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Effect of Sodium Dodecyl Sulfate Concentration on Photooxidation of Methyl Orange Sensitized by Tetraphenylporphyrin

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The effect of sodium dodecyl sulfate (SDS) concentration on the efficiency of the photooxidation of Methyl Orange sensitized by tetraphenylporphyrin (TPP) in the surfactant solution was investigated. It was found that the efficiency of the photooxidation is lower above the critical micelle concentration of SDS–TPP mixed solution ($\mathrm{cmc}_{\mathrm{mix}}$) than below the $\mathrm{cmc}_{\mathrm{mix}}$. By taking into consideration the formation of an oligomer complex between one TPP molecule and several SDS molecules below the $\mathrm{cmc}_{\mathrm{mix}}$, it was possible to explain the relatively lower efficiency above the $\mathrm{cmc}_{\mathrm{mix}}$ in terms of the lower reactivity between singlet oxygen produced by the excited TPP and methyl orange (MO) in the less polar environment on the micellar surface.

Keywords——tetraphenylporphyrin; methyl orange; sodium dodecyl sulfate; photooxidation; micellar solution; micellar environment; singlet oxygen

Photooxidations using porphyrin derivatives as photosensitizers have been utilized in the photochemotherapy of various tumors.[1] Certain porphyrins accumulate in tumor cells.[2-5] Irradiation of porphyrin-loaded cells with light results in the formation of singlet oxygen ($^1\Delta_g$). This potent oxidizing agent interacts with cellular amino acids, unsaturated acyl chains of phospholipids, cholesterol and nucleic acids, leading to tumor regression.[6-9] In our previous studies, photooxidation of methyl orange sensitized by tetraphenylporphyrin in a variety of surfactant micelles was examined as a model for biological systems and the effects of the microenvironment around the sensitizer on the photooxidation were investigated. It was suggested that singlet oxygen contributes to the oxidation of methyl orange.[10] It was also found that the efficiency in the photooxidation of methyl orange is the highest when tetraphenylporphyrin is solubilized in anionic micelles composed of sodium dodecyl sulfate.[11]

In the present paper, we investigated the effect of sodium dodecyl sulfate concentration on the efficiency of the photooxidation of methyl orange sensitized by tetraphenylporphyrin in the micellar solution.

Experimental

Materials——An anionic surfactant, sodium dodecyl sulfate (SDS) was obtained from Nakarai Chemical Co.,

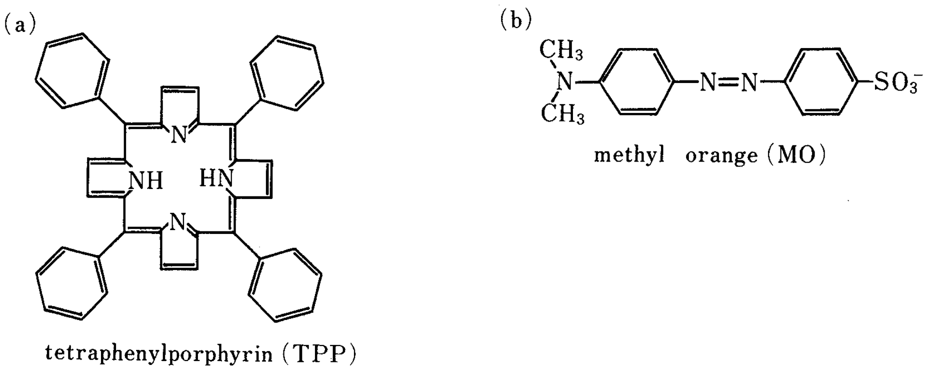

Fig. 1. Structures of (a) TPP and (b) MO

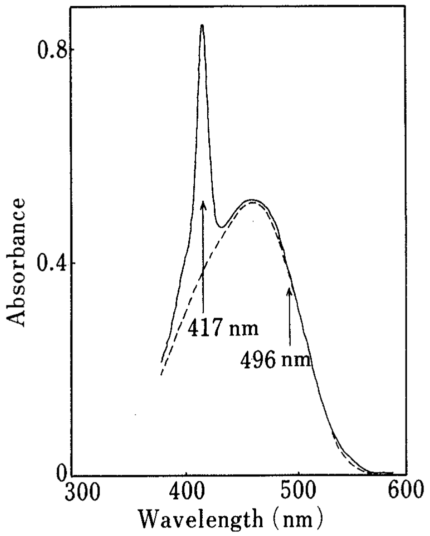

Fig. 2. Absorption Spectrum of 21 μM MO in 20 mM SDS with 1.0 μM TPP (——) and without TPP (------)

