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Laser-induced formation of pyrenyloxy radical from 1-hydoxypyrene and further oxidation: Micellar effects

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Abstract

Laser photolysis of POH was investigated in aqueous ethanol, three organic solvents with different polarity, SDS micellar and AOT reverse micellar solutions. The analysis of the laser-induced transient absorption (TA) spectra showed that the pyrenyloxy radical ($PO^{\bullet}$) was formed via a prompt deprotonation from the cation radical $(POH)^{\bullet+}$. The observed micellar effects on the formation of the pyrenyloxy radical were consistent with this mechanism of formation. Based on the shift as well as the intensity of the TA peak attributable to the pyrenyloxy radical, information on the microenvironment surrounding POH (i.e., hydrophobic or hydrophilic, contact or no contact with water phase) was obtained. The secondary photochemical reaction after laser irradiation was investigated with UV/vis absorption spectroscopy. 1,6- and 1,8-pyrenediones were found to be produced as the main photoproducts.

Keywords: Laser photolysis; Micelle; Pyrenyloxy radical; Transient absorption spectroscopy; 1-Hydroxypyrene; Solvent effects

1. Introduction

Pyrene derivatives have been used extensively to probe the microenvironment contained in micellar or membrane systems [1,2]. The suitability of these derivatives is mainly based on a remarkable change in fluorescent properties depending on the surrounding environment. Recently, we found that several laser-induced transient species other than the fluorescent species (i.e., singlet excited state) can also sensitively probe the micellar microenvironment [3,4]. The triplet species, the cation radical, the anion radical and the solvated (hydrated) electron are transiently produced by a moderate or intense laser irradiation from pyrene derivatives such as 1,3,6,8-pyrenetetrasulfonate ($PS_4{}^{4-}$) and 1-pyrenesulfonate ($PS_4{}^{4-}$) [5]. The micellar dynamics involving the fusion–fission process as well as the percolation phenomena were successfully monitored based on the decay behavior of the triplet species from $PS_4{}^{4-}$ [4]. The success is greatly due to the following two experimental efforts. The first step is to separate the spectrum of the specific transient species used as a probe molecule, for example, the triplet species or cation radical, from the observed spectra consisting of multiple transient species. The second step is to elucidate the decay mechanism of the corresponding transient species. The amount and quality of information gained with these transient species may be comparable with that from fluorescence measurements. However, this method has the disadvantage that the probe molecule itself is photochemically decomposed during repeated laser exposure because the laser used is intense. For example, the $PS_4{}^{4-}$ anion is transformed into pyranine after several hundred laser shots [6]. In the case of PS^- ion, hydroxypyrenesulfonate (PSOH) was initially formed and this was followed by desulfonation, yielding hydroxypyrene [6]. At this stage, the characteristics of the dominant transient species generated from $PS_4{}^{4-}$ and PS^- have been made clear. The former, with marked hydrophilicity, is useful to probe a water nano- or microdroplet such as water pool in a reversed micellar solution [3,4]. The latter, having an amphiphilic property due to the hydrophilic sulfonate anion and the hydrophobic pyrenyl group, can probe the interface region between the water phase and hydrophobic phase [3]. The transient species from these molecules show well-defined behaviors in each location. Such transient species being successfully used as a microenvironment probe, however, are limited in number. Herein, we investigate the laser-induced photochemistry of 1-hydroxypyrene (POH) to develop a new probe.

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This molecule is essentially hydrophobic and only slightly soluble in pure water, whereas, in alkaline solution, deprotonation easily occurs. The resulting PO^- ion is soluble in water. Similar deprotonation can be expected to occur photochemically as in pyrenecarboxylic acid [7] and pyranine [8,9]. Proton transfer in an excited state was also observed in several hydroxyaromatic compounds such as 1-naphtol, strongly depending on the environment [10,11]. Thus, the probing characteristic of this molecule may be controlled with chemical and/or physical treatments. The excitation of POH, however, seems to induce little heterolytic dissociation, leading to the formation of electronically excited PO^- ($(PO^-)^*$) + H^+ ions [12]. The photochemistry and photophysics of POH remains unresolved.

