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Photochemistry of Flavins in Micelles: Specific Effects of Anionic Surfactants on the Monomerization of Thymine Cyclobutane Dimers Photosensitized by Tetra-*O*-acyl Riboflavins*

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ABSTRACT

It was found that tetra-*o*-acyl riboflavins efficiently photosensitize the monomerization of the *cis, syn*-cyclobutane dimers of 1,3-dimethylthymine and 1,3-dimethyluracil in aqueous solution in the presence of such anionic surfactants as sodium dodecyl sulfate and sodium hexadecyl sulfate at concentrations higher than their critical micelle concentration, while little monomerization of the dimers was photosensitized by the flavins in the absence of the surfactants and even in the presence of cationic and nonionic surfactants.

INTRODUCTION

Flavins (FL)‡ are biologically important molecules that have versatile catalytic capabilities in oxidation–reduction reactions *via* either electron transfer or net hydride transfer (1,2). Electron-transfer catalysis is particularly important in the photochemistry of FL (3), because an additional driving force by photoexcitation is available to allow electron transfer with a variety of reactants. The excited-state properties of the oxidized-form FL have been well documented (4), allowing them to be used as electron-transfer photocatalysts, whereas fully reduced FL (FLH_2) are much less attractive as photocatalysts because of chemical instabilities toward air oxidation and little available information about the exited states.

Among electron-transfer photocatalyses of FL in biological systems, a unique photobiological phenomenon is the enzyme-dependent photorepair of UV-damaged DNA. Upon exposure of DNA to UV, adjacent pyrimidine bases in the same strand form the *cis,syn*-cyclobutane dimer as a major UV-induced lesion of DNA, which can be efficiently photorepaired after binding of the dimer with DNA photolyase from *Escherichia coli* (5). An essential chemical process of the photorepair is the efficient monomerization of pyrimidine cyclobutane dimers that proceeds through photochemical electron transfer with the FL chromophore of DNA photolyase (6). In early studies on model reactions of photorepair, nonbiological photosensitizers were used to explore molecular mechanisms for dimer splitting, and dimer models were found to be monomerized by photochemical electron transfer at various efficiencies depending upon the photosensitizers used (7–10).

Attempts have been made to construct more realistic model reaction systems using FL as effective photosensitizers. However, either the oxidized or reduced form of various FL is ineffective in photosplitting of dimer models in aqueous and polar organic solutions under neutral and weakly acidic or basic conditions (1,8–12). It was recently found that splitting of dimer models is efficiently photosensitized by the oxidized form of FL in MeCN in the presence of $HClO_4$ (13–15) or $Mg(ClO_4)_2$ (16) and by the reduced form (FLH_2) under highly basic conditions (17). An essential mechanistic pathway of these photoreactions is excited-state electron transfer from dimer models to the protonated species of FL (13–15), or to $FL{\cdot}Mg^{2+}$ complexes (16) or from the deprotonated FLH_2 to a dimer model (17). In photorepair, it is known that DNA photolyase is active only with the fully reduced FL chromophore but is inactive with the oxidized form (6,18). This fact seems to be unusual in FL photochemistry, because the nonemissive excited states of FLH_2 are perhaps very short-lived, in contrast to the rich photochemistry of the oxidized-form FL (3,4). It may be implied that oxidized-form FL would be inherently incapable of photocatalyzing the cycloreversion of dimer models under neutral conditions. We have found that the oxidized form of tetra-*O*-acyl riboflavins ($[RCO]_4Fl$) can efficiently photosensitize splitting of the 1,3-dimethylthymine cyclobutane dimer (**1a**) in the presence of sodium dodecyl sulfate (SDS) in

*Dedicated to Prof. Sang Chul Shim, Korean Advanced Institute of Science and Technology, in commemoration of his 60th birthday.

