BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN VOL. 42 1231—1239 (1969)

Reactions of Singlet Oxygen and Half-reduced Oxygen which are Produced Simultaneously by the Interaction of Triplet Dye and Oxygen

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(Received September 11, 1968)

Under experimental conditions in which the interaction between triplet dye and oxygen is an initiating process (D–O mechanism), the photosensitized oxidation of leucofluorescein in the aqueous solution (reaction (1)) has been found to decrease in rate with the increasing oxygen concentration and that the magnitude of the retarding effect of oxygen is almost the same independent of sensitizing dye. This is in sharp contrast to the kinetic feature of the oxidative photobleaching of dye (reaction (2)), where the rate becomes constant beyond a certain small oxygen concentration. On the basis of these findings, it has been proposed that in reaction (1), $^1O_2$ plays a dominant role and it is deactivated by process $^1O_2+^3O_2\rightarrow 2^3O_2$. As to reaction (2), strong evidence has been presented for the view that the interaction of triplet dye and oxygen in the aqueous solution produces a rather stable $D^+\cdots O_2^-$ of which only a small portion decomposes irreversibly. The principal evidence is as follows; 1) the free half-oxidized dye (produced by the reaction between triplet dye and $[Co(Ox)_3]^{3-}$ was found to oxidize FH_2 with a high rate and its decay was found to be accelerated by the addition of FH_2; 2) the decay of the half-oxidized species (first order decay!) produced in the aerated aqueous solution, on the other hand, was not affected by FH_2 under otherwise similar conditions as in (1).

It is well known that the following three mechanisms have long been proposed for the photosensitized oxidation, initiated by the interaction between triplet dye and oxygen.[1]

a) Formation of singlet oxygen (Kautsky, Gaffron)
b) Electron transfer (Weiss, Franck)
c) Formation of molecular complex between dye and oxygen (Schenck).

At present, it seems that special interest is being focused on the first mechanism. Since the appearance of Foote and Wexler's work,[2] a number of papers have been published in favor of the singlet oxygen theory. In spite of such a trend, the authors believe that the other two mechanisms must also be taken into account. In fact, the existence of half-oxidized and half-reduced dye found by Lindqvist,[3,4] Grossweiner[5] and by us[6,7] has naturally led to the possible occurrence of photooxidation by virtue of the half-oxidized dye, *i. e.*, by the second mechanism; Lindqvist[8a] for instance, has found such an example in the eosine-EDTA-Fe^{3+} system. Other examples have also been reported by us[19] and by Kasche.[8b]

The third mechanism which Schenck has long been advocating, was modified recently as a charge transfer type complex,[9] and this mechanism is now thought to be closely related with the second one.

The first object of the present paper is to report a new kind of reaction which proceeds *via* singlet oxygen; it is the photosensitized oxidation of leucofluorescein (FH_2) initiated by D–O mechanism, some thiazine and xanthene dyes being used as sensitizer. The second object is to make it clear that the photooxidative bleaching of such dyes does not occur by singlet oxygen but *via* electron transfer reaction or alternatively *via* radical ion pair. This has been substantiated partly, by the

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[1] For instance, Th. Forster, *Z. Elektrochem.*, **56**, 716 (1952).

[2] C. S. Foote and S. Wexler, *J. Am. Chem. Soc.*, **86**, 3879, 3880 (1964); C. S. Foote, S. Wexler and W. Ando, *Tetrahedron Letters*, **1965**, 4111.

[3] L. Lindqvist, *Arkiv. for Kemi.*, **16**, 79 (1960).

[4] V. Kasche and L. Lindqvist, *Photochem. Photobiol.*, **4**, 923 (1965).

[5] E. F. Zwicker and L. I. Grossweiner, *J. Phys. Chem.*, **67**, 549 (1963).

[6] S. Kato, M. Morita and M. Koizumi, This Bulletin, **37**, 117 (1964).

[7] T. Ohno, S. Kato and M. Koizumi, *ibid.*, **39**, 232 (1966).

[8] a) L. Lindqvist, *Acta Chem. Scand.*, **20**, 2967 (1966). b) V. Kasche, *Photochem. Photobiol.*, **6**, 643 (1967).

[9] K. Gollnick and G. O. Schenck, *Pure and App. Chem.*, **9**, 507 (1964).

finding of a quite different dependence of the two types of reaction on the oxygen concentration. Thirdly we would like to compare the oxidative behavior of all the conceivable transient species, $D^+\cdots O_2^-$, O_2^-, $D^+\cdots D^-$ and D^+ [*1] in addition to singlet oxygen $^1O_2$, choosing FH_2 as an oxidizable substance. A remarkable finding is that $D^+\cdots O_2^-$ is less reactive than singlet oxygen, whereas the free D^+ is more reactive. This may be the first chemical evidence for the formation of a rather stable complex, $D^+\cdots O_2^-$.

Experimental

Purification of materials and most of the experimental procedures are similar to those in previous papers. All the experiments were performed at 30°C. pH of the solution was fixed at 6.2 by using the buffer of acetic acid-acetate, except otherwise mentioned.