Furthermore, POH is one of the biologically interesting molecules because it is the initial product in the metabolic processing of pyrene in many living things [13–17]. Pyrene is a typical polyaromatic hydrocarbon (PAH), with PAHs being well-known procarcinogens. Their metabolites as well as reaction products formed chemically or photochemically are known to act as potent carcinogens and/or mutagens. For example, hydroxylated pyrenes involving POH and further oxidized pyrenes such as pyrenediones act as mutagens [18] or significantly enhance the mutagenicity of potent mutagens such as aromatic amines [19–21]. Based on this experimental background, POH contained in urine specimens has been used as a biomarker for exposure to PAH [22–24]. Studies on the metabolism and mutagenicity of pyrene derivatives have greatly contributed to the development of the chemistry of pyrene derivatives. We expect that the present work will contribute to the biological study of pyrene derivatives.

2. Experimental

2.1. Materials and sample preparation

POH was purchased from molecular probe and used as received. Bis (2-ethylhexyl) sodium sulfosuccinate (AOT (Tokyo Kasei)) was purified with the method reported by Politi et al. [9]. The other reagents involving sodium dodecylsulfate (SDS) and solvents commercially available were used without further purification. Two micellar solutions were prepared: 0.1 M AOT/isooctane reverse micellar solution and 0.1 M SDS micellar solution. The former micellar solutions with a molar ratio of $[H_2O]/[AOT]$ (= W) equal to 10 or 50 were used in the experiments. An aqueous ethanol solution with ethanol/water (v/v) = 3:2 was used since POH was little solubilized in neutral water. The concentration of POH, [POH], was adjusted to 1×10^{-4} M or $2–3 \times 10^{-5}$ M for TA measurements or photochemical experiments, respectively. The sample solution was deaerated via several freeze – pump – thaw cycles and then displaced by Ar, O_2 or N_2O gas.

2.2. TA and fluorescence measurements

Transient absorption (TA) spectra and the decay behavior were measured using an apparatus reported previously [5,25]. The excitation was performed with the third harmonics of a Nd:YAG laser (355 nm, pulse width ≅ 13 ns). The laser energy was kept at 4–5 mJ/0.2 cm^2 unless mentioned otherwise. A short arc Xe lamp was used as the monitor light source. The signal at each wavelength, detected with the photomultiplier, was stored on a digital oscilloscope and transferred to a personal computer. The observed temporal range after laser irradiation was from 0.1–0.2 to 10–90 μs at 5–50 ns per point. Each signal was averaged for 5–20 shots of laser irradiation. Fluorescence spectra and the decay curves were measured with a pulse-sampling method under excitation conditions similar to those used in the TA measurements.

2.3. Photochemistry

The photochemical reaction was carried out by irradiating a constant volume (ca. 3.5 cm^3) of sample solution with the third harmonics of the Nd:YAG laser at 355 nm [6]. A quartz cell (1 cm × 1 cm) was used for the reaction cell. Laser irradiation conditions were as follows: energy; about 5 mJ/pulse, repetition frequency; 0.2–0.4 Hz, effective area of laser irradiation; ca. 0.2 cm^2. Under these conditions, the effective irradiation volume was estimated to be less than 5% of the whole sample solution. The conditions were similar to those used for measurements of TA spectra. The solution was sufficiently stirred and a fresh batch was available for each pulse of irradiation. Photoproducts were monitored with UV/vis absorption spectroscopy as a function of the number of laser shots. The observed spectra were considered to reflect the final products (not intermediates) because the interval required for the measurement of UV/vis spectra was in the order of minutes.