Ltd. The surfactant was washed with ether and recrystallized twice from hot ethanol. The surface tension *vs.* concentration curve for the aqueous SDS solution gave no minimum around the critical micelle concentration (cmc). The cmc value obtained was 8 mM, in agreement with the reported value.[13] *meso*-$\alpha,\beta,\gamma,\delta$-Tetraphenylporphyrin (TPP) was purchased from Dojindo Laboratories. The purity of TPP was checked by thin layer chromatography (TLC) on Silica gel 70 with *n*-hexane–acetone (1 : 1, v/v) as the eluting solvent. A single spot was obtained. TPP in acetone was stored at below 4 °C as a stock solution. Methyl orange (MO), obtained from Wako Pure Chemical Industries Ltd., was used without further purification. Tris(hydroxymethyl)aminomethane (Tris) of analytical grade was used as received. The structures of TPP and MO are represented in Fig. 1. Water was doubly distilled from a quartz still.

Measurements——The sample solution was prepared as follows. Aliquots of the stock solutions of TPP in acetone and of SDS in water were mixed. Then, the solvent of the mixed solution was evaporated off at 40 °C and the residue was dissolved in 1 mM Tris–HCl buffer solution containing 2.1 μM MO. The pH of the aqueous solution was maintained at 7.3.

Irradiations were carried out by using a Kondo–Sylvania 1 kW tungsten–halogen lamp. A Toshiba KL-42 interference filter (transmittance characteristics: $\lambda_{\max}=417.5$ nm and half-wavelength = 18.0 nm) was used to obtain monochromatic light. The intensity of light incident upon the cell was 3.21×10^{15} quanta s^{-1} as determined by ferrioxalate chemical dosimetry.[14] The sample solution was contained in a quartz cell and kept at 25 °C by circulation of thermostated water. The sample solution was stirred well with a magnetic stirrer during irradiation.

As shown in Fig. 2, TPP has a sharp absorption band at about 417 nm and MO has a rather broad absorption band at around 450 nm. The decomposition of MO by the photooxidation was monitored in terms of the decrease of absorbance at 496 nm by using a Hitachi 100-10 spectrophotometer. The absorbance of TPP at 496 nm is negligible.

The fluorescence intensities of TPP excited at 417 nm were monitored at 653 nm by using a Jasco FP-550 spectrofluorometer at 25 °C.

Results

Photooxidation rates of MO sensitized by TPP were measured in the SDS micellar solution. The oxidation mechanism, which was proposed in our previous paper[10)] is represented as follows.

$$^1\mathrm{TPP} \xrightarrow{h\nu} {}^1\mathrm{TPP}^* \tag{1}$$

$$^1\mathrm{TPP}^* \longrightarrow {}^1\mathrm{TPP} + h\nu \tag{2}$$

$$^1\mathrm{TPP}^* \longrightarrow {}^3\mathrm{TPP}^* \tag{3}$$

$$^3\mathrm{TPP}^* \longrightarrow {}^1\mathrm{TPP} + h\nu \tag{4}$$

$$^3\mathrm{TPP}^* + {}^3\mathrm{O}_2 \longrightarrow {}^1\mathrm{TPP} + {}^1\mathrm{O}_2 \tag{5}$$

$$^1\mathrm{O}_2 \longrightarrow {}^3\mathrm{O}_2 + \Delta \tag{6}$$

$$^1\mathrm{O}_2 + \mathrm{MO} \longrightarrow \mathrm{MO(ox)} \tag{7}$$

Here, ^{1}TPP and MO(ox) represent the ground-state sensitizer and the oxidized MO, respectively.