Results and Discussion

Photosensitized Oxidation of Leucofluorescein. This reaction was studied by one of the present authors (M. K.) in collaboration with Uchida and Kato.[10–12] It was found in this series of work that the oxidation of FH_2 by acridine in the presence of such a large quantity of oxygen as to make the attack of oxygen on the triplet acridine predominant (D–O mechanism), decreases in rate with the increase of the oxygen concentration. Any attempt to clarify the cause of this phenomenon, however, was not made at that time. A few years later, a similar phenomenon was found in the photooxidation of acridan by acridine in the benzene solution containing oxygen.[13] In these papers, the phenomenon was tentatively interpreted by the scheme

$$A^T + {}^3O_2 \rightarrow A + {}^1O_2$$

$${}^1O_2 + AH_2 \rightarrow AH + HO_2$$

$${}^1O_2 + {}^3O_2 \rightarrow 2{}^3O_2,$$

in which A^T, $^1O_2$ and AH_2 are respectively triplet acridine, singlet oxygen and acridan. If a similar scheme holds for the photooxidation of FH_2, then the ratio of the rate constants for reaction, $^1O_2 + FH_2 \rightarrow HO_2 + FH$ and $^1O_2 + {}^3O_2 = 2{}^3O_2$ should be constant irrespective of the sensitizing dye. To support the above scheme, it is necessary to make experiments using various sensitizers. Therefore, in the present paper, the reaction was investigated quantitatively in a somewhat modified way, using methylene blue (MB), eosine (Es), and thionine (Th), as sensitizers. Before this, some experiments were performed using MB in order to reconfirm the previous result and to find a more suitable condition for the quantitative investigation ((A) and (B)).

A) *Reaction in the Degassed Solution.* The photochemical oxidation of FH_2 sensitized by MB, in the degassed aqueous solution (pH, 6.2) was reconfirmed. As reported in the previous paper, MB is partially photobleached accompanied with the oxidation of FH_2. To simplify the kinetic treatment, it is desirable to keep the concentration of MB as constant; the addition of a certain quantity of H_2O_2 was attempted to reconvert the reduced MB back to the original dye. It was found that 8.8×10^{-3}M of H_2O_2 restores MB very efficiently without affecting any essential feature of the reaction.[*2] In fact, the reaction was found to occur approximately as first order in FH_2 (when $[FH_2]$ is small), as expected from the following rate formula which can be derived directly from reaction scheme I.

$$\Phi_{D-F} = \varphi_{ST}\frac{k^r{}_L[FH_2]}{k_d{}^S + k_L{}^S[FH_2]} \qquad (I)$$

Scheme I

$$\begin{array}{l}
MB \xrightarrow{I_{ab}\varphi_{ST}} MB^T \overset{MB}{\rightarrow} 2MB \text{ or } MB^+ + MB^- \quad k_D{}^s \quad (1) \\
\qquad\quad {}_{k_d}\!\swarrow \; \overset{FH_2}{\rightarrow} \left\{ \begin{array}{lll} MB + FH_2 & k_L{}^d & (2) \\ MB^- + FH_2{}^+ & k_L{}^r & (3) \end{array} \right\} k_L{}^s \\
\qquad\quad MB \qquad\qquad k_d{}^s = k_d + k_D{}^s\,[MB]
\end{array}$$

φ_{ST}, $k^r{}_L$, $k^S{}_L$ and k_d are respectively the probability for intersystem crossing, rate constant for the genuine reaction, the total rate constant including deactivation and the rate constant for (spontaneous) deactivation of triplet MB.

Keeping the concentration of MB as 7.8×10^{-6}M,

TABLE 1. DEPENDENCE OF THE QUANTUM YIELD ON THE CONCENTRATION OF FH_2 IN DEGASSED SOLUTION

$[FH_2]\times10^5$ M	Φ	$[FH_2]\times10^5$ M	Φ
0.3	0.022	4.0	0.16_5
0.5	0.034	5.0	0.19_4
1.0	0.058	10.0	0.22
1.5	0.094		
2.5	0.105		
3.0	0.12_6		

[*1] Throughout the present paper, D and D^+ denote respectively the original dye and the half-oxidized dye, irrespective of its real charge.

10) K. Uchida, S. Kato and M. Koizumi, This Bulletin, **35**, 16 (1962).

11) K. Uchida and M. Koizumi, *ibid.*, **35**, 1871, 1875 (1962).

12) K. Uchida, *ibid.*, **36**, 1097 (1963).

13) S. Niizuma, Y. Ikeda and M. Koizumi, *ibid.*, **40**, 2249 (1967); A. Kira and M. Koizumi, *ibid.*, **40**, 2486 (1967).

[*2] Hence the similar quantity of H_2O_2 was added throughout the present research.

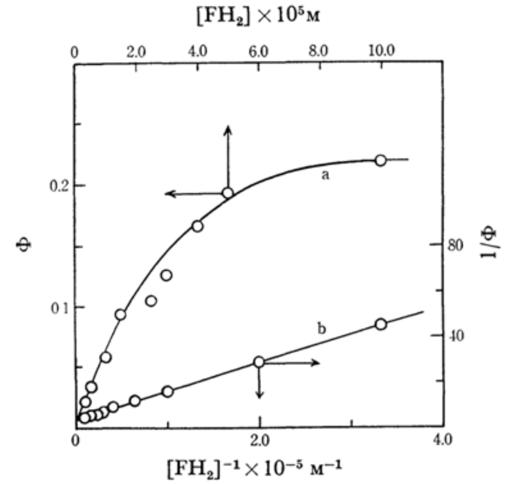

Fig. 1. Dependence of the quantum yield on the concn. of FH$_2$ ([MB]=7.8×10^{-6}M) in degassed soln.

a ---- Φ *vs.* [FH$_2$] plot
b ---- Φ^{-1} *vs.* [FH$_2$]$^{-1}$ plot

the dependence of the quantum yield on the FH$_2$ concentration was examined. The results are given in Table 1 and in Fig. 1.