3. Results and discussion

3.1. Laser-induced transient species from POH

Fig. 1A and B show the TA spectra of POH and PO observed in the aqueous ethanol solution and 0.1 M NaOH aqueous solution, respectively. Two TA peaks at 410 and 490 nm observed in the aqueous ethanol solution showed different quenching behaviors for O_2 and N_2O. The former peak was sensitively quenched by O_2 but little affected by N_2O, while the latter peak and the 495 nm peak in 0.1 M NaOH (Fig. 1B) were quenched partially by N_2O and thoroughly by O_2. Based on the results of this quenching experiment [5], 410 and 490 nm (495 nm in 0.1 M NaOH) peaks were mainly assigned to the triplet–triplet (T–T) absorption and the absorption of the anion radical, respectively. The T–T absorption peak at 410 nm is in agreement with the T–T absorption spectra of POH in 1,2-dichloroethane reported previously [26]. The TA peak of the anion radical from POH was observed at about 490 nm in common with several pyrene derivatives as well as pyrene [5]. The intense peak around 440 nm, observed in both the aqueous ethanol and 0.1 M NaOH solutions, showed unusual quenching behavior. More than half of the intensity contributing to this peak was affected by neither O_2 nor N_2O. It was little quenched by I^- either (see spectra 3 and 4 in Fig. 1A). Hence, this transient species was not the cation radical, being still less the anion radical or the triplet. Neverthe-

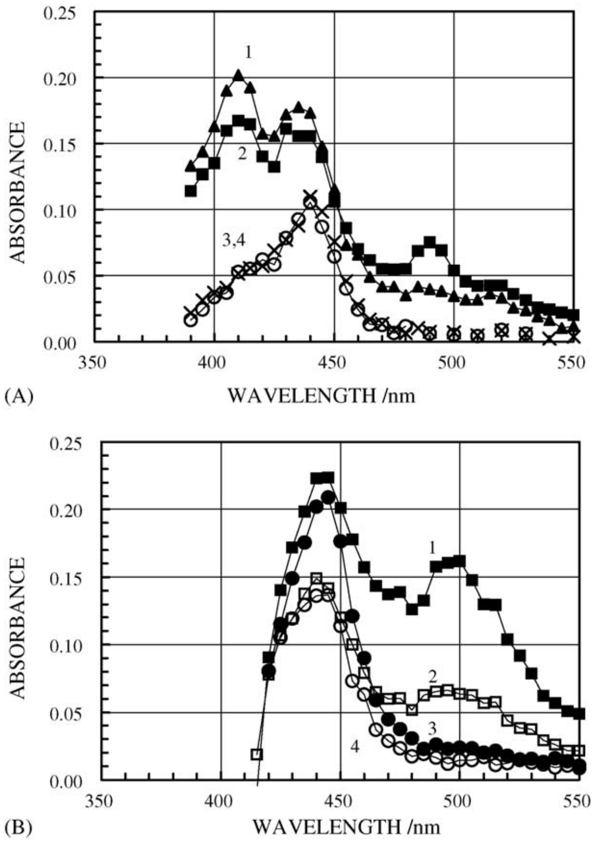

Fig. 1. TA spectra of POH in aqueous ethanol solution (top, A) and of PO in 0.1 M NaOH solution (bottom, B). The TA spectra at wavelengths shorter than 390 nm in Fig. 1A and 415 nm in Fig. 1B were not detected due to overlap of the intense absorption from POH and PO^-, respectively. (Top, 1 A) 1 (solid triangles); 1.0 μs after laser irradiation under N_2O (1.0 μs/N_2O), 2 (solid squares); 1.0 μs/N2, 3 (open circles); 10 μs/O_2, 4 (cross); $[KI] = 2 \times 10^{-3}$ M, 10 μs/O_2. (Bottom, 1B) 1 (solid squares); 1.0 μs/N_2, 2 (open squares); 10 μs/N_2, 3 (solid circles); 1.0 μs/O_2, 4 (open circles); 10 μs/O_2.

