photogenerated protons may be important for understanding the light-induced biological activity of hypericin.[4] As we have discussed elsewhere,[8,41−43] this H-atom transfer is not observed when hypericin is bound to HSA. Indeed, as we indicate below, this transient is not observed when hypericin is bound to either A1-1 or P1-1, which leads us to speculate upon the relative roles of singlet oxygen and photoinduced proton transfer in these systems.

Materials and Methods

GST. The expression and purification of the GSTs, the determination of their binding constants to hypericin, and the quantification of their photoinduced oxidative damage via hypericin have been thoroughly discussed by Lu and Atkins.[7]

Steady-State and Time-Resolved Measurements. Steady-state absorbance spectra were obtained on Perkin-Elmer Lambda 18 double beam UV−vis spectrophotometer with 1-nm resolution. Steady-state fluorescence spectra were obtained on a Spex Fluoromax with a 4-nm band-pass and corrected for lamp spectral intensity and detector response. For both fluorescence and absorption measurements, a 3-mm path length quartz cuvette was used.

The apparatus for time-correlated single photon counting is described elsewhere.[26,27] Fluorescence decay kinetics were recorded by exciting at 580 nm and collecting emission at >610 nm with a cutoff filter for a maximum of 10 000 counts at the peak channel. The polarized fluorescence traces were used to obtain fluorescence anisotropy decay parameters collected to a maximum of 10 000 counts at the peak channel. Fits were considered acceptable for $\chi^2 \leq 1.2$.

Flash Photolysis. Flash photolysis experiments were performed with a system based on a 10-Hz Surelite I-10 (Continuum, Santa Clara, CA) Nd:YAG laser. The fundamental, 1064 nm, was frequency doubled, and the resulting radiation at 532 nm with a 100 mW of average power was used as the pump source. A 75-W Xe lamp (Photon Technology International, NJ) was used as a probe source. The UV from this Xe source was filtered off with a benzene solution filter. Fluorescence and scattered light were blocked by an 8-nm band-pass monochromator at 520 nm before a 1P-28 photomultiplier. Transient absorbance data were collected by a dual-channel 50-MHz Picoscope 3000 (Pico Technology Ltd., U.K.).

Time-Resolved Pump−Probe Absorption Spectroscopy. The instrument function of our time-correlated single photon counting system has a full width at half maximum (fwhm) of 80 ps. To investigate a more rapid phenomenon, better time resolution was required. This was provided by a homemade regeneratively amplified Ti−sapphire system, providing 130-fs pulses at 814 nm which was then frequency doubled and used to perform pump−probe measurements.[36,43,44] For the pump−probe measurements, 407 nm was used as the pump source and a white light continuum, as the probe source. The probe wavelength was 600 nm.

Sample Preparation. A DMSO solution of hypericin was used as the stock solution for sample preparation; the total organic content of the final sample solution was <1% for both hypericin/HSA and hypericin/A1-1 or hypericin/P1-1 in phosphate−EDTA buffer (pH 6.9). The hypericin/protein solutions were incubated for about 4−7 h in the dark prior to steady-state, time-resolved, and flash photolysis experiments. For steady-state fluorescence measurements, the solutions were

TABLE 1: Fluorescence Lifetimes of Hypericin Bound to GSTs[a]

	τ_1	a_1	τ_2	a_2	τ_3	$\langle\tau\rangle^b$
P1-1/hyp						
10/1	140	0.24	1860	0.18	5400	3500
1/1	170	0.24	1550	0.14	5200	3480
1/1.7	160	0.31	1560	0.15	5200	3100
1/3	210	0.31	1890	0.15	5250	3180[c]
1/7	160	0.51	1460	0.14	5230	2120
1/10	130	0.72	1100	0.10	5200	1140
A1-1/hyp						
10/1	300	0.28	2800	0.17	5900	3800
1/1	300	0.34	2000	0.14	5400	3200
1/1.7	280	0.43	2100	0.13	5650	2900
1/3	250	0.68	1200	0.15	5450	1250
1/10	150	0.86	820	0.12	4200	300