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‡*Abbreviations:* CMC, critical micelle concentration; DTAC, dodecyltrimethylammonium chloride; FL, flavin; **1a**, *cis,syn*-cyclobutane dimer of 1,3-dimethylthymine; **1b**, *cis,syn*-cyclobutane dimer of 1,3-dimethyluracil; PEDE, poly(ethyleneglycol) dodecyl ether; $(RCO)_4Fl$, tetra-*O*-acyl riboflavin; SDS, sodium dodecyl sulfate; SHS, sodium hexadecyl sulfate; SOS, sodium octyl sulfate; **2a**, 1,3-dimethylthymine; **2b**, 1,3-dimethyluracil.

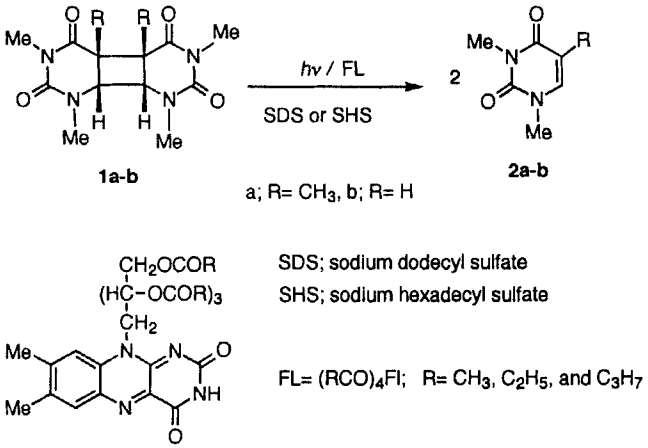

Figure 1. 1,3-Dimethylthymine (**2a**), 1,3-dimethyluracil (**2b**), their *cis,syn*-cyclobutane dimers (**1a**,**1b**) and tetra-*O*-acyl riboflavins ([RCO]$_4$Fl). The half-peak oxidation potentials *vs* Ag/AgNO$_3$ in CH$_3$CN are +1.32 V for **1a** and +1.31 V for **1b**, and the half-peak reduction potentials for (RCO)$_4$Fl are −1.12 V (R = CH$_3$), −1.11 V (R = CH$_2$CH$_3$) and −1.11 V (R = CH$_2$CH$_2$CH$_3$).

aqueous solution under neutral conditions (19). This finding appears to be important for the exploration of inherent reactivities in photocatalysis of FL. In this paper, we report details of the specific surfactant effects on splitting of **1a** and the 1,3-dimethyluracil cyclobutane dimer (**1b**) photocatalyzed by (RCO)$_4$Fl.

MATERIALS AND METHODS

Materials. All the surfactants used were purchased from Tokyo Kasei and were used as received. The preparation of **1a** and **1b** was carried out according to the literature methods (20–22). Irradiation of 1,3-dimethylthymine (**2a**) in ice at −20°C with a high-pressure mercury lamp gave **1a** and the *cis,anti*-dimer, which were separated by column chromatography on alumina; details of the procedure

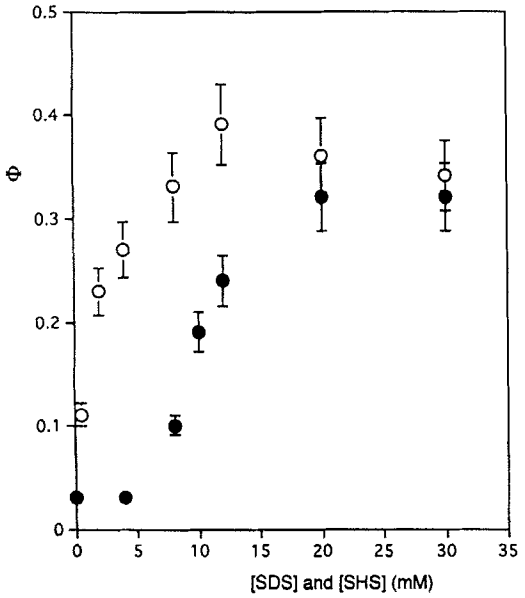

Figure 2. Dependence of Φ_{2a} on surfactant concentrations for (CH$_3$CO)$_4$Fl-photosensitized monomerization of **1a** in the presence of SDS (-●-) and SHS (-○-): [**1a**] = 20 m*M*.