less, it is very likely that this species is generated from the cation radical based on the following two results: (1) absorbance data at 440 nm observed in both aqueous ethanol and 0.1 M NaOH solutions depend on laser energy with exponents greater than 1, as shown in Fig. 2, suggesting a two-photon formation process; (2) an intense transient absorption due to hydrated electrons generated via the two-photon process was detected at around 700 nm. These experimental results indicate that the two-photon ionization of the pyrenyl frame occurs in both POH and PO^- to generate a hydrated electron associated with the cation radicals, $(POH)^{\bullet +}$ and $(PO^-)^{\bullet +}$, respectively. Although the cation radicals were not detected under the experimental conditions investigated, we propose that the transient absorption at 440 nm is attributable to the pyrenyloxy radicals formed by Eqs. (1) and (2) in aqueous ethanol and 0.1 M NaOH solutions, respectively:

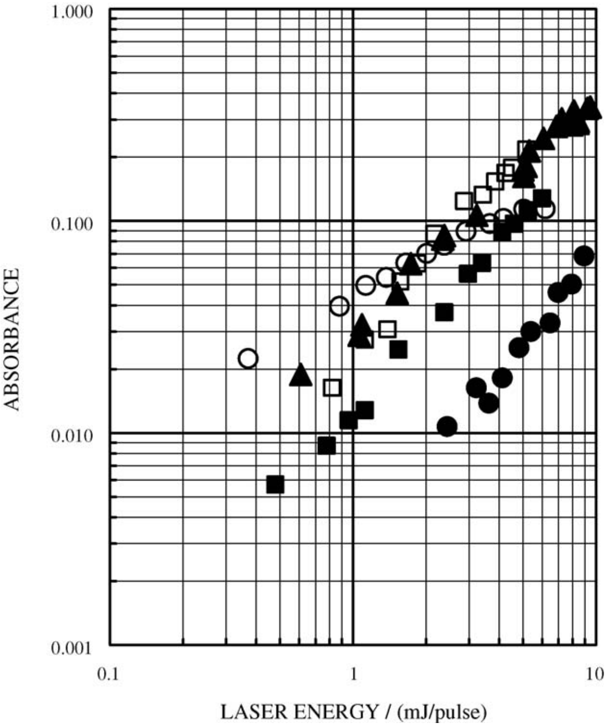

Fig. 2. Laser energy dependence of the formation of pyrenyloxy radical and hydrated electron. The solid squares and solid triangles show the absorbance data at 440 nm (pyrenyloxy radical) observed in aqueous ethanol solution at 2 μs and 0.1 M NaOH solution at 1 μs after laser irradiation under O_2, respectively. The solid circles show the absorbance data at 700 nm (hydrated electron) observed in 0.1 M NaOH solution at 0.15 μs under N2. These data are compared with the laser energy dependence of the cation radical (open squares) and the triplet (open circles) of PS^-, where the two transient species were found to be generated via two-photon and one-photon processes, respectively [5,25].

$$\mathrm{POH} + 2h\nu \rightarrow (\mathrm{POH})^{\bullet +} + (\mathrm{e}^-)\mathrm{aq},\ (\mathrm{POH})^{\bullet +} \rightarrow \mathrm{PO}^{\bullet} + \mathrm{H}^+ \qquad (1)$$

$$\mathrm{PO}^- + 2h\nu \rightarrow (\mathrm{PO}^-)^{\bullet +} + (\mathrm{e}^-)\mathrm{aq},\ (\mathrm{PO}^-)^{\bullet +} \rightarrow \mathrm{PO}^{\bullet} \qquad (2)$$

This assignment is consistent with the previous works [12,27]. The TA spectra attributable to the pyrenyloxy radical were also observed in several pure organic solvents. The absorption maximum wavelength (λ_{max}) and the absorbance at λ_{max} relative to that in aqueous ethanol solution (RA_{max}) were found to markedly depend on the nature of the solvent used as shown in Table 1.

Transient absorption due to the cation radical, $(POH)^{\bullet +}$, that was assumed to be the precursor species in Eq. (1), was detected in an acetonitrile solution. Figs. 3 and 4 show the TA spectra

Table 1
Solvent dependence of λ_{max} and RA_{max} observed under O_2

	0.1 M NaOH	Aq. EtOH[a]	EtOH	CH_3CN	Iso-octane	0.1 M SDS	$W = 50$[b]	$W = 10$[b]
λ_{max}/nm	442	440	436	430	425	440	426	425
RA_{max}	1.3	1.0	0.57	0.52	0.38	1.8	0.92	0.80

[a] $EtOH:H_2O = 3:2$ (v/v).
[b] 0.1 M AOT.

