indicate that additional decay routes, not included in the present analysis, play a significant role in the process. For example the observation of intramolecular exciplex fluorescence emission is a manifestation of a decay route. In addition, the correction procedure based on the O-4,4 standard is likely to fail for compounds other than the P-*n,m* series for which direct excitation of acceptor fluorescence at 266 nm cannot be neglected. For those compounds for which $\xi^{T-T} > 1$, we expect to observe complementarity between singlet–singlet intra-EET and triplet–triplet intra-EET. Only P-8,2 deviates from this general behavior. This can be understood since for P-8,2 the interchromophoric distance is shorter than that for the other molecules of the P-*n,m* series, promoting both modes of intra-EET.

While the above analysis shows the existence of the triplet–triplet intra-EET process, it is incomplete. It does not reveal the exact structural dependence of triplet–triplet intra-EET in bichromophoric molecules. For singlet–singlet intra-EET we were able to demonstrate that the Dexter[18] exchange interaction can serve as a good approximation for the mechanism of this process. Since triplet–triplet intra-EET is usually described by the same mechanism, Q_{ET}^{T-T} should be expressed in a form similar to eq 30. Yamamoto and co-workers have shown recently[25] that indeed this is the case for a different series of bichromophoric molecules. In their series,[25] the triplet–triplet intra-EET process could be evaluated by following the intramolecular quenching of the donor chromophore phosphorescence. Unfortunately, due to the negligible phosphorescence of the aromatic chromophore in our series of compounds, such an approach could not be applied.

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5. Conclusions

We have shown that exchange interaction controls short-range intramolecular electronic energy transfer in bichromophoric molecules for which the spectral overlap integral is low. Special design of bichromophoric molecular structure made it possible to determine the contributions of interchromophore distance and relative chromophore orientation to exchange interaction. It is concluded that in general the exchange integral and singlet–singlet intra-EET are dependent upon the relative orientation of the interacting orbitals, resulting in a preferred orientation for electron exchange. However, the simple Dexter formulation of exchange interaction still holds in some cases.

In addition, we have demonstrated the triplet–triplet intra-EET process in bichromophoric molecules. This process complements our observation of singlet–singlet intra-EET. In contrast to singlet–singlet intra-EET, complete quantitative analysis of the structural dependence of this process requires the separation of the singlet–singlet and triplet–triplet routes from the intersystem crossing processes. Thus, the complete molecular structural dependence of the triplet–triplet intra-EET process was not established for our series of bichromophoric molecules.

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Flavin-Photosensitized Monomerization of Dimethylthymine Cyclobutane Dimer: Remarkable Effects of Perchloric Acid and Participation of Excited-Singlet, Triplet, and Chain-Reaction Pathways

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Abstract: We report the efficient monomerization of the cis-cisoid cyclobutane dimer of 1,3-dimethylthymine photosensitized by riboflavin tetraacetate (r-Fl), 3-methyl-8-chloro-10-phenyl-5-deazaflavin (d-Fl), and lumiflavin in acetonitrile in the presence of $HClO_4$, as a model reaction of photorepair. The limiting quantum yields are unity for the r-Fl-photosensitized monomerization in degassed and aerated solution and also for the d-Fl-photosensitized run in degassed solution. Mechanistic studies involving kinetic analysis of the reactions, fluorescence quenching, and laser-flash photolysis demonstrate that the photosensitized monomerization proceeds through electron transfer from the dimer to the protonated flavins in both the excited-singlet and triplet states. It is suggested that the singlet and triplet radical pairs initially formed by the electron transfer exclusively undergo the net monomerization with little participation of geminate recombination back to the precursors. A chain reaction has been found to participate as another mechanistic channel in the r-Fl-photosensitized monomerization of Ar-purged solution and in the d-Fl-photosensitized reaction of Ar-purged and aerated solution; the limiting quantum yields are much greater than unity. It is suggested that a chain carrier is photochemically generated from the protonated form of r-Fl or d-Fl to catalyze the monomerization of the dimer complexed with O_2.

Introduction

The ultraviolet (UV) induced cyclodimerization of pyrimidine bases in DNA is a major origin of lethal and/or mutagenic UV effects of biological systems,[1] which can be repaired upon exposure of UV-irradiated biological systems to near-UV and/or visible light.[1,2,3a] The photorepair of UV-damaged DNA requires enzyme

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Table I. Quantum Yields for Flavin-Photosensitized Monomerization of DT_2[a]

FL	with no acid	with 10 mM $HClO_4$		
		Φ_{spl}(degas)[b]	Φ_{spl}(Ar)[c]	Φ_{spl}(air)[d]
r-Fl	$<10^{-3}$	0.74 (1.0)	0.94 (~10)	0.43 (1.0)
d-Fl	$<10^{-3}$	0.77 (1.0)	1.8 (~6)	2.7 (~10)
l-Fl	*e*	0.75	*f*	0.55

[a] At 366 nm for MeCN solution at 30 ± 1 °C; [FL] = 0.5 mM, $[DT_2]$ = 20 mM, and $[HClO_4]$ = 10 mM. In parentheses are limiting quantum yields obtained from plots of Φ_{spl}^{-1} vs $[DT_2]^{-1}$. [b] For solution degassed by four freeze–pump–thaw cycles under $<10^{-3}$ Torr. [c] For solution bubbled with Ar (99.99%) for 15 min. [d] For aerated solution. [e] Insoluble in dry MeCN. [f] Not determined.

called DNA photolyase, occurring through photochemical monomerization of pyrimidine dimers with nearly unit[3] or relatively high[4] quantum efficiencies. Extensive studies on model reactions using various photosensitizers and dimer models[5-8] indicate that electron transfer is a probable mechanism for photorepair. An electron-transfer mechanism for enzymic photorepair was indeed demonstrated by picosecond laser photolysis of *Escherichia coli* photolyase in the presence of the deoxyuridine dinucleotide cyclobutane dimer.[9] In most model reactions, the monomerization quantum yields are significantly or much lower than unity, mainly arising from geminate recombination of photosensitizer–dimer ion–radical pairs which competes with or predominates over the ring splitting of the dimer cation or anion radicals.[5b,6d] In enzymic photorepair, geminate recombination of the photogenerated chromophore–dimer redox pair should be minor or negligible, perhaps due to unique, but unknown, functions of photolyase.