Figure 1 shows that a linear relationship holds between 1/Φ and 1/[FH$_2$], as is expected from Eq. (I). From the intercept and the slope, the values of $\varphi_{ST}\ k^{r}{}_{L}/k^{S}{}_{L}$ and $k^{S}{}_{L}/k^{S}{}_{d}$ were evaluated respectively as 0.30 and 2.9×10^{4}M^{-1}. The value of $k^{S}{}_{d}$ reported by us is 1.5—3×10^4 sec^{-1}.[6] The recent value of Danziger *et al.*[14] is 2.5×10^4 sec^{-1}. Using the latter value we get $k^{S}{}_{L}$ as 7.2×10^{8}M^{-1}·sec^{-1}. Thus reaction $MB^T+FH_2\rightarrow MBH+FH\cdot$ occurs with a rate constant which is a little smaller than the diffusion-controlled one. The above value is more than ten times larger than the one for reaction $MB^T+EDTA\rightarrow MB^-+EDTA^+$.[14]

B) *Reaction in the Aerated Solution and in the Solution Equilibrated with one Atmosphere of Oxygen* (hereafter abbreviated oxygenated solution). It was established that the photosensitized oxidation of FH$_2$ by MB, occurs in the aerated solution as well as in the oxygenated solution. The results are given in Table 2 and in Fig. 2.

TABLE 2. QUANTUM YIELD OF FH$_2$ OXIDATION IN THE AERATED AND IN THE OXYGENATED SOLUTION

[FH$_2$]×10^{5}M	Φ (air saturated)	Φ (1 atm. of oxygen)
0.3	0.011	—
0.5	0.016	0.008
1.0	0.029	0.017
2.5	0.075	—
3.0	0.083	0.052
4.0	0.104	—
5.0	0.142	0.090
6.0	0.155	0.097
10.0	0.167	0.10

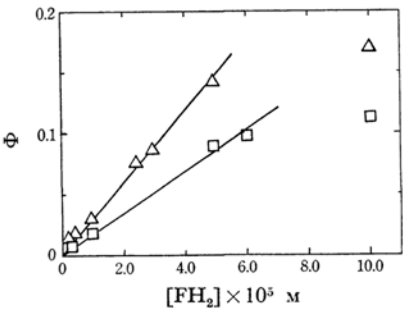

Fig. 2. Dependence of the quantum yield on the concn. of FH$_2$ in the aerated (△) and in the oxygenated (○) acetate buffer solution. ([MB]=7.7×10^{-6} M)

Figure 2 shows that the rate is proportional to [FH$_2$] below 6×10^{-5}M of FH$_2$. The quantum yield in the aerated solution is about one half that of the degassed solution under otherwise similar conditions, when the FH$_2$ concentration is less than 1×10^{-5}M. In the oxygenated solution the rate is much smaller than in the aerated solution.

Now triplet MB is attacked by oxygen with a rate constant of 3×10^{9}M^{-1} sec^{-1}. This value was evaluated by combining the value of $k^{S}{}_{d}/k^{S}{}_{O2}$=7.75×10^{-6} ($k^{S}{}_{O2}$, rate constant for the entire reaction including the mere deactivation between MB^T and O$_2$ reported already[15]) with the $k^{S}{}_{d}$ value mentioned above. Comparison of this value with that of $k^{S}{}_{L}$ (7.2×10^{8}M^{-1} sec^{-1}) leads to the expectation that in the aerated solution, triplet MB is mainly attacked by oxygen instead of by FH$_2$ as long as the latter concentration is less than 6×10^{-5}M. Accordingly there is no doubt that the above reaction is due to a certain oxidative species that is produced by the reaction between MB^T and oxygen. Moreover, the smaller Φ in the oxygenated solution suggests that this species is deactivated by the ground state oxygen. Such a possibility was investigated more quantitatively by changing the concentration of dissolved oxygen in a wider range.

C) *Dependence of the Quantum Yield on the Concentration of Oxygen.* Fixing the concentration of FH$_2$ and MB respectively at 1.0×10^{-5} and 8.0×10^{-6}M, the dependence of the quantum yield of the reaction on the oxygen concentration was examined from [O$_2$]=0 to 1.2×10^{-3}M. The results are given in Table 3 and in Fig. 3.

As Fig. 3 shows, the 1/Φ *vs.* [O$_2$] plot consists of two linear portions, which can be explained as

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[14] R. M. Danziger, K. H. Bar-Eli and K. Weiss, *J. Phys. Chem.*, **71**, 2633 (1967).

[15] H. Obata, K. Kogasaka and M. Koizumi, This Bulletin, **32**, 125 (1959).

Table 3. Dependence of Φ on the oxygen concentration $[FH_2]=1.0\times10^{-5}$M

$[O_2]\times10^4$ M	Φ	$[O_2]\times10^4$ M	Φ
0	0.058	6.5	0.016
0.2	0.049		(0.018)
1.0	0.038	8.0	0.018
	0.036		0.019
1.7	0.032	10.0	0.015
2.4	0.027	12.0	0.014
4.0	0.025	14.0	0.011
5.0	0.021		