Table 1. Quantum yields of **2a**, **b** formation ($\Phi_{2a,b}$) at 405 nm in (CH$_3$CO)$_4$Fl-photosensitized monomerization of **1a**, **b** in the presence of SHS*

	SHS (m*M*)			
	Φ_{2a}			Φ_{2b}
1a, **b** (m*M*)	1.0	5.0	30	5.0
50	0.29	0.49	0.59	0.63
25	0.18	0.28	0.44	0.42
20	0.14	0.24	0.37	0.36
17	0.13	0.23	0.32	0.35
13	0.10	0.18	0.25	0.30

*For degassed aqueous solutions containing (CH$_3$CO)$_4$Fl (0.5 m*M*), **1a**, **b** and SHS.

were described in previous papers (21). For the preparation of **1b**, a 150 cm^3 acetone solution of 1,3-dimethyluracil (**2b**) (9.0 g, 64 mmol) was bubbled with Ar for 20 min and then irradiated at room temperature with a high-pressure mercury lamp through a Pyrex glass for 40 h. A precipitate formed by the irradiation was collected by filtration and then recrystallized from acetonitrile to give **1b** (4.0 g, 44%) (22), whereas the *trans,syn*- and *cis,anti*-dimers were isolated by column chromatography of alumina (Fuji Silysia BW-300). The flavin photosensitizers (RCO)$_4$Fl were obtained by the acylation of riboflavin with the corresponding acid anhydrides in dry pyridine (23) and recrystallized from ethanol/chloroform mixtures.

Instruments. The ^{1}H and ^{13}C NMR spectra were obtained on a Bruker AC 250P spectrometer. Steady-state and time-resolved fluorescence measurements were performed on a Hitachi F-4500 spectrofluorometer and a Horiba NAES 550 single-photon-counting instrument, respectively. The oxidation potentials were measured on a Hokuto Denko HA 501G potentiostat using an HB-105 function generator and an Ag/AgNO$_3$ reference electrode in acetonitrile.

Photoreactions. Aqueous solutions (2 cm^3) containing (RCO)$_4$Fl (0.5 m*M*), **1a**,**b** (20 m*M* in most runs or varying amounts) and a surfactant at given concentrations placed in Pyrex vessels were degassed by four freeze–pump–thaw cycles under high vacuum or bubbled with a stream of Ar or O$_2$ for 10 min or used as prepared without any deaeration treatment and then irradiated at 405 nm using a high-pressure mercury lamp. The progress of the photoreactions was followed by HPLC using a Wakosil 5C18 RS column and an MeOH-H$_2$O eluent, and a ferrioxalate actinometer was used for the determination of quantum yield ($\Phi_{2a,b}$).

RESULTS

Irradiation was performed at 405 nm for degassed aqueous solutions containing (RCO)$_4$Fl (0.5 m*M*,), **1a** or **1b** (20 m*M* or varying amounts) and a surfactant (0–50 m*M*) (Fig. 1). In the absence of a surfactant, little formation of **2a** occurred as reported previously (1,8–12); the formation quantum yield (Φ_{2a}) was <0.03. In the presence of SDS or sodium hexadecyl sulfate (SHS), on the other hand, Φ_{2a} increased with an increase of surfactant concentration, particularly at concentrations higher than the critical micelle concentration (CMC = 8 m*M* for SDS or 0.5 m*M* for SHS), to reach a maximum at ~20 m*M* for SDS or ~10 m*M* for SHS, as shown in Fig. 2. Similarly, the FL-photosensitized splitting of **1b** was remarkably enhanced by the anionic surfactants. By contrast, such enhancing effects were not observed at all with the cationic surfactant, dodecyltrimethylammonium chloride (DTAC; CMC = 15 m*M*), and with the nonionic one, poly(ethyleneglycol) dodecyl ether (PEDE; CMC = 0.1 m*M*), even at concentrations higher than their CMC.

