Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 2943–2946

Novel fluorescent probe for detecting hydroperoxides with strong emission in the visible range

Nobuaki Soh,* Tomoyuki Ariyoshi, Tuyoshi Fukaminato, Koji Nakano, Masahiro Irie and Toshihiko Imato*

Department of Applied Chemistry, Graduate School of Engineering, Kyushu University, 744, Moto-oka, Nishi-ku, Fukuoka 819-0395, Japan

Received 3 November 2005; revised 11 February 2006; accepted 28 February 2006
Available online 22 March 2006

Abstract—A novel fluorescent probe, a swallow-tailed perylene derivative for detecting hydroperoxides (Spy-HP), containing perylene 3,4,9,10-tetracarboxyl bisimide as the main skeleton in the structure, was developed. Spy-HP reacted rapidly with hydroperoxides such as *m*-chloroperbenzoic acid (MCPBA) and cumene hydroperoxide to form its oxidized derivative, Spy-HPOx, and emitted an extremely strong fluorescence ($\Phi \sim 1$) in the visible range (λ_{ex} = 524 nm and λ_{em} = 535 nm), as the result of canceling the photoinduced electron transfer (PET) effect. The reaction between Spy-HP and hydroperoxides proceeded quantitatively in strict stoichiometry, without being affected by autoxidation or photobleaching. Because of these prominent properties, Spy-HP is expected to be a novel and useful fluorescent probe to 'spy' on hydroperoxides in biosamples.

Lipid peroxidation is generally thought to be an important causative factor for some diseases and for aging.[1–6] Since large quantities of hydroperoxides are produced in the first stage of lipid peroxidation, hydroperoxides have attracted a great deal of considerable attention as a marker of oxidative stress. Several methods for detecting hydroperoxides have been reported to date.[7–18] Among them, a method using diphenyl-1-pyrenylphosphine (DPPP)[11–16] appears to be one of the most successful. Indeed, DPPP has been applied not only to the determination of various hydroperoxides using an HPLC post-column detection system,[13,14] but also to the monitoring of lipid peroxidation within cell membranes by utilizing as a fluorescent probe.[15,16] Unfortunately, however, the both excitation and emission wavelengths of DPPP are in such a short range (λ_{ex} = 352 nm and λ_{em} = 380 nm) that detection using DPPP frequently suffers from undesirable effects such as autofluorescence from biomatrices and damage to living cells.

Basic fluorescent properties of fluorescent probe are heavily dependent on the fluorophore, that is selected as the main skeleton of the fluorescent probe. The short wavelengths of excitation and emission of DPPP are attributed to the pyrenyl group. If the pyrenyl group is substituted by a more highly conjugated polycyclic group, the wavelengths of the probe would be shifted to longer wavelengths. Although 4-(2-diphenylphosphinoethylamino)-7-nitro-2,1,3-benzoxadiazole containing a benzofurazan skeleton was developed for the detection of hydroperoxides at longer wavelengths,[17] the excitation wavelength of the reagent would not be sufficiently long (λ_{ex} = 458 nm) for desirable detection and the fluorescence quantum yields of the fluorescent derivative formed after the reaction with hydroperoxides would not be sufficiently high (0.44 in acetonitrile and 0.21 in methanol). Very recently, a fluorescent probe containing normal perylene was also developed for determination of hydroperoxides, however, the excitation and emission wavelengths are not in sufficiently long range (λ_{ex} = 440 nm and λ_{em} = 470 nm).[18] On the other hand, some fluorescent 'perylene derivatives' are known to have excellent fluorescent properties such as long excitation and emission wavelengths, a high photochemical stability, a high fluorescence quantum yield, a low quantum yield of intersystem crossing, and versatile reactivity. Such perylene derivatives would be promising candidates for use in the design of analytical fluorescent reagents, especially in designing fluorescent probes for biosamples. However, perylene derivatives

Keywords: Hydroperoxide; Fluorescent probe; Perylene 3,4,9,10-tetracarboxyl bisimide; Photoinduced electron transfer; Lipid peroxidation.
* Corresponding authors. Tel.: +81 92 802 2891; fax: +81 92 802 2889 (N.S.); tel./fax: +81 92 802 2889 (T.I.); e-mail addresses: soh@cstf.kyushu-u.ac.jp; imato@cstf.kyushu-u.ac.jp