[a] All times are given in picoseconds. Fluorescence lifetimes are fit to a sum of three exponentials. In all cases, [hyp] $\sim 5.0 \times 10^{-6}$ M. [b] The error in the average fluorescence lifetime is given by $\langle\tau\rangle = \sum_i a_i\tau_i$. It is estimated to be accurate to ca. ±15%. [c] This value is higher than expected given the P1-1/hypericin ratio and the corresponding results for A1-1/hypericin. Nevertheless, as we indicate in the text, under these conditions, the fluorescence anisotropy decay departs from a single exponential, indicating surface binding of the hypericin.

prepared with a stoichiometry of 10:1 protein-to-hypericin with [hyp] $\sim 1.0 \times 10^{-6}$ M. For time-resolved fluorescence studies, [hyp] $\sim 5.0 \times 10^{-6}$ M, varying amounts of protein concentrations as indicated in Table 1 were employed. Flash photolysis experiments were performed with [hyp] $\sim 5.0 \times 10^{-5}$ M with a 3:1 protein-to-hypericin ratio. For pump−probe experiments, [hyp] was kept at $\sim 8.0 \times 10^{-5}$ M and a 1:1 protein-to-hypericin ratio was maintained. (The spectra were found to be similar in the case of the 10:1 and 1:1 protein-to-hypericin ratios.)

Results and Discussion

Binding of Hypericin to GSTs and Singlet Oxygen Generation. GST binds hypericin very tightly:[7] A1-1 with $K_D = 0.65\ \mu$M and P1-1 with $K_D = 0.51\ \mu$M. Binding of hypericin to GSTs also inhibits their catalytic activity.[7] The submicromolar affinity with which hypericin binds to GST is the first case of high-affinity binding of hypericin to a biologically important molecule that has been documented.

Our data suggest that one hypericin molecule binds to each of the two subunits comprising GST (Table 1). Binding is determined by monitoring the average fluorescence lifetime of hypericin as a function of the GST/hypericin ratio. When GST/hypericin $<^1/_2$, the average fluorescence lifetime decreases significantly from a constant value of ~3.3 ns. An even more compelling indication of this binding ratio is that when it is less than $^1/_2$, the fluorescence anisotropy decay is no longer a simple single exponential with a ≥20-ns depolarization time but takes on a second much more rapid component indicative of a chromophore loosely bound to the surface.[8] For example, when P1-1/hypericin is $^1/_3$, $r(t) = 0.02\ \exp(-t/0.98\ \text{ns}) + 0.27\ \exp(-t/19\ \text{ns})$.

An intriguing observation is that the photophysical properties of hypericin can be altered upon binding to GSTs. In particular, when hypericin binds to A1-1, light-induced oxidative damage is produced, as evidenced by the mass spectrometry data obtained after 30 min of optical irradiation. On the other hand, no such oxidative damage is observed for P1-1. Figure 3 compares photosensitized oxidation by hypericin of HSA, A1-1, and P1-1.[7] While P1-1 is essentially impervious to oxidative damage, both HSA and A1-1 are extremely susceptible.

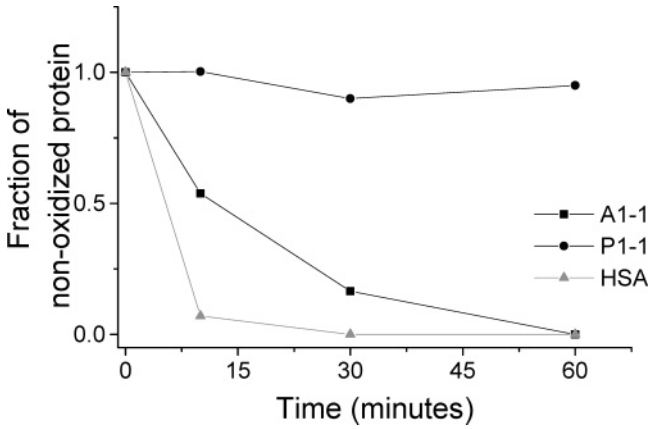

Figure 3. Protein oxidation from light-activated bound hypericin. Details of the experiment are given by Lu and Atkins.[7]