In the initial process, TPP in the SDS micellar phase is excited by light and TPP in the singlet excited state (^{1}TPP*) is formed (process 1). Then, ^{1}TPP* is inactivated through the radiative (fluorescence) relaxation process (process 2), but some intersystem crossing occurs, and TPP in the triplet excited state (^{3}TPP*) is generated (process 3). Though ^{3}TPP* disappears through the radiative (phosphorescence) relaxation process (process 4), a part of ^{3}TPP* reacts with oxygen $\mathrm{O}_2(^3\Sigma_g^-)$, ($^3\mathrm{O}_2$) and this reaction result in the formation of singlet oxygen $\mathrm{O}_2(^1\Delta_g)$, ($^1\mathrm{O}_2$), (process 5). $^1\mathrm{O}_2$ is inactivated through the thermal interaction with environmental molecules (process 6),[15)] but most of $^1\mathrm{O}_2$ react with MO, causing irreversible oxidation of MO (process 7).[10)]

If k' is defined as the second-order rate constant of the oxidation of MO (process 7), the oxidation rate can be written as

$$-\mathrm{d[MO]}/\mathrm{d}t = k'[^1\mathrm{O}_2][\mathrm{MO}] \tag{8}$$

As the production processes of ^{1}TPP* and $^1\mathrm{O}_2$ are much faster than the oxidation process represented by Eq. 7 in the stationary state, $k'[^1\mathrm{O}_2]$ can be regarded as a constant. Therefore, Eq. 8 can be rewritten as

$$-\mathrm{d[MO]}/\mathrm{d}t = k[\mathrm{MO}] \tag{9}$$

and

$$\ln[\mathrm{MO}] = \ln[\mathrm{MO}]^0 - kt \tag{10}$$

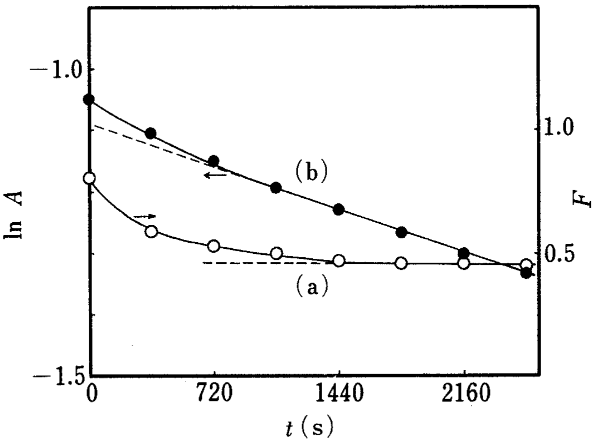

Fig. 3. Time Courses of Fluorescence Intensity of TPP (a) and of the Logarithm of Absorbance of MO (b)

SDS, 8 mM; TPP, 1.0 μM.

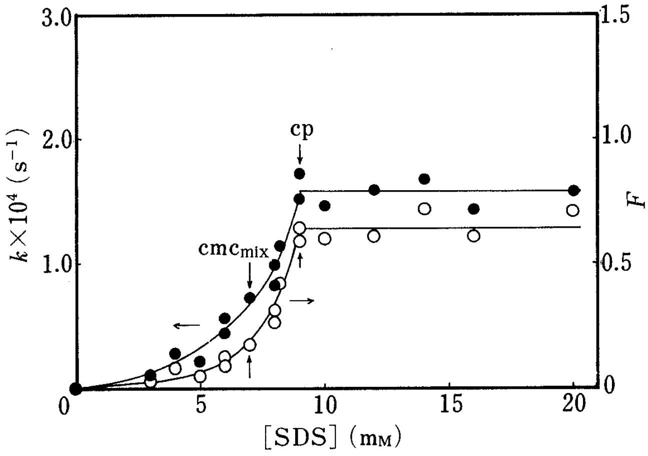

Fig. 4. Effects of SDS Concentration on the Rate Constant k and on the Fluorescence Intensity of TPP

TPP, 1.0 μM.

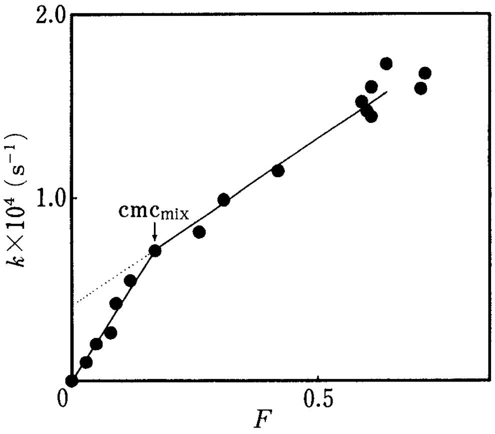

Fig. 5. Relationship between Rate Constant k and Fluorescence Intensity F of TPP

TPP, 1.0 μM.

where $k = k'[^1O_2]$. Here, t stands for the irradiation time and $[MO]^0$ shows the initial concentration of MO. Since $[^1O_2]$ is proportional to $[^1TPP^*]$, the value of k is proportional to $[^1TPP^*]$ and also to the fluorescence intensity of $^1TPP^*$.

