

**Table III.** Dioxygen and Carbon Monoxide Affinities (mmHg) of Selected Hemoglobins

hemoglobin	ref	$P_{1/2}(\text{O}_2)$	$P_{1/2}(\text{CO})$	temp, °C
Hb ascaris	36	$1-4 \times 10^{-3}$	$1.0 \times 10^{-1}$	20
	37 <sup>a</sup>	$4.7 \times 10^{-3}$	$6.3 \times 10^{-2}$	27.5
	38 <sup>b</sup>	$4.7 \times 10^{-2}$	$7.4 \times 10^{-4}$	20
leg Hb	38 <sup>c</sup>	$4.5 \times 10^{-2}$	$7.1 \times 10^{-4}$	25
	39	$7.0 \times 10^{-1}$	$1.8 \times 10^{-2}$	20
Hb aphrodite	40 <sup>d</sup>	1.1	$6.5 \times 10^{-3}$	20
Hb <sup>AR</sup>	35a <sup>e</sup>	0.15-1.5	$1-4 \times 10^{-3}$	
Hb <sup>T</sup>	35a <sup>e</sup>	9-160	$1-2.8 \times 10^{-1}$	

<sup>a</sup> These values are obtained from the ratios for the rate of binding and of dissociation,  $k_{\text{on}}/k_{\text{off}}$ , and converted by using the following values for the solubility of  $\text{O}_2$  and  $\text{CO}$  in water;  $1.82 \times 10^{-6}$  and  $1.36 \times 10^{-6}$  M/torr, respectively.<sup>41</sup> <sup>b</sup> The value for  $P_{1/2}(\text{O}_2)$  was calculated from  $P_{1/2}(\text{CO})$  and the partition coefficient,  $M = 64$ . A value of  $4 \times 10^{-2}$  torr was measured directly.<sup>42</sup> <sup>c</sup> Values are obtained from the ratio of  $k_{\text{on}}/k_{\text{off}}$ . See footnote a for conversion. <sup>d</sup>  $P_{1/2}(\text{CO})$  is calculated from  $P_{1/2}(\text{O}_2)/M$ , where  $M = 167$ . <sup>e</sup> The values reported therein are a compilation from the original literature and are sensitive to pH, ionic strength, phosphate concentration, etc. The temperatures are in the range 15-25 °C.

(4) Even with small amounts of charge transfer onto the  $\text{O}_2$  ligand, a substantial attraction exists at distances much longer than conventionally taken as indicative of strong hydrogen bonds. Thus, hydrogen bonding and the absence or minimization of repulsive contacts with other groups, such as the porphyrin, may be a major factor in the means by which hemoglobin ascaris, for example, achieves a dioxygen affinity nearly 3 orders of magnitude

greater than vertebrate hemoglobins, such as myoglobin (see Table III).

(5) In the maximization of electrostatic attractions between coordinated dioxygen and an H-X moiety, where X is an electronegative atom or group, substantial distortions (up to 25°) of M-O-O bond angles from expected angles of 120° may result.

**Acknowledgment.** R. S. Drago acknowledges the partial support of this research by the National Science Foundation Grant CHE 82 13398. G. B. Jameson acknowledges the support of the Research Corporation and the donors of the Petroleum Research Fund, administered by the American Chemical Society.

**Registry No.**  $\text{O}_2$ , 7782-44-7;  $\text{CO}$ , 630-08-0; pivalamide, 754-10-9.

(35) (a) Collman, J. P.; Brauman, J. I.; Doxsee, K. M. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 6035-6039. (b) Collman, J. P.; Brauman, J. I.; Doxsee, K. M.; Halbert, T. R.; Suslick, K. S. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 564-568.

(36) Okazaki, T.; Wittenberg, J. B. *Biochim. Biophys. Acta* **1965**, *111*, 503-511.

(37) Gibson, Q. H.; Smith, M. H. *Proc. R. Soc. London, Ser. B.* **1965**, *163*, 206-214.

(38) Imamura, A.; Riggs, A.; Gibson, Q. H. *J. Biol. Chem.* **1972**, *247*, 521-526.

(39) Antonini, E.; Brunori, M. "Hemoglobin and Myoglobin in their Reactions with Ligands; Elsevier: North Holland, 1971.

(40) Wittenberg, B. A.; Briehl, R. W.; Wittenberg, J. B. *Biochem. J.* **1965**, *96*, 363-369.

(41) Schafer, K.; Lax, E. In "Landolt-Bornstein Tables"; Springer-Verlag: West Berlin, 1962; Vol. 2, Part 2, pp 1-89.

(42) (a) Appleby, C. A. *Biochim. Biophys. Acta* **1962**, *60*, 226-235. (b) Wittenberg, J. B.; Bergersen, F. J.; Appleby, C. A.; Turner, G. L. *J. Biol. Chem.* **1974**, *249*, 4057-4066.

## Flavin Analogue-Metal Ion Complexes Acting as Efficient Photocatalysts in the Oxidation of *p*-Methylbenzyl Alcohol by Oxygen under Irradiation with Visible Light

Shunichi Fukuzumi, Sadaki Kuroda, and Toshio Tanaka\*

Contribution from the Department of Applied Chemistry, Faculty of Engineering, Osaka University, Suita, Osaka 565, Japan. Received October 9, 1984

**Abstract:** Flavin analogues (3-methyl-10-phenylisoalloxazine and 3-methyl-10-phenyl-5-deazaisoalloxazines) have been found to catalyze the photooxidation of *p*-methylbenzyl alcohol by oxygen efficiently in the presence of  $\text{Mg}^{2+}$  or  $\text{Zn}^{2+}$  ion in acetonitrile to yield *p*-methylbenzaldehyde and hydrogen peroxide under irradiation of the visible light of  $\lambda > 360$  nm. The  $\text{Mg}^{2+}$  or  $\text{Zn}^{2+}$  ion forms a 1:1 complex with flavin analogues in acetonitrile. Such a complex formation between flavin analogues and the metal ion not only increases the oxidizing ability of the excited states of flavin analogues as indicated by the significant acceleration in the fluorescence quenching rate of flavin analogues with electron donors (methyl- and methoxy-substituted benzenes) by the complex formation but also stabilizes flavin analogues against irradiation with visible light to prevent the photodegradation of flavin analogues. The photocatalytic oxidation of *p*-methylbenzyl alcohol by oxygen is shown to proceed mainly via the singlet-excited states of flavin analogue-metal ion complexes acting as efficient photocatalysts.