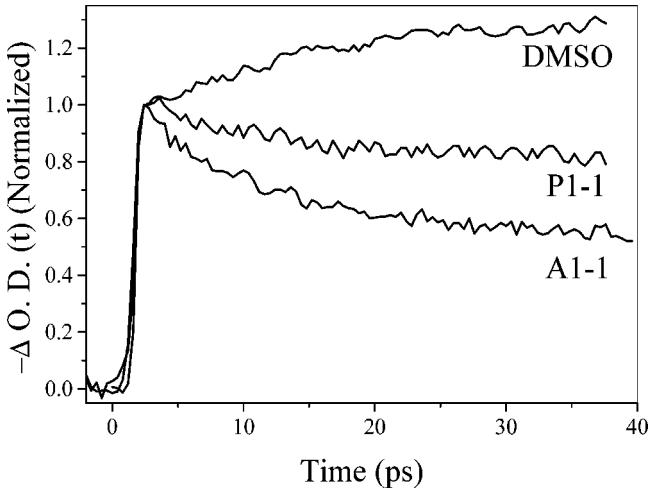

Figure 4. Singlet kinetics of hypericin. The probe wavelength is 600 nm. Only hypericin in DMSO exhibits the ~10-ps rise time attributed to excited-state intramolecular H-atom transfer. Note that the hypericin/P1-1 trace is nearly flat on the 40-ps time scale, more like that of hypericin/HSA [8] than that of hypericin/A1-1.

Photophysics of Hypericin Bound to GSTs. As noted in the Introduction, we have argued that one of the primary processes deactivating the excited-state singlet of hypericin in bulk organic solvent is intramolecular hydrogen-atom transfer. The signature of this process is the rise time in the kinetics, as indicated in Figure 4 for hypericin in DMSO. This process is not, however, observed when hypericin is bound to HSA.[8] We interpreted this result in terms of hypericin binding to a tryptophan residue in HSA that inhibited its H-atom transfer. Given that intramolecular H-atom transfer is impeded in hypericin/HSA and that light-induced protein oxidation of hypericin/HSA is more similar to that of hypericin/A1-1 than to that of hypericin/P1-1, it was our original expectation that the photophysics of hypericin/A1-1 should more nearly resemble that of hypericin/HSA than that of hypericin/P1-1. This expectation was not, however, borne out uniformly by subsequent experiment: the singlet kinetics (Figure 4) and fluorescence intensities (Figure 5) of hypericin/P1-1 more closely resemble those of hypericin/HSA. On the other hand, the long-time kinetics (Figure 6) indicate that the population of hypericin radical ion in A1-1 is essentially identical to that generated in complex with HSA and about a factor of 2 less than that obtained in complex with P1-1.

We note that the data presented in Figure 4 are transient absorption data, which detect both stimulated emission, induced

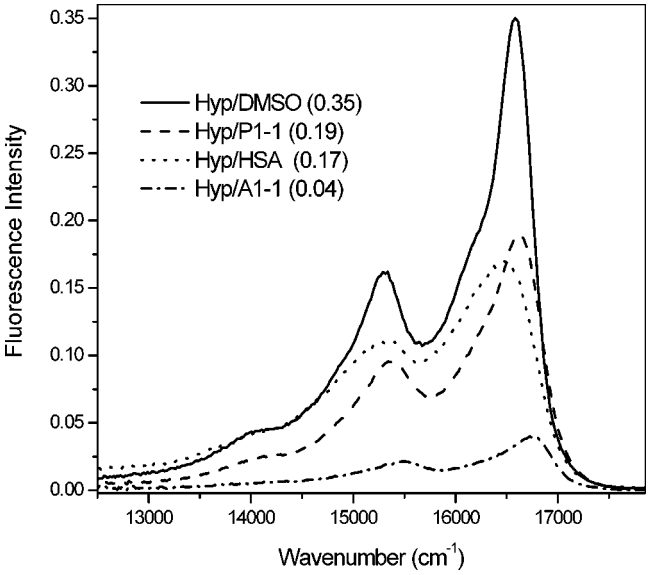

Figure 5. Absolute hypericin fluorescence intensity vs wavenumber. The integrated intensities on the energy (wavenumber) scale provide the fluorescence quantum yields (determined from these spectra), which are given in parentheses in the figure. Again, note that the fluorescence quantum yield of hypericin/P1-1 is more like that of hypericin/HSA than that of hypericin/A1-1. The samples were excited at 550 nm.