Recently, the principal chromophore of photolyase in some biological systems was identified as fully reduced FAD ($FADH_2$),[2,10] whereas a pteridine[11] or 8-hydroxy-5-deazaflavin[12] is also involved, perhaps, as complementary chromophore. On the basis of fluorescence quenching experiments, it was suggested that excited-singlet $FADH_2$ donates an electron to pyrimidine dimer to initiate the monomerization.[13] However, the direction of electron transfer is still controversial since picosecond laser photolysis failed to detect $FADH_2^{\cdot+}$ even in a time domain of >20 ps.[9a] Model reactions indicate that dimer cation radicals are usually much more reactive toward the ring splitting than the corresponding anion radicals[5-8] with one exception,[14] while electron abstraction from pyrimidine dimer by excited $FADH_2$ is thought to be energetically unfavorable. Moreover, model reactions using a variety of reduced or oxidized flavins and deazaflavins as photosensitizer are inefficient ($\Phi \leq 10^{-2}$),[5,15-17] and little has been

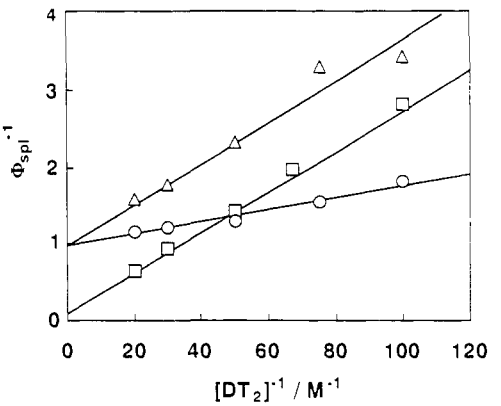

Figure 1. Double-reciprocal plots of Φ_{spl} vs $[DT_2]$ for r-Fl-photosensitized monomerization of DT_2 in degassed (–○–), aerated (–△–), and Ar-purged (–□–) MeCN at 30 ± 1 °C; [r-Fl] = 0.5 mM, and $[HClO_4]$ = 10 mM; irradiation at 366 nm.

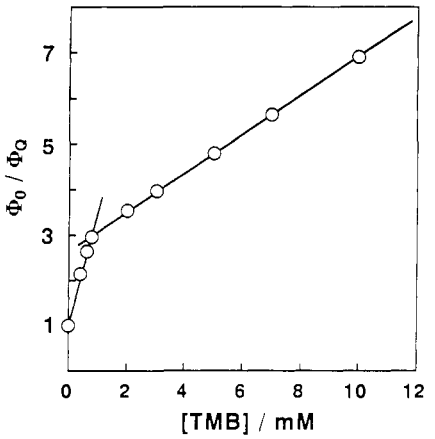

Figure 2. A Stern–Volmer plot for quenching of r-Fl-photosensitized monomerization of DT_2 by TMB in Ar-purged MeCN at 30 ± 1 °C; [r-Fl] = 0.5 mM, $[DT_2]$ = 20 mM, and $[HClO_4]$ = 10 mM; irradiation at 366 nm; TMB = 1,2,4-trimethoxybenzene.

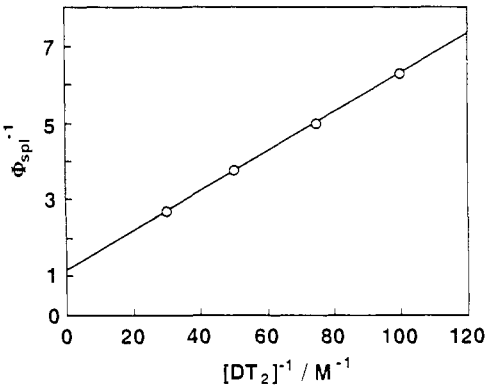

Figure 3. A double-reciprocal plot of Φ_{spl} vs $[DT_2]$ for r-Fl-photosensitized monomerization of DT_2 in the presence of 0.8-mM TMB in Ar-purged MeCN at 30 ± 1 °C; [r-Fl] = 0.5 mM and $[HClO_4]$ = 10 mM; irradiation at 366 nm; TMB = 1,2,4-trimethoxybenzene.

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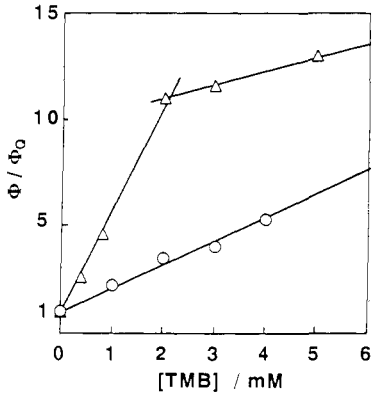

Figure 4. Stern–Volmer plots for quenching of d-Fl-photosensitized monomerization of DT_2 by TMB in degassed (–O–) and aerated (–Δ–) MeCN at 30 ± 1 °C; [d-Fl] = 0.5 mM, [DT_2] = 20 mM, and [$HClO_4$] = 10 mM; irradiation at 366 nm; TMB = 1,2,4-trimethoxybenzene.

investigated on the reaction mechanisms. Therefore, a crucial question arises as to whether or not the efficient photorepair process might be attributable to inherent functions of the flavin chromophores in electron-transfer photosensitization.

In order to understand chemical roles of the flavin chromophores in photorepair, it is required to explore the photosensitization capabilities of flavins as well as the mechanistic details in the monomerization of dimer models under various conditions. It is known that oxidized flavins reveal versatile photocatalytic capabilities in oxidation of organic substrates in the presence of an acid.[18] We wish to report that the monomerization of the cis-cisoid cyclobutane dimer (DT_2) of 1,3-dimethylthymine is efficiently photosensitized by such flavin photosensitizers (FL) as riboflavin tetraacetate (r-Fl), lumiflavin (l-Fl), and a 5-deazaflavin derivative (d-Fl) *in the presence of perchloric acid to proceed through divergent mechanistic channels involving electron transfer in either the excited-singlet or triplet state and a chain-reaction pathway.*[19,20]

Results

Photoreactions. Table I lists quantum yields for the FL-photosensitized monomerization of DT_2 at 20 mM in degassed and aerated MeCN in the absence and the presence of $HClO_4$. In most runs, the photosensitized reactions of DT_2 were carried out in the presence of 10 mM $HClO_4$ by irradiation at 366 nm. As shown in Figure 1, double-reciprocal plots of splitting quantum yield (Φ_{spl}) vs concentration of DT_2 ([DT_2]) for the r-Fl/$HClO_4$ pair in degassed and aerated solutions give common intercepts of unity within experimental errors, i.e. common limiting quantum yields of unity (Φ_{spl}^{∞}(degas) ≃ Φ_{spl}^{∞}(air) ≃ 1). For Ar-purged solution, on the other hand, Φ_{spl} exceeds unity at [DT_2] ≥ 30 mM to reach a limiting value of Φ_{spl}^{∞}(Ar) ≃ 10 (Figure 1). Figure 2 shows a Stern–Volmer plot for quenching of the photoreaction in Ar-purged solution by 1,2,4-trimethoxybenzene (TMB), which has a break at ~1 mM in TMB. Figure 3 shows a double-reciprocal plot of Φ_{spl} vs [DT_2] in the presence of 0.8 mM TMB for Ar-purged solution. In the case of d-Fl, the following values of Φ_{spl}^{∞} depending on O_2 concentration in solution were obtained from double-reciprocal plots of Φ_{spl} vs [DT_2] (supplementary material); Φ_{spl}^{∞}(degas) ≃ 1, Φ_{spl}^{∞}(Ar) ≃ 6, and Φ_{spl}^{∞}(air) ≃ 10. Figure 4 shows Stern–Volmer plots for TMB quenching of the d-Fl-photosensitized reaction in degassed and Ar-purged solutions.