Photochemistry of flavin and its analogues has been the subject of intense research, since photochemical activation of flavin analogues makes it possible to oxidize substrates which cannot be oxidized thermally.<sup>1-6</sup> As such, photoreduction of flavin

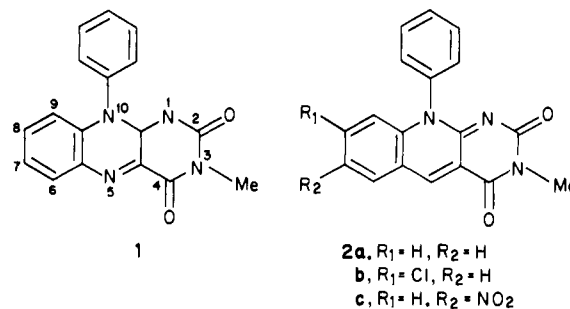
analogues by a variety of substrates such as amino acids, amines, carboxylic acids, and  $\text{NADH}^{1-5}$  or intramolecularly by the ribityl

(1) (a) Penzer, G. R.; Radda, G. K. *Q. Rev.* **1967**, *21*, 43. (b) Heelis, P. F. *Chem. Soc. Rev.* **1982**, *11*, 15. (c) Müller, F. *Photochem. Photobiol.* **1981**, *34*, 753. (d) Penzer, G. R.; Radda, G. K. In "Methods in Enzymology"; McCormick, D. B., Wright, L. D., Eds.; Academic Press: New York, 1971; Vol. XVIII, p 479. (e) Traber, R.; Werner, T.; Schreiner, S.; Kramer, H. E. A.; Knappe, W.-R.; Hemmerich, P. In "Flavins and Flavoproteins"; Yagi, K., Yamano, T., Eds.; Japan Scientific Society Press: Tokyo, 1980; p 431.

(2) (a) Frisell, W. R.; Chung, C. W.; Mackenzie, C. G. *J. Biol. Chem.* **1959**, *234*, 1297. (b) Enns, K.; Burgess, W. H. *J. Am. Chem. Soc.* **1965**, *87*, 5766. (c) Radda, G. K. *Biochim. Biophys. Acta* **1966**, *112*, 448. (d) McCormick, D. B.; Koster, J. F.; Veeger, C. *Eur. J. Biochem.* **1967**, *2*, 387. (e) Penzer, G. R.; Radda, G. K. *Biochem. J.* **1968**, *109*, 259. (f) Penzer, G. R. *Ibid.* **1970**, *116*, 733. (g) Massey, V.; Stankovich, M.; Hemmerich, P. *Biochemistry* **1978**, *17*, 1. (h) Heelis, P. F.; Parsons, B. J.; Phillips, G. O.; McKellar, J. F. *Photochem. Photobiol.* **1979**, *30*, 343. (i) Traber, R.; Krammer, H. E. A.; Hemmerich, P. *Biochemistry* **1982**, *21*, 1687.

side chain of the flavin<sup>6</sup> has been known for many years. On the other hand, dehydration of substrates catalyzed by flavin analogues has merited considerable interest in relation to flavoenzyme-catalyzed dehydrogenation reactions,<sup>7,8</sup> although thermally oxidizable substrates under mild conditions have been limited to rather strong reductants such as alcoholate anions.<sup>7-11</sup> In order to use flavin analogues as efficient photocatalysts in the dehydrogenation of substrates which cannot be oxidized thermally, however, there are two difficult problems to be solved: one is the photodegradation of flavin analogues themselves<sup>6,12</sup> resulting in facile deactivation of the catalyst system and the other is that the oxidizing ability of the excited states of simple flavin analogues may still be insufficient to oxidize neutral alcohols because of the predominant photodegradation of flavin analogues. Thus, no photocatalytic conversion of neutral alcohols to aldehydes with simple flavin analogues as catalysts has so far been reported.<sup>13</sup>

In this study,<sup>14</sup> we have resolved these two difficult problems altogether by using flavin analogues-metal ion complexes as photocatalysts which are found to have much stronger oxidizing ability as well as much improved stability against the visible light than free flavin analogues. First, we report the successful photocatalytic oxidation of *p*-methylbenzyl alcohol by oxygen using flavin analogues (3-methyl-10-phenylisoalloxazine, **1**, and 3-methyl-10-phenyl-5-deazaalloxazines, **2a-c**) in the presence of Mg<sup>2+</sup> or Zn<sup>2+</sup> ion as catalysts in acetonitrile. Next, the unprecedented effect of metal ions in the photocatalytic reaction has been delineated by analyzing the fluorescence quenching of flavin analogues by methyl- and methoxy-substituted benzenes in the absence and presence of metal ions in acetonitrile to evaluate the change of the oxidizing ability of flavin analogues by the complex formation with metal ions. Finally, the reaction mechanism for the photooxidation of *p*-methylbenzyl alcohol by flavin ana-



logue-metal ion complexes will be discussed.