absorption, and ground-state bleaching. Consequently, there is always the possibility that the interpretation of the observed kinetics is complicated by several simultaneous phenomena. The interpretation of the GST data is based upon our failure to see a rise time for the 1:1 complex of hypericin/HSA in not only transient absorption but also fluorescence upconversion experiments,[8] the latter of which only detect emission from the lowest singlet state.

The long-time kinetics do *not* reflect the decay of the triplet, T_1. Indeed, we do not detect a transient triplet species for hypericin bound to these proteins in our experiments. The lifetime of hypericin triplet in an air-saturated solution at room temperature is expected to be ≤ 1 μs, depending on the solvent (see the caption to Figure 6).

No component of this duration is observed. We have previously observed such long-lived species of hypericin and its analogues in micelles.[45] Jardon and co-workers have discussed long-lived hypericin transients in terms of deprotonation of the hypericin triplet to form the anion,[46,47] and we[38] have shown that triplet hypericin is a proton donor. It thus seems reasonable to attribute the long-lived transient to a deprotonated species originating from the triplet, Hyp^-, and in the following discussion we refer to this species as a deprotonated or anionic form of hypericin.

It may be argued that hypericin bound to P1-1 is not accessible to oxygen and that this explains the absence of oxidative damage in the complex. This is unlikely. First, the structural homology of the two proteins (Figure 2) suggests that hypericin binds similarly to both. Second, a more quantitative indication is given by Stern−Volmer fluorescence quenching experiments.[7] Through the use of iodide as a quencher, quenching constants of 4.3, 8.3, and 1.9 M^{-1} were obtained for hypericin bound to A1-1, P1-1, and HSA, respectively.[7] These results indicate that hypericin bound to all three of these proteins is accessible to fluorescence quenchers and that, in fact, it is even slightly more accessible when bound to P1-1 than to the other two proteins.

