Singlet Oxygen Production Photosensitized by Fluorescein in Reversed Micellar Solutions

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The quantum yield of singlet oxygen production was investigated in dodecylammonium propionate reversed micellar solutions containing 1,3-diphenylisobenzofuran and fluorescein as photosensitizer. The quantum yield was highly enhanced by the binding of fluorescein to reversed micelles, and increased with decreasing the solubilized water content in reversed micelles. Results obtained was discussed with the optical properties of fluorescein (absorption, fluorescence and phosphorescence) in reversed micellar solutions.

Introduction

Recently, chemical reactions in micellar systems attract increasingly various worker's attention from the interest of bio-mimic chemistry. The DF oxidation by singlet oxygen produced by the photosensitization of pyrene has been studied by us in various micellar solutions [1-3]. The DAP reversed micellar solutions provide a hydrophilic environment in the interior of micelles in non-polar phase. Therefore, various interesting reaction systems can be constructed in these solutions different from aqueous micellar ones. It is known that the micellar size depends on the solubilized water content [4]. At the stoichiometric H_2O concentrations below 0.37 M, all the water molecules are bound to the surfactant head group (bound water range). With increasing the stoichiometric H_2O concentration above 0.37 M, the average aggregation number and the number of free water molecules entrapped in a reversed micelle increased rapidly (free water range) [4].

We found that F acted as considerably good photosensitizer for singlet oxygen production in DAP reversed micellar cyclohexanic solutions and the quantum yield for singlet oxygen production was highly enhanced by DAP reversed micelles depending on the solubilized water content.

In the present investigation, the DF photo-oxidation in DAP reversed micellar solutions containing F and DF, and the optical properties

(absorption, fluorescence and phosphorescence) of F bound to DAP reversed micelles were dealt with changing the solubilized water content, and the information about the mechanism of the enhancement of the quantum yield for singlet oxygen production by DAP reversed micelles was sought for.

Materials and Methods

Cyclohexane, $KCr(NH_3)_2(NCS)_4$ (Raineck's salt), DAP and F purchased from Katayama Co. were guaranteed or extra pure reagents. DF obtained from Aldrich Chemical Co. was of special grade. Laboratory-distilled water was further distilled from alkaline $KMnO_4$ solutions.

Reversed micellar solution was prepared by dissolving DAP in cyclohexanic solution containing DF at room temperature. The DAP cyclohexanic solution was stirred for ten minutes in the dark at 40 °C after adding powdered F or aqueous F solutions at various water concentrations.

The concentration of F and DF in reversed micellar solutions were determined by absorbances at 500 and 415 nm, respectively. The absorbances were measured with a Hitachi spectrophotometer type 356. The reaction mixture in a quartz vessel ($1 \times 1 \times 4$ cm³) was irradiated with the yellow light isolated from a 150 W xenon lamp through a cut filter (colour glass filter, type V-Y 48, Toshiba Electric Co.) at 40 °C.

The number of photons absorbed by F was determined by the chemical actinometry method (sensitive to 504 nm light) of Wegner and Adamson [5]. The actinometer cell containing a $5.0 \cdot 10^{-3}$ M $KCr(NH_3)_2(NCS)_4$ aqueous solution at pH 5.3 was placed just behind the target cell, and was illuminated for 20 min in the presence and absence of the target cell.

The fluorescence spectrum of F at room temperature was measured with a Hitachi type 203 spectrofluorimeter.

A glass tube with a diameter of 5 mm and a length of 20 cm was employed for the fluorescence

Abbreviations: F, fluorescein sodium; DF, 1,3-diphenylisobenzofuran; DAP, dodecylammonium propionate.

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and phosphorescence measurements at 77 K. Anaerobic solutions were prepared by the freeze-pump-thaw cycles.