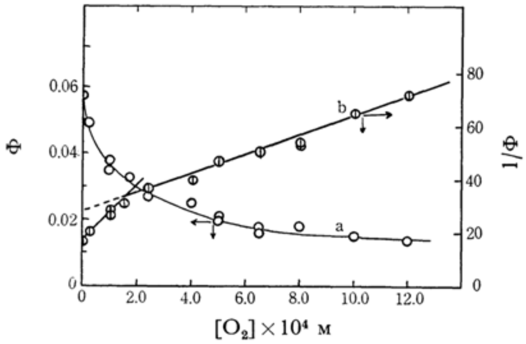

Fig. 3. Effect of oxygen concn. on the quantum yield of fluorescein formation. (Sens. ---- MB, 8.0×10^{-6}M)

a ---- Φ *vs.* $[O_2]$ plot
b ---- Φ^{-1} *vs.* $[O_2]$ plot

follows. In the low concentration region of oxygen, Φ decreases with the increase of oxygen concentration due to the competition of the reaction between MB^T and FH_2 and that between MB^T and oxygen. In the higher concentration region of oxygen where D–O mechanism prevails, the quantum yield still maintains the value of $\sim10^{-2}$ but it gradually decreases with the oxygen concentration. Thus a quite similar interpretation as in the oxidation of acridan with acridine in benzene[13] has been found to hold in the case of MB. If one assumes that Scheme II (D–O mechanism) holds for the high oxygen concentration region, one can easily derive Eq. (II).

Scheme II

$$MB \xrightarrow{I_{ab}\varphi_{ST}} MB^T \xrightarrow[k^s_{O_2}]{O_2} \begin{cases} {}^1O_2 + MB & k^r_{O_2} \quad (4) \\ {}^3O_2 + MB & k^d_{O_2} \quad (5) \end{cases}$$

$${}^1O_2 \xrightarrow{\text{(Solvent)}} {}^3O_2 \qquad k_d' \qquad (6)$$

$${}^1O_2 + {}^3O_2 \rightarrow 2\,{}^3O_2 \qquad k'_{d,O_2} \qquad (7)$$

$${}^1O_2 + FH_2 \rightarrow HO_2 + FH \quad k_L' \qquad (8)$$

$$\Phi_{D-O} = \varphi_{ST}\frac{k^r_{O_2}}{k^s_{O_2}}\frac{k_L'[FH_2]}{k_d'+k'_{d,O_2}[O_2]+k_L'[FH_2]}$$

$$\approx \varphi_{ST}\frac{k^r_{O_2}}{k^s_{O_2}}\frac{k_L'[FH_2]}{k_d'+k'_{d,O_2}[O_2]} \qquad (II)$$

Applying Eq. (II) to the linear portion in the higher concentration region of oxygen, one can estimate the value of $k'_{d,O_2}/k'_d$ as $1.1—1.2\times10^3$M^{-1}. The linear part in the lower oxygen concentration region consists of the contribution from Φ_{D-F} and Φ_{D-O} and cannot be analysed in a simple way.

D) Comparison of the Values of $k'_{d,O_2}/k'_d$ for Several Sensitizers. If Scheme II really holds, the value of $k'_{d,O_2}/k'_d$ should be independent of a sensitizer used. To examine this, oxygen concentration effect on Φ_{D-O} in the higher concentration region of oxygen was examined using thionine

Table 4. Φ_{D-O} of the oxidation of FH_2 by using thionine and eosine as a sensitizer

a) Thionine $[Th]=9\times10^{-6}$M $[FH_2]=1.0\times10^{-5}$M

$[O_2]\times10^4$ M	$\Phi_{D-O}\times10^2$
0	38.2
2.4	3.8
3.9	3.1
7.0	1.6
9.5	1.3
12.0	1.2

b) Eosine $[Es]=1.0\times10^{-5}$ M $[FH_2]=1.0\times10^{-5}$M

$[O_2]\times10^4$ M	$\Phi_{D-O}\times10^2$
0	4.6
2.4	~0.46
4.0	0.38
5.0	0.33, 0.36
7.5	0.25, 0.28
10.0	0.19
12.0	0.18

Table 5. Values of $k'_{d,O_2}/k'_d$

Sensitizer	Oxidized sub.	Solvent	E^T_{sens} (cm^{-1})†	$(k'_{d,O_2}/k'_d)\times10^{-3}$	Φ^0_{D-O}‡
MB	FH_2	water	—	1.1—1.2	0.036 0.045
Th	FH_2	water	—	1.6—2.2	0.033
Es	FH_2	water	14900	1.8—2.5	0.0067
Acridine	FH_2	water	15800$\pi\pi^*$	(0.6)	0.03
(Acridine	Acridan	benzene	21400nπ^*	(0.4—0.6))	

† Triplet level of a sensitizer
‡ The quantum yield when reaction (7) is absent

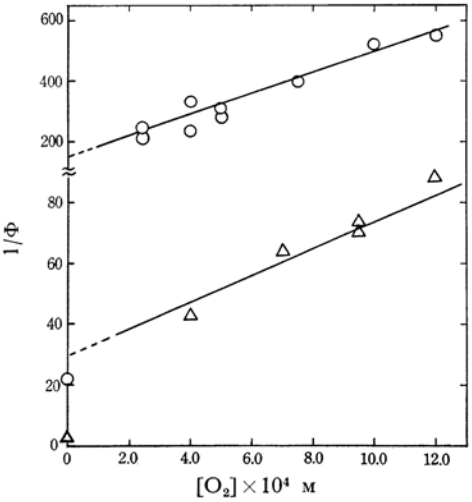

Fig. 4. Φ^{-1} *vs.* $[O_2]$ plot by using eosine (1.0×10^{-5}M, ○) and thionine (9.0×10^{-6}M, △) as a sensitizer.

and eosine as a sensitizer. The results are given in Tables 4, 5 and in Fig. 4. In Table 5 are added the values for acridine calculated from the old data.[12,13]

E) Assignment of the Oxidative Species in the Above Reaction. From the above result, one can say that in the photosensitized oxidation of FH_2 *via* D–O mechanism, the rate decreases with the increase of oxygen concentration in a quite similar way irrespective of a sensitizer used. This implies that the oxidative species reacting with FH_2 is the same one species for all the dyes. Further, one can conclude that this species does not participate in the oxidative bleaching of dye, because in contrast to the retarding effect of excess oxygen in the above reaction, the rate of oxidative bleaching of dye increases with the oxygen concentration and becomes constant when the concentration is high enough,[15,16] as shown in Fig. 5. The limiting quantum yields reported by our group are given in Table 6.