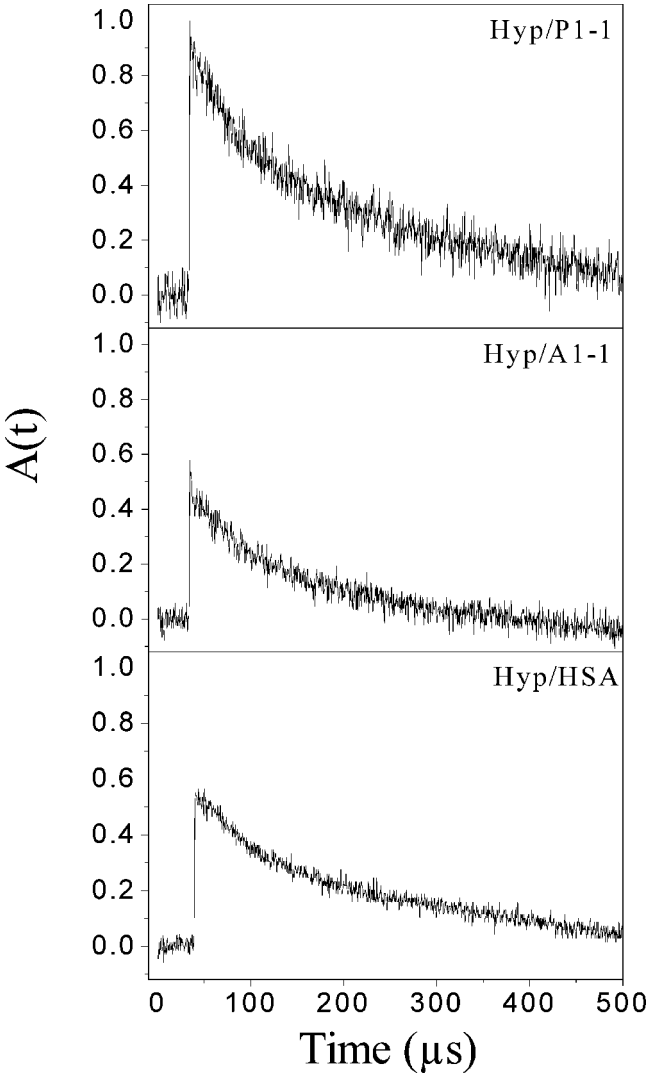

Figure 6. Room-temperature, long-time kinetics of hypericin, reflecting the behavior of the hypericin anion. For laser flash photolysis experiments, samples were pumped at 532 nm and the transient was probed after a monochromator set at 520 nm (see experimental details). For all the samples [hyp] $\sim 5.0 \times 10^{-5}$ M with 3:1 protein-to-hypericin ratio. The solutions were prepared in phosphate−EDTA buffer (pH 6.9) for both GSTs and HSA. The traces are scaled to their relative intensity, with the trace for hyp/P1-1 normalized to unit intensity at time zero. These results were reproduced three times using a different protein preparation for each experiment. For the long-time kinetics, the intensity of the hypericin/A1-1 signal is essentially identical to that of hypericin/HSA. In the context of earlier work by ourselves,[38] Falk and co-workers,[48] and especially Jardon and co-workers,[46,47] we attribute the origin of these long-lived transients to the deprotonation of the hypericin triplet. Jenks and co-workers[49] have observed similar transients arising from reduction and oxidation of triplet by electron acceptors and donors. It is unlikely that the long-lived kinetics can be attributed to the triplet. The time constant for triplet decay resulting from oxygen quenching is estimated to be on the order of 5 μs assuming a diffusion-controlled reaction and an oxygen concentration at atmospheric pressure of ~0.2 mM.[50]

The unanticipated behavior of the singlet kinetics and the fluorescence quantum yields originally proved quite puzzling in light of the hypericin activity. They, however, can be rationalized by considering the following points:

1. The intersystem crossing rate, k_{ISC}, must be five times larger for hypericin/A1-1 than for hypericin/P1-1 in order to explain the fluorescence quantum yield and the singlet decay data, $\Phi_T^{A1-1} = 5\Phi_T^{P1-1}$ (Figures 4 and 5).

2. Note that the anion intensity of hypericin/A1-1 is about two times smaller than that of hypericin/P1-1, $2\Phi_{ion}^{A1-1} = \Phi_{ion}^{P1-1}$ (Figure 6).

3. The data in Figure 3 highlighting photosensitized oxidation of protein imply that hypericin/A1-1 makes at least ten times more singlet oxygen than hypericin/P1-1, $\Phi_{\Delta}^{A1-1} = 10\Phi_{\Delta}^{P1-1}$. Consequently, we can rationalize hypericin/A1-1 being about ten times more reactive than hypericin/P1-1.

4. We make the reasonable assumption that singlet oxygen and hypericin anion are produced only by the triplet and that their respective yields, Φ_{ion} and Φ_{Δ}, are given by the standard expressions

$$\Phi_{ion} = \Phi_T \frac{k_{ion}}{k[O_2] + k_{ion}}$$

and

$$\Phi_{\Delta} = \Phi_T \frac{k[O_2]}{k[O_2] + k_{ion}}$$

where we assume that $k[O_2]$ is the same for all the proteins considered here. Taking the ratios of the respective yields

$$\frac{\Phi_{ion}^{A1-1}}{\Phi_{ion}^{P1-1}} = \frac{\Phi_T^{A1-1}}{\Phi_T^{P1-1}} \frac{k_{ion}^{A1-1}}{k_{ion}^{P1-1}} \frac{k[O_2] + k_{ion}^{P1-1}}{k[O_2] + k_{ion}^{A1-1}}$$