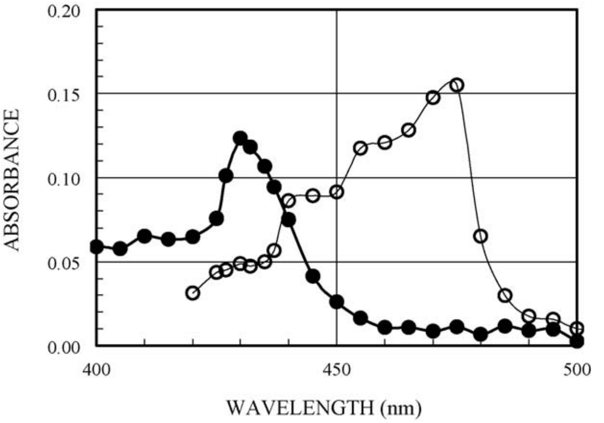

Fig. 3. TA spectra of POH in CH_3CN at 0.2 μs (open circles) and 10 μs (solid circles) under O_2. This measurement was performed under the laser irradiation at higher energy (7–8 mJ/0.2 cm^2).

and the decay behaviors of the cation radical in comparison with those of the pyrenyloxy radical, respectively. The cation radical was observed at $\lambda_{max} \cong 475$ nm. This TA peak was quenched completely by adding KI in accordance with the quenching properties of the cation radical [5], while the decay rate of the pyrenyloxy radical detected at 430 nm was little affected by the iodide anion. The lifetime of the cation radical in acetonitrile was estimated to be 840 ± 80 ns under O_2. Fig. 4 shows that the apparent rise time of the pyrenyloxy radical seems to be nearly equal to the decay time of the cation radical. This agreement also supports the formation mechanism of the pyrenyloxy radical presented by Eq. (1). When only a trace amount of water (about 0.5% v/v) was added to the acetonitrile solution the lifetime of the cation radical became very short (<50 ns) and then the TA attributable to the cation radical became absent with a further increase in water content under our experimental conditions. On the other hand, the absorbance at 430 nm attributable to the pyrenyloxy radical increased and the rise time decreased with an increase of water content. The absorbance became constant (about 0.24 at 1 μs) when water content reaches a few % v/v. These results suggest that the presence of water affects both the yield and the lifetime of the cation radical. Several quenching mechanisms are known for the pyrenyl cation radical: a bimolecular process with the parent molecule [5] and an electron accepting process from electron donors such as the iodide anion (see Fig. 4) and hydrated electron. In the case of $(POH)^{\bullet +}$, the most dominant quenching process is believed to be the proton dissociation process yielding the pyrenyloxy radical. Any quenching mechanisms other than the proton dissociation process are improbable or less contributive. The presence of water can markedly accelerate this main quenching process. Therefore, the yield of the pyrenyloxy radical should be determined by the initial concentration of the cation radical formed via charge separation process after two-photon ionization. Since the charge separation can efficiently occurs in the presence of water, the yield of the cation radical, and therefore, the pyrenyloxy radical, increases by adding water.