### Experimental Section

**Materials.** Flavin analogues (3-methyl-10-phenylisoalloxazine (**1**), 3-methyl-10-phenyl-5-deazaalloxazine (**2a**), 3-methyl-8-chloro-10-phenyl-5-deazaalloxazine (**2b**), 3-methyl-7-nitro-10-phenyl-5-deazaalloxazine (**2c**)) were prepared according to the procedure in the literature.<sup>15</sup> **1**: Anal. Calcd for C<sub>17</sub>H<sub>12</sub>N<sub>4</sub>O<sub>2</sub>: C, 67.1; H, 4.0; N, 18.4. Found: C, 66.6; H, 4.0; N, 18.3. **2a**: Anal. Calcd for C<sub>18</sub>H<sub>13</sub>N<sub>3</sub>O<sub>2</sub>: C, 71.4; H, 4.3; N, 13.9. Found: C, 71.0; H, 4.3; N, 13.8. **2b**: Anal. Calcd for C<sub>18</sub>H<sub>12</sub>N<sub>3</sub>O<sub>2</sub>Cl: C, 64.0; H, 3.6; N, 12.4. Found: C, 63.9; H, 3.7; N, 12.6. **2c**: Anal. Calcd for C<sub>18</sub>H<sub>12</sub>N<sub>4</sub>O<sub>4</sub>: C, 62.1; H, 3.5; N, 16.1. Found: C, 61.7; H, 3.6; N, 16.4. *p*-Methylbenzyl alcohol and benzene derivatives (methyl- and methoxy-substituted benzenes) were obtained commercially and purified by the standard method.<sup>16</sup> Anhydrous metal perchlorates, Mg(ClO<sub>4</sub>)<sub>2</sub> and NaClO<sub>4</sub>, also were obtained commercially and dried before use. Zinc perchlorate Zn(ClO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O obtained from Alpha Inorganics was purified by successive recrystallization from a mixture of acetonitrile and dichloromethane in order to remove the trace amount of perchloric acid. Potassium ferrioxalate used as an actinometer was prepared according to the literature<sup>17</sup> and purified by recrystallization from hot water. Acetonitrile (MeCN) used as a solvent was purified and dried by the standard procedure<sup>16</sup> and stored on calcium hydride under nitrogen atmosphere.

**Photochemical Reactions of Flavin Analogues.** Typically, *p*-methylbenzyl alcohol (12 mg) was added to a Schlenk tube which contained an MeCN solution (2 cm<sup>3</sup>) of a flavin analogue (1.9 × 10<sup>-4</sup> M) and Mg(ClO<sub>4</sub>)<sub>2</sub> (0.10 M). The Schlenk tube is equipped with a sidearm fused to a square quartz cuvette (1 or 10 mm i.d.). After the reactant solution in the tube was thoroughly degassed in vacuum by the successive freeze-pump-thaw cycles, the solution was transferred to the quartz cuvette, which was then irradiated for a few hours with visible light from a Ushio Model U1-501 Xenon lamp through a Toshiba filter L-39 transmitting light of λ > 360 nm. The rate of the photochemical reaction of flavin analogues with *p*-methylbenzyl alcohol under a degassed condition was measured from the decay of the absorption band due to the flavin analogues (λ<sub>max</sub>: **1** 439 nm, **2a** 403 nm, **2b** 404 nm, **2c** 401 nm in the absence of Mg(ClO<sub>4</sub>)<sub>2</sub> in MeCN; **1** 424 nm, **2a** 388 nm, **2b** 388 nm, **2c** 387 nm in the presence of 0.10 M Mg(ClO<sub>4</sub>)<sub>2</sub> in MeCN) by using a Union SM-401 spectrophotometer.

The photochemical reaction under an atmospheric pressure of oxygen also was carried out in a similar manner by introducing oxygen into the Schlenk tube after degassing the system. The oxidation product (*p*-methylbenzaldehyde) was analyzed by GLC as well as the UV spectrophotometry (λ<sub>max</sub> = 254 nm, ε = 1.60 × 10<sup>4</sup> M<sup>-1</sup> cm<sup>-1</sup>) in a similar manner reported in the literature.<sup>18</sup> The amount of reduction product H<sub>2</sub>O<sub>2</sub> was determined by the standard method (titration by iodide ion);<sup>19</sup> the diluted MeCN solution (×100) of the product mixture was treated with excess amounts of NaI and the amount of I<sub>3</sub><sup>-</sup> formed was determined by the visible spectrum (λ<sub>max</sub> = 361 nm, ε = 2.50 × 10<sup>4</sup> M<sup>-1</sup> cm<sup>-1</sup>). It was confirmed that the formation rate of I<sub>3</sub><sup>-</sup> agreed with that in the reaction of H<sub>2</sub>O<sub>2</sub> under the same conditions.

**Flavin Analogue-Metal Ion Complexes.** The formation of flavin analogue-metal ion complexes was examined from the change of the visible spectra of flavin analogues in the presence of various concentrations of metal ions. The IR spectra of flavin analogue-metal ion complexes in

