Dynamic Behavior of a Fluorescent Probe, a Swallow-tailed Perylene Derivative for Detecting Hydroperoxides

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Dynamic behavior of a fluorescent probe, a swallow-tailed perylene derivative (Spy-Hp) for detecting hydroperoxides was studied by fluorescence lifetime and transient absorption measurements. The MO calculations suggest the intramolecular charge transfer for Spy-Hp. The excited state lifetime of Spy-Hp is shorter than that of the oxidized form, which was caused by intramolecular fluorescence quenching. These results elucidate the mechanism of Spy-Hp for detecting hydroperoxides in biosamples.

There have been many reports and development of molecular fluorescent probes in the field of chemical biology.[1] Fluorescent probes are quite useful for clarifying the functions of target biomolecules in biological systems. Temporal and spatial information concerning the target molecule can be obtained with high sensitivity in vivo cellular systems.

We recently developed a novel fluorescent probe, swallow-tailed perylene derivatives for detecting hydroperoxides (Spy-Hp, Figure 1a).[2] The detection mechanism is presumed as follows. Fluorescence from perylene moiety is quenched by photo-induced electron transfer from triphenylphosphine to perylene. Oxidized Spy-Hp (Spy-Ox) shows strong emission from perylene by cancellation of the electron transfer. We report here the dynamic behavior of Spy-Hp and Spy-Ox in toluene solution as studied by fluorescence lifetime and transient absorption measurements.

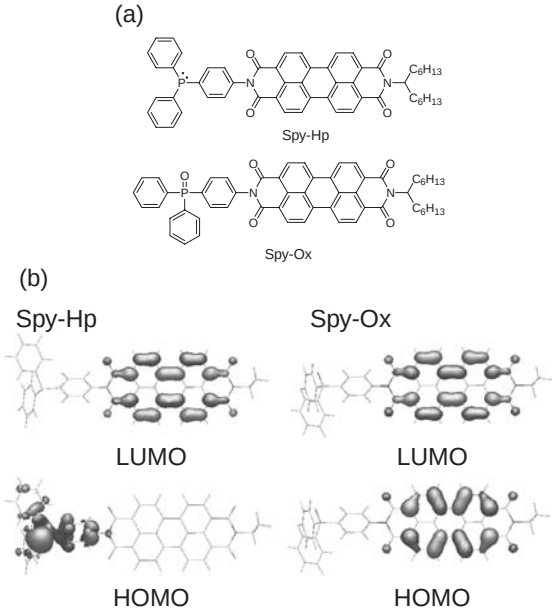

Figure 1. Molecular structure and MO results calculated by the PM3 method.

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 as reported previously.[2] Experimental details of spectral measurements were described previously.[3–5]

First, we evaluated the electronic properties of Spy-Hp and Spy-Ox by molecular orbital (MO) calculations at PM3 level.[6] The MO of Spy-Hp is localized on the perylene bisimide moiety in the LUMO, and on the triphenyl phosphate moiety in HOMO as shown in Figure 1b. These observations suggest the existence of a charge-transfer (CT) state, which is generated by transferring an electron from the triphenyl phosphate moiety to the perylene moiety. On the other hand, HOMO and LUMO of Spy-Ox are localized at the perylene moiety. The MO calculation implies that electron-transfer reaction takes place only in Spy-Hp.

The absorption and fluorescence spectra of Spy-Hp and Spy-Ox are identical to the perylene bisimide derivatives which have been reported previously.[7] The absorption spectra in the visible region of Spy-Hp and Spy-Ox in toluene solution shows peaks around $\lambda = 480$ and 530 nm, and a shoulder at $\lambda = 450$ nm. The absorption spectra with the vibrational structure are identical to and characteristic of perylene bisimide absorption.[7] The CT absorption band was not observed in Spy-Hp, which is probably attributed to the high extinction coefficient of the perylene moiety ($\approx$88000) compared with that of CT band.[2] The fluorescence spectra of Spy-Ox shows two vibronic structures at $\lambda = 535$ and 580 nm in toluene solution as shown in Figure 2. The emission from Spy-Hp, on the other hand, is very weak. The fluorescence quantum yields of Spy-Hp and Spy-Ox are 0.08 and 0.87 in toluene solution, respectively.

The fluorescence decay of Spy-Ox excited at 440 nm is reproduced by a single-exponential function with $\tau = 3.7$ ns. On the other hand, the fluorescence decay of Spy-Hp is reproduced by a double-exponential function with $\tau = 0.16$ and 3.7 ns. We confirmed dynamic fluorescence quenching of Spy-Hp. The faster component can be assigned to the fluorescence lifetime of Spy-Hp. The long lifetime components are identical to the S_1

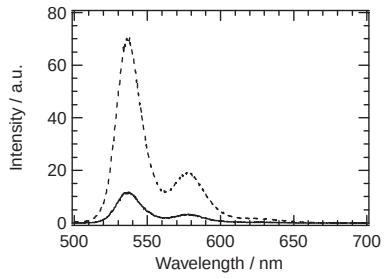

Figure 2. Fluorescence spectra of Spy-Hp (solid line) and Spy-Ox (dashed line) in toluene solution excited at $\lambda = 460$ nm. The solution concentration was 2.5×10^{-7} mol·dm^{-3}.