Table 1 lists the $\Phi_{2a,b}$ values at 405 nm for (CH$_3$CO)$_4$Fl-

Table 2. Quantum yields ($\Phi_{2a,b}$) at 405 nm for the formation of **2a**, **b** in $(RCO)_4Fl$-photosensitized monomerization of **1a**, **b** in the presence of SDS*

$(RCO)_4Fl$							
$(CH_3CO)_4Fl$				$(C_2H_5CO)_4Fl$		$(C_3H_7CO)_4Fl$	
1a (m*M*)	Φ_{2a}	**1b** (m*M*)	Φ_{2b}	**1a** (m*M*)	Φ_{2a}	**1a** (m*M*)	Φ_{2a}
50	0.54	40	0.40	40	0.22	40	0.33
25	0.37	20	0.31	20	0.20	20	0.20
20	0.32	13	0.21	13	0.15	13	0.17
17	0.28	10	0.19	10	0.11	8	0.11
13	0.24	8	0.16	8	0.08	7	0.10

*For a degassed aqueous solution containing $(RCO)_4Fl$ (0.5 m*M*), **1a**, **b** and SDS (50 m*M*).

Table 3. Intercept-to-slope ratios (I/S) and reciprocal intercepts ($\Phi_{2a,b}^{\infty}$) obtained from double-reciprocal plots of $\Phi_{2a,b}$ *vs* concentration of **1a**, **b** for FL-photosensitized monomerization of **1a**, **b** in the presence of SDS and SHS and Stern–Volmer constants (K_{SV}) for quenching of $(RCO)_4Fl$ fluorescence by **1a** in the presence of SDS

$(RCO)_4Fl$	**1a**, **b**	Surfactant (m*M*)	K_{SV} (M^{-1})	I/S (M^{-1})	$\Phi_{2a,b}^{\infty}$
$(CH_3CO)_4Fl$	**1a**	SDS (50)	5.7	31	0.85
$(C_2H_5CO)_4Fl$	**1a**	SDS (50)	7.2	35	0.58
$(C_3H_7CO)_4Fl$	**1a**	SDS (50)	3.0	37	0.52
$(CH_3CO)_4Fl$	**1b**	SDS (50)		39	0.66
$(CH_3CO)_4Fl$	**1a**	SHS (50)		36	0.87
		(5.0)		21	0.87
		(1.0)		10	0.87
$(CH_3CO)_4Fl$	**1b**	SHS (5.0)		38	0.87

photosensitized splitting of **1a,b** at various concentrations in the presence of SHS at 1.0, 5.0 and 30 m*M*, and those for the photosensitized reactions of **1a,b** in the presence of SDS at a fixed concentration (50 m*M*) are shown in Table 2. Double reciprocal plots of the $\Phi_{2a,b}$ values *vs* the concentrations of **1a,b** are linear to give the intercept-to-slope ratios (I/S) and reciprocal intercepts (limiting quantum yields, $\Phi_{2a,b}^{\infty}$) that are summarized in Table 3. It is of mechanistic significance to note that the reciprocal intercepts are relatively high (~0.87) and almost constant for the $(CH_3CO)_4Fl$-photosensitized reactions of **1a** in the presence of either SDS at 50 m*M* or SHS at the three different concentrations. On the other hand, the I/S values are higher at higher concentrations of SHS for the $(CH_3CO)_4Fl$-photosensitized reaction of **1a** but are almost constant at a fixed concentration of SDS for the photoreaction of **1a** using the three $(RCO)_4Fl$.

While the values shown in Tables 1 and 2 were obtained for solutions that had been thoroughly degassed by freeze–pump–thaw cycles, it was found that the photoreactions are extremely sensitive to O_2 concentrations in solution. As shown in Table 4, the Φ_{2a} value was only ~0.05 for aerated solutions (as-prepared solution before deaeration treatment) and increased to 0.17 after Ar bubbling of the aerated solution for 10 min. However, this value is still significantly smaller than that for a fully degassed solution. Probably, Ar bubbling of an aerated solution leaves O_2 at a very low concentration, allowing significant quenching of the photoreaction. Upon O_2 saturation of the solution, the photoreactions were almost completely quenched ($\Phi^{2a} \leq 0.02$).