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.02.078

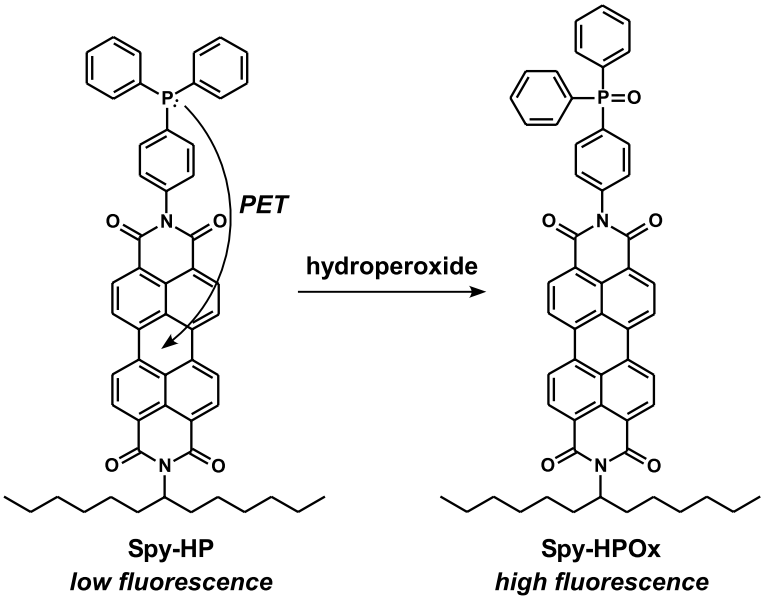
have not been extensively examined as a skeleton for fluorescent probes in spite of their excellent fluorescent properties, while many other fluorophores such as fluorescein derivatives are widely used for this purpose. In this paper, we wish to report on a novel perylene-based fluorescent probe, a swallow-tailed perylene derivative for detecting hydroperoxides (Spy-HP), which reacts with hydroperoxides and emits a strong fluorescence in the visible range. As the perylene fluorophore in the main skeleton of the probe, perylene 3,4,9,10-tetracarboxyl bisimide was selected because the fluorophore has prominent properties such as sufficiently long excitation and emission wavelengths, a high fluorescence quantum yield (~1), and high molar coefficient for the extinction maximum (88,000).[19] Furthermore, the fluorophore can be excited using frequently used laser and the fluorophore is useful for single-molecule fluorescence-based studies because of such excellent properties.[20,21]

The structure of Spy-HP and its detection mechanism for hydroperoxides are shown in Scheme 1. Spy-HP was synthesized by coupling *N*-(1-hexylheptyl)perylene-3,4,9,10-tetracarboxyl-3,4-anhydride-9,10-imide and 4-(diphenylphosphino)benzeneamine.[22] As shown in Scheme 1, we expected that Spy-HP would react with hydroperoxides to form an oxidized derivative (Spy-HPOx) and, as a result, emit a strong fluorescence based on cancellation of photoinduced electron transfer (PET) effect[23] from the donor moiety (triphenylphosphine moiety) to the acceptor moiety (perylene moiety).

The fluorescent properties of Spy-HP and Spy-HPOx were initially investigated. The excitation and emission wavelengths of both Spy-HP and Spy-HPOx were found to be sufficiently long (λ_{ex} = 524 nm and λ_{em} = 535 nm in ethanol). The fluorescence of Spy-HP was efficiently suppressed due to the PET effect ($\Phi \sim 0.05$ in ethanol), on the other hand, Spy-HPOx emitted an extremely strong fluorescence ($\Phi \sim 1$ in ethanol). Since the value of the fluorescence quantum yield of Spy-HPOx (~1) was nearly equal to that of perylene 3,4,9,10-tetracarboxyl bisimide itself, it appeared likely that the PET effect would be cancelled in Spy-HPOx. Similar results were obtained for other organic solvents, such as methanol and CH_2Cl_2.