- (3) (a) Hemmerich, P.; Massey, V.; Weber, G. *Nature (London)* **1967**, *213*, 728. (b) Brustlein, M.; Knappe, W.-R.; Hemmerich, P. *Angew. Chem., Int. Ed. Engl.* **1971**, *10*, 804. (c) Hemmerich, P.; Knappe, W.-R.; Kramer, H. E. A.; Traber, R. *Eur. J. Biochem.* **1980**, *104*, 511. (d) Traber, R.; Kramer, H. E. A.; Knappe, W.-R.; Hemmerich, P. *Photochem. Photobiol.* **1981**, *33*, 807.
- (4) (a) Sun, M.; Song, P. S. *Biochemistry* **1973**, *12*, 4663. (b) Hore, P. J.; Volbeda, A.; Dijkstra, K.; Kaptein, R. *J. Am. Chem. Soc.* **1982**, *104*, 6262.
- (5) (a) Massey, V.; Hemmerich, P. *Biochemistry* **1978**, *17*, 9. (b) Duchstein, H.-J.; Fenner, H.; Hemmerich, P.; Knappe, W.-R. *Eur. J. Biochem.* **1979**, *95*, 167. (c) Visser, A. J. W. G.; Fendler, J. H. *J. Phys. Chem.* **1982**, *86*, 2406.
- (6) (a) Holmström, B.; Oster, G. *J. Am. Chem. Soc.* **1961**, *83*, 1867. (b) Moore, W. M.; Spence, J. T.; Raymond, F. A.; Colson, S. D. *Ibid.* **1963**, *85*, 3367. (c) Holmström, B. *Ark. Kemi.* **1964**, *22*, 329. (d) Yang, C. S.; McCormick, D. B. *J. Am. Chem. Soc.* **1965**, *87*, 5763. (e) Cairns, W. L.; Metzler, D. E. *Ibid.* **1971**, *93*, 2772.
- (7) (a) Bruce, T. C. *Acc. Chem. Res.* **1980**, *13*, 256. (b) Walsh, C. *Ibid.* **1980**, *13*, 148. (c) Bruce, T. C. *Prog. Bioorg. Chem.* **1976**, *4*, 1. (d) Hemmerich, P. *Prog. Chem. Org. Nat. Prod.* **1976**, *33*, 29. (e) Hemmerich, P.; Massey, V.; Fenner, H. *FEBS Lett.* **1977**, *84*, 5.
- (8) (a) Skibo, E. B.; Bruce, T. C. *J. Am. Chem. Soc.* **1983**, *105*, 3304, 3316. (b) Yuan, L.-C.; Bruce, T. C. *Ibid.* **1984**, *106*, 1530. (c) Eberlein, G.; Bruce, T. C. *Ibid.* **1983**, *105*, 6679, 6685. (d) Loechler, E. L.; Hollocher, T. C. *Ibid.* **1980**, *102*, 7312, 7322, 7328.
- (9) (a) Yoneda, F.; Sakuma, Y.; Hemmerich, P. *J. Chem. Soc., Chem. Commun.* **1977**, 825. (b) Yoneda, F.; Nakagawa, K. *Ibid.* **1980**, 878. (c) Yoneda, F.; Sakuma, Y.; Kadokawa, Y.; Koshiro, A. *Chem. Lett.* **1979**, 1467. (d) Yoneda, F.; Yamato, H.; Ono, M. *J. Am. Chem. Soc.* **1981**, *103*, 5943. (e) Yoneda, F.; Mori, K.; Matsuo, S.; Kadokawa, Y.; Sakuma, Y. *J. Chem. Soc., Perkin Trans. 1* **1981**, 1836.
- (10) (a) Shinkai, S.; Kuroda, H.; Manabe, O.; Yoneda, F. *J. Chem. Soc., Chem. Commun.* **1981**, 391. (b) Shinkai, S.; Nakao, H.; Tsuno, T.; Manabe, O.; Ohno, A. *Ibid.* **1984**, 809. (c) Shinkai, S.; Ishikawa, Y.; Manabe, O. *Chem. Lett.* **1982**, 809.
- (11) (a) Yano, Y.; Yatsu, I.; Oya, E.; Ohshima, M. *Chem. Lett.* **1983**, 775. (b) Yano, Y.; Ohshima, M.; Sutoh, S. *J. Chem. Soc., Chem. Commun.* **1984**, 695.
- (12) (a) Gladys, M.; Knappe, W.-R. *Chem. Ber.* **1974**, *107*, 3658. (b) Knappe, W.-R. *Ibid.* **1975**, *108*, 2422. (c) Hemmerich, P. *Fortschr. Chem. Org. Naturst.* **1976**, *33*, 451. (d) Moore, W. M.; Ireton, R. C. *Photochem. Photobiol.* **1977**, *25*, 347.
- (13) Photooxidation of cyclopentanol catalyzed by 1,3,9,11,12,14-hexaazapentacene-2,4,8,10(1H,3H,9H,11H)-tetraones (double-headed 5-deazaflavins) has recently been reported: Yoneda, F.; Kuroda, K.; Koga, M.; Ibuka, T. *J. Chem. Soc., Chem. Commun.* **1984**, 872.
- (14) Preliminary reports have appeared: Fukuzumi, S.; Kuroda, S.; Tanaka, T. *Chem. Lett.* **1984**, 417, 1375.

- (15) (a) Yoneda, F.; Shinozuka, K.; Tsukuda, K.; Koshiro, A. *J. Heterocycl. Chem.* **1979**, *16*, 1365. (b) Nagamatsu, T.; Hashiguchi, Y.; Higuchi, M.; Yoneda, F. *J. Chem. Soc., Chem. Commun.* **1982**, 1085.

- (16) Perrin, D. D.; Armarego, W. L. F.; Perrin, D. R. "Purification of Laboratory Chemicals"; Pergamon Press: Elmsford, NY, 1966.

- (17) (a) Hatchard, C. G.; Parker, C. A. *Proc. R. Soc. London, Ser. A* **1956**, *235*, 518. (b) Calvert, J. G.; Pitts, J. N. "Photochemistry"; Wiley: New York, 1966; p 783.

- (18) Ogata, Y.; Sawaki, Y.; Matsunaga, F.; Tezuka, H. *Tetrahedron* **1966**, *22*, 2655.

- (19) Mair, R. D.; Graupner, A. *J. Anal. Chem.* **1964**, *36*, 194.

a mixture of chloroform and acetonitrile (3:1 v/v) was measured by using a Hitachi 215 spectrophotometer.

**Fluorescence-Quenching Experiments.** Fluorescence measurements were carried out on a Hitachi 650-10S fluorescence spectrophotometer. The fluorescence quantum yield ( $\Phi_f$ ) of a flavin analogue **1** upon excitation of the absorption maximum ( $\lambda_{\max} = 424$  nm) in MeCN was determined as 0.41 by using fluorescein in 0.10 N NaOH as a standard<sup>20</sup> in a similar manner described elsewhere.<sup>21</sup> The fluorescence quantum yield of the standard with 424-nm excitation was adopted as 0.92 from the literature value<sup>12</sup> with a similar excitation (435.8 nm).