and

$$\frac{\Phi_{\Delta}^{A1-1}}{\Phi_{\Delta}^{P1-1}} = \frac{\Phi_T^{A1-1}}{\Phi_T^{P1-1}} \frac{k[O_2] + k_{ion}^{P1-1}}{k[O_2] + k_{ion}^{A1-1}}$$

from which we conclude that

$$20 = \frac{\Phi_{\Delta}^{A1-1}}{\Phi_{\Delta}^{P1-1}} \frac{\Phi_{ion}^{P1-1}}{\Phi_{ion}^{A1-1}} = \frac{k_{ion}^{P1-1}}{k_{ion}^{A1-1}}$$

In other words, because the triplet state of hypericin is much more susceptible to decay into the anion when it is bound to P1-1 than to A1-1 or HSA, it generates considerably less singlet oxygen when complexed to P1-1 than to the two latter proteins. The origin of this difference can only be attributed to the amino acids surrounding hypericin in the various proteins and their effect on the excited-state photophysics of hypericin. An energy level diagram summarizing these processes is given in Figure 7.

Conclusions

While the argument given above provides a pleasing rationalization of the kinetic and steady-state data and renders it consistent with the light-induced biological reactivity of hypericin in different protein environments, it is important to note that the atomistic details of A1-1 and P1-1 giving rise to these differences must still be resolved. This is a main goal of the current work in progress.

An interesting consequence of this work is that it suggests that insofar as the long-lived transient species depicted in Figure 6 can be attributed to the hypericin anion originating from the triplet, Hyp^-, even if there is no *intramolecular* H-atom transfer in the singlet state for hypericin bound to HSA, A1-1, or P1-1, the corresponding triplet state is still able to liberate a proton.

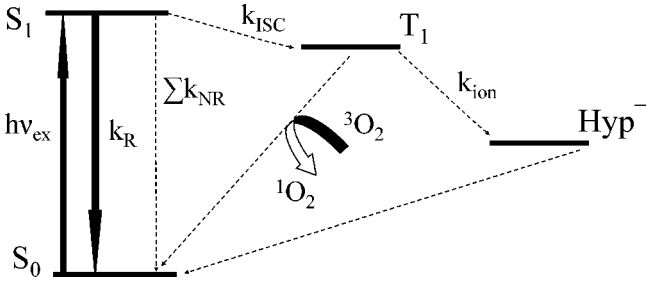

Figure 7. Electronic energy levels of hypericin and their deactivation processes. k_R is the radiative rate of the first excited singlet and Σk_{NR} is the sum of all the nonradiative processes deactivating this state except for k_{ISC}, the intersystem crossing rate, which is specifically indicated, taking the singlet to the triplet. At room temperature, we suggest that the triplet state decays predominantly by deprotonation to form the anion, k_{ion}, or by interaction with ground-state oxygen to form singlet oxygen, $k[^3O_2]$.

To date, the bulk of the photophysical studies of hypericin has been carried out in homogeneous organic solution. Studying the light-induced activity of hypericin in complex with biologically important proteins will contribute to an appreciation of both its therapeutic utility as well as its possible toxicity.

Acknowledgment. J.W.P. was supported by Grant No. P01 ES012020 from the National Institute of Environmental Health Sciences (NIEHS) and the Office of Dietary Supplements (ODS), National Institutes of Health (NIH). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIEHS and NIH. W.M.A. was supported by the UW NIEHS sponsored Center for Ecogenetics and Environmental Health, Grant NIEHSP30ES07033 and by NIH, Grant GM62284. We thank Mr. Weiya D. Lu for providing GST for this work.