To evaluate the applicability of Spy-HP as a fluorescent probe for hydroperoxides, Spy-HP (1 μM) was treated with various concentrations (50, 100, 200, 400, 800 nM and 1–4 μM) of *m*-chloroperbenzoic acid (MCPBA), which is a lipid-soluble hydroperoxide and was used as a representative hydroperoxide in this study. Figure 1a shows the fluorescence spectra of Spy-HP, after a 5 min incubation with MCPBA in ethanol at 37 °C. As expected, the fluorescence intensity of Spy-HP increased with an increase in the concentration of the hydroperoxide and a good linear relationship was observed between the fluorescence intensity at 535 nm and the concentration of the hydroperoxide in the range of 0–1 μM, as can be seen from Figure 1b. The fluorescence intensity was saturated at concentrations higher than 1 μM and a sharp breakpoint was observed at a concentration of 1 μM. Taking into consideration the fact that the concentration of the Spy-HP solution before the addition of hydroperoxide was 1 μM, the result would be quite reasonable and strongly suggests that Spy-HP reacts with the hydroperoxide rapidly at a stoichiometric molar ratio of 1:1 strictly without being affected by autoxidation or photobleaching. Spy-HP also increased the fluorescence intensity upon the addition of cumene hydroperoxide. The results indicate that Spy-HP reacted with hydroperoxides quantitatively and Spy-HP would, therefore, be useful for the determination of hydroperoxides.

Figure 2 shows fluorescent images of solutions containing Spy-HP (sample a: 10 μM Spy-HP), Spy-HPOx (sample b: 10 μM Spy-HPOx), and Spy-HP with hydroperoxide (sample c: 10 μM Spy-HP and 20 μM



Scheme 1. Chemical structure of Spy-HP and a schematic illustration of the mechanism of detection of hydroperoxides using the probe.

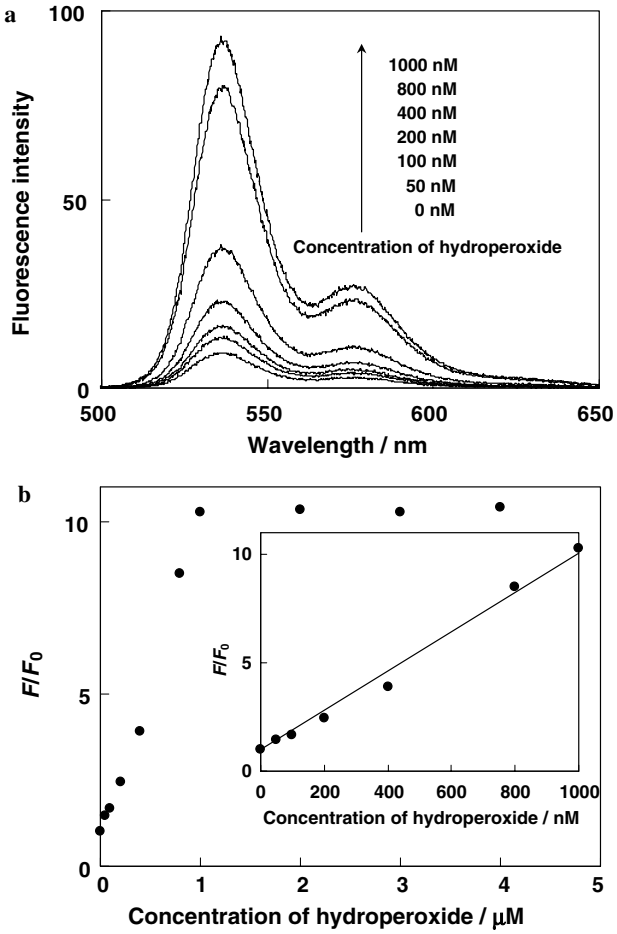


Figure 1. (a) Emission spectra for Spy-HP at 37 °C in ethanol in the presence of hydroperoxide (MCPBA) at various concentrations. The emission spectra were obtained 5 min after the addition of the hydroperoxide to the Spy-HP (1 μM) under aerobic conditions. (b) Relationship between the concentration of the hydroperoxide and the ratio of the fluorescence intensity. F_0 and F denote the fluorescence intensities at 535 nm before and after the addition of the hydroperoxide, respectively.