Quenching experiments of the flavin analogue fluorescence in the absence and presence of  $\text{Mg}(\text{ClO}_4)_2$  in MeCN were carried out by using methyl- and methoxy-substituted benzenes as quenchers. Relative fluorescence intensities of a flavin analogue at the emission maximum ( $\lambda_{\max}$ : **1** 506 nm, **2a-c** 451 nm in the absence of  $\text{Mg}(\text{ClO}_4)_2$ ; **1** 493 nm, **2a** 439 nm, **2b** 434 nm, **2c** 443 nm in the presence of 0.10 M  $\text{Mg}(\text{ClO}_4)_2$ ) were measured for MeCN solutions of a flavin analogue and a quencher at various concentrations. There has been no change in the shape but rather the intensity of the fluorescence spectrum by the addition of the benzene derivatives. The Stern-Volmer relationship (eq 1, where  $K_q$  is the quenching constant) was obtained between the ratio of the fluores-

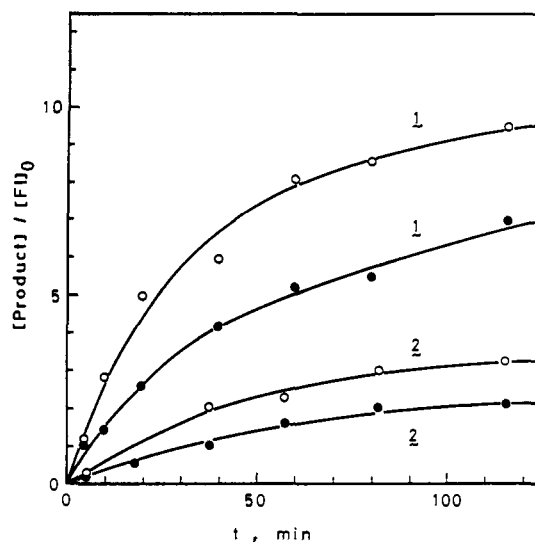
$$\Phi_f^0 / \Phi_f = 1 + K_q [Q] \quad (1)$$

cence quantum yields in the absence and presence of a quencher  $\Phi_f^0 / \Phi_f$  and the quencher concentration  $[Q]$ . The quenching rate constant  $k_q$  ( $=K_q \tau^{-1}$ ) was determined from the quenching constant  $K_q$  and the fluorescence lifetime  $\tau$ . The  $\tau$  values of flavin analogues ( $\tau$ : **1** 2.4, **2a** 1.7, **2b** 1.8, **2c** 0.38 ns in the absence of metal ion; **1** 1.7, **2a** 1.6, **2b** 1.0, **2c** 0.19 ns in the presence of 0.10 M  $\text{Mg}^{2+}$  ion; **1** 1.8, **2a** 1.8 ns in the presence of 0.10 M  $\text{Zn}^{2+}$  ion) were evaluated with the maximum  $K_q$  values which became constant with increasing the donor ability of quenchers, when the  $k_q$  value is well assumed to be equal to the diffusion rate constant;  $2.0 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ .<sup>22</sup>

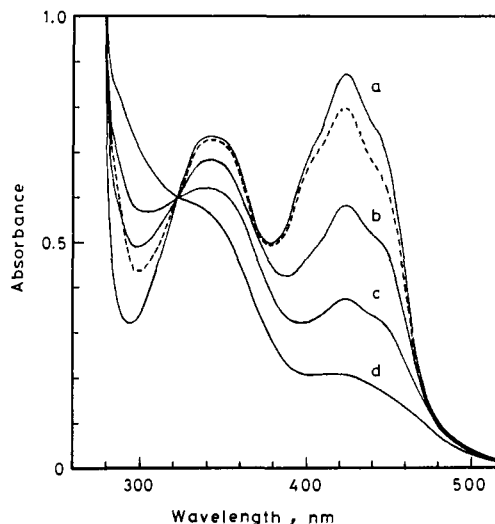
**Quantum-Yield Determinations.** A standard actinometer (potassium ferrioxalate)<sup>17</sup> was used for the quantum-yield determinations on the photochemical reactions of flavin analogues with *p*-methylbenzyl alcohol under a degassed condition as well as the photooxidation of *p*-methylbenzyl alcohol by oxygen catalyzed by flavin analogues in the presence of  $\text{Mg}^{2+}$  ion in MeCN. The quantum yield was determined in the region of visible light through a Toshiba C-39 A glass filter transmitting light of  $350 \text{ nm} < \lambda < 470 \text{ nm}$ . The actinometry experiments were carried out under the conditions such that both an actinometer and a flavin analogue absorb essentially all the incident light (>95%). The quantum yields of the photochemical reactions of flavin analogues with *p*-methylbenzyl alcohol under a degassed condition were determined from the rate of disappearance of the absorption band due to flavin analogues in the absence and presence of 0.10 M  $\text{Mg}(\text{ClO}_4)_2$  and those of the photooxidation under an atmospheric pressure of oxygen were determined from the rate of formation of *p*-methylbenzaldehyde.

## Results and Discussion

**Photooxidation of *p*-Methylbenzyl Alcohol Catalyzed by Flavin Analogues.** Irradiation of an oxygen-saturated MeCN solution containing a flavin analogue **1**, *p*-methylbenzyl alcohol, and  $\text{Mg}(\text{ClO}_4)_2$  with visible light of  $\lambda > 360$  nm results in the formation of *p*-methylbenzaldehyde and hydrogen peroxide, as shown in Figure 1. Yields of *p*-methylbenzaldehyde based on the initial amount of **1** exceed 1000% after 150 min, demonstrating an efficient recycling of **1** in the photooxidation of *p*-methylbenzyl alcohol, although the rate of formation of *p*-methylbenzaldehyde decreased gradually with an increase in the irradiation time (Figure 1), probably because of concomitant photodegradation of **1**. The amount of hydrogen peroxide formed by the photooxidation of *p*-methylbenzyl alcohol is somehow smaller than that of *p*-methylbenzaldehyde, but the ratio of the concentration of *p*-methylbenzaldehyde to hydrogen peroxide remains fairly constant throughout the photolysis. When a 5-deazaflavin **2a** is used as a photocatalyst instead of a flavin **1**, yields of *p*-methylbenzaldehyde based on the initial amount of **2a** exceed 300% after 120 min, but yields of hydrogen peroxide as well as *p*-methylbenzaldehyde become lower than those in the case of **1**, indicating a lower catalytic efficiency of a 5-deazaflavin **2a** than a flavin **1**.