Spectroscopic Measurements of Protonation. Upon addition of $HClO_4$ to MeCN solution of r-Fl, the absorption spectrum (λ_{max} 345, 442, and 468 (sh) nm) was changed to that of protonated r-Fl (r-FlH^+, λ_{max} 390 nm) with isosbestic points at 350 and 420 nm. Similar acid-induced spectral changes occurred with d-Fl.

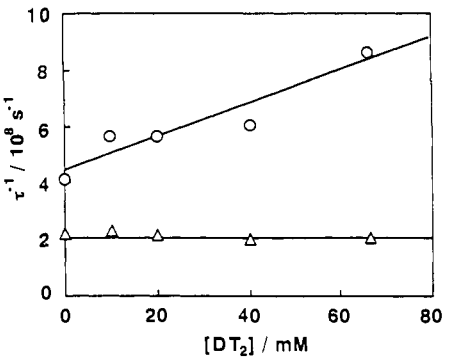

Figure 5. Effects of DT_2 concentration on the τ_1 (–O–) and τ_2 (–Δ–) components in the biexponential fluorescence decay of r-Fl in the presence of 10 mM $HClO_4$ in Ar-purged MeCN at 30 ± 1 °C; [r-Fl] = 25 μM; excitation at 366 nm.

Table II. Photophysical Properties of r-Fl and d-Fl

	r-Fl		d-Fl	
	[$HClO_4$] = 0 mM	10 mM	0 mM	10 mM
	absorption			
λ_{max}, nm	345 (8.8)	390	319 (9.6)	350 (21.5)
(ϵ, 10^3 M^{-1} cm^{-1})	442 (12.6)	(19.4)	400 (12.5)	362 (sh)
	468 (sh)		422 (sh)	
	fluorescence			
λ_{max}, nm	520	518	469	380
ϕ_F	0.12	~0.03		
τ_F, ns	7.2	2.4 (τ_1, 50%[a])	2.3	0.6
		5.6 (τ_2, 50%[a])		
	T–T absorption			
λ_{max}, nm	~680[b]	~660[b]	530[c]	500[c]
τ_T, μs	~100[b]	32[b]	6.0[c]	16[c]

[a] Quantum yields of the two components in a biexponential decay. [b] Determined by conventional Xe-flash photolysis. [c] Determined by laser-flash photolysis using the third harmonic of an Nd-YAG laser.

From the spectral changes, the equilibrium constant (K_G) was determined to be ~10^4 M^{-1} at 25 °C for either r-Fl or d-Fl.[21] On the other hand, all the ^1H and ^{13}C NMR signals of DT_2 (10 mM) in CD_3CN solution were essentially unchanged upon addition of 10 mM $DClO_4$. No indication was obtained for formation of charge-transfer complexes, since the absorption spectra and the aromatic ^1H NMR signals of r-FlH^+ and d-FlH^+ in the presence of 10 mM $HClO_4$ were not affected at all by addition of 20 mM DT_2.

The fluorescence of r-Fl in Ar-purged MeCN showed a broad spectrum (λ_{max} 520 nm) with a quantum yield (Φ_F) of 0.12 and a decay lifetime of 7.2 ns (χ^2 = 1.29 for single-exponential fits). Upon addition of $HClO_4$, the fluorescence quantum yield decreased with little change in the spectral shape accompanied by only a few nanometer blue shift, reaching a value of Φ_F ≃ 0.03 at 10 mM in the acid. Quenching of r-Fl fluorescence by DT_2 occurred only in the presence of $HClO_4$; the Stern–Volmer constants in the presence of 10 mM $HClO_4$ are 28 M^{-1} (K_{SV}) in Ar-purged solution and 20 M^{-1} (K_{SV}') in aerated solution. The fluorescence of r-Fl in the presence of 10 mM $HClO_4$ showed a biexponential decay with lifetimes of 2.4 (τ_1) and 5.6 ns (τ_2) (χ^2 = 1.07). Upon addition of DT_2, the τ_1 component was shortened without a significant change of τ_2, as shown in Figure 5. Table II lists photophysical properties of r-Fl in the absence and presence of $HClO_4$. In the case of d-Fl, the fluorescence in Ar-purged MeCN showed a single exponential decay in either the absence or presence of 10 mM $HClO_4$ with a lifetime of 2.3 or 0.6 ns each. Quenching of the fluorescence by DT_2 again occurred only in the presence of $HClO_4$; K_{SV} = K_{SV}' = 5.8 M^{-1} at 10 mM in the acid.

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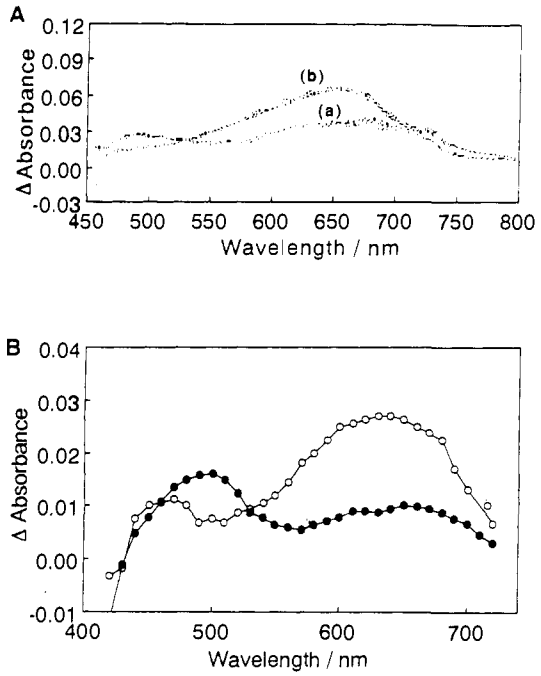

Figure 6. Transient absorption spectra taken (A) at a delay time of 5 μs after the flash of an Xe-flash lamp at >350 nm for Ar-purged MeCN solution of r-Fl (6 μM) in the absence (a) and presence (b) of 10 mM $HClO_4$ at 30 ± 1 °C and (B) at a delay time of 0.5 μs after the flash of the third harmonic of an Nd-YAG laser for Ar-purged MeCN solution of the r-Fl (25 μM)/$HClO_4$ (10 mM) pair in the absence (–O–) and presence (–●–) of 20 mM DT_2 at 30 ± 1 °C.