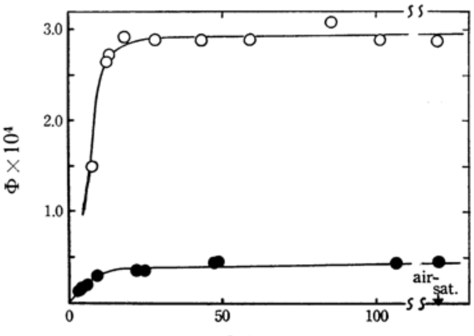

Fig. 5. Effect of the dissolved oxygen concn. on the quantum yield of photobleaching of eosine (○) and methylene blue (●).

TABLE 6. QUANTUM YIELD FOR THE OXIDATIVE BLEACHING OF DYE

	$\Phi \times 10^4$
Flourescein	1.3 [16]
Eosine	2.8 [16]
Erythrosine	3.7 [16]
Methylene blue	0.47[15]

Table 6 shows that Φ for the oxidative photobleaching of dye is about two orders of magnitude less than that of the sensitized oxidation of FH_2.

The conceivable oxidative species produced *via* D–O mechanism are $^1O_2$, O_2^-, D^+ and $D^+\cdots O_2^-$ which are produced by the following processes.

$$D^T + {}^3O_2 \rightarrow D + {}^1O_2 \tag{9}$$

$$D^T + {}^3O_2 \rightarrow D^+\cdots O_2^- \tag{10}$$

$$\rightarrow D^+ + O_2^- \tag{11}$$

The production of half-oxidized dye has been fully established by Lindqvist,[3,17] Grossweiner[5] and by us.[6,7] To interpret the result that $k'_{d,O_2}/k'_d$ is independent of sensitizing dyes, only $^1O_2$ or O_2^- should be a reactive species. But the latter can be ruled out because D^+ which is produced accompanied by O_2^- (reaction (11)) is very reactive toward FH_2, as will be shown below. Furthermore half-oxidized dye is thought to be rather insensitive to oxygen.[6,7] Thus the most plausible interpretation is that the oxidation of FH_2 occurs *via* $^1O_2$ and the oxidative bleaching of sensitizing dyes occurs *via* electron transfer between D^T and oxygen. That acridine sensitized photooxidation of acridan, in which there is no evidence of the formation of half-oxidized form, occurs with about equal $k'_{d,O_2}/k'_d$ is also consistent with this view.

Further evidence for the electron transfer mechanism of the oxidative bleaching is supplied by our previous result that in the high concentration of dye the D–D mechanism occurs and in this case oxidative bleaching of dye as well as the rate of oxygen consumption becomes very much larger than in the D–O mechanism[18]; thus in the D–D mechanism, the reaction proceeds as follows. $D^T + D \rightarrow D^+ + D^-$ or $D^+\cdots D^-$, $D^- + O_2 \rightarrow D + O_2^-$, $D^+ \rightarrow$ destruction. If a reactive species were $^1O_2$ for the photobleaching, the larger Φ in the D–D mechanism cannot be explained.

The present results in conjunction with the results so far obtained previously lead to the conclusion that the oxidation of FH_2 and the oxidative

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[16] M. Imamura and M. Koizumi, This Bulletin, **28**, 117 (1955).

[17] V. Kasche and L. Lindqvist, *Photochem. Photobiol.*, **4**, 923 (1965).

[18] Y. Usui, K. Itoh and M. Koizumi, This Bulletin, **38**, 1015 (1965).

bleaching of sensitizing dyes occur as two different processes and that it is most likely that the former proceeds *via* $^1O_2$ while the latter *via* electron transfer.

This view will be further supported when the experimental condition is controlled in such a way that a particular species such as $D^+\cdots O_2^-$, O_2^-, $D^+\cdots D^-$ or D^+ is predominantly produced, and we compare the reactivity of these species toward FH_2. Such a comparison will, furthermore, allow us to make a more distinct discrimination of the reactive species in the oxidative bleaching of dye.

Experiments for Comparing the Reactivity of Various Transient Species to FH_2. A) $D^+\cdots O_2^-$. Strong support for the existence of such a complex has been presented already in the case of eosine.[19] Thus this dye gives the transient spectrum due to the half-oxidized dye in the aerated solution, which decays in the first order ($k=10^3$ sec^{-1}) independent of the oxygen concentration (phosphate buffer). For MB there is still no clear-cut evidence for the existence of such a complex, although this is plausible.[6,14] Hence, choosing eosine, the effect of the addition of FH_2 on the decay of $D^+\cdots O_2^-$ was examined at 458 nm (the absorption peak of the half-oxidized dye) at which the absorption due to half-reduced fluorescein is not appreciable.[3] The results are given in Table 7.

Table 7. The effect of the addition of FH_2 on the decay of $D^+\cdots O_2^-$

[Eos]$=1.0\times10^{-5}$M; aerated solution of pH$=6.2$ (0.1M Ac-buffer)

Concn. of FH_2M	Decay const.* of $D^+\cdots O_2^-$ (sec^{-1})$\times10^{-4}$
0	7
1.0×10^{-5}	5.3
4.0×10^{-5}	6.1
8.0×10^{-5}	4.9
20.0×10^{-5}	3.9—5.0

* The values have unexpectedly, been found to be more than the ones for the phosphate buffer solution. The decay constants in the 0.01M AcOH-AcONa buffer solution are nearly equal to the latter. The phenomenon is worthy of further investigation.