The phosphorescence spectrum and intensity of F were measured by the phosphoroscope method. The excitation light was provided from a 150 W tungsten lamp through an interference filter (peak, 479 nm; halfwidth, 16 nm). The phosphoroscope (rotating cylinder type) was used for separating the phosphorescence from the fluorescence. A cylinder rotated at the constant rate of $3.6 \cdot 10^3$ rpm. The observation of the emission was delayed by 0.6 msec after excitation. The durations of the observation and excitation were designed to be equally 2 msec per one rotation of the cylinder. The phosphorescence intensity was detected with a photomultiplier (RCA 1C31034A) through a Nikon P-250 type monochromator, and its signal was fed to a home-made lock-in-amplifier. The phosphorescence decay was measured with a wave memory (NF model E-5001) and an oscilloscope (Hewlett Packard model 1725A).

Results and Discussion

When the DAP cyclohexanic solution (air-bubbled) containing F and DF was irradiated with the yellow light (> 480 nm) absorbed by F, the DF concentration exhibited an initial, linear decrease with the irradiation time followed by a saturation.

The decrease in DF concentration was caused by the oxidation of DF by singlet oxygen produced by the F photosensitization. DF is known to be highly reactive with singlet oxygen.

The initial rate (R) of the DF oxidation increased linearly with increasing DF concentration in low DF concentration range as shown in Fig. 1. The initial slope in the curve of R vs DF concentration

Fig. 1. The initial rate (R) of DF oxidation as a function of DF concentration at various concentrations of solubilized water. R, initial rate; concentrations of F and DAP, $3.6 \cdot 10^{-6}$ and $8.0 \cdot 10^{-2}$ M, respectively; H_2O concentrations for Curves 1, 2, 3, 4, 5 and 6, 0, 0.2, 0.3, 0.4, 0.5 and 0.6 M, respectively; temperature, 40 °C.

Fig. 2. Simplified diagram for the photochemical reaction in DAP reversed micellar solutions containing F and DF. 3F , triplet state of F; Φ^1O_2 , quantum yield for singlet oxygen production in cyclohexanic phase; k_r , rate constant for DF oxidation by singlet oxygen; k_d , rate constant for physical decay of singlet oxygen in cyclohexane; k_q , rate constant for the quenching of singlet oxygen by empty DAP reversed micelles.

increased with decreasing the solubilized H_2O content.

Simplified diagram for the photochemical reaction in DAP reversed micellar solutions containing F and DF is shown in Fig. 2, assuming that singlet oxygen is consumed by the DF oxidation, the quenching by empty micelles and the physical decay to triplet oxygen. H_2O and F are bound to the interior of micelles (F, hydrophilic), and DF may be dispersed in cyclohexanic phase (DF, hydrophobic). The quantum yield (Φ_{DF}) for the DF oxidation is represented as follows.

$$\Phi_{DF} = \Phi^1O_2 \left(\frac{k_r[DF]}{k_d + k_r[DF] + k_q[M]} \right), \quad (1)$$

where Φ^1O_2 , k_r , k_d and k_q are illustrated in the legend of Fig. 2, and $[M]$ is the empty micellar concentration. The k_q values were obtained by us at various H_2O concentrations in the previous paper [3], and the $k_q[M]$ value was estimated to be $3.0 \cdot 10^4 \sim 5.1 \cdot 10^4 \text{ sec}^{-1}$ for the H_2O concentrations of 0 ~ 0.6 M. We assumed that one micelle bound one F molecule. The F concentration ($3.6 \cdot 10^{-6}$ M) used was much less than that of reversed micelles (order of 10^{-3} M). The micellar concentration was determined using the aggregation number obtained by Correll *et al.* [4]. Since the k_r and k_d values were $5.3 \cdot 10^8 \text{ M}^{-1} \cdot \text{sec}^{-1}$ and $5.8 \cdot 10^4 \text{ sec}^{-1}$ [3], respectively, $k_r[DF]$ ($\leq 5.3 \cdot 10^3 \text{ sec}^{-1}$) could be neglected compared with $k_d + k_q[M]$ in eq. (1) in the DF concentration range below $1.0 \cdot 10^{-5}$ M. Then, Φ_{DF} is proportional to the DF concentration, and the slope is represented as follows.

$$\text{Slope} = \frac{\Phi^1\text{O}_2 \cdot k_r}{k_d + k_q[M]} \quad (2)$$

The experimental values of Φ_{DF} also exhibited a linear relation with the DF concentration below $1.0 \cdot 10^{-5}$ M. Accordingly, the quantum yield of singlet oxygen production ($\Phi^1\text{O}_2$) could be calculated at various H_2O concentrations from eq. (2). The results obtained were shown in Fig. 3.