Table III. Dependence of Transient Absorbance and Triplet Lifetime of r-Fl on DT_2 Concentration[a]

	ΔA		
$[DT_2]$, mM	at 510 nm[b]	at 690 nm[c]	τ_T,[d] μs
0	0.153	0.070	32
1.0	0.238	0.063	
2.0	0.263	0.056	
5.0	0.272	0.049	27
10	0.306	0.035	24
20	0.363	0.023	20

[a] Determined by laser-flash photolysis using the third harmonic of an Nd-YAG laser; [r-Fl] = 6 μM and $[HClO_4]$ = 10 mM in Ar-purged solution. [b] Difference absorbances mainly due to formation of r-$FlH_2^{\cdot+}$ at 1 μs after the flash. [c] Difference absorbances mainly due to formation of ^{3}r-Fl*H^+ at 1 μs after the flash. [d] Triplet lifetimes monitored at 690 nm.

Flash Photolysis. Figure 6A shows transient absorption spectra of r-Fl in the absence and presence of 10 mM $HClO_4$ taken at a delay time of 5 μs after the flash of a conventional Xe-flash lamp at >350 nm. In either the absence or presence of 10 mM $HClO_4$, the transient showed a single-exponential decay with each lifetime of 100 or 32 μs (Table II). Laser-flash photolysis of the r-Fl/DT_2/$HClO_4$ system using the third harmonic (355 nm) of an Nd:YAG laser gave a new transient absorbing at 450–550 nm immediately after the flash (≤0.5 μs), as shown in Figure 6B. The increase of DT_2 concentration resulted in an increase of the absorbance at 450–550 nm at compensation of the absorbance at >600 nm and also in a shortening of the decay lifetime of the longer-wavelength transient (Table III). In the case of d-Fl, the absorption maxima and lifetimes of the triplets were also obtained by laser-flash photolysis in the absence and presence of $HClO_4$ and are summarized in Table II. Attenuation of the triplet by DT_2 in the presence of 10 mM $HClO_4$ occurred to leave a longer-lived transient absorbing at 390 and 520 nm (supplementary material).

Discussion

Protonation of Flavin Photosensitizer. Essential processes associated with protonation of the flavins (FL) are shown in eq 1–5. In the case of r-Fl, the biexponential fluorescence decay

$$FL + H^+ \overset{K_G}{\rightleftharpoons} FLH^+ \xrightarrow{h\nu\ (366\ nm)} {}^1FL^*H^+ \quad (1)$$

$$^1FL^*H^+ \overset{K_S}{\rightleftharpoons} {}^1FL^* + H^+ \quad (2)$$

$$^1FL^*H^+ \xrightarrow{^pk_{ST}} {}^3FL^*H^+ \quad (3)$$

$$^1FL^* \xrightarrow{k_{ST}} {}^3FL^* \quad (4)$$

$$^3FL^* + H^+ \overset{K_T}{\rightleftharpoons} {}^3FL^*H^+ \quad (5)$$

in the presence of 10 mM $HClO_4$ indicates the involvement of two emitting species irrespective of complete protonation in the ground state. Since excited-singlet riboflavin was reported to have a lower pK_a than the ground and triplet states in aqueous solution,[22,23] it is reasonable to assume that partial deprotonation of ^{1}r-Fl*H^+ occurs to give ^{1}r-Fl* in equilibrium (eq 2). The τ_1 (2.4 ns) and τ_2 (5.6 ns) components can be attributed to ^{1}r-Fl*H^+ and ^{1}r-Fl*, respectively. At higher acid concentration (100 mM), the fluorescence decay is still biexponential with a greater contribution (80%) of τ_1 = 2.4 ns and only a minor one (20%) of τ_2 = 4.2 ns. In the case of d-Fl, ^{1}d-Fl*H^+ should be dominant, as suggested by the short-lived, single-exponential fluorescence decay.

Intersystem crossing of ^{1}FL*H^+ and ^{1}FL* should occur to generate ^{3}FL*H^+ and ^{3}FL*. Formation of ^{3}r-Fl* in the absence of $HClO_4$ is demonstrated by the transient spectrum, which is very similar to reported T–T absorption spectra of flavins.[24] In the presence of 10 mM $HClO_4$, on the other hand, ^{3}r-Fl*H^+ should be the exclusive triplet species, as shown by the considerably different transient spectrum and by the single-exponential decay with a much shorter lifetime. This appears to be in line with the reported pK value of triplet lumiflavin in aqueous solution, which is 4.4, larger than that in the ground state (~0).[25] It is therefore conceivable that ^{3}r-Fl* formed through process 4 should be rapidly protonated at 10 mM in $HClO_4$, i.e. $K_T > K_G > K_S$. Similarly, ^{3}d-Fl*H^+ should be the exclusive triplet species in the presence of 10 mM $HClO_4$, as shown by the T–T absorption spectra and the decay lifetimes. It was reported that the triplet pK value (2.5) of a 5-deazaflavin is slightly greater than the ground-state value (1.8).[26] However, the protonation sites are probably different, N-5 in the case of ^{3}r-Fl*H^+ but N-1 in the case of ^{3}d-Fl*H^+.[27]