**Figure 1.** Molar ratios of the product [*p*- $\text{MeC}_6\text{H}_4\text{CHO}$  (O) and  $\text{H}_2\text{O}_2$  (●)] to the initial amount of flavin analogue (**1**,  $1.9 \times 10^{-4}$  M; **2a**,  $1.2 \times 10^{-4}$  M) vs. irradiation time for the photooxidation of *p*- $\text{MeC}_6\text{H}_4\text{CH}_2\text{OH}$  ( $4.9 \times 10^{-2}$  and  $1.0 \times 10^{-1}$  M catalyzed by **1** and **2a**, respectively) by oxygen in the presence of  $\text{Mg}^{2+}$  ion ( $1.0 \times 10^{-1}$  M) in MeCN at 298 K.



**Figure 2.** Electronic spectra observed in the photochemical reaction of **1** ( $1.9 \times 10^{-4}$  M) with *p*- $\text{MeC}_6\text{H}_4\text{CH}_2\text{OH}$  ( $1.2 \times 10^{-1}$  M) in the presence of  $\text{Mg}^{2+}$  ion ( $1.0 \times 10^{-1}$  M) in MeCN under a degassed condition; irradiation time: (a) 0, (b) 2, (c) 4, (d) 22 min. The broken-line spectrum is observed upon introducing oxygen to the solution after irradiation with visible light of  $\lambda > 360$  nm for 22 min.

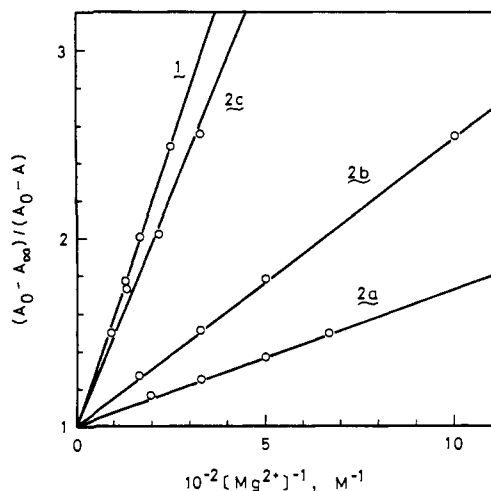
It was confirmed that no thermal reaction of *p*-methylbenzyl alcohol with **1** or **2a** has occurred in the absence or presence of  $\text{Mg}^{2+}$  ion in MeCN at 298 K.

When **1** is allowed to react with *p*-methylbenzyl alcohol under a degassed condition by irradiation of the absorption band of **1** in the presence of 0.10 M  $\text{Mg}^{2+}$  ion ( $\lambda_{\max} = 424$  nm), the absorbance at 424 nm decreases with an increase in the irradiation time as shown in Figure 2. Upon introducing oxygen to the resulting solution, **1** is regenerated in more than 90% yield as shown by the broken line in Figure 2. Thus, an efficient recycling of **1** occurs in the photooxidation of *p*-methylbenzyl alcohol in the presence of 0.10 M  $\text{Mg}^{2+}$  ion. In the absence of  $\text{Mg}^{2+}$  ion, however, the rate of disappearance of the absorption band due to **1** was much slower than that in the presence of 0.10 M  $\text{Mg}^{2+}$  ion. Moreover, the regeneration of **1** has hardly been observed in the absence of  $\text{Mg}^{2+}$  ion by introducing oxygen to the resulting solution, in contrast to the result in Figure 2. In fact, *p*-methylbenzaldehyde has scarcely been formed in the absence of  $\text{Mg}^{2+}$  ion under otherwise the same conditions as those in Figure

(20) Dawson, W. R.; Windsor, M. W. *J. Phys. Chem.* **1968**, *72*, 3251.

(21) Fukuzumi, S.; Hironaka, K.; Tanaka, T. *J. Am. Chem. Soc.* **1983**, *105*, 4722.

(22) (a) Rehm, D.; Weller, A. *Isr. J. Chem.* **1970**, *8*, 259. (b) Rehm, D.; Weller, A. *Ber. Bunsenges. Phys. Chem.* **1969**, *73*, 834.



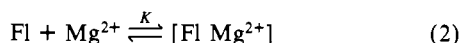
**Figure 3.** Plots of  $(A_0 - A_\infty)/(A_0 - A)$  vs.  $[Mg^{2+}]^{-1}$  for the change of absorption spectra of flavin analogues (**1**, **2a-c**) in the presence of various concentrations of  $Mg^{2+}$  ion in MeCN at 298 K; see eq 3.

**Table I.** Formation Constants  $K$  of the  $Mg^{2+}$  or  $Zn^{2+}$  Complexes with Flavin Analogues in MeCN at 298 K

flavin analogue	$K, M^{-1}$	
	$Mg^{2+}$	$Zn^{2+}$
<b>1</b>	$1.7 \times 10^2$	$4.8 \times 10$
<b>2a</b>	$1.1 \times 10^3$	$1.5 \times 10^2$
<b>2b</b>	$6.4 \times 10^2$	$9.9 \times 10$
<b>2c</b>	$1.8 \times 10^2$	$7.7 \times 10$

1. Thus,  $Mg^{2+}$  ion acts as a "catalyst" in the photooxidation of *p*-methylbenzyl alcohol catalyzed by a flavin analogue.<sup>23</sup> A similar "catalytic" effect also was observed by using  $Zn^{2+}$  ion instead of  $Mg^{2+}$  ion. Such a "catalytic" effect of metal ions is not ascribed to a salt effect since the same concentration of  $Na^+$  ion has caused no effect on the photochemical reaction of **1** with *p*-methylbenzyl alcohol. In order to elucidate the important role of metal ions such as  $Mg^{2+}$  and  $Zn^{2+}$  in the photooxidation of *p*-methylbenzyl alcohol catalyzed by a flavin analogue, we first examine the interaction between flavin analogues and metal ions in MeCN.

