Available online at www.sciencedirect.com

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 16 (2006) 1414–1417

Bioorganic & Medicinal Chemistry Letters

Anion of hypericin is crucial to understanding the photosensitive features of the pigment

Liang Shen, Hong-Fang Ji and Hong-Yu Zhang*

Shandong Provincial Research Center for Bioinformatic Engineering and Technique, Center for Advanced Study, Shandong University of Technology, Zibo 255049, PR China

Received 15 September 2005; revised 26 October 2005; accepted 10 November 2005
Available online 6 December 2005

Abstract—Photosensitive behaviors of hypericin (HYP) have attracted much attention, because of HYP's great potential in photodynamic therapy. It has been found that HYP differs from homologous pigments, such as hypocrellin A (HA), in photosensitive features. For instance, despite the comparable triplet state quantum yields, HYP holds a much lower singlet oxygen yield than HA. To understand the unique photosensitive behaviors of HYP, time-dependent density functional theory is employed to calculate a series of excited-state properties of HYP and its anion (dominant in polar solvents), which are then compared with excited-state properties of HA. It is revealed that the stronger electron-donating power of HYP anion than that of HA is responsible for the HYP's photosensitive features.

Hypericin (HYP, Fig. 1) is a naturally occurring phenanthroperylene quinine pigment, which can be found in plants of genus *Hypericum* and some insect species.[1–3] HYP has gained much attention in the past decades, because of its multiple pharmacological effects, such as antidepressant, antiviral, antiretroviral, and in particular photosensitizing activities.[4–9]

Owing to the great potential of being developed as photodynamic medicine, the photosensitive behaviors of HYP have been extensively investigated.[8,10–13] It is interesting to note that HYP differs from homologous pigments, such as hypocrellin A (HA, Fig. 1), a naturally occurring perylenequinonoid pigment, in photosensitive features. For instance, although both pigments have comparable triplet state quantum yields,[12] the singlet oxygen yield of HYP (about 0.34 in DMSO, 0.35 in ethanol, and 0.25 in acetonitrile)[12,14,15] is much lower than that of HA (0.88 in ethanol and 0.79 in acetonitrile).[12] Considering the principle that structure determines property, the different photosensitive behaviors of HYP and HA can be explained in terms of their distinct structures.

Thanks to the vicinal hydroxyls in the bay region and the intramolecular hydrogen bond between them,[16,17] HYP tends to dissociate one proton in the region in polar solvents to give birth to HYP anion (HYP^-, Fig. 2).[16,18,19] In contrast, HA exists mainly in neutral form. Thus, HYP^- is likely responsible for the unique photosensitive behaviors of HYP. Indeed, the bay-region anion has been neglected in most of the previous researches on HYP's photosensitization.[10–13]

In view of the successful use of time-dependent density functional theory (TD-DFT) method in exploring the excited-state behaviors of dyes and pigments,[13,20–22] we attempt to elucidate the photosensitive features of HYP by calculating the photo-physicochemical proper-

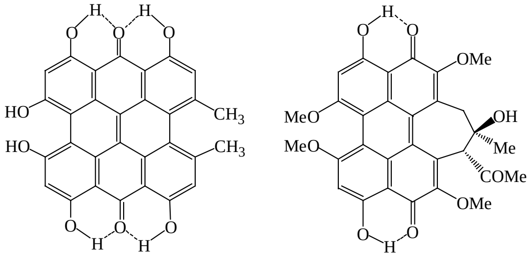

Figure 1. Molecular structures of hypericin and hypocrellin A.

Keywords: Hypericin; Hypocrellin A; Anion; Photosensitive mechanism; Time-dependent density functional theory.

* Corresponding author. Tel./fax: +86 533 278 0271; e-mail: zhanghy@sdut.edu.cn

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.11.056

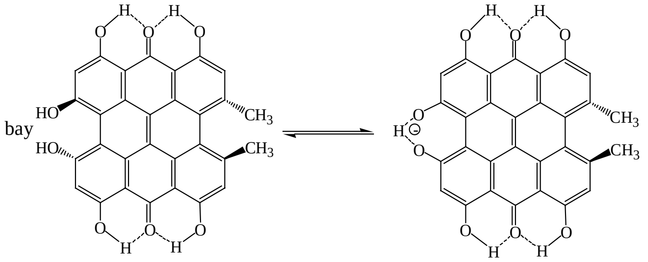

Figure 2. 7,14-Diketo tautomer of hypericin and the corresponding anion derived from proton dissociation in the bay region.

ties of HYP and HYP$^-$ with the TD-DFT method and then to compare them with those of HA.