Fig. 3. Quantum yield ($\Phi^1\text{O}_2$) for singlet oxygen production as a function of H_2O concentration. Concentrations of F and DAP, $3.6 \cdot 10^{-6}$ and $8.0 \cdot 10^{-2}$ M, respectively; temperature, 40°C .

The quantum yield ($\Phi^1\text{O}_2$) decreased rapidly with increasing the H_2O concentration below about 0.4 M H_2O (bound water range) and showed a gradual decrease above this H_2O concentration (free water range). The $\Phi^1\text{O}_2$ value at 0 M H_2O was about 0.15. This value was 1.5 times higher than that at 0.6 M H_2O .

Usui [6] described that the quantum yield of singlet oxygen production by F photosensitization was 0.06 in aqueous solutions. The value obtained by Gollnick and Schenck [7] was 0.03 in water. It is notable that $\Phi^1\text{O}_2$ was highly enhanced compared with that in water phase and strongly dependent on the solubilized H_2O content, when F was introduced into DAP reversed micelles.

To seek for the information about the effects of DAP micelles and solubilized H_2O on $\Phi^1\text{O}_2$, the following experiments were carried out. The absorption and fluorescence spectra of F were measured in DAP reversed micellar solutions (Fig. 4). At room temperature, the absorption band at 490 nm in bulk aqueous F solutions red-shifted in DAP reversed micellar solutions. The band positions at 0 (Curve a) and 0.6 M H_2O appeared at 500 nm and 499 nm, respectively. The red-shift of the absorption band in DAP micellar solutions may be caused by the binding interaction between F and DAP micelle.

Fig. 4. Absorption and fluorescence spectra of F. Concentrations of F at room temperature and 77 K, $3.6 \cdot 10^{-6}$ and $1.0 \cdot 10^{-5}$ M, respectively; concentration of DAP, $8.0 \cdot 10^{-2}$ M; Curve 1, fluorescence spectrum of F solubilized in DAP micellar solutions containing 0 M H_2O at room temperature; Curve 2, fluorescence spectrum of F solubilized in DAP micellar solutions containing 0 M H_2O at 77 K; Curve a, absorption spectrum of F solubilized in DAP micellar solutions containing 0 M H_2O at room temperature.

The fluorescence band appeared at 526 and 525 nm in DAP reversed micellar solutions containing 0 (Curve 1) and 0.6 M H_2O , respectively, at room temperature. The band position in aqueous F solution was 498 nm at room temperature. The fluorescence band red-shifted largely at 77 K and appeared at 530 and 525 nm for the H_2O contents of 0 (Curve 2) and 0.6 M, respectively.

Next, the fluorescence intensity at room temperature are plotted against the H_2O content in Fig. 5. The fluorescence intensity decreased slightly with increasing the H_2O content, but it was highly quenched compared with that in bulk aqueous solutions. The phosphorescence could not be measured at room temperature.

Fig. 5. Fluorescence intensity as a function of H_2O concentration at room temperature. Concentrations of F and DAP, $3.6 \cdot 10^{-6}$ and $8.0 \cdot 10^{-2}$ M, respectively.

Fig. 6. Phosphorescence spectra of F at 77 K. Concentrations of F and DAP, $1.0 \cdot 10^{-5}$ and $8.0 \cdot 10^{-2}$ M, respectively; H_2O concentrations for Curves 1, 2, 3 and 4, 0, 0.2, 0.6 M in anaerobic samples and 0 M in O_2 -bubbled sample, respectively.