References and Notes

(1) Duran, N.; Song, P. S. *Photochem. Photobiol.* **1986**, *43*, 677.
(2) Lown, J. W. *Can. J. Chem.* **1997**, *75*, 99.
(3) Diwu, Z. *Photochem. Photobiol.* **1995**, *61*, 529.
(4) Kraus, G. A.; Zhang, W. J.; Fehr, M. J.; Petrich, J. W.; Wannemuehler, Y.; Carpenter, S. *Chem. Rev.* **1996**, *96*, 523.
(5) Falk, H. *Angew. Chem., Int. Ed.* **1999**, *38*, 3117.
(6) Adman, E. T.; Le Trong, I.; Stenkamp, R. E.; Nieslanik, B. S.; Dietze, E. C.; Tai, G.; Ibarra, C.; Atkins, W. M. *Proteins: Struct., Funct., Genet.* **2001**, *42*, 192.
(7) Lu, W. D.; Atkins, W. M. *Biochemistry* **2004**, *43*, 12761.
(8) Das, K.; Smirnov, A. V.; Wen, J.; Miskovsky, P.; Petrich, J. W. *Photochem. Photobiol.* **1999**, *69*, 633.
(9) Meruelo, D.; Lavie, G.; Lavie, D. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 5230.
(10) Lenard, J.; Rabson, A.; Vanderoef, R. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 158.
(11) Hudson, J. B.; Zhou, J.; Chen, J.; Harris, L.; Yip, L.; Towers, G. H. N. *Photochem. Photobiol.* **1994**, *60*, 253.
(12) Anker, L.; Gopalakrishna, R.; Jones, K. D.; Law, R. E.; Couldwell, W. T. *Drugs Future* **1995**, *20*, 511.
(13) Couldwell, W. T.; Gopalakrishna, R.; Hinton, D. R.; He, S.; Weiss, M. H.; Law, R. E.; Apuzzo, M. L. *Neurosurgery* **1994**, *35*, 705.
(14) Zhang, W.; Anker, L.; Law, R. E.; Hinton, D. R.; Gopalakrishna, R.; Pu, Q.; Gundimeda, U.; Weiss, M. H.; Couldwell, W. T. *Clin. Cancer Res.* **1996**, *2*, 843.
(15) Zhang, W.; Law, R. E.; Hinton, D. R.; Couldwell, W. T. *Cancer Lett.* **1997**, *120*, 31.
(16) Linde, K.; Ramirez, G.; Mulrow, C. D.; Pauls, A.; Weidenhammer, W.; Melchart, D. *Br. Med. J.* **1996**, *313*, 253.
(17) Suzuki, O. K.; Oya, M.; Bladt, S.; Wagner, H. *Planta Med.* **1984**, *50*, 272.
(18) Takahashi, I. N. S.; Kobayashi, E.; Nakano, H.; Suziki, K.; Tamaoki, T. *Biochem. Biophys. Res. Commun.* **1989**, *165*, 1207.