DISCUSSION

The remarkable effects of SDS and SHS on the FL-photosensitized splitting of **1a,b** are clearly attributable to the prior incorporation of FL and **1a,b** in micelles (Eqs. 1–3), but not to the general salt effects due to the increase of ionic strength nor to special salt effects observed for some electron-transfer photoreactions of organic molecules (8). This is supported by the following observations: (1) the surfactant effects critically appear at concentrations higher than CMC and (2) sodium octyl sulfate (SOS) having high CMC (130 m*M*) showed no enhancing effect even at ~100 m*M*. It has been reported that 53% of 0.27 m*M* lumiflavin is incorporated in SDS micelles at 27 m*M* of the surfactant (24). The FL photosensitizers used in the present investigation are less soluble in water than lumiflavin but become substantially soluble upon addition of each of the surfactants used, particularly at concentrations higher than the CMC. It is, therefore, reasonable to assume that the FL should be largely solubilized in SDS and SHS micelles and perhaps in the cationic and nonionic micelles as well.

The incorporation of **1a** into the anionic micelles was shown by a study of SDS effects on the ^1H NMR behavior of **1a.** Figure 3 shows chemical-shift changes in the ^1H NMR signals of **1a** with varying amounts of SDS and SOS at <100 m*M* in D_2O. In the case of SOS where micelles are not formed, the increase of SOS concentration resulted in common up-field shifts of all the proton signals, probably due to the increase of ionic strength in solution. In the case of SDS where the micelles are formed, on the other hand, a down-field shift occurred for the signal of the cyclobutane methine proton (Ha) upon addition of SDS at concentrations above CMC, clearly indicating that **1a** molecules should be located, at least in part, in a domain different from the bulk water phase. Although the other proton signals showed common up-field shifts, the chemical-shift changes by addition of 60 m*M* SDS are considerably greater than those by addition of 60 m*M* SOS. The ^1H NMR behavior in the presence of SDS might be attributable to combined effects by the incorporation of **1a** in SDS micelles and by increases of ionic strength in solution. Presumably, **1a** appears to be incorporated in an outer-sphere domain of the SDS micelles. The reactants in the bulk water phase, $(FL)_{aq}$ and $(\mathbf{1a,b})_{aq}$, and those in the micelles, $(FL)_m$ and $(\mathbf{1a,b})_m$, should exist in equilibrium (Eqs. 1 and 2).

Table 4. The $(CH_3CO)_4Fl$-photosensitized reactions of **1a** under degassed, aerated and O_2-saturated conditions*

Conditions	Φ_{2a}
Freeze–pump–thaw degassing	0.35
Ar bubbling for 10 min	0.19
Aerated (as prepared)	0.05
O_2 bubbling for 1 min	0.02
O_2 bubbling for 10 min	0.01

*For aqueous solutions of $(CH_3CO)_4Fl$ (0.5 m*M*), **1a** (20 m*M*) and SDS (50 m*M*).