**Flavin Analogue-Metal Ion Complexes.** The electronic spectra of flavin analogues **1** and **2a-c** in dry MeCN were significantly affected by the addition of  $Mg(ClO_4)_2$ ; the absorption bands of **1** and **2a-c** in the absence of  $Mg^{2+}$  ion are blue shifted in the presence of 0.10 M  $Mg^{2+}$  ion (see Experimental Section). Similar blue shifts were observed for the fluorescence maxima of **1** and **2a-c** by the addition of  $Mg^{2+}$  ion. When  $Mg^{2+}$  ion is assumed to form a complex with a flavin analogue (Fl) with a 1:1 stoichiometry (eq 2),<sup>4b,14,24</sup> such a change of the electronic spectrum



by the addition of a large excess of  $Mg^{2+}$  ion (more than 10 times than the concentration of Fl) may be expressed by eq 3, where

$$\frac{A_0 - A_\infty}{A_0 - A} = 1 + \frac{1}{K[Mg^{2+}]} \quad (3)$$

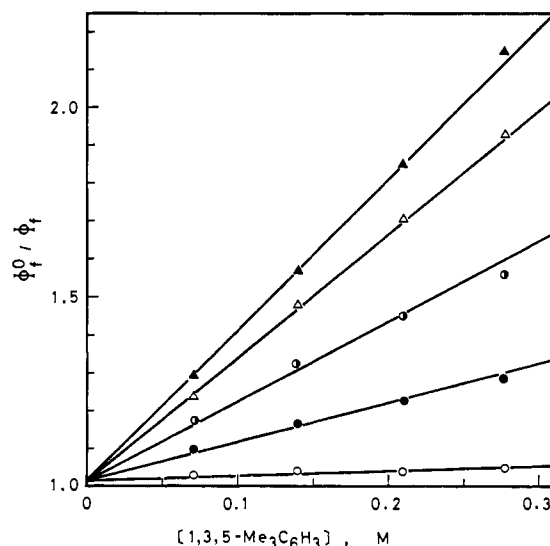
(23) The role of  $Mg^{2+}$  ion may be called as a "catalyst" of the flavin analogue photocatalyst system.

(24) For other metalloflavin complexes, see: (a) Hemmerich, P.; Lauterwein, J. In "Inorganic Biochemistry"; Eichhorn, G., Ed.; Elsevier: Amsterdam, 1973; p 1168. (b) Lauterwein, J.; Hemmerich, P.; Lhoste, J.-M. *Inorg. Chem.* **1975**, *14*, 2152, 2161. (c) Amos, L. W.; Sawyer, D. T. *Ibid.* **1974**, *13*, 78. (d) Kierkegaard, P.; Leijonmarck, M.; Werner, P.-E. *Acta Chem. Scand.* **1972**, *26*, 2980. (e) Fritchie, C. J., Jr. *J. Biol. Chem.* **1972**, *247*, 7459. (f) Clarke, M. J.; Dowling, M. G.; Garafalo, A. R.; Brennan, T. F. *Ibid.* **1980**, *255*, 3472. (g) Dowling, M. G.; Clarke, M. J. *Inorg. Chim. Acta* **1983**, *78*, 153. (h) Shinkai, S.; Ishikawa, Y.; Shinkai, H.; Tsuno, T.; Manabe, O. *Tetrahedron Lett.* **1983**, *24*, 1539. (i) Shinkai, S.; Ishikawa, Y.; Shinkai, H.; Tsuno, T.; Makishima, H.; Ueda, K.; Manabe, O. *J. Am. Chem. Soc.* **1984**, *106*, 1801.

**Table II.** The  $\nu(C=O)$  Frequencies of the  $C^2$ - and  $C^4$ -Carbonyl Groups of Flavin Analogues in the Absence and Presence of  $Mg^{2+}$  or  $Zn^{2+}$  Ion in MeCN/ $CHCl_3$  (1:3 v/v)

flavin analogue	$\nu(C^2=O), cm^{-1}$			$\nu(C^4=O), cm^{-1}$		
	none <sup>a</sup>	$[Mg^{2+}]^b$	$[Zn^{2+}]^b$	none <sup>a</sup>	$[Mg^{2+}]^b$	$[Zn^{2+}]^b$
<b>1</b>	1663	1625	1625	1707	1705	1706
<b>2a</b>	1639	1602	1595	1697	1702	1700
<b>2b</b>	1643	1602		1700	1702	
<b>2c</b>	1648	1610		1704	1708	

<sup>a</sup> In the absence of metal ion. <sup>b</sup> 0.10 M.



**Figure 4.** Stern-Volmer plots for the fluorescence quenching of a flavin analogue **2a** by 1,3,5-trimethylbenzene in the presence of various concentrations of  $Mg^{2+}$  ion [0 (O),  $5.0 \times 10^{-4}$  (●),  $1.5 \times 10^{-3}$  (○),  $8.0 \times 10^{-3}$  (Δ),  $1.0 \times 10^{-1}$  M (▲)] in MeCN.