(19) Andreoni, A.; Colasanti, A.; Colasanti, P.; Mastrocinique, M.; Riccio, P.; G., R. *Photochem. Photobiol.* **1994**, *59*, 529.
(20) Thomas, C.; MacGill, R. S.; Miller, G. C.; Pardini, R. S. *Photochem. Photobiol.* **1992**, *55*, 47.
(21) Vandenbogaerde, A. L.; Cuveele, J. F.; Proot, P.; Himpens, B. E.; Merlevede, W. J.; deWitte, P. A. *J. Photochem. Photobiol., B* **1997**, *38*, 136.
(22) Weller, M.; Trepel, M.; Grimmel, C.; Schabet, M.; Bremen, D.; Krajewski, S.; Reed, J. C. *Neurol. Res.* **1997**, *19*, 459.
(23) Mirossay, L.; Mirossay, A.; Kocisova, E.; Radvakova, I.; Miskovsky, P.; Mojzis, J. *Physiol. Res.* **1999**, *48*, 135.
(24) Park, J.; English, D. S.; Wannemuehler, Y.; Carpenter, S.; Petrich, J. W. *Photochem. Photobiol.* **1998**, *68*, 593.
(25) Gai, F.; Fehr, M. J.; Petrich, J. W. *J. Am. Chem.Soc.* **1993**, *115*, 3384.
(26) Gai, F.; Fehr, M. J.; Petrich, J. W. *J. Phys. Chem.* **1994**, *98*, 8352.
(27) Gai, F.; Fehr, M. J.; Petrich, J. W. *J. Phys. Chem.* **1994**, *98*, 5784.
(28) Das, K.; English, D. S.; Fehr, M. J.; Smirnov, A. V.; Petrich, J. W. *J. Phys. Chem.* **1996**, *100*, 18275.
(29) Das, K.; English, D. S.; Petrich, J. W. *J. Phys. Chem. A* **1997**, *101*, 3241.
(30) Das, K.; English, D. S.; Petrich, J. W. *J. Am. Chem. Soc.* **1997**, *119*, 2763.
(31) Das, K.; Smirnov, A. V.; Snyder, M. D.; Petrich, J. W. *J. Phys. Chem. B* **1998**, *102*, 6098.
(32) Das, K.; Dertz, E.; Paterson, J.; Zhang, W.; Kraus, G. A.; Petrich, J. W. *J. Phys. Chem. B* **1998**, *102*, 1479.
(33) Das, K.; Ashby, K. D.; Wen, J.; Petrich, J. W. *J. Phys. Chem. B* **1999**, *103*, 1581.
(34) English, D. S.; Das, K.; Ashby, K. D.; Park, J.; Petrich, J. W.; Castner, E. W. *J. Am. Chem. Soc.* **1997**, *119*, 11585.
(35) 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.
(36) English, D. S.; Zhang, W.; Kraus, G. A.; Petrich, J. W. *J. Am. Chem. Soc.* **1997**, *119*, 2980.
(37) Smirnov, A. V.; Das, K.; English, D. S.; Wan, Z.; Kraus, G. A.; Petrich, J. W. *J. Phys. Chem. A* **1999**, *103*, 7949.
(38) Fehr, M. J.; Mccloskey, M. A.; Petrich, J. W. *J. Am. Chem. Soc.* **1995**, *117*, 1833.
(39) Sureau, F.; Miskovsky, P.; Chinsky, L.; Turpin, P. Y. *J. Am. Chem. Soc.* **1996**, *118*, 9484.
(40) Chaloupka, R.; Sureau, F.; Kocisova, E.; Petrich, J. W. *Photochem. Photobiol.* **1998**, *68*, 44.
(41) Petrich, J. W. *Int. Rev. Phys. Chem.* **2000**, *19*, 479.
(42) Chowdhury, P. K.; Das, K.; Datta, A.; Liu, W. Z.; Zhang, H. Y.; Petrich, J. W. *J. Photochem. and Photobiol., A* **2002**, *154*, 107.
(43) Halder, M.; Chowdhury, P. K.; Gordon, M. S.; Petrich, J. W.; Das, K.; Park, J.; Alexeev, Y. *Adv. Photochem.* **2005**, *28*, in press.
(44) English, D. S.; Das, K.; Petrich, J. W. *Abstr. Pap. Am. Chem. Soc.* **1997**, *213*, 405.
(45) Wen, J.; Chowdhury, P.; Wills, N. J.; Wannemuehler, Y.; Park, J.; Kesavan, S.; Carpenter, S.; Kraus, G. A.; Petrich, J. W. *Photochem. Photobiol.* **2002**, *76*, 153.
(46) Dumas, S.; Eloy, D.; Jardon, P. *New J. Chem.* **2000**, *24*, 711.
(47) Dumas, S. Thesis, Universite Joseph Fourier- Grenoble 1, 2000.
(48) Immitzer, B.; Etzlstorfer, C.; Obermuller, P. A.; Sonnleitner, M.; Schutz, G. J.; Falk, H. *Monatsh. Chem.* **2000**, *131*, 1039.
(49) Darmanyan, A. P.; Jenks, W. S.; Eloy, D.; Jardon, P. *J. Phys. Chem. B* **1999**, *103*, 3323.
(50) Murov, S. L.; Carmichael, I.; Hug, G. L. *Handbook of Photochemistry,* 2nd ed.; Marcel Dekker: New York, 1993.