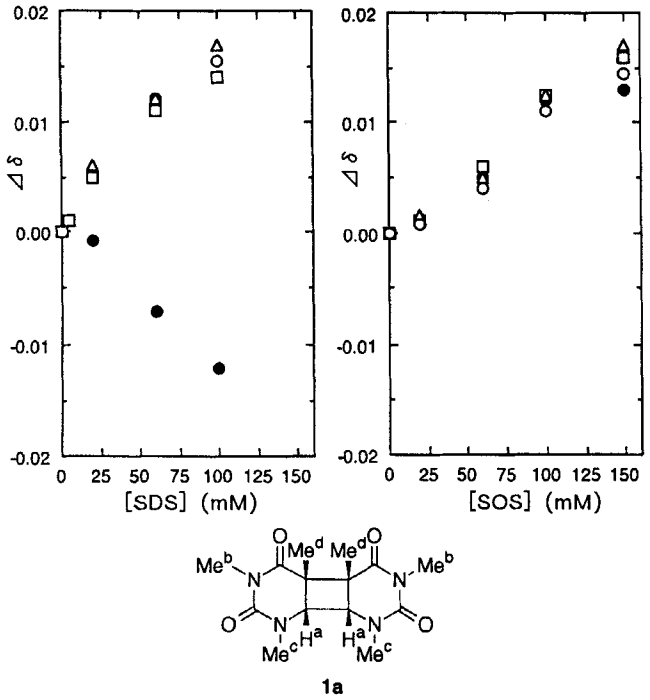

Figure 3. Chemical-shift changes (in ppm) of the ^{1}H NMR signals of **1a** in D_2O in the presence of SDS (left) and SOS (right); the chemical shifts (δ) in the absence of surfactants are 3.93 (H^a: –●–), 2.88 (N-Me^b: –○–), 2.81 (N-Me^c: –△–) and 1.30 (Me^d: –□–).

Concomitant incorporation of FL and **1a,b** in a micellar particle may occur between $(FL)_m$ and either $(\mathbf{1a,b})_{aq}$ or $(\mathbf{1a,b})_m$ and also between $(FL)_{aq}$ and $(\mathbf{1a,b})_m$ (eq. 3).

$$(FL)_{aq} + \text{micelle} \rightleftharpoons (FL)_m \quad (1)$$

$$(\mathbf{1a, b})_{aq} + \text{micelle} \rightleftharpoons (\mathbf{1a, b})_m \quad (2)$$

$$(FL)_m + (\mathbf{1a, b})_{aq}/(\mathbf{1a, b})_m \quad \text{and}$$

$$(FL)_{aq} + (\mathbf{1a, b})_m \rightleftharpoons (FL\text{–}\mathbf{1a, b})_m \quad (3)$$

The photoexcitation of FL in the micelles gives the excited singlet and triplet states of FL incorporated in the micelles, $(^{1,3}FL^*)_m$ and $(^{1,3}FL^*\text{–}\mathbf{1a,b})_m$ (Eqs. 4 and 5). Possible incorporation of excited-state FL from the water phase ($[^{1,3}FL^*]_{aq}$) into the micelles should also be considered for the FL-photosensitized reactions. In the exited singlet state, however, the incorporation of $(^1FL^*)_{aq}$ should be negligible (Eq. 6) because of the short lifetime (5–6 ns). The micelle concentration can be calculated by using the reported aggregation number (60–100) (24) to be <1 m*M* at 50 m*M* of SDS, thus giving the maximum rate ($\sim 10^7$ s^{-1}) for the incorporation of a molecule from the bulk water phase into a micellar particle under the assumption of diffusion-controlled limits. This rate is smaller by an order of magnitude than the decay of $(^1FL^*)_{aq}$($>10^8$ s^{-1}). In the long-lived triplet state (~100 μs) (14,16), on the other hand, the incorporation of $(^3FL^*)_{aq}$ into micellar particles can occur in pseudoequilibrium with the dissociation of the micelle-incorporated $^3FL^*$ (Eq. 7). The increase of I/S with the increase in SHS concentration shown in Table 3 reflects an increase in the number of micellar particles. However, the dependence of the photoreaction on SHS concentrations shown in Fig. 1 and Table 3 seems not to be relevant to detailed discussions, because micellar particles are changed in both size and shape depending upon surfactant concentrations (25).

$$(FL)_m + h\nu \rightarrow (^1FL^*)_m \rightarrow (^3FL^*)_m \quad (4)$$

$$(FL\text{–}\mathbf{1a, b})_m + h\nu \rightarrow (^1FL^*\text{–}\mathbf{1a, b})_m \rightarrow (^3FL^*\text{–}\mathbf{1a, b})_m \quad (5)$$

$$(^1FL^*)_{aq} + \text{micelle or } (\mathbf{1a, b})_m \overset{\#}{\rightleftharpoons} (^1FL^*)_m \text{ or } (^1FL^*\text{–}\mathbf{1a, b})_m \quad (6)$$

$$(^3FL^*)_{aq} + \text{micelle or } (\mathbf{1a, b})_m \rightleftharpoons (^3FL^*)_m \text{ or } (^3FL^*\text{–}\mathbf{1a, b})_m \quad (7)$$