MCPBA). A mercury–xenon lamp (Moritex, MUV-202U) was used to acquire the images. As seen from Figure 2, it can be clearly confirmed with the naked eye that weakly fluorescent Spy-HP reacts with hydroperoxide to give strongly fluorescent Spy-HPOx, owing to the extremely high fluorescence quantum yields (~1) of Spy-HPOx and the efficient PET effect induced in Spy-HP.

In conclusion, we report on the successful development of a novel fluorescent probe for monitoring hydroperoxides, Spy-HP, containing perylene 3,4,9,10-tetracarboxyl bisimide as the main skeleton in the structure. Spy-HP reacted quantitatively with hydroperoxides to form its oxidized derivative, Spy-HPOx, which emits a strong fluorescence in the visible range, without being affected by autoxidation or photobleaching. Due to these unique properties, Spy-HP is expected to be a novel and useful fluorescent probe to 'spy' on hydroperoxides, particularly in biosamples. Furthermore, Spy-HP is also the first

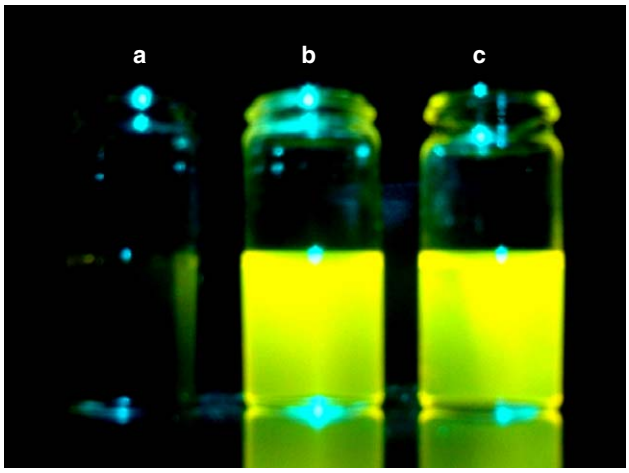


Figure 2. Photographs of CH_2Cl_2 solutions of Spy-HP (sample a: 10 μM Spy-HP), Spy-HPOx (sample b: 10 μM Spy-HPOx), and Spy-HP treated with hydroperoxide (sample c: 10 μM Spy-HP and 20 μM MCPBA). Photographs were obtained using a mercury–xenon lamp.

prototype of fluorescent probes, having perylene-derivative skeletons with prominent fluorescent properties. Although many fluorescent probes have been reported to date, most were designed and developed to function in the cytoplasm. Different from ordinary fluorescent probes, highly hydrophobic fluorescent probes based on a perylene-derivative skeleton are promising candidates for use as new fluorescent probes, which could be localized to monitor biomolecules or bioprocesses in hydrophobic regions of living cells such as cell membranes.

Acknowledgments

This work was supported by Industrial Technology Research Grant Program in 2005 (Grant No. 05A01507a to N.S.) from New Energy and Industrial Technology Development Organization (NEDO) of Japan, and by the Iketani Science and Technology Foundation (Grant No. 0171030A to N.S.).

References and notes

1. Glavind, J.; Hartmann, S.; Clemmesen, J.; Jessen, K. E.; Dam, H. *Acta Pathol. Microbiol. Scand.* **1952**, *30*, 1.
2. Yoshikawa, T.; Yamaguchi, K.; Kondo, M.; Mizukawa, N.; Ohta, T.; Hirakawa, K. *Arch. Gerontol. Geriatr.* **1982**, *1*, 209.
3. Hashimoto, M.; Hosssain, S.; Masumura, S. *Exp. Gerontol.* **1999**, *34*, 687.
4. Markesbery, W. R. *Free Radical Biol. Med.* **1997**, *23*, 134.
5. Butterfield, D. A.; Drake, J.; Pocernich, C.; Castegna, A. *Trends Mol. Med.* **2001**, *12*, 548.
6. Halliwell, B.; Gutteridge, J. M. C. *Free Radicals in Biology and Medicine*, 3rd ed.; Oxford University Press: Oxford, 1999.
7. Yagi, K. *Methods Enzymol.* **1984**, *105*, 328.
8. Marshall, P. J.; Warso, M. A.; Lands, W. E. M. *Anal. Biochem.* **1985**, *145*, 192.
9. Health, L.; Tappel, A. *Anal. Biochem.* **1976**, *76*, 184.
10. Yamamoto, Y.; Brodsky, M. H.; Baker, J. C.; Ames, B. N. *Anal. Biochem.* **1987**, *160*, 7.