Figure 3 shows plots of the logarithm of the absorbance of MO ($\ln A$) and the fluorescence intensity of TPP (F) as a function of irradiation time (t). As can be seen from Fig. 3, there is a linear relationship between $\ln A$ and the irradiation time when the F value is constant. In this study, the slope of the straight line was employed as the rate constant k in Eq. 9. The values of k and F for various concentrations of SDS at 1.0 μM TPP are shown in Fig. 4. The F value increases with the concentration of SDS below 9 mM SDS and is constant above 9 mM. The variation of k value with the concentration of SDS corresponds to that of the F value. The k values are plotted as a function of the F values in Fig. 5. These plots show two straight lines that intersect at the cmc of SDS–TPP mixed solution (cmc$_{mix}$), which was determined to be 7 mM by measuring the surface tensions of mixed solutions. The slopes of the two lines in Fig. 5 can be regarded as indicating the reaction efficiencies of TPP sensitizer below and above the cmc$_{mix}$. These slopes indicate that the efficiency of TPP for the photooxidation of MO is lower above the cmc$_{mix}$ than below the cmc$_{mix}$.

Discussion

Solubilized States of TPP Molecules in SDS Solution

It is known that porphyrins do not fluoresce in the dimer state but do in a monomer state.

Mukerjee *et al.* suggested that below the cmc of SDS, water-insoluble dye molecules can exist as a monomer dye in the aqueous phase by forming a complex with some SDS molecules, that is, by forming a pre-micelle.[16-19] It is presumed that most of the TPP is dispersed as complexes (dimer, trimer, *etc.*) or small crystals, which do not fluoresce. A small part of the TPP is solubilized in the aqueous phase as monomers by forming a complex with SDS molecules (TPP–SDS oligomer complex). As shown in Fig. 5, below the $\mathrm{cmc_{mix}}$, only the fluorescence intensity corresponding to the amount of the TPP in complex form can be observed. On the other hand, if the number of the SDS micelles is small above the $\mathrm{cmc_{mix}}$, TPP molecules may be considered to be concentrated and hence to dimerize partly in the SDS micellar phase. Therefore, with increase of the concentration of SDS, the dimerizing TPP molecules dissociate into monomer and the F value is increased. When the concentration of SDS reaches 9 mM, the F value becomes maximum, because all the TPP molecules are dissociated into monomer and the F value becomes constant above 9 mM. This critical concentration of SDS is indicated by critical point (cp) in Fig. 4. The dissolved states of TPP in the SDS aqueous solution are schematically depicted in Fig. 6, by dividing the SDS concentration range into three regions.

Difference in Photooxidation Efficiencies below and above $\mathrm{cmc_{mix}}$

It may be considered that sensitizers participating in this photooxidation are monomer TPP in the micellar phase and/or monomer TPP forming TPP–SDS oligomer complex in the aqueous phase. Therefore, the slope of line above the $\mathrm{cmc_{mix}}$ in Fig. 5 corresponds to the reaction activity of monomer TPP in the SDS micellar phase and the slope of the line below the $\mathrm{cmc_{mix}}$ can be regarded as the reaction activity of TPP forming TPP–SDS oligomer complex in the aqueous phase. The rate constant k may be represented by Eqs. 11a and 11b above and below $\mathrm{cmc_{mix}}$, respectively.

$$k = k_\mathrm{b}[\mathrm{TPP}]_\mathrm{b} \quad \text{at} \quad [\mathrm{SDS}] \leqq \mathrm{cmc_{mix}} \tag{11a}$$

$$k = k_\mathrm{b}[\mathrm{TPP}]_\mathrm{b(max)} + k_\mathrm{m}[\mathrm{TPP}]_\mathrm{m} \quad \text{at} \quad [\mathrm{SDS}] > \mathrm{cmc_{mix}} \tag{11b}$$

Here, $[\mathrm{TPP}]_\mathrm{b}$ and $[\mathrm{TPP}]_\mathrm{m}$ are the concentration of monomer TPP solubilized as TPP–SDS oligomer complex and that of monomer TPP in the micellar phase, and k_b and k_m are the rate constants of the MO degradation by TPP sensitizer forming TPP–SDS oligomer complex and by TPP sensitizer solubilized in the micellar phase, respectively. $[\mathrm{TPP}]_\mathrm{b(max)}$ shows the concentration of TPP sensitizer solubilized as TPP–SDS oligomer complex at the $\mathrm{cmc_{mix}}$,

