Ultrafast Excited-State Processes in the Antiviral Agent Hypericin

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Hypericin (Figure 1) has been shown by Lavie et al. to inactivate mature and properly assembled retroviruses, notably human immunodeficiency virus (HIV).[1] Recently Kraus, Carpenter, and co-workers demonstrated the antiretroviral activity of hypericin against equine infectious anemia virus (EIAV), a lentivirus closely related to HIV, and have determined that light is required for activity.[2] The requirement of light leads to the fundamental questions, *what is the mechanism of action of hypericin and what is the role of light*?

Hypericin produces singlet oxygen.[3-5] But investigations of the photoreceptors, the stentorins, of the protozoan ciliate *Stentor coeruleus* cause one to question the relative importance of photosensitized formation of singlet oxygen by hypericin for its antiviral activity. The structure of stentorin[6] and its absorption and emission spectra are nearly identical with those of hypericin. Song and co-workers observed a light-induced pH change across the cell membranes of *S. coerulus* in vivo and in model systems and suggested the possibility of proton release from stentorin.[7] Under conditions of sufficiently high light flux ($\sim$5000 W/m^2), stentorin can produce enough singlet oxygen to kill *S. coerulus*.[4]

Given the structural and spectral similarities of hypericin and stentorin and given the involvement of proton generation at "natural" light fluxes and the production of levels of singlet oxygen that are toxic at high light fluxes, our goal is to elucidate the photophysical processes responsible for the antiviral activity of hypericin and, in the process, to identify the significant pathways of nonradiative decay of the excited singlet state of hypericin.

Using a white-light continuum generated by an amplified laser system providing $\lesssim$1-ps resolution, transient absorption spectra at given time delays and decay kinetics at various wavelengths were obtained.[8] Identical results are obtained using 588 or 294 nm as the excitation source. The major observations and conclusions obtained from these preliminary studies are the following: (1) Transient absorption spectra indicate the presence of a short-lived excited-state species that is formed immediately upon excitation of ground-state hypericin (Figure 1). (2) This short-lived excited state decays in $\sim$5 ps as determined either by direct measurement of the excited-state absorbance at 630 nm (Figure 2) or by the finite rise time for the bleaching at 600 nm. We propose that this species forms an excited-state tautomer whose deprotonation would be responsible for the light-dependent pH decrease observed by Song and co-workers.[7] The possibility for ultrafast proton transfer in hypericin is based on its local structural analogy (proximity of hydroxyl groups to carbonyl groups) with the well-characterized prototype for proton transfer, 3-hydroxyflavone.[9] The $\sim$5-ps state cannot be the fluorescent state, whose lifetime is $\sim$5 ns, but may indeed be a precursor to it. (3) Another strong transient absorbance is observed farther in the red immediately upon excitation of hypericin (Figure 3). This transient absorbance is attributed to a solvated electron because it can be quenched by the electron scavenger acetone.[10]

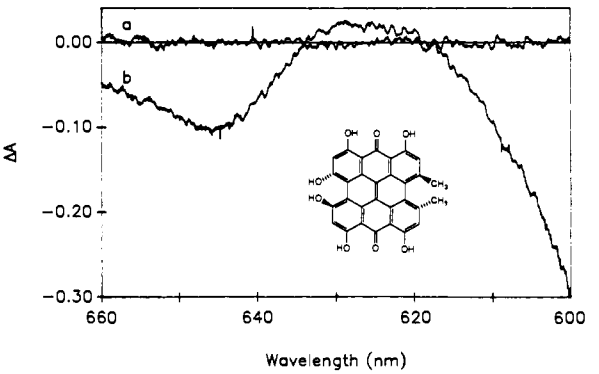

Figure 1. Transient absorption spectrum of hypericin in methanol at 20 °C using a charge-coupled device (Princeton Instruments 1152UV). λ_{ex} = 588 nm. (a) Delay time of −20 ps between the pump and the probe pulse. This is the control experiment that demonstrates that the observed signal is due to the excitation or pump pulse. (b) "Zero" delay between pump and probe. Three distinct phenomena are observed. From lower wavelength to higher wavelength, they are (1) bleaching of the ground-state absorption of hypericin; (2) a new transient species whose absorbance can be clearly seen at $\sim$630 nm; and (3) a transmission increase in a region where there is no ground-state absorbance (this corresponds to stimulated emission from excited-state hypericin). The short-lived absorbance at 630 nm is attributed to formation of a species with a protonated carbonyl (see text). This assignment is based on our result that the hypericin along with no hydroxyl groups, mesonaphthobianthrone, exhibits essentially no fluorescence in the aprotic solvent DMSO. In H_2SO_4, however, its absorption and fluorescence spectra are nearly identical to those of hypericin.