The fluorescence of $(CH_3CO)_4Fl$ is not appreciably quenched by **1a** in aqueous and polar organic solutions (14,16). In the presence of 50 m*M* SDS, however, fluorescence quenching by **1a** occurred with a Stern–Volmer constant (K_{SV}) of 5.7 M^{-1}, thus suggesting that the photoreaction might proceed in the excited singlet state of micelle-incorporated FL, $(^1FL^*)_m$ and $(^1FL^*\text{–}\mathbf{1a,b})_m$, at least in part. However, the I/S value listed in Table 3 is largely different from the K_{SV} value, indicating that the participation of the exited-singlet species in the net photoreaction of **1a** should be minor, if any. The triplet state of micelle-incorporated FL, $(^3FL^*)_m$ and $(^3FL^*\text{–}\mathbf{1a,b})_m$ should play an important role, a mechanism supported by the efficient O_2 quenching of the photoreaction. In this regard, it should be noted that the fluorescence of $(CH_3CO)_4Fl$ in Ar-purged solution in the presence of 50 m*M* SDS is almost identical in intensity with that of thoroughly degassed solution and is attenuated only by 10% upon air saturation. The concentration of O_2 left in solution after Ar purging should be sufficient for the substantial quenching of the long-lived triplet state but too low to quench the short-lived excited singlet state. On the basis of the published mechanisms (6–17), therefore, it is reasonable to assume that the FL-photosensitized monomerization of **1a,b** proceeds in the micelles *via* electron transfer from **1a,b** to $^3FL^*$, for the most part, and also to $^1FL^*$ to a minor extent, as shown in Eqs. 8–11 where $(Fl^{\cdot-}//\mathbf{1a,b}^{\cdot+})_m$ and $(Fl^{\cdot-}//\mathbf{2a,b}^{\cdot+}/\mathbf{2a,b})_m$ denote, respectively, the radical ion pairs formed by excited-state electron transfer and by splitting of $\mathbf{1a,b}^{\cdot+}$ in the former. Provided that O_2 in aerated solution can quench all of triplet-state FL and ~10% of excited-singlet FL, the singlet pathway would participate to an ~15% extent, at most, in the photoreaction of 20 m*M* **1a** at 50 m*M* of SDS.

$$(^{1,3}FL^*\text{–}\mathbf{1a, b})_m \rightarrow (FL^{\cdot-}//\mathbf{1a, b}^{\cdot+})_m \quad (8)$$

$$(^3FL^*)_m + (\mathbf{1a, b})_{aq/m} \rightarrow (FL^{\cdot-}//\mathbf{1a, b}^{\cdot+})_m \quad (9)$$

$$(FL^{\cdot-}//\mathbf{1a, b}^{\cdot+})_m \rightarrow (FL^{\cdot-}//\mathbf{2a, b}^{\cdot+}/\mathbf{2a, b})_m \rightarrow FL + 2\ \mathbf{2a, b} \quad (10)$$

$$(FL^{\cdot-}//\mathbf{1a, b}^{\cdot+})_m \rightarrow (FL\text{–}\mathbf{1a, b})_m \quad (11)$$

The splitting efficiency (Φ_{10}) after the excited-state electron transfer should be determined by competition between the ring cleavage of $\mathbf{1a,b}^{\cdot+}$ (Eq. 10) and back electron transfer of the radical ion pair (Eq. 11). Provided that the singlet

pathway would be negligible, the $\Phi_{2a,b}^{\infty}$ values should equal $2\Phi_{10} \times \Phi_T \times \Phi(T_m)$ where Φ_T and $\Phi(T_m)$ denote, respectively, the intersystem crossing yield (Eqs. 4 and 5) and the fraction of $(^3FL^*)_m$ in the total triplet FL. If $\Phi_T \sim 0.7$, a value reported for riboflavin in water (26), calculations using the $\Phi_{2a,b}^{\infty}$ value (0.87) give a minimum value of Φ_{10} (~0.62) under the assumption of $\Phi(T_m) = 1.0$. This Φ_{10} value seems to be relatively high among usual organic photoreactions, implying the importance of environmental effects on the electron-transfer photochemistry of FL.