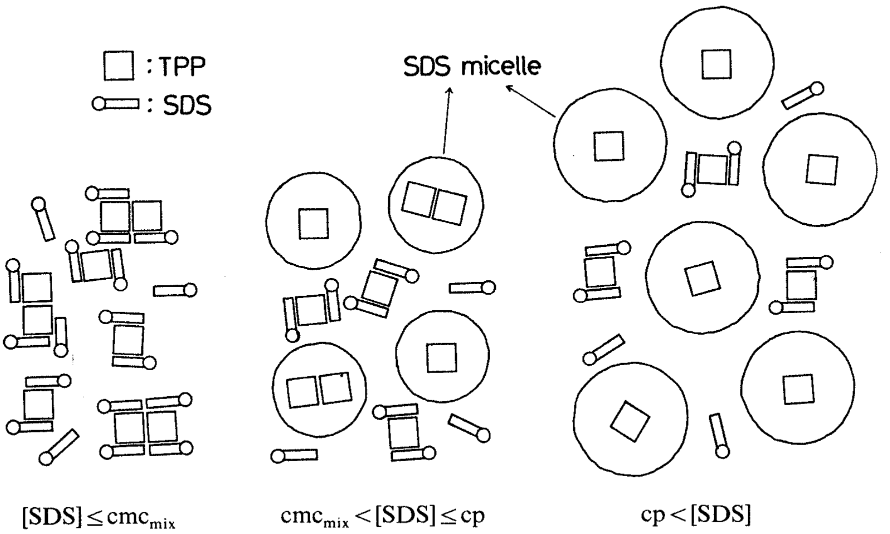

Fig. 6. Solubilized States of TPP Molecules in SDS Solution

corresponding to the maximum of $[\mathrm{TPP}]_b$. On the other hand, if the extinction coefficient and the fluorescence quantum yield of TPP are equal in these two solubilized states, the following equations hold between fluorescence intensity F and TPP concentration.

$$F=\alpha[\mathrm{TPP}]_b \quad \text{at} \quad [\mathrm{SDS}]\leqq \mathrm{cmc}_{\mathrm{mix}} \tag{12a}$$

$$F=\alpha([\mathrm{TPP}]_{b(\max)}+[\mathrm{TPP}]_m) \quad \text{at} \quad [\mathrm{SDS}]>\mathrm{cmc}_{\mathrm{mix}} \tag{12b}$$

From Eqs. 11a, 11b, 12a and 12b, the following relationships are derived.

$$k=(k_b/\alpha)F \quad \text{at} \quad [\mathrm{SDS}]\leqq \mathrm{cmc}_{\mathrm{mix}} \tag{13a}$$

$$k=(k_b-k_m)[\mathrm{TPP}]_{b(\max)}+(k_m/\alpha)F \quad \text{at} \quad [\mathrm{SDS}]>\mathrm{cmc}_{\mathrm{mix}} \tag{13b}$$

Since the maximum of the F value, 0.64 as shown in Fig. 4, may be regarded as the fluorescence intensity corresponding to 1 μM monomer TPP, the α value is calculated from Eq. 12a, being $0.64\times10^6\,\mathrm{M}^{-1}$. By using this α value, the monomer TPP concentration at the $\mathrm{cmc}_{\mathrm{mix}}$ may be evaluated to be $[\mathrm{TPP}]_{b(\max)}=0.27\,\mu\mathrm{M}$, corresponding to the fluorescence intensity of 0.17. According to Eqs. 13a and 13b, from the slopes of the two straight lines in Fig. 5, the k_b/α and k_m/α values are obtained to be 4.23×10^{-4} and $1.82\times10^{-4}\,\mathrm{s}^{-1}$, respectively. From the α value, the values of k_b and k_m are calculated to be 2.71×10^2 and $1.16\times10^2\,\mathrm{s}^{-1}\,\mathrm{M}^{-1}$, respectively. These values are comparable with the values obtained from the intercept of the line based on Eq. 13b with the ordinate in Fig. 5. This agreement suggests that the k_b and k_m values are reasonable. Thus, the reaction activity of monomer TPP in the micellar phase is lower than that of monomer TPP forming TPP–SDS oligomer complex in the aqueous phase.