From Table 7, it appears that the addition of FH_2 causes a slight decrease of the decay, but this may perhaps be due to the overlap of the absorption due to F which is produced by the simultaneous oxidation of FH_2 by $^1O_2$. Thus the above experiment definitely indicates that $D^+...O_2^-$ is indifferent to FH_2, when the latter concentration is 10^{-5}—10^{-4}M.

B) O_2^-. According to Usui and Koizumi,[19] the following reaction occurs when a suitable quantity of EDTA is added to the aerated solution of eosine (phosphate buffer).

$$D^+\cdots O_2^- + \text{EDTA} \xrightarrow{k_R} D + \text{EDTA}^+ + O_2^- \quad (12)$$

Flash experiments were made in order to know the exact rate constant of the above reaction. Figure 6 gives the plot of the first order decay constant at 460 nm against the EDTA concentration. From this plot k_R was evaluated as $k_R=7.0\times10^5$M^{-1} sec^{-1}. This value is consistent with the results previously reported.[19] If the free O_2^- were to react with FH_2. Then the addition of a suitable quantity of EDTA is expected to increase the rate of oxidation of FH_2. The result, as shown in Table 8, is contrary to this expectation.

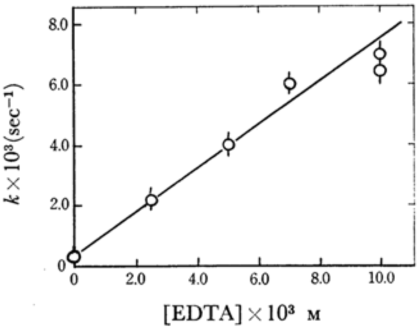

Fig. 6. Plot of the first order decay constant of $D^+\cdots O_2^-$ against [EDTA)]. (phos. buffer, pH$=6.8$)

Table 8. effect of the addition of EDTA Φ_{D-O} [Es]$=1.0\times10^{-5}$M [FH_2]$=1.0\times10^{-5}$M pH$=6.2$

[EDTA]M	Φ_{D-O}
0	0.0046
4×10^{-3}	0.0042
4×10^{-2}	0.0026

There is no sign of increase in Φ_{D-O} values.*3 A moderate decrease in the case of 4×10^{-2}M of EDTA may be due to the accompanied photobleaching of eosine due to reaction, $D^T+\text{EDTA}\rightarrow D^-+\text{EDTA}^+$.

C) $D^+\cdots D^-$. The formation of $D^+\cdots D^-$ has not been fully established. In the case of eosine in the aqueous solution, the formation of the free D^+ and D^- seems rather predominant, since the half-oxidized and half-reduced species decay in the

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[19] Y. Usui and M. Koizumi, This Bulletin, **40**, 440 (1967).

*3 We feel that the concentrations of EDTA were too low for reaction (12) to compete with the spontaneous deactivation of $D^+\cdots O_2^-$ in the present buffer solution. Further increase of EDTA may, however, change the initial process from $D^T+O_2\rightarrow D^+\cdots O_2^-$ ($k=2.5$—3.1×10^8M^{-1} sec^{-1} [19]) to $D^T+\text{EDTA}\rightarrow D^-+\text{EDTA}$ ($k=5\times10^5$M^{-1} sec^{-1} [7]).

second order with approximately the same rate constant.[7),*4] On the other hand, according to Danziger *et al.*[14)] the existence of $D^+\cdots D^-$ is likely to occur in the case of MB. Hence choosing MB, the dependence of the oxidation reaction of FH_2 on the concentration of MB was examined in the aerated solution. If the switch-over from D–O to D–D mechanism occurs with the increase in [D], it is expected that $D^+\cdots D^-$ will probably be attacked first by oxygen when $[FH_2]$ is not so large, as in the oxidative photobleaching and also as in the oxygen absorption experiment,[18)] leaving D^+ as free. The free D^+ may then react with FH_2. Hence it is possible that the rate of the oxidation of FH_2 increases with the concentration of dye. Since an appreciable quantity of dimer exists in the high concentrated solution of dye, chiefly monomer was excited using a suitable cut-off filter.[*5] The concentration of monomer was calculated by using the dissociation constant $K=2.8\times10^{-4}$ [21)] (at 26.7°C). The results are shown in Table 9 and in Fig. 7.

As Fig. 7 shows, the quantum yield really increases with [MB] and reaches about a constant value above 2×10^{-5}M of MB. Thus the switch-over from D–O to D–D mechanism seems to occur around 2×10^{-5}M. This value is about one order smaller than the oxygen concentration, and it is suggested that the rate constant for $D^T+D\rightarrow$ is appreciably larger than that for $D^T+O_2\rightarrow$. There is, however, some possibility that the contribution of the ground state dimer still remains. In addition, it is to be noted that in the experiment described in A), D–D mechanism participates to some extent in the low concentration region of oxygen. But, for the plots drawn in Figs. 3 and 4 in the range $[O_2]>2.4\times10^{-4}$M, the contribution of D–D mechanism may be neglected. The above result indicates that a species $D^+\cdots D^-$ in the presence of oxygen is more effective than $^1O_2$.