Excited-State Electron Transfer in the Photosensitized Monomerization. A general mechanism of the FL-photosensitized monomerization of DT_2 is shown by processes 6–12, where

$$^1FL^*H^+ + DT_2 \xrightarrow{k_6} {}^1\overline{FLH^{\cdot}//DT_2^{\cdot+}} \quad (6)$$

$$^3FL^*H^+ + DT_2 \xrightarrow{k_7} {}^3\overline{FLH^{\cdot}//DT_2^{\cdot+}} \quad (7)$$

$$^{1,3}\overline{FLH^{\cdot}//DT_2^{\cdot+}} \xrightarrow{^{1,3}k_8} {}^{1,3}\overline{FLH^{\cdot}//DT^{\cdot+}/DT} \rightarrow FLH^+ + 2DT \quad (8a)$$

$$\rightarrow FLH^{\cdot} + DT^{\cdot+} + DT \quad (8b)$$

$$^{1,3}\overline{FLH^{\cdot}//DT_2^{\cdot+}} \xrightarrow{^{1,3}k_9} FLH^+ + DT_2 \quad (9)$$

$$^{1,3}FL^*H^+ + O_2 \longrightarrow FLH^+ + O_2 \quad (10)$$

$$DT^{\cdot+} \xrightarrow{+e^-} DT \quad (11)$$

$$r\text{-}FlH^{\cdot} + H^+ \rightarrow r\text{-}FlH_2^{\cdot+} \quad (12)$$

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$^{1 \text{ or } 3}\overline{\text{FLH}^{\bullet}//\text{DT}_2^{\bullet+}}$, FLH$^{\bullet}$, and r-Fl$H_2^{\bullet+}$ denote singlet or triplet geminate radical pairs, the semiquinone radical of FL, and the protonated semiquinone radical of r-Fl, respectively. The transient absorption spectrum at 450–550 nm in Figure 6B is very similar to that reported for r-Fl$H_2^{\bullet+}$ [18c] and the protonated semiquinone radical of riboflavin,[28] providing the evidence for excited-state electron transfer (eq 6 and 7). Similarly, laser-flash photolysis of the d-Fl/DT_2/$HClO_4$ system gave a transient assignable to d-FlH$^{\bullet}$. Excited-singlet electron transfer (eq 6) is shown by fluorescence quenching by DT_2 in the presence of $HClO_4$ and more clearly, in the case of r-Fl, by the rapid formation of r-Fl$H_2^{\bullet+}$ within 0.5 μs, which is much faster than quenching of ^{3}r-Fl*H$^+$ by DT_2. The plot in Figure 5 gives a value of $k_6 = 5.8 \times 10^9$ M^{-1} s^{-1} while a slight larger value (1.2×10^{10} M^{-1} s^{-1}) was obtained from K_{SV}/τ_1 without taking into account equilibrium 2. In the case of d-Fl, k_6 was determined from K_{SV}/τ_F to be 1.0×10^{10} M^{-1} s^{-1}. The reduction potential of either ^{1}r-Fl*H$^+$ or ^{1}d-Fl*H$^+$ should be positive enough for electron abstraction from DT_2 to occur with ease.

Triplet-state electron transfer (eq 7) is shown by the DT_2-induced shortening of τ_T. Plots of τ_T^{-1} vs [DT_2] give the values of k_7 which are $\simeq 1 \times 10^6$ M^{-1} s^{-1} for r-Fl and 4.8×10^8 M^{-1} s^{-1} for d-Fl, suggesting that process 7 is endergonic for ^{3}r-Fl*H$^+$ but almost isoergonic for ^{3}d-Fl*H$^+$. The "slow formation" of r-Fl$H_2^{\bullet+}$ due to the triplet-state electron transfer could not be detected because of the complex time-dependent behavior of the transient absorption at 450–550 nm, which involves the dominant rapid rise followed by a slow decay together with attenuation of the partially overlapped T–T absorption. In the case of d-Fl, the behavior of the transient at 380 and 530 nm is still more complex since the absorption is very weak at 530 nm and overlapped with both the T–T absorption and the ground-state absorption at 380 nm.

The involvement of both the excited-singlet and triplet mechanisms in the r-Fl-photosensitized reaction of *degassed solution* is shown by the reciprocal slope of the plot in Figure 1, which is ~110 M^{-1}, far from K_{SV} (28 M^{-1}) for fluorescence quenching. In contrast, the reciprocal slope for *aerated solution* is 36 M^{-1}, much closer to K_{SV}' (20 M^{-1}), clearly indicating that the singlet pathway is left by virtually complete quenching of ^{3}r-Fl*H$^+$ by O_2. The discrepancy between the reciprocal slope and K_{SV}' mainly arises from experimental errors in quantum-yield measurements. Similarly, the d-Fl-photosensitized reaction of *degassed solution* involves both the singlet and triplet pathways, as discussed above. Contributions of the singlet pathway are significant, but not large at DT_2 concentration used (≤33 mM), because of the extremely short lifetime of ^{1}d-Fl*H$^+$. For instance, the fraction of the fluorescence quenched by 20 mM DT_2 is 0.10, considerably smaller than Φ_{spl}(degas) = 0.74 at [DT_2] = 20 mM.

Splitting of $DT_2^{\bullet+}$ occurs prior to diffusive separation of the radical pairs ($k_8 \gg 10^8$ s^{-1}).[6d] The monomerization of DT_2 would complete in a solvent cage (eq 8a) to substantial extents. Presumably, the flavin radicals detected by laser-flash photolysis should be those having escaped from a solvent cage after splitting of $DT_2^{\bullet+}$ (eq 8b). In general, however, geminate recombination of radical pairs may compete with rapid splitting of cyclobutane cation radicals in a solvent cage.[6d,29] However, Φ_{spl}^{∞}(air) is unity in the case of r-Fl, strongly suggesting that geminate recombination of $^1\overline{\text{r-FlH}^{\bullet}//\text{DT}_2^{\bullet+}}$ is much slower than splitting of $DT_2^{\bullet+}$, i.e. $^1k_8 \gg {}^1k_9$. In the case of d-Fl, this might be again true as implied by Φ_{spl}(degas) = 1.

In order to discuss the triplet pathway for the r-Fl-photosensitized reaction, we use the differences between the quantum yields for degassed and aerated solution represented by eq 13 and 14,

$$\Phi_{spl}(\text{degas}) = \left(\frac{K_{SV}[DT_2]}{1 + K_{SV}[DT_2]}\right) + \Phi_{spl}(T)\left(\frac{\Phi_{isc}}{1 + K_{SV}[DT_2]}\right)\left(\frac{k_7\tau_T[DT_2]}{1 + k_7\tau_T[DT_2]}\right) \quad (13)$$

$$\Phi_{spl}(\text{air}) = \left(\frac{K_{SV}'[DT_2]}{1 + K_{SV}'[DT_2]}\right) \quad (14)$$

$$\Phi_{spl}(T)\cdot\Phi_{isc} = \left[(\Phi_{spl}(\text{degas}) - \Phi_{spl}(\text{air})) - \left(\frac{K_{SV}[DT_2]}{1 + K_{SV}[DT_2]} - \frac{K_{SV}'[DT_2]}{1 + K_{SV}'[DT_2]}\right)\right](1 + K_{SV}[DT_2])\left(\frac{1 + k_7\tau_T[DT_2]}{k_7\tau_T[DT_2]}\right) \quad (15)$$