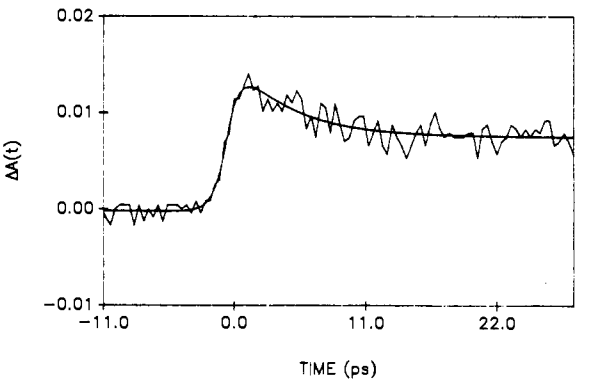

Figure 2. Transient absorption spectrum of hypericin in methanol at 20 °C using separate diodes to monitor the transmitted and reference beams obtained from a white-light continuum. λ_{ex} = 588 nm, and λ_{probe} = 630 nm. The transient absorbance rises within the duration of our $\sim$1-ps pulse and decays according to the law $\Delta A(t) = 0.0047 \exp(-t/4.8\text{ ps}) + 0.0043 \exp(-t/\infty)$. The second component does not decay on the time scale of the experiment. The observation of the $\sim$5-ps transient in this region is consistent with the one-color pump–probe measurements of Struve and co-workers, who observed a *finite rise time* in the ground-state bleaching of stentorin.[12] We interpret this finite rise time (see text) as arising from an excited-state species that has oscillator strength in the same spectral region as the ground-state species.

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(1) Lavie, G.; Valentine, F.; Levin, B.; Mazur, Y.; Gallo, G.; Lavie, D.; Weiner, D.; Meruelo, D. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 5963.
(2) Kraus, G. A.; Pratt, D.; Tossberg, J.; Carpenter, S. *Biochem. Biophys. Res. Commun.* **1990**, *172*, 149. Carpenter, S.; Kraus, G. A. *Photochem. Photobiol.* **1991**, *53*, 169.
(3) Durán, N.; Song, P.-S. *Photochem. Photobiol.* **1986**, *43*, 677.
(4) Yang, K.-Ch.; Prusti, R. K.; Walker, E. W.; Song, P.-S.; Wanatabe, M.; Furuya, M. *Photochem. Photobiol.* **1986**, *43*, 305.
(5) Racinet, H.; Jardon, P.; Gautron, R. *J. Chim. Phys.* **1988**, *85*, 971. Bouirig, H.; Eloy, D.; Jardon, P. *J. Chim. Phys.* **1992**, *89*, 1391.
(6) Tao, N.; Orlando, M.; Hyon, J.-S.; Gross, M.; Song, P.-S., *J. Am. Chem. Soc.*, in press.
(7) Song, P.-S.; Walker, E. B.; Auerbach, R. A.; Robinson, G. W. *Biophys. J.* **1981**, *35*, 551. Walker, E. B.; Lee, T. Y.; Song, P.-S. *Biochim. Biophys. Acta* **1979**, *587*, 129.
(8) Gai, F.; Rich, R. L.; Petrich, J. W. *J. Phys. Chem.*, submitted for publication.
(9) Sengupta, P. K.; Kasha, M. *Chem. Phys. Lett.* **1979**, *68*, 382. Strandjord, A. J. G.; Courtney, S. H.; Friedrich, D. M.; Barbara, P. F. *J. Phys. Chem.* **1983**, *87*, 1125. Schwartz, B. J.; Peteanu, L. A.; Harris, C. B. *J. Phys. Chem.* **1992**, *96*, 3591. Frey, W.; Laermer, F.; Elsaesser, T. *J. Phys. Chem.* **1991**, *95*, 10395.

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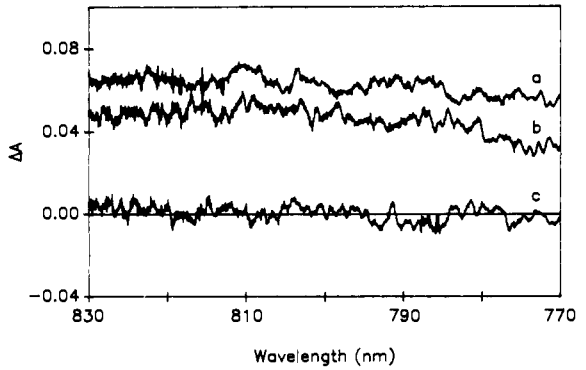

Figure 3. Transient absorption spectrum of hypericin in methanol. λ_{ex} = 588 nm. (a) "Zero" delay. (b) "Zero" delay, but the solution is 1.0 M in acetone, an electron scavenger.[10] (c) Delay time of −20 ps between pump and probe.

Whether the solvated electron arises from a monophotonic event—as we have demonstrated for 7-azaindole and indole[8,11]—or a multiphotonic event is not yet clear. Although the log ϕ_e vs log I_{ex} curve can be fit to a line with a slope of 0.9 ± 0.3, the small but nonnegligible contribution of stimulated emission (there is still fluorescence at 790 nm) could artificially reduce the slope from, for example, 2 to 1.

We are currently investigating the relative importance of photogenerated protons and electrons as well as singlet oxygen for the antiviral activity of hypericin.

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(10) Gai, F.; Chen, Y.; Petrich, J. W. *J. Am. Chem. Soc.* **1992**, *114*, 8343.
(11) Tao, N.; Song, P.-S.; Savikhin, S.; Struve, W. S. *Biochim. Biophys. Acta*, submitted for publication.
(12) Wiesenfeld, J. M.; Ippen, E. P. *Chem. Phys. Lett.* **1980**, *73*, 47.