It is of significance, associated with the electron-transfer photochemistry of FL, to discuss why the FL-photosensitized monomerization of **1a,b** efficiently proceeds in anionic micelles in contrast to little occurrence of any reactions in water and polar organic solvents. In water and polar organic solvents, the polar solutes $(RCO)_4Fl$ and **1a,b** should be strongly solvated, particularly by hydrogen bonding with water molecules. Electron transfer between such strongly solvated reactants might suffer relatively high intrinsic barriers associated with large reorganization of solvent molecules in solvation shells. Consequently, the driving force for a relevant electron transfer process must be large enough to overcome such reorganization energies. This situation should be particularly unfavorable for isoergonic electron transfer and even more for weakly endoergonic reactions. The excited-state electron transfer of $(RCO)_4Fl$ with **1a,b** appears to fit the case, as implied by calculations of the free energy changes (-2.8 ± 0.1 kcal mol^{-1} in the excited singlet state and $+8.3 \pm 0.1$ kcal mol^{-1} in the triplet state) using the half-peak redox potentials in Fig. 1 and the excitation energies (3,4).

In a hydrophobic domain of the micelle where the reactants would be free from strong solvation with water molecules, solvational reorganization energies might be low enough to allow the excited-state electron transfer to proceed at a reasonable rate constant. The incorporation of $(RCO)_4Fl$ in a hydrophobic domain of the micelle was suggested by the observation that the fluorescence of $(CH_3CO)_4Fl$ was enhanced by $\geq$30% in intensity upon addition of 30 m*M* SDS, as reported for lumiflavin (24). Moreover, the incorporation of both $(RCO)_4Fl$ and **1a,b** in a micellar particle is certainly beneficial to effective collisions for electron transfer to occur within the excited-state lifetimes. For the same reason, on the other hand, similar micellar effects could be expected for the back electron transfer of the radical ion pair (Eq. 11). This is clearly not the case, because the net effects of the anionic surfactants are remarkably positive. Presumably, the exterior negative charges surrounding the micellar particles might effectively work as an electrostatic force, *e.g.* to separate $FL^{\cdot-}$ and $\mathbf{1a,b}^{\cdot+}$ in distance long enough to retard back electron transfer.

A serious question should emerge as to why the FL-photosensitized reactions of **1a,b** can be specifically enhanced by the anionic surfactants but not at all by either cationic (DTAC) nor nonionic (PEDE) surfactants. An attractive speculation is that the exterior anionic sulfate groups surrounding the SDS and SHS micelles should be susceptible to hydrogen bonding with water molecules to form relatively rigid solvation shells associated with stabilization of the micellar particles having a dense hydrophobic domain. As a result, water molecules cannot penetrate into the micelles in depth for strong solvation of the reactants to occur. This is clearly not the case for DTAC, because the trimethylammonium group is inherently not capable of hydrogen bonding. Moreover, the steric bulkiness of the hydrophilic groups of DTAC and PEDE seems to be unfavorable for that formation of stable micelles with a dense hydrophobic domain. Perhaps, water molecules can penetrate into the micelles of DTAC and PEDE so that the reactants may not be free from strong solvation with water molecules even in the micellar particles. In line with this speculation, the flavin fluorescence was unchanged upon addition of either DTAC or PEDE, unlike the substantial enhancing effect of SDS.

CONCLUSION

The present investigation clearly demonstrates that $(RCO)_4Fl$ are potentially active in photosensitizing the monomerization of **1a,b** in the hydrophobic domain of anionic micelles under neutral conditions. The lack of FL photosensitization of dimer splitting in water or very polar organic solvents was attributed to strong solvation of the reactants. This should be significantly associated with the electron-transfer photochemistry of "naked" FL. In this regard, it is of interest to note that the FL chromophore of DNA photolyase is not situated in an exterior region exposed to bulk water (27,28). Nevertheless, DNA photolyase with an oxidized FL chromophore is inactive in photorepair (6,18). A crucial question emerges as to whether the inactive nature of DNA photolyase with the oxidized FL chromophore originates not from the inherent lack of photocatalytic capability of the oxidized FL but from other properties of the enzyme.

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