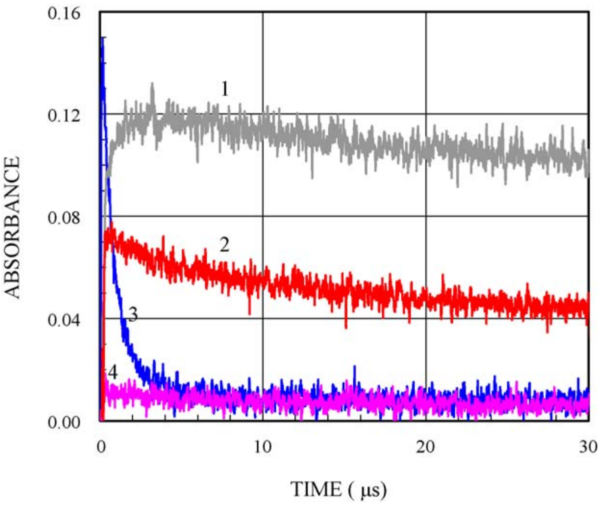

Fig. 4. TA decay profiles observed at 430 nm (1 (grey) and 2 (red)) and 470 nm (3 (blue) and 4 (purple)) in CH_3CN in the presence (2 and 4) and absence (1 and 3) of iodide anion under O_2.

Another pathway presented by Eqs. (1′) + (1″) may be possible, leading to the $PO^{\bullet}$ radical, when the excited POH ($(POH)^*$) produced by one-photon excitation could so rapidly dissociate into a proton and an excited PO^- ($(PO)^*$) within the excitation laser pulse. Such a heterolytic dissociation producing proton and the excited anion via singlet excited state was known in pyrene derivatives [7–9].

$$POH + h\nu \rightarrow (POH)^* \rightarrow H^+ + (PO^-)^* \qquad (1')$$

$$(PO^-)^* + h\nu \rightarrow (PO^-)^{\bullet +} + (e^-)aq,\ (PO^-)^{\bullet +} \rightarrow PO^{\bullet} \qquad (1'')$$

In this case, fluorescence from the intermediate species $(PO^-)^*$ as well as from $(POH)^*$ would be detected. However, the fluorescence spectrum of POH was found to involve only a small contribution from (PO^-) (<10%) even when the spectra were observed under irradiated conditions similar to those used in the TA measurements. Therefore, the formation of $PO^{\bullet}$ via this pathway, i.e., the deprotonation in $(POH)^*$, is considered negligible.

3.2. Environmental effects on formation of pyrenyloxy radical

Fig. 5 shows the TA spectra of POH observed in two micellar solutions: a 0.1 M SDS micellar solution and a 0.1 M AOT/isooctane reverse micellar solution with $W = 50$. The TA spectra in a reverse micellar solution with $W = 10$ was very similar to that in the $W = 50$ solution. The observed peaks can be ascribed to the pyrenyloxy radical because these spectra were measured at 10 μs after laser irradiation under O_2 where the two dominant transient species from POH, triplet and anion radical, completely disappeared. No significant peaks were detected under the same conditions in a wavelength range (450–500 nm) where the precursor cation radical could show the peak. Two marked features were observed in these micellar solutions: a large peak shift and a marked intensity difference. The absorption maximum was found to markedly shift from 425 nm in the AOT reverse micelle to 440 nm in SDS micelle. Such a large shift was compared to the shift due to the change of solvent

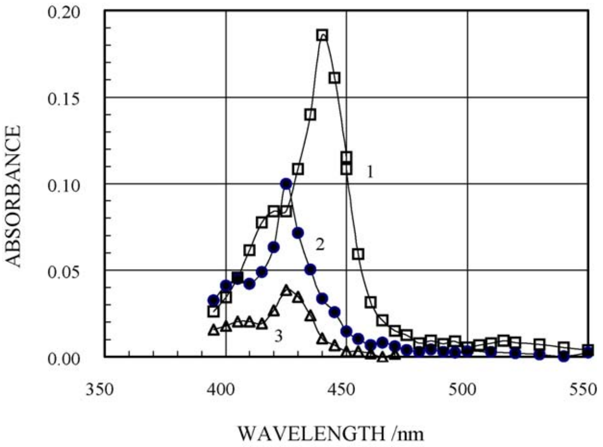

Fig. 5. Micellar effects on the TA spectra of POH. The three spectra, 1 (open squares), 2 (solid circles), and 3 (open triangles), show the TA spectra of POH observed in 0.1 M SDS, 0.1 M AOT with $W = 50$ and isooctane solutions, respectively. All the spectra were observed at 10 μs after laser irradiation under O_2.

from isooctane (nonpolar; $\lambda_{max} = 425$ nm) to aqueous ethanol solution ($\lambda_{max} = 440$ nm).