lifetime of Spy-Ox.[8] The long lifetime component therefore is probably identical to a small amount of the oxidized species with a high fluorescence quantum yield (≈1) existed in the solution, which were generated during repeated laser excitation. The fluorescence quenching rate constant (k_q) was estimated to be $6.0 \times 10^9\ s^{-1}$ by $k_q = 1/\tau(\text{Spy-Hp}) - 1/\tau(\text{Spy-Ox})$.[9]

Figure 3a shows transient absorption spectra of Spy-Hp in toluene solution excited with femtosecond laser pulse at 400 nm. The transient absorption spectra show negative absorption signals at 550 and 580 nm and positive absorption in the range of 600 to 780 nm immediately upon excitation. The negative absorption is ascribed to the stimulated emission of perylene moiety. The time profile of transient absorbance at 580 nm in toluene solution is reproduced by a single-exponential function with $\tau = 130 \pm 20$ ps as shown in Figure 3b. This value is comparable to the time constant obtained from the fluorescence lifetime measurements ($\tau = 160$ ps). On the other hand, the positive absorption remains for a few nanoseconds after excitation as shown in Figure 3a. The positive absorption band in the range of 600 to 780 nm can be ascribed to the absorption of $S_n \leftarrow S_1$ and the anion radical of perylene bisimide, which has a peak around 725 and 700 nm, respectively.[10,11] The time profile of transient absorbance at 690 nm in toluene solution indicates double-exponential decay with $\tau = 150 \pm 10$ ps and 4.8 ns as shown in Figure 3c. The faster component can be assigned to the S_1 state lifetime of Spy-Hp because it corresponds to the fluorescence lifetime of Spy-Hp and the recovery of the transient absorbance at 580 nm originating from the stimulated emission. Therefore the transient absorption spectra in the early stage (400 ps) after the excitation indicate that the main component of the transient species is the excited singlet state of perylene bisimide moiety. The transient absorption up to 1 ns mainly comprises the perylene anion radical. Thus the slower component will correspond to the charge recombination of the perylene anion radical.

Figure 4 shows transient absorption spectra of Spy-Hp and Spy-Ox in toluene solution observed at 1 ns after excitation. The shape of the transient absorption spectrum of Spy-Ox is identical to that of Spy-Hp at the initial state after excitation (≈1 ps). In this time region, the photoinduced charge separation

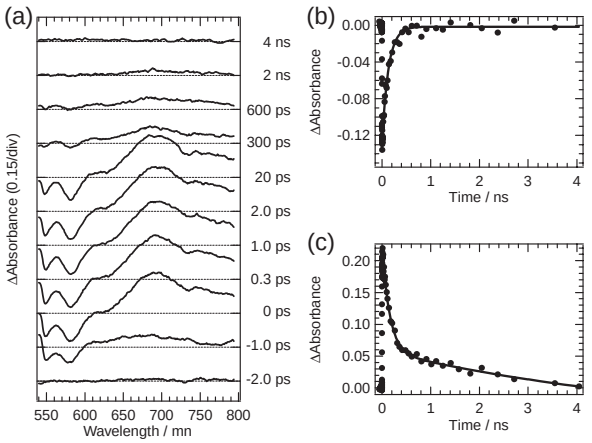

Figure 3. (a) Transient absorption spectra of Spy-Hp in toluene solution excited at $\lambda = 400$ nm femtosecond laser pulse. Time profiles of transient absorbance of Spy-Hp in toluene solution monitored at (b) $\lambda = 580$ nm and at (c) $\lambda = 690$ nm.

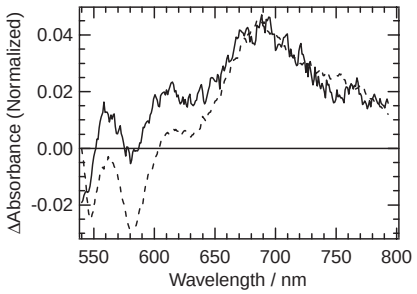

Figure 4. Transient absorption spectra of Spy-Hp (solid line) and Spy-Ox (dashed line) in toluene solutions observed at 1 ns after the excitation.

is scarcely observed in Spy-Hp as mentioned above. Thus the transient spectra of Spy-Ox can be assigned to the $S_n \leftarrow S_1$ absorption of perylene bisimide. On the other hand, positive absorption was observed around 560 to 630 nm for Spy-Hp system. The absorption band of the cation radical of triphenyl phosphate is located around 560 nm[12] which overlaps with the bleaching due to stimulated emission of the perylene portion. Comparison of the transient absorption spectra suggests that the electron transfer takes place only in Spy-Hp. Needless to say, the photoinduced electron-transfer reaction of Spy-Hp in polar solvents such as acetonitrile solution was also observed.

In conclusion, we confirmed the dynamic quenching process in Spy-Hp, which results in charge separation. The emission from Spy-Hp is quenched dynamically even in low dielectric media. These findings indicate Spy-Hp is useful for detecting hydroperoxides in biosamples.

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