11. Akasaka, K.; Suzuki, T.; Ohrui, H.; Meguro, H. *Anal. Lett.* **1987**, *20*, 731.
12. Akasaka, K.; Suzuki, T.; Ohrui, H.; Meguro, H. *Anal. Lett.* **1987**, *20*, 797.
13. Akasaka, K.; Ijichi, S.; Watanabe, K.; Ohrui, H.; Meguro, H. *J. Chromatogr.* **1992**, *596*, 197.
14. Akasaka, K.; Ohrui, H.; Meguro, H. *J. Chromatogr.* **1993**, *628*, 31.
15. Okimoto, Y.; Watanabe, A.; Niki, E.; Yamashita, T.; Noguchi, N. *FEBS Lett.* **2000**, *474*, 137.
16. Okimoto, Y.; Warabi, E.; Wada, Y.; Niki, E.; Kodama, T.; Noguchi, N. *Free Radical Biol. Med.* **2003**, *35*, 576.
17. Onoda, M.; Uchiyama, S.; Endo, A.; Tokuyama, H.; Santa, T.; Imai, K. *Org. Lett.* **2003**, *5*, 1459.
18. Chotimarkorn, C.; Nagasaka, R.; Ushio, H.; Ohshima, T.; Matsunaga, S. *Biochem. Biophys. Res. Commun.* **2005**, *338*, 1222.
19. Langhals, H. *Heterocycles.* **1995**, *40*, 477.
20. Zang, L.; Liu, R.; Holman, M. W.; Nguyen, K. T.; Adams, D. M. *J. Am. Chem. Soc.* **2002**, *124*, 10640.
21. Holman, M. W.; Liu, R.; Adams, D. M. *J. Am. Chem. Soc.* **2003**, *125*, 12649.
22. *N*-(1-Hexylheptyl)perylene-3,4,9,10-tetracarboxyl-3,4-anhydride-9,10-imide was synthesized in four steps referring to a previous report (Wescott, L. D.; Mattern, D. L. *J. Org. Chem.* **2003**, *68*, 10058). 4-(Diphenylphosphino)benzeneamine was synthesized in one step according to a previous report (Herd, O.; Heßler, A.; Hingst, M.; Tepper, M.; Stelzer, O. *J. Organomet. Chem.* **1996**, *522*, 69). Experimental details for the synthesis of Spy-HP are as follows: *N*-(1-hexylheptyl)perylene-3,4,9,10-tetracarboxyl-3,4-anhydride-9,10-imide (104.5 mg, 0.182 mmol), 4-(diphenylphosphino)benzeneamine (60 mg, 0.215 mmol), and $(AcO)_2Zn$ (8.62 mg, 0.047 mmol) were dissolved in quinoline (372 μl, 3.15 mmol) under a N_2 atmosphere, and the solution was refluxed at 155 °C for 4 h. After the addition of 2 N HCl, the mixture was allowed to stand overnight at room temperature. The resulting precipitate was dissolved in $CHCl_3$, washed with 2 N HCl and water, and concentrated by rotary evaporation. The crude compound was purified by chromatography on silica gel with $CHCl_3$ as the eluent to afford a dark red powder (63.6 mg, 63% yield). ^1H NMR (400 MHz, $CDCl_3$): δ (ppm) 0.7–0.8 (t, 6H, $2CH_3$), 1.1–1.2 (m, 16H, $8CH_2$), 1.7–1.9 (m, 2H, CH_2CHN), 2.1–2.3 (m, 2H, CH_2CHN), 5.15–5.25 (m, 1H, CHN), 7.3–7.5 (m, 14H, phenyl), 8.5–8.7 (m, 8H, perylene). FAB-MS: 833.3510 $(M+H)^+$.
23. Soh, N.; Sakawaki, O.; Makihara, K.; Odo, Y.; Fukaminato, T.; Kawai, T.; Irie, M.; Imato, T. *Bioorg. Med. Chem.* **2005**, *13*, 1131.