The calculation procedures are as follows. Initial structures of HYP, HYP$^-$, and derived molecules were fully optimized in solution (ethanol and DMSO systems) by the hybrid B3LYP[23,24] functional with 6-31G(d) Gaussian basis set. Then, the excited-state properties in ethanol and DMSO were calculated by TD-DFT formalism with the same basis set,[25] by which the absorption spectra and triplet (T_1) state energies were obtained. On the basis of the ground (S_0) state and T_1 state properties, the vertical electron affinities (VEAs) and vertical ionization potentials (VIPs) of HYP$^-$ on both states were estimated. During the calculations, the solvent effects were taken into consideration by employing the self-consistent reaction field (SCRF) method with polarizable continuum model (PCM).[26–28] All of the calculations were performed with Gaussian 03 package of programs.[29]

Table 1 lists the B3LYP/6-31G(d)-calculated five lowest singlet excitation energies (*E*) and oscillator strengths (*f*) of HYP and HYP$^-$ in ethanol. It can be found that the deprotonation of HYP causes a bathochromic shift of ~20 nm for the absorption maximum. Interestingly, the two theoretical absorption bands of HYP$^-$ (corresponding to the first ($S_0 \rightarrow S_1$) and second ($S_0 \rightarrow S_2$) lowest excitations) are closer to the experimental values than those of HYP (Table 1),[19,30] which provides solid evidence to support the finding that HYP anion is dominant form in polar solvents.[18,19]

As known to all, during photosensitization, S_0 state photosensitizers (PSs) are initially excited to singlet excited (S_1) state and then intersystem cross to T_1 state. Residing in the long-lived T_1 state, PS can photogenerate reactive oxygen species through various pathways. First, T_1 state PS may react with ground state oxygen ($^3O_2$) through energy transfer to generate $^1O_2$ (Eq. 1). Second, T_1 state PS may pass one electron to $^3O_2$ to give birth to $O_2^{\bullet-}$ (Eq. 2). Third, through autoionization reactions between T_1 and S_0 states (Eq. 3) or both T_1 states (Eq. 4), the PS anion radical can be generated, which could then pass its electron to surrounding $^3O_2$ to form $O_2^{\bullet-}$ (Eq. 5). As the photosensitive pathways are competitive, how each pathway is modulated will determine the relative yields of $^1O_2$ and $O_2^{\bullet-}$.

$$\mathrm{PS(T_1)} + {}^3\mathrm{O_2} \rightarrow \mathrm{PS(S_0)} + {}^1\mathrm{O_2} \tag{1}$$

$$\mathrm{PS(T_1)} + {}^3\mathrm{O_2} \rightarrow \mathrm{PS}^{\cdot+} + \mathrm{O_2}^{\cdot-} \tag{2}$$

$$\mathrm{PS(T_1)} + \mathrm{PS(S_0)} \rightarrow \mathrm{PS}^{\cdot+} + \mathrm{PS}^{\cdot-} \tag{3}$$

$$\mathrm{PS(T_1)} + \mathrm{PS(T_1)} \rightarrow \mathrm{PS}^{\cdot+} + \mathrm{PS}^{\cdot-} \tag{4}$$

$$\mathrm{PS}^{\cdot-} + {}^3\mathrm{O_2} \rightarrow \mathrm{PS(S_0)} + \mathrm{O_2}^{\cdot-}. \tag{5}$$

According to the above photosensitive picture, the photosensitive mechanisms of HYP$^-$ are examined as below.

First, pathway (1) is explored. The triplet excitation energy (E_{T_1}) of HYP$^-$ is estimated as 1.52 eV, close to the experimental value (1.64 eV) in polar solvents.[30,31] It is obvious that the E_{T_1} of HYP$^-$ is higher than the excited-state energy of singlet oxygen ($^1O_2$), 1.06 eV,[20] suggesting that HYP$^-$ can, in principle, generate $^1O_2$ through energy transfer (Eq. 1) in DMSO. This agrees well with the experimental finding that the pigment can give birth to $^1O_2$ in polar solvents when irradiated by light.[12,14,15]

Second, pathway (2) is examined. The prerequisite of the reaction is that the summation of vertical ionization potentials for T_1 state (VIP_{T_1}) of HYP$^-$ and the adiabatic electron affinity (AEA) of $^3O_2$ is negative. In DMSO, the summation of VIP_{T_1} (3.36 eV, Table 2) and AEA_{O_2} (−3.65 eV)[21] is negative, suggesting that $O_2^{\bullet-}$ can be generated through this pathway in DMSO. However, according to previous research,[22] reaction (2) is forbidden for HA in the same solvent, which results from the weaker electron-donating ability of HA than that of HYP$^-$ on T_1 state (reflected from their VIP_{T_1}, 4.44 vs 3.36 eV, Table 2).