TABLE 9. DEPENDENCE OF THE QUANTUM YIELD OF THE OXIDATION OF FH_2 ON [MB]
$[O_2]=2.4\times10^{-4}$M, $[FH_2]=1.0\times10^{-5}$M

$[MB]_{total}\times10^5$M	$[MB]_{monomer}$	$I_{ab}\times10^5$mol sec^{-1}	Φ
12.0	7.7	3.5_8	0.027
10.0	6.7	3.5_2	0.025
8.0	5.8	3.4_6	0.029
7.2	5.2	3.4_0	0.025
6.0	4.5	3.2_0	0.028
4.0	3.0	2.7_6	0.030
2.0	1.8	2.1_6	0.031
1.0	0.9_5	1.3_3	0.026
0.8	0.7_6	1.1_4	0.019
0.5	0.48	2.3_2	0.0066

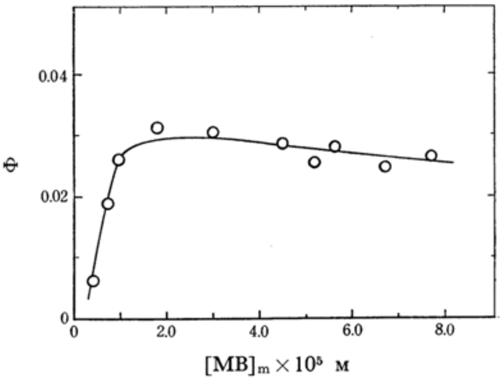

Fig. 7. Effect of the MB concn. on the quantum yield of fluorescein formation.

The investigation of the influence of the oxygen concentration on the rate in the high [MB] region where D–D mechanism prevails, is thought to be interesting, because it may provide information on the reactivity of $D^+\cdots D^-$ and the free D^+ toward FH_2; the latter species may perhaps be produced by reaction $D^+\cdots D^-+O_2\rightarrow D^++D+O_2^-$. Fixing $[MB]=8.0\times10^{-5}$M ($[MB_m]=5.2\times10^{-5}$M) and $[FH_2]=1.0\times10^{-5}$M, the concentration of oxygen was changed from zero to 12.0×10^{-4}M. The results are shown in Fig. 8. It is seen that in the high concentration region of oxygen, the plot essentially coincides with that given in Fig. 3. This is natural because the D–O mechanism prevails in this region. But in the low concentration region of oxygen, the increase of Φ with the decrease in oxygen concentration is not so large as in the case of 8.0×10^{-6}M of MB. The value of $\Phi=0.041$ in the absence of oxygen is undoubtedly due to the reaction between $D^+\cdots D^-$ and FH_2, because under present experimental conditions the reaction of D^T with D prevails over that with FH_2. In the presence of a small amount of oxygen, reactions, $D^T+O_2\rightarrow D+{}^1O_2$, $D^T+O_2\rightarrow D^+\cdots O_2^-$ will compete with reaction $D^T+D\rightarrow D^+\cdots D^-$ on the one hand, and reaction $D^+\cdots D^-+FH_2\rightarrow$ and $D^+\cdots D^-+O_2\rightarrow D^++D+O_2^-$ will compete on the other hand. The last reaction may possibly increase the overall rate of FH_2-oxidation. The plot shown in Fig. 8 is consistent with this view although not conclusive, because of the scantiness of the data. At any rate,

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[*4] In the alcoholic solution, however, some evidence exists for the formation[20)] of $D^+\cdots D^-$.

20) A. Kira and S. Kato, *Sci. Rep. of Tohoku Univ., Ser. I*, **48**, 1964 (1965).

[*5] Danziger *et al.*[14)] claimed that $D^+\cdots D^-$ is mainly produced by the excitation of the ground state dimer. However, we believe their result does not eliminate the possibility of $D^T+D\rightarrow D^+\cdots D^-$. The first order decay of D^T never excludes reaction $D_T+D\rightarrow 2D$, as has already been domonstrated in the case of eosine.[7)] Moreover, the present result cannot be explained unless reaction $D^T+D\rightarrow D^+...D^-$ occurs.

21) E. Rabinowitch and L. F. Epstein, *Ind. Eng. Chem.*, **63**, 69 (1941).

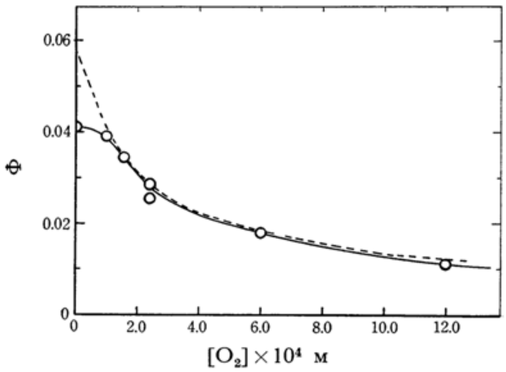

Fig. 8. Effect of oxygen concn. on the quantum yield of fluorescein formation in high MB concn. (8.0×10^{-5}M). *cf.* ---- [MB]$=8.0 \times 10^{-6}$M (Dotted line)

there is scarcely any doubt that in the case of MB, D–R mechanism is the most effective for the photo-oxidation of FH_2, D–D mechanism next and D–O mechanism the least effective.