where $\Phi_{spl}(T) = {}^3k_8/({}^3k_8 + {}^3k_9)$ and $\Phi_{isc} = {}^Pk_{ST}\tau_1 + k_{ST}\tau_2$. For simplicity, we assume that the participation of a chain reaction can be neglected in both degassed and aerated solutions (vide infra). This assumption leads to eq 15, which gives values of $\Phi_{spl}(T)\cdot\Phi_{isc}$ of 0.8 ± 0.2 by calculations using the known parameters and the observed quantum yields at [DT_2] = 20, 33, and 50 mM. Although the deviations are relatively large because of errors involved in the quantum yields, the values are similar to the intersystem-crossing quantum yield (Φ_{isc} = 0.7) reported for riboflavin in aqueous solution.[24] Therefore, $\Phi_{spl}(T)$ might be close to unity or relatively high, i.e. ${}^3k_8 \gg {}^3k_9$. In the case of d-Fl, this appears to be again true, since a value of $\Phi_{spl}(T)\cdot\Phi_{isc}$ = 0.70 is obtained by calculations using K_{SV}, $k_7\tau_T$, and Φ_{spl}(degas) at [DT_2] = 20 mM according to eq 13 under the assumption that ${}^1k_8 \gg {}^1k_9$.

For degassed solution of 0.5 mM r-Fl, 20 mM DT_2, and 0.3 mM $HClO_4$, where both r-Fl and r-FlH$^+$ are present in a ~1.3:1 ratio, selective photoexcitation of *unprotonated* r-Fl at 470 nm caused the monomerization of DT_2 with a quantum yield of 0.30, showing a sharp contrast with the lack of photosensitization ability of r-Fl in the absence of the acid. Interestingly, this photoreaction was almost completely quenched upon air saturation, suggesting that the triplet electron-transfer pathway (eq 7) is exclusively involved. Probably, protonation of ^{1}r-Fl* is too slow at 0.3 mM in $HClO_4$ to compete with the decay and intersystem crossing (eq 16). Under similar conditions, on the other hand, no splitting

$$\text{r-FlH}^+ \rightleftarrows \text{r-Fl} + \text{H}^+ \xrightarrow{h\nu\ (470\ \text{nm})} {}^1\text{r-Fl}^* + \text{H}^+ \rightarrow {}^3\text{r-Fl}^* + \text{H}^+ \rightleftarrows {}^3\text{r-Fl}^*\text{H}^+ \quad (16)$$

of DT_2 occurs at all upon selective photoexcitation of *unprotonated* d-Fl at 400 nm. Presumably, both ^{1}d-Fl* and ^{3}d-Fl* might not be protonated at 0.3 mM in $HClO_4$. The different behavior of r-Fl and d-Fl would arise from the stronger basicity of N-5 of ^{3}r-Fl* compared with N-1 of ^{3}d-Fl*.[25]

Participation of a Chain-Reaction Mechanism and Effects of Oxygen. A chain-reaction mechanism is clearly involved in the photosensitized reactions by r-Fl *in Ar-purged solution* and by d-Fl *in Ar-purged* and *aerated solution* in the presence of $HClO_4$, since the limiting quantum yields are much greater than unity. In *degassed solution*, on the other hand, the participation of a chain-reaction mechanism should be negligible or only minor, if applicable at all, since Φ_{spl}^{∞}(degas) ≃ 1 for the runs with either r-Fl or d-Fl. In a previous paper,[8] we reported that DT_2 forms a molecular complex with O_2 (eq 17) to become highly activated

$$DT_2 + O_2 \overset{K_C}{\rightleftharpoons} DT_2{}^{\bullet}O_2 \quad (17)$$

toward the catalytic monomerization by the photogenerated cation radical of aromatic hydrocarbons and that $DT_2{}^{\bullet}O_2$ exists to a substantial extent even after flushing with pure Ar. It is therefore suggested that $DT_2{}^{\bullet}O_2$ is formed to open up a channel for the catalytic monomerization of DT_2 by chain carrier X (eq 18). Oxidation of the flavin radicals by O_2 (eq 21) is also taken into consideration for the effects of O_2.

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$$DT_2{}^{\cdot}O_2 \xrightarrow{\text{X (chain reaction)}} 2DT + O_2 \quad (18)$$

$$X + O_2 \rightarrow \text{others} \quad (19)$$

$$FLH^+ \xrightarrow{h\nu,\ O_2\ (?)} X \quad (20)$$

$$FLH^{\cdot}\ (\text{r-}FlH_2{}^{\cdot+},\ \text{d-}FlH^{\cdot},\ \text{etc.}) \xrightarrow{O_2} FLH^+ \quad (21)$$

Since O_2 usually acts as a potential inhibitor of chain and catalytic processes, interception of X by O_2 (eq 19) may also occur to control the net effects of O_2. This appears to fit the case of r-Fl, since $\Phi_{spl}^{\infty}(Ar) \gg \Phi_{spl}^{\infty}(air) \simeq 1$. Probably, flushing of solution with Ar removes excess O_2 but leaves enough $DT_2{}^{\cdot}O_2$ for the catalytic process to proceed, while excess O_2 present in aerated solution might intercept X for the most part. In the case of d-Fl, on the other hand, interception of X by excess O_2 is not important, since the photosensitized reaction occurs more efficiently in aerated solution than in Ar-purged solution. The different behavior of X in O_2 interception depending on the flavins suggests the involvement of different chain carriers in the r-Fl and d-Fl-photosensitized reactions. It is therefore reasonable to assume that X is generated not from a common precursor such as DT_2 but from r-Fl or d-Fl (eq 20), though details of X are still unknown. Identification of X is now under investigation involving close analysis of possible photoproducts from the flavins.

Alternatively, the chain reaction could be explained by a usual cation-radical chain mechanism, i.e. $DT^{\cdot+} + DT_2 \rightarrow DT + DT_2{}^{\cdot+} \rightarrow 2DT + DT^{\cdot+}$,[20] That the presence of O_2 is essential for the chain reaction would be attributable to O_2 oxidation of the half-reduced flavin radicals which might minimize the chain-termination reaction between $DT^{\cdot+}$ and the flavin radicals to ensure the chain propagation by electron exchange between $DT^{\cdot+}$ and DT_2. If this were the case, the electron-exchange process should be much slower than bimolecular reactions between the transient redox species at very low steady-state concentration. Otherwise, it cannot be understood why no chain reaction occurs in degassed solution. Since r-$FlH_2{}^{\cdot+}$ detected by laser-flash photolysis of r-Fl (0.5 mM), DT_2 (20 mM), and $HClO_4$ (10 mM) in Ar-purged MeCN showed a half-life of >50 μs, the rate constant for the electron exchange should be $<10^6$ M^{-1} s^{-1}, too small for the isoergonic or exergonic process. According to this simple mechanism, moreover, it is very difficult to explain why the participation of a chain reaction is negligible or minor in the r-Fl-photosensitized reaction of aerated solution but dominant in the case of d-Fl.