Third, as mentioned above (Eqs. 3 and 4) T_1 state HYP$^-$ may also react with a neighboring S_0 state (Eq. 3) or T_1 state HYP$^-$ (Eq. 4). According to theoretical parameters listed in Table 2, the total energies of reaction (3) ($\mathrm{VEA}_{T_1} + \mathrm{VIP}_{S_0}$ or $\mathrm{VIP}_{T_1} + \mathrm{VEA}_{S_0}$) are positive, while that of reaction (4) ($\mathrm{VEA}_{T_1} + \mathrm{VIP}_{T_1}$) is negative. Thus, the anion radical of HYP$^-$ (HYP$^{\cdot 2-}$) can be generated

Table 1. Five lowest singlet excitation energies (*E*) and oscillator strengths (*f*) of hypericin and its anion in ethanol

HYP	^1E (eV)	2.22	2.76	2.87	3.10	3.20
	λ (nm)	560	449	433	399	387
	f	0.35	0.32	0.02	0.02	0.03
HYP$^-$	^1E (eV)	2.14	2.54	2.88	3.04	3.14
	λ (nm)	581 (590)[a]	489 (480)[a]	431	408	395
	f	0.27	0.14	0.09	0.02	0.06

[a] Experimental data are indicated in parentheses.[19,30]

Table 2. Lowest triplet excitation energies (E_{T_1}, in eV), vertical electron affinities (VEAs, in eV), and vertical ionization potentials (VIPs, in eV) of hypericin anion and hypocrellin A in DMSO

	E_{T_1}	VEA_{S_0}	VEA_{T_1}[a]	VIP_{S_0}	VIP_{T_1}[b]
HYP^-	1.52	−2.68	−4.20	4.88	3.36
HA[c]	1.54	−3.39	−4.93	5.98	4.44

[a] $VEA_{T_1} = VEA_{S_0} - E_{T_1}$.

[b] $VIP_{T_1} = VIP_{S_0} - E_{T_1}$.

[c] Data from Ref. 22.

through reaction (4). $HYP^{\bullet 2-}$ then can pass its electron to surrounding ${}^3O_2$ to form $O_2^{\bullet -}$ (Eq. 5), owing to the negative reaction energy $(VIP(HYP^{\bullet 2-}) + AEA_{O_2} = 3.03 + (-3.65) = -0.62\ \text{eV})$. This reveals another $O_2^{\bullet -}$-generating mechanism of HYP^- in polar solvents. However, this pathway is less possible than pathway (2), because it depends on the interaction of two T_1 state HYP^- molecules. Once $O_2^{\bullet -}$ is given, other ROS, such as H_2O_2 and ${}^{\bullet}OH$, can be produced through the Fenton reaction or Haber–Weiss reaction,[32,33] which will efficiently amplify the photosensitizing activity of HYP.

In conclusion, HYP^- predominates in polar solvents. Both ${}^1O_2$ and $O_2^{\bullet -}$ can be photogenerated by HYP^- through energy transfer or electron transfer. However, the quite strong electron-donating potential of HYP^- enables its T_1 state to react directly with molecular oxygen to give birth to $O_2^{\bullet -}$ (Eq. 2), which is not permitted for HA. Considering the fact that the different photosensitive pathways are competitive and the electron transfer is favored in polar solvents, reaction (2) will get dominant in polar systems comparing with reaction (1) for HYP^-, which accounts well for the experimentally observed lower ${}^1O_2$ yield of HYP than that of HA in polar solvents. Thus, anion is crucial to understanding the photosensitive features of HYP. As HYP is also a model molecule to explore intramolecular hydrogen transfer (IHT),[34] we suggest that the HYP^- should be taken into consideration to explain its intriguing IHT behaviors, because there has been much debate on this topic.[35,36]

Acknowledgments

This work was supported by the National Basic Research Program of China (2003CB114400) and the National Natural Science Foundation of China (Grant Nos. 30100035 and 30570383).