This result indicates that the pyrenyloxy radical formed in a SDS micelle probably exists in a water-like environment, maybe in contact with the water phase, while the pyrenyloxy radical in an AOT reverse micelle, showing an absorption peak at 425 nm, exists in the hydrophobic domain of the micelle. The observed A_{max} in the SDS micellar solution was about two times larger than that in AOT reverse micellar solutions. The latter was comparable to the A_{max} in aqueous ethanol solution as shown in Table 1. This difference is not due to the difference in life span of the radical in the different environments, indicating that the decay curves are very similar in both micellar solutions. Therefore, such a marked difference in the intensity is mainly due to the difference of the yield of the pyrenyloxy radical if the solvent-dependence of the molar absorption coefficient is neglected. The intensity increases in the following order: isooctane < CH_3CN < ethanol < AOT reverse micelle with $W = 10 \leq W = 50 \leq$ aqueous ethanol solution < 0.1 M SDS micelle. The most probable process to limit the yield of the pyrenyloxy radical is considered to be the formation of the cation radical, $(POH)^{\bullet+}$, via the two-photon ionization of POH. The yield of the cation radical is known to strongly depend on the local environment surrounding the probe molecule [3,5]. In a nonpolar solvent such as isooctane, a nascent photoelectron can easily recombine with the nascent cation radical before the complete charge separation. Such an efficient recombination results in a decrease in the yield of the cation radical and thereby the pyrenyloxy radical. In a reverse micelle the water pool can act as a good scavenger for the nascent photoelectron to generate a hydrated electron. In a 0.1 M AOT reverse micelle with $W = 50$ or 10, the electron emitted into the water pool can be efficiently trapped in the water pool but the electron emitted toward the oily solvent can easily recombine with the cation radical. Therefore, the yield of pyrenyloxy radical may be not so great even in such a large water pool. On the other hand, in the 0.1 M SDS micellar solution, the POH molecule is considered to exist near the micellar surface and partially in contact with the water phase. A nascent photoelectron could promptly be trapped in the water and the charge separation can be completely achieved. Thus, the prominent intensity of the 440 nm peak in 0.1 M SDS as shown in Fig. 5 can be explained. In the aqueous ethanol solution, the absorbance at 440 nm was found to be not so intense although water was involved. This result suggests that a probe molecule (POH) is sheathed with many ethanol molecules (POH is insoluble in water!!). In this environment, water molecules or clusters seem not to work as an efficient electron trap. In conclusion, the shift as well as the intensity of the TA peak of the pyrenyloxy radical generated via two-photon ionization of POH can sensitively probe the properties of the microenvironment: hydrophobic or hydrophilic and contact or no contact with the water phase. Furthermore, the decay behavior of the cation radical, $(POH)^{\bullet+}$, which was estimated to be the precursor of the pyrenyloxy radical, was also dependent of the microenvironment. Several transient species are produced by laser excitation of only one probe molecule such as POH and each of these species can simultaneously act as a probing species for a different microenvironment. This is an advantage of using transient species to probe a microenvironment [3–5].

3.3. Photochemistry

When a solution containing POH was repeatedly irradiated with a pulse laser at 355 nm, the color of the solution changed to yellow and finally yellowish brown with increases in the number of laser shots. The photoproducts were analyzed by UV/vis absorption spectroscopy. As shown in Fig. 6, a new absorption band similar to a plateau appeared in the wavelength range from 400 to 550 nm after several hundred shots of laser irradiation. The observed spectra were very similar in aqueous ethanol, SDS micellar and AOT reverse micellar solutions. This indicates that the same photoproducts were formed in these solutions. The absorption spectra of the photoproducts that were roughly estimated by subtracting the contribution from the surviving POH appeared similar to a trapezoid with the upper side ranging from 400 to 460 nm. Broad absorption spectra with a trapezoidal shape were previously observed in the spectra of 1,6-pyrenedione and/or its mixture with 1,8-pyrenedione [14,28–30]. An intense trapezoidal absorption band with the upper side ranging from 400 to 460 nm was noted for 1,6-pyrenedione, while this band is weakened and separates into two peaks at −350 and −460 nm in 1,8-pyrenedione.