The FL-photosensitized reactions are quenched by 1,2,4-trimethoxybenzene (TMB) through electron transfer with $^{1,3}Fl^*H^+$ (eq 22 and 23)[26] or interception of X (eq 24). In the case of d-Fl,

$$^1FL^*H^+ + TMB \xrightarrow{k_{22}} FLH^{\cdot} + TMB^{\cdot+} \rightarrow FLH^+ + TMB \text{ and/or others} \quad (22)$$

$$^3FL^*H^+ + TMB \xrightarrow{k_{23}} FLH^{\cdot} + TMB^{\cdot+} \rightarrow FLH^+ + TMB \text{ and/or others} \quad (23)$$

$$X + TMB \longrightarrow FLH^+ + TMB \text{ and/or others} \quad (24)$$

$$^1\text{r-}Fl^* + TMB \xrightarrow{k_{25}} \text{r-}Fl^{\cdot-} + TMB^{\cdot+} \rightarrow \text{r-}Fl + TMB \text{ and/or others} \quad (25)$$

quenching of ^{1}d-Fl^*H^+ by TMB at ≤4 mM is negligible (≤6%), though $k_{22} = 2 \times 10^{10}$ M^{-1} s^{-1} as calculated from the fluorescence-quenching constant (13 M^{-1}). Provided that TMB quenching of the d-Fl-photosensitized reaction *in degassed solution* exclusively proceeds through process 23, k_{23} can be calculated from the slope (1.05×10^3 M^{-1}) of the linear Stern–Volmer plot in Figure 4 to be 1.0×10^{10} M^{-1} s^{-1}. This value is quite reasonable for electron transfer from TMB to ^{3}d-Fl^*H^+,[26] again supporting the conclusion that the photoreaction *in degassed solution* mainly involves the triplet electron-transfer mechanism with no or little participation of a chain reaction. In contrast, the Stern–Volmer plot for TMB quenching of the reaction in aerated solution shows a break at ~2 mM in TMB. Moreover, the slope of the plot at <2 mM in TMB is 5.1×10^3 M^{-1}, 5 times greater than that for *degassed solution* even in the presence of efficient triplet quencher O_2. It is therefore reasonable to conclude that the photoreaction *in aerated solution* mainly proceeds through the chain-reaction process. The efficient interception of X by TMB (eq 24) suggests that X is a long-lived oxidative species capable of catalyzing DT_2 monomerization like the cation radicals of arenes.[8]

In the case of r-Fl, the Stern–Volmer constants for fluorescence quenching by TMB in the presence and absence of 10 mM $HClO_4$ are 86 and 67 M^{-1}, from which k_{22} and k_{25} were estimated to be a diffusion-controlled limit and 9.3×10^9 M^{-1} s^{-1}, respectively. TMB quenching of the r-Fl-photosensitized monomerization in *Ar-purged solution* gives again a Stern–Volmer plot with a break at ~1 mM in TMB (Figure 2). The slope of the plot at <1 mM in TMB is $\sim 2.5 \times 10^3$ M^{-1}, greater by 1 order of magnitude than the fluorescence-quenching constants, indicating dominant quenching of long-lived ^{3}r-Fl^*H^+ and chain carrier X by TMB. In the presence of 0.8 mM TMB, where quenching of ^{1}r-Fl* and ^{1}r-Fl^*H^+ is not important (~6%), a double-reciprocal plot of Φ_{spl} vs $[DT_2]$ (Figure 3) gives the intercept-to-slope ratio of 23 M^{-1}, a value very similar to K_{SV} (28 M^{-1}) for fluorescence quenching by DT_2. Moreover, the limiting quantum yield obtained from the intercept of the plot is 0.85, almost identical with that for *aerated solution within* experimental errors. It appears that virtually complete quenching of both ^{3}r-Fl^*H^+ and X by 0.8 mM TMB occurs to leave the excited-singlet pathway. In the case of r-Fl, X is also an oxidative species capable of catalyzing DT_2 monomerization but susceptible to efficient interception by TMB even at <1 mM.

Conclusions

The oxidized form of flavin photosensitizers r-Fl, d-Fl, and l-Fl has been found to be potentially capable of photosensitizing the monomerization of dimer model DT_2 in the presence of $HClO_4$ in MeCN through divergent mechanistic channels, in contrast to the lack of the photosensitization capabilities in the absence of the acid.[30] The protonated flavins play essential roles in the photosensitization. In the case of r-Fl, it has been proved that electron transfer occurs from DT_2 to the short-lived excited-singlet state of protonated r-Fl (^{1}r-Fl^*H^+) at a diffusion-controlled limit and to the long-lived triplet state (^{3}r-Fl^*H^+) at $\sim 10^6$ M^{-1} s^{-1} to cause the net monomerization of DT_2 in ~100% quantum yields; the participation of such an energy-dissipating process as geminate recombination of the initially formed radical pair is negligible. This seems to be true for the d-Fl-photosensitized monomerization in the presence of $HClO_4$ as well, though details of the singlet pathway have not been established. Table IV summarizes the kinetic parameters of the elementary processes.

Moreover, a chain-reaction does occur in the coexistence of O_2, depending on both the flavin photosensitizers and concentration of O_2. An essential effect of O_2 originates from the formation of a molecular complex between DT_2 and O_2 to activate DT_2 toward the catalyzed monomerization by a chain carrier. Although details of the chain-reaction process are still unknown, the chain carrier is considered to be photochemically generated from r-FlH^+ or d-FlH^+.

The present investigation has exemplified that the protonated flavins reveal unique photosensitization capabilities unlike other nonbiological photosensitizers, implying the chemical importance of the flavin chromophores in photorepair, even though the principal chromophore is $FADH_2$. If any proton source is present in *Streptomyces griseus* photolyase, it would be interesting to speculate a partial contribution of the deazaflavin chromophore to efficient photorepair after protonation, though its major function has been attributed to that as a photoantenna in *Anacystis nidulans* photolyase.[31] On the other hand, it seems to be generally

(30) The observed acid effects are not particular effects of $HClO_4$ since HBF_4 was found to be as effective as $HClO_4$: Yasuda, M.; Orihata, K.; Shima, K. Personal communication.