Fig. 6 shows the phosphorescence spectra of F at various H_2O concentrations at 77 K. The phosphorescence band blue-shifted from 640 to 630 nm with the increase of the H_2O content from 0 to 0.6 M. The phosphorescence intensity depended on the H_2O content, and it decreased with increasing the H_2O content. Furthermore, the phosphorescence intensity was highly quenched by O_2 . The fluorescence and phosphorescence intensities are shown in

Fig. 7. Fluorescence and phosphorescence intensities as a function of H_2O concentration at 77 K. Concentrations of F and DAP, $1.0 \cdot 10^{-5}$ and $8.0 \cdot 10^{-2}$ M, respectively; Curves 1, 2 and 3, phosphorescence intensities of anaerobic samples and O_2 -bubbled samples, and fluorescence intensity of anaerobic (or O_2 -bubbled) sample, respectively.

Fig. 7 as a function of the H_2O concentration at 77 K.

The phosphorescence intensity of anaerobic samples increased rapidly in the bound water range (<0.4 M H_2O) with decreasing the water content (Curve 1), but O_2 -bubbled samples had low phosphorescence intensities (strong quenching by O_2) nearly independent of the H_2O content (Curve 2).

Further, the phosphorescence was highly enhanced in anaerobic micellar solutions compared with that in anaerobic water solutions as seen in Fig. 7. The fluorescence was not quenched by O_2 .

As seen in Fig. 4, F bound to the interior of DAP reversed micelle had the absorption and fluorescence bands at longer wavelengths than those of free F. The fluorescence intensity was highly quenched by binding of F to micelle (Fig. 5). Moreover, the absorption and fluorescence band positions, and the fluorescence intensity changed by the solubilized H_2O content (Figs. 4 and 5). These indicate that the electronic state of F is highly affected by the adsorptive interaction between F and DAP micelle. Singlet oxygen is considered to be produced through the transfer of the triplet energy of F to oxygen. For this reason, the singlet oxygen formation depends strongly on the population of the triplet state of F. At 77 K, the increase in the phosphorescence intensity and the decrease in the fluorescence intensity with decreasing the solubilized water content, and the enhancement of the phosphorescence intensity by the binding of F to DAP micelle as shown in Fig. 7 suggest that the intersystem crossing from the excited singlet state of F to the triplet state was enhanced by the binding interaction between F and DAP micelle. This interaction may be weakened with increasing the solubilized water content. These may cause high values of Φ^1O_2 in DAP micellar solutions and the dependence of Φ^1O_2 on the solubilized H_2O content as seen in Fig. 3.

The lifetime of the triplet state of F has been obtained as 20 msec by Lidqvist using the T-T absorption method [8] in bulk aqueous solutions at room temperature. The lifetime of the phosphorescent state obtained by us in DAP micellar solutions at 77 K was about 450 msec at the H_2O content of 0~0.6 M. We could not determine the lifetime of the F triplet state at room temperature owing to extreme weakness of the phosphorescence, but the lifetime of the F triplet state might be highly lengthened inside DAP micelle compared with that of free F. This is also another important factor responsible for the high value of Φ^1O_2 in DAP micellar solutions.

At present, we can not go into further mechanism for the effects of DAP micelle and solubilized water on Φ^1O_2 . We must make clear up the structure of

DAP micelle depending on the solubilized water, the binding site and electronic state of F in DAP micelle, and the polarity and viscosity inside DAP micelle in future.

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- [1] N. Miyoshi and G. Tomita, *Z. Naturforsch.* **33b**, 622 (1978).
 - [2] N. Miyoshi and G. Tomita, *Photochem. Photobiol.* **29**, 527 (1979).
 - [3] N. Miyoshi and G. Tomita, *Z. Naturforsch.* **34b**, 339 (1979).
 - [4] G. Correll, R. N. Chesser, III, F. Nome, and J. H. Fendler, *J. Am. Chem. Soc.* **100**, 1254 (1978).
 - [5] E. E. Wegner and A. W. Adamson, *J. Am. Chem. Soc.* **88**, 394 (1966).
 - [6] Y. Usui, *Chemistry Letters (Japan)* **1973**, 743.
 - [7] K. Gollnick and G. O. Schenck, *Pure Appl. Chem.* **9**, 507 (1964).
 - [8] L. Lindqvist, *J. Phys. Chem.* **67**, 1701 (1963).