It is known that the concentration of oxygen molecules solubilized in the micellar phase is higher than in the aqueous phase.[20] Therefore, the number of singlet oxygen species produced in unit time by TPP sensitizer forming TPP–SDS oligomer complex may be considered to be larger than that produced by monomer TPP in the micellar phase. However this consideration is contradictory to our finding that the reactivity of TPP in the aqueous phase is higher than that of TPP in the micellar phase. This discrepancy may be interpreted as follows. All singlet oxygens produced by TPP–SDS oligomer complex interact with MO in the aqueous phase. On the other hand, some of the singlet oxygen produced by monomer TPP in the micellar phase interacts with MO adsorbed on the micellar surface. But it has been shown that the activity of the singlet oxygen is diminished in a less polar environment.[21] Therefore, it may be considered that the singlet oxygen produced in the micellar phase can not effectively oxidize MO in the less polar environment on the micellar surface[10,11] and is rather inactivated by MO adsorbed on the micellar surface. The inactivation of singlet oxygen by MO in the micellar phase was suggested from our previous findings that the reaction activity of photooxidation in the SDS micellar solution is larger than in heptaethylene glycol monododecyl ether and cetyltrimethylammonium chloride micellar solutions, where the micelles may adsorb MO molecule more strongly than do the SDS micelles owing to the absence of the electrostatic repulsion between MO and the micellar surface.[11] The values of k_b and k_m are proportional to the number of singlet oxygen species released from the TPP–SDS oligomer complex and the SDS micelles, respectively, in unit time. Since the value of $(k_b-k_m)/k_b$ is 0.57, this indicates that 57% of singlet oxygen produced in the SDS micellar phase is inactivated by MO without oxidizing MO owing to the less polar environment on the micellar surface. As a result, the reaction activity of monomer TPP in the micellar phase is lower than that of TPP forming TPP–SDS oligomer complex in a more polar environment. These considerations are shown schematically in Fig. 7.

In conclusion, the effect of SDS concentration on the efficiency of the photooxidation of MO sensitized by TPP in SDS solution was studied. It was found that the efficiency is lower

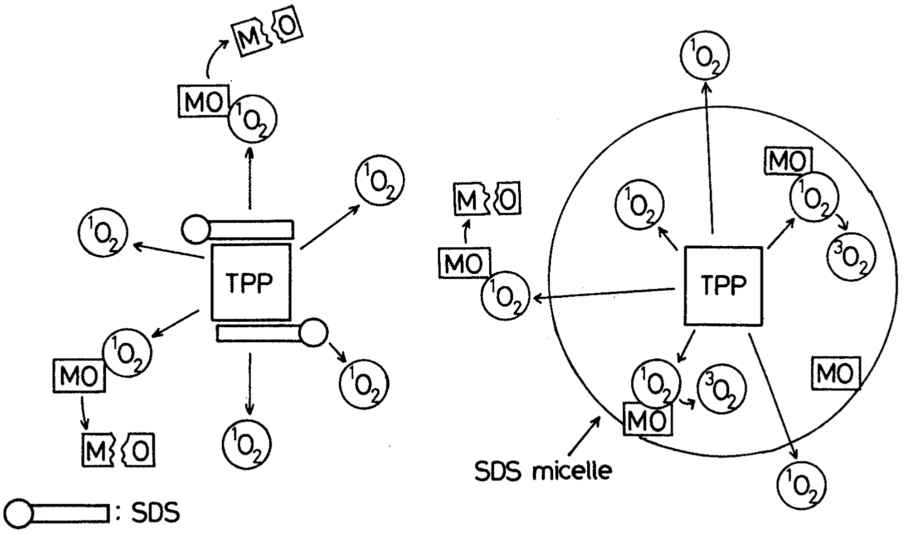

Fig. 7. Differences in Photooxidation Efficiencies between Monomer TPP in the Micellar Phase and TPP–SDS Oligomer Complex in the Aqueous Phase

above the $\mathrm{cmc_{mix}}$ than below the $\mathrm{cmc_{mix}}$, and concluded that the relatively lower efficiency above the $\mathrm{cmc_{mix}}$ could be ascribed to the lower reactivity between singlet oxygen and MO in the less polar environment on the micellar surface. According to this view, it may be expected that, if much amount of monomer TPP can be solubilized not in micelle but as the oligomer complex with surfactants, more efficient photooxidation of MO will be achieved.

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