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Table IV. Kinetic Parameters Associated with FL-Photosensitized Splitting of DT_2

processes	parameters
$FL + H^+ \rightleftarrows FLH^+$	$K_G \simeq 10^4\ M^{-1}$ (FL = r-Fl and d-Fl)
$^{1,3}FL^* + H^+ \rightleftarrows {}^{1,3}FL^{\bullet}H^+$	$K_S < K_G < K_T$ (FL = r-Fl)
$^1FL^*H^+ + DT_2 \rightarrow {}^1FLH^{\bullet}//DT_2^{\bullet+}$	$k_6 = \begin{cases} 5.8 \times 10^9\ M^{-1}\ s^{-1} & (FL = r\text{-}Fl) \\ 1.0 \times 10^{10}\ M^{-1}\ s^{-1} & (FL = d\text{-}Fl) \end{cases}$
$^3FL^*H^+ + DT_2 \rightarrow {}^3FLH^{\bullet}//DT_2^{\bullet+}$	$k_7 = \begin{cases} 1 \times 10^6\ M^{-1}\ s^{-1} & (FL = r\text{-}Fl) \\ 4.8 \times 10^8\ M^{-1}\ s^{-1} & (FL = d\text{-}Fl) \end{cases}$
$^{1,3}FLH^{\bullet}//DT_2^{\bullet+} \rightarrow FLH^+ + 2DT$	$\Phi_{spl}(S) = 100\%$, $\Phi_{spl}(T) \simeq 100\%$ (FL = r-Fl)
	$\Phi_{spl}(S) = 100\%$ (?), $\Phi_{spl}(T) = 100\%$ (FL = d-Fl)

true that the oxidized form of flavins without protonation is inherently incapable of photosensitizing the monomerization of dimer models. It is therefore understandable that *E. coli* photolyase is inactive with the oxidized FAD cofactor,[32] if any proton source is unavailable in or is lost from photolyase.

Experimental Section

Materials and General Methods. Melting points were taken on a hot plate (Yanagimoto) and are uncorrected. ^1H NMR spectra were recorded on a JEOL JNM-PMX100 spectrometer, ^{13}C NMR spectra on a JEOL FX-100 spectrometer, and UV–vis absorption spectra on a Hitachi 220-A spectrometer. Fluorescence spectra were recorded on a Hitachi 850 spectrofluorometer after correction of instrument responses, and fluorescence quantum yields were determined by using benzene solution of zone-refined anthracene as reference. Fluorescence lifetimes were measured for Ar-purged MeCN solution (4 mL) of r-Fl (25 μM) or d-Fl (30 μM) in the absence or presence of 10 mM $HClO_4$ in a quartz cuvette (10-mm pathlength) on a Horiba NAES-1100 time-resolved spectrofluorimeter after deconvolution. Quantitative analysis of both the consumption of DT_2 and the formation of DT was performed by HPLC on a TOSO CCPD dual pump and a TOSO UV-8010 spectromonitor using a Wakosil 5C18 column; the mobile phase was 40% methanol in $NaOH/KH_2PO_4$ buffer (pH 7) at a flow rate of 0.35 mL/min, and the wavelength of the spectromonitor was set at 274 nm.

Perchloric acid (70%) was used as received. Acetonitrile was distilled three times from P_2O_5 and then from CaH_2. 1,2,4-Trimethoxybenzene (Nakarai Tesque) was distilled from Na under a reduced pressure. The preparation of DT_2 was carried out according to the literature method[33] after modification, details of which were described in a separate paper.[6d] Lumiflavin (Merck) was recrystallized from ethanol. Riboflavin tetraacetate and 3-methyl-8-chloro-10-phenyl-5-deazaflavin were kindly provided by Dr. Fukuzumi of Osaka University; the former had been prepared by acetylation of riboflavin with acetic anhydride in dry pyridine,[34] whereas the latter had been obtained by a reaction of 6-(phenylamino)-3-methyluracil with 2-bromo-4-chlorobenzaldehyde in *N,N*-dimethylformamide.[35] Purification of these flavins was performed by recrystallization from an ethanol/chloroform mixture for r-Fl and from *N,N*-dimethylformamide for d-Fl.

Flavin-Photosensitized Monomerization of DT_2. Acetonitrile solutions (1.0 mL) containing a flavin photosensitizer (0.5 mM), $HClO_4$ (0.3 or 10 mM), and DT_2 (usually 20 mM or appropriate concentration) were introduced into quartz cells (5-mm pathlength) and were degassed by four freeze–pump–thaw cycles under $<10^{-3}$ Torr for *degassed runs* or bubbled with a stream of Ar (99.99%) for 15 min for *Ar-purged runs*. For aerated runs, the solutions were used as prepared without these procedures. Photoreactions were carried out by irradiation of the solutions in the quartz cells at 366, 400, and 470 nm using a Wacom KXL-500F Xe lamp (300 W) combined with a Shimadzu monochromator. The light intensities were determined by potassium tris(oxalato)iron(III) actinometry to be (1.36–4.53) $\times 10^{-7}$ einstein/min. The progress of the photoreactions was followed by HPLC analysis.

Transient Absorption Experiments. The transient absorption spectra were taken with a conventional Xe flash photolysis and a Quantel YG580 Nd-YAG laser. Details of the equipments were described elsewhere.[36] For Xe-flash photolysis, acetonitrile solution (10 mL) containing r-Fl (6 μM) and $HClO_4$ (10 mM) was placed in a quartz cell of 100-mm pathlength, bubbled with a stream of Ar (99.99%) for 15 min, and then irradiated with the 2-μs pulse of the Xe-flash lamp through a glass filter (>350 nm). For laser-flash photolysis, acetonitrile solution (4 mL) containing r-Fl (25 μM) or d-Fl (30 μM), DT_2 (usually 20 mM), and $HClO_4$ (10 mM) was placed in a quartz cuvette (10-mm pathlength), bubbled with a stream of Ar(99.99%) for 15 min, and then irradiated by the 10-ns pulses at the third harmonic (355 nm) of the Nd-YAG laser. Data analysis was performed by using an Electronica ELK5120 wave memory and an NEC PC9801 personal computer.

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Supplementary Material Available: Double-reciprocal plots of Φ_{spl} vs $[DT_2]$ for d-Fl-photosensitized reaction in degassed, Ar-purged, and aerated MeCN (Figure 7) and transient absorption spectra obtained by laser-flash photolysis of the d-Fl/DT_2/$HClO_4$ system in Ar-purged MeCN (Figure 8) (2 pages). Ordering information is given on any current masthead page.

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