Hammel et al. reported similar spectral changes during the enzymatic oxidation of pyrene with ligninase [30]. They observed a new broad absorption band with a trapezoidal shape in the wavelength range from 400 to 460 nm (upper side). This absorption band was assigned to a mixture of 1,6-pyrenedione and 1,8-pyrenedione in combination with product analysis by HPLC. Hammel et al. proposed the mechanism by which oxidation of pyrene yields 1,6-pyrenedione and/or 1,8-pyrenedione. The first step is the catalytic one-electron oxidation of pyrene, forming the cation radical of pyrene, with ligninase in the presence of H_2O_2. The first water molecule attacks the cation radical yielding monohydroxypyrene. The monohydroxypyrene is also

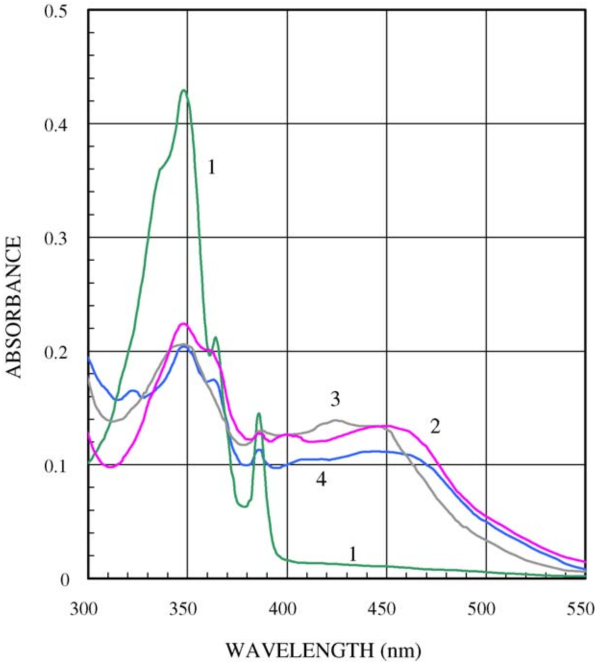

Fig. 6. UV/vis spectra of the photoproducts in laser-photolysis of POH in aqueous ethanol and micellar solutions. Spectrum 1 (green) was observed before laser irradiation. Spectra 2 (purple), 3 (grey) and 4 (blue) were observed after 400 shots of laser irradiation under O_2 in aqueous ethanol, 0.1 M SDS micellar and 0.1 M AOT/isooctane ($W = 50$) reverse micellar solutions, respectively. The spectrum observed under the same condition in a reverse micellar solution with $W = 10$ overlapped with spectrum 3 ($W = 50$).

one-electron oxidized, then hydroxylated. The mono- and dihydroxypyrene are easily oxidized by ligninase and converted into the corresponding pyrenequinones. This pathway was also proposed in the electrochemical and/or chemical hydroxylation and further oxidation of polycyclic aromatic compounds [31,32]. Hammel et al. obtained evidence in $H_2{}^{18}$ O-labeling experiments that the source of the pyrenedione oxygens is water and molecular oxygen does not supply quinone oxygens [30].

This mechanism likely dominates our photochemical oxidation process of pyrene derivatives. Here the starting molecule is POH. Although we could not necessarily confirm the formation of the $(POH)^{\bullet+}$ cation radical in every case investigated, a relatively long-lived pyrenyloxy radical was detected. The pyrenyloxy radical is likely to be hydroxylated at position C6 or C8 because it involves several resonance structures in which the C6 or C8 carbon is positively charged. The resulting 6- or 8-hydroxy pyrene derivatives can be deprotonated thermally or photochemically to yield the corresponding pyrenediones.

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