References and notes

1. Falk, H. *Angew. Chem., Int. Ed.* **1999**, *38*, 3116.
2. Vogel, G. *Science* **2001**, *291*, 35.
3. Britton, G. *The Biochemistry of Natural Pigments*; Cambridge University Press: Cambridge, 1984.
4. Lavie, G.; Valentine, F.; Levin, B.; Mazur, Y.; Gallo, D.; Lavie, D.; Weiner, D.; Meruelo, D. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 5963.
5. Lavie, G.; Mazur, D.; Lavie, D.; Meruelo, D. *Med. Res. Rev.* **1995**, *15*, 111.
6. Hudson, J. B.; Harris, L.; Towers, G. H. N. *Antiviral Res.* **1993**, *20*, 173.
7. Kraus, G. A.; Zhang, W.; Fehr, M. J.; Petrich, J. W.; Wannemuehler, Y.; Carpenter, S. *Chem. Rev.* **1996**, *96*, 523.
8. Duran, N.; Song, P. S. *Photochem. Photobiol.* **1986**, *43*, 677.
9. Diwu, Z. *Photochem. Photobiol.* **1995**, *61*, 529.
10. Ehrenberg, B.; Anderson, J. L.; Foote, C. S. *Photochem. Photobiol.* **1998**, *68*, 135.
11. Weiner, L.; Mazur, Y. *J. Chem. Soc., Perkin Trans. 2* **1992**, 1439.
12. Dumas, S.; Lepretre, J.-C.; Lepellec, A.; Darmanyan, A.; Jardon, P. *J. Photochem. Photobiol., A: Chem.* **2004**, *163*, 297.
13. Guedes, R. C.; Eriksson, L. A. *J. Photochem. Photobiol., A: Chem.* **2005**, *172*, 293.
14. Wills, N. J.; Park, J.; Wen, J.; Kesavan, S.; Kraus, G. A.; Petrich, J. W.; Carpenter, S. *Photochem. Photobiol.* **2001**, *74*, 216.
15. Darmanyan, A. P.; Burel, L.; Eloy, D.; Jardon, P. *J. Chim. Phys.* **1994**, *91*, 1774.
16. Freeman, D.; Frolow, F.; Kapinus, E.; Lavie, D.; Lavie, G.; Meruelo, D.; Mazur, Y. *Chem. Commun.* **1994**, 891.
17. Wang, D.-P.; Kong, D.-X.; Chen, D.-Z.; Zhang, H.-Y. *Dyes Pigments* **2001**, *51*, 71.
18. Skalkos, D.; Tatsis, E.; Gerothanassis, I. P.; Troganis, A. *Tetrahedron* **2002**, *58*, 4925.
19. Freeman, D.; Konstantinovskii, L.; Mazur, Y. *Photochem. Photobiol.* **2001**, *74*, 206.
20. Llano, J.; Raber, J.; Eriksson, L. A. *J. Photochem. Photobiol., A: Chem.* **2003**, *154*, 235.
21. Shen, L.; Ji, H.-F.; Zhang, H.-Y. *Chem. Phys. Lett.* **2005**, *409*, 300.
22. Shen, L.; Ji, H.-F.; Zhang, H.-Y. *J. Photochem. Photobiol., A: Chem.*, in press.
23. Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B.* **1988**, *37*, 785.
24. Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 1372.
25. Stratmann, R. E.; Scuseria, G. . E.; Frisch, M. J. *J. Chem. Phys.* **1998**, *109*, 8218.
26. Miertus, S.; Scrocco, E.; Tomasi, J. *Chem. Phys.* **1981**, *55*, 117.
27. Miertus, S.; Tomasi, J. *Chem. Phys.* **1982**, *65*, 239.
28. Cossi, M.; Barone, V.; Cammi, J. *Chem. Phys. Lett.* **1996**, *255*, 327.
29. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Cli.ord, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Salvador, P.; Dannenberg, J. J.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaroni, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 03*, Revision A 11.; Gaussian, Inc: Pittsburgh, PA, **2003**.
30. Arabei, S. M.; Galaup, J. P.; Jardon, P. *Chem. Phys. Lett.* **1997**, *270*, 31.
31. Angerhofer, A.; Falk, H.; Meyer, J.; Schoppel, G. *J. Photochem. Photobiol., B: Biol.* **1993**, *20*, 133.
32. Fenton, H. J. H. *J. Chem. Soc.* **1894**, *65*, 899.

33. Haber, F.; Weiss, J. *Proc. R. Soc. London, Ser. A.* **1934**, *147*, 332.
34. Petrich, J. W. *Int. Rev. Phys. Chem.* **2000**, *19*, 479, and references therein.
35. Zhang, H.-Y.; Chen, D.-Z. *Dyes Pigments* **2000**, *46*, 17.
36. Plaza, P.; Mahet, M.; Tchaikovskaya, O. N.; Martin, M. M. *Chem. Phys. Lett.* **2005**, *408*, 96.