D) Free D^+. In the case of the deaerated aqueous eosine solution, half-oxidized and half-reduced dye seem to exist mainly as free species.[7] But this is not yet completely conclusive. According to our result, not yet published, electron transfer reaction between triplet dye and $[Co(Ox)_3]^{3-}$ occurs very efficiently, yielding D^+ and $[Co(Ox)_3]^{4-}$. Hence the reactivity of free D^+ toward FH_2 was examined by using the system consisting eosine $\sim 1 \times 10^{-5}$M, $K_3[Co(Ox)_3]$, 6.0×10^{-4}M and FH_2, 6.0×10^{-5}M (pH=6.2). Preliminary experiments by means of the steady light illumination has shown that the oxidation of FH_2 occurs very efficiently with $\Phi = ca.$ 0.15. This value is about the same as the quantum yield of the decomposition of eosine by $K_3[Co(Ox)_3]$ under similar conditions. It was ascertained that the bleaching of eosine never occurs in the early stage of the reaction. Thus one can conclude that the free D^+ produced by the reaction between D^T and $[Co(Ox)_3]^{3-}$ oxidizes FH_2 very quickly.

To confirm this further, the decay rate of the free D^+ was compared in the two systems, a) [Es]$=5.0 \times 10^{-6}$M, $K_3[Co(Ox)_3]=5.0 \times 10^{-4}$M, b) [Es]$=5.0 \times 10^{-6}$M, $K_3[Co(Ox)_3]=5.0 \times 10^{-4}$M, $[FH_2]=1.0 \times 10^{-5}$M, both in the deaerated solution. The preliminary experiment has shown that the decay for system (a) obeys rather the second order rate formula, but the apparent first order rate constant evaluated from the initial slope was 2—9×10^2 sec^{-1}. On the other hand, the first order rate constant for system (b) was 0.9—1.0×10^4 sec^{-1}, which implies that the rate constant for $D^+ + FH_2 \rightarrow D + FH + H^+$, is of the magnitude of 10^9M$^{-1}\cdot$sec^{-1}.[*6] Thus one can conclude that the free D^+ reacts with FH_2 very quickly.

Summary and Conclusion

In the present investigation, it has been proposed that FH_2 in the aerated solution, is oxidized by the singlet oxygen originating from the interaction of D^T with O_2 (D–O mechanism). This view was supported by the finding that the retarding effect of the excess oxygen is independent of a sensitizer. On the other hand, the oxidative photobleaching of dye occurs most (probably) *via* the electron transfer reaction. Hence the quantum yield of the two types of reaction depends on the occurrence of the following two processes.

$$D^T + {}^3O_2 \rightarrow D^+\cdots O_2^-, \tag{10}$$

$$D^T + {}^3O_2 \rightarrow D + {}^1O_2. \tag{9}$$

TABLE 10. QUANTUM YIELD OF THE OXIDATION OF FH_2 (at 1×10^{-5}M) IN THE AERATED SOLUTION

		MB	Th	Es	Acridine
${}^1O_2$		0.035	0.038	0.006	(0.007)
	$\Phi^0_{D\text{-}O}$*	0.036—0.045	0.033—0.038	0.0067	
O_2^-				0	
$D^+\cdots O_2^-$				0	
D^+				0.15	
$D^+\cdots D^-$		0.04			
D^T	Φ_0**	0.058	0.38	0.046	
	Φ_{bleach}***	0.47×10^{-4}		2.8×10^{-4}	0

* Quantum yield when the deactivation of ${}^1O_2$ by ${}^3O_2$ is absent.
** Deaerated solution (for MB $\Phi_{0,max} = \varphi_{ST} k^r_L/k^s_L = 0.3$).
*** Quantum yield for the oxidative photobleaching by oxygen.

[*6] According to Ref. 7 the first order decay constant of D^+ (maybe partly exists as $D^+\cdots D^-$) is *ca.* 9×10^2 sec^{-1}. The addition of 1.0×10^{-5}M of FH_2 does not change it so much in the absence of $K_3[Co(Ox)_3]$, the observed value being 1.6×10^3 sec^{-1}. This is consistent with the view that some part of half-oxidized dye exists as $D^+\cdots D^-$.

That thiazine dyes have larger quantum yields for the oxidation of FH_2 than xanthene dyes and smaller quantum yields for the oxydative bleaching, may be attributed partly to the more favorable occurrence of (9) than (10). This seems to be well correlated with the oxidation-reduction potential of the two types of dye. In the case of acridine, the only reaction is perhaps (9), since there is no evidence of the occurrence of reaction (10).

As to the reactivity of various oxidative species toward FH_2, O_2^- and $D^+\cdots O_2^-$ have been found to be ineffective, although the evidence for the former is rather weak. Both the free D^+ and $D^+\cdots D^-$ are effective. The free D^+ is perhaps more effective than $D^+\cdots D^-$ but the quantitative comparison is a future problem and such studies will be helpful for the discrimination of the two species.

The low reactivity of $D^+\cdots O_2^-$, in sharp contrast to the free D^+, may be considered to be an unambiguous proof of the formation of such a complex by reaction $D^T+O_2\rightarrow D^+\cdots O_2^-$. This we, believe, is the first chemical evidence for the formation of transient molecular compound between dye and oxygen having the nature of ion-pair. It has now become almost certain that the half-oxidized dye produced by triplet eosine and oxygen, does not exist as free radical ion but exists as a rather stable ion pair $D^+\cdots O_2^-$. That the oxidative bleaching starts from the electron transfer process is further supported by a larger value of Φ for D–D mechanism than for D–O mechanism. In the D–O mechanism, it is most likely that most of $D^+\cdots O_2^-$ goes back to D and O_2 with only a very small portion resulting in destruction of dye. In the D–D mechanism on the other hand, oxygen will attack $D^+\cdots D^-$ or D^-, producing D and O_2^- and the remaining D^+ will give colourless product(s).

The quantitative data, some of which are still preliminary are summarized in Table 10.

The authors would like to thank Professor H. Kokubun for helpful discussions.