$A$  is the absorbance at a fixed wavelength of interest in the 380–430 nm region in the presence of a certain concentration of  $Mg^{2+}$  ion, and  $A_0$  and  $A_\infty$  are the absorbances at the same wavelength in the absence of  $Mg^{2+}$  ion and in the presence of a large excess  $Mg^{2+}$  ion, sufficient to form the complex to >99%, respectively. Linear plots between  $(A_0 - A_\infty)/(A_0 - A)$  and  $[Mg^{2+}]^{-1}$  are shown in Figure 3, indicating the formation of the 1:1 complex (eq 2). The formation constants  $K$  obtained from the slopes in Figure 3 are listed in Table I. When  $H_2O$  was added to the flavin analogue **1**- $Mg^{2+}$  ion system, the  $K$  values ( $1.5 \times 10^2$  and  $6.5 \times 10 M^{-1}$  in the presence of  $2.8 \times 10^{-2}$  and  $8.3 \times 10^{-2}$  M  $H_2O$ , respectively) became smaller than that ( $1.7 \times 10^2 M^{-1}$ ) in dry MeCN where the  $H_2O$  concentration is less than  $1 \times 10^{-3}$  M. Such a decrease of the  $K$  value by the addition of  $H_2O$  may be ascribed to the hydration of  $Mg^{2+}$  ion. In fact, no complex formation of **1** with  $Mg^{2+}$  ion has been observed in  $H_2O$ . It was found that  $Zn^{2+}$  ion also forms a 1:1 complex with flavin analogues with smaller formation constants than those of  $Mg^{2+}$  ion (Table I).<sup>25</sup>

The IR spectra of a flavin **1** and 5-deazaflavins **2a-c** in MeCN/ $CHCl_3$  (1:3 v/v)<sup>26</sup> show two  $C=O$  stretching bands assignable to the  $C^2$ - and  $C^4$ -carbonyl groups as reported in the literature.<sup>27</sup> When an excess amount of  $Mg^{2+}$  or  $Zn^{2+}$  ion such that most flavin molecules form the 1:1 complex with the metal ion is added to the MeCN/ $CHCl_3$  solutions of flavin analogues, only the  $C=O$  stretching bands due to the  $C^2$ -carbonyl group of a flavin **1** and 5-deazaflavins **2a-c** are significantly red shifted,

(25) The  $K$  values of flavin analogue- $Zn^{2+}$  ion complexes may be reduced by the water contained in  $Zn(ClO_4)_2 \cdot 6H_2O$ .

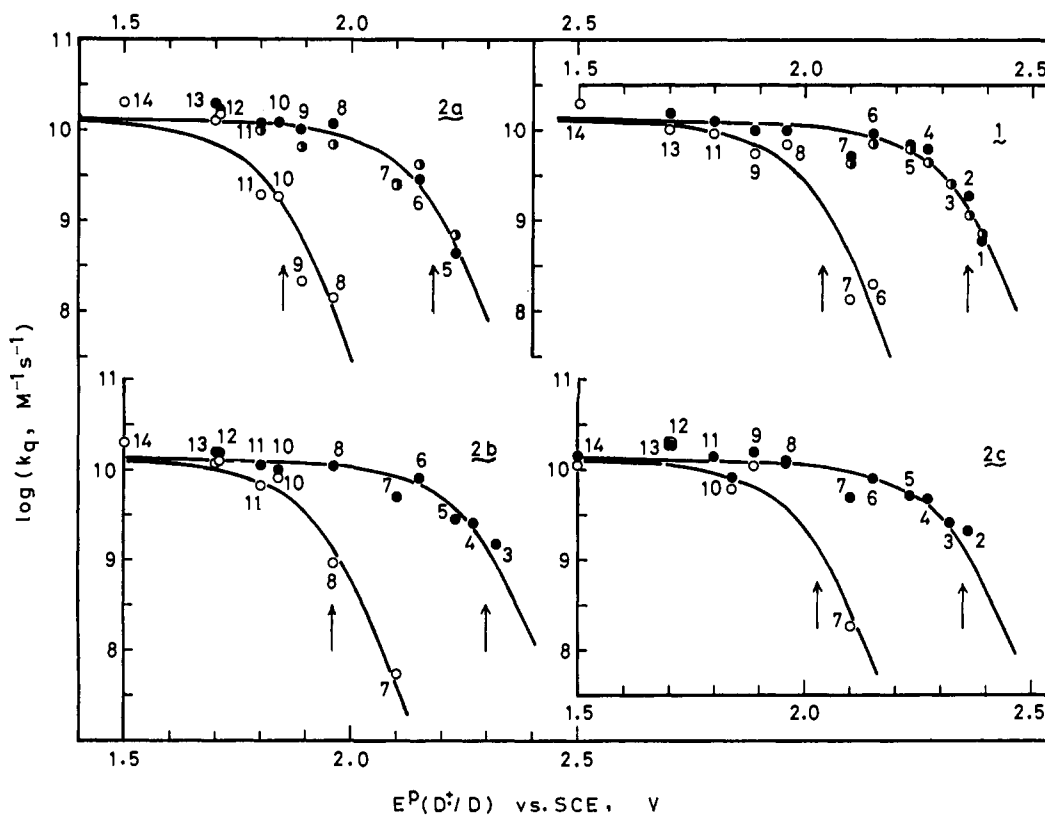
(26) A solvent mixture (MeCN/ $CHCl_3$ , 1:3 v/v) was used in order to increase the solubilities of flavin analogues.

(27) (a) Dudley, K. H.; Ehrenberg, A.; Hemmerich, P.; Müller, F. *Helv. Chim. Acta* **1964**, *47*, 1354. (b) Wessiak, A.; Bruce, T. C. *J. Am. Chem. Soc.* **1983**, *105*, 4809. (c) Pokola, A.; Jorns, M. S.; Vargo, D. *Ibid.* **1982**, *104*, 5466.

**Table III.** Quenching Constants  $K_q$  for the Fluorescence Quenching of **1** and **2a-c** by Methyl- and Methoxy-Substituted Benzenes in the Absence and Presence of 0.10 M  $Mg^{2+}$  Ion and Those of **1** and **2a** in the Presence of 0.10 M  $Zn^{2+}$  Ion in MeCN

no.	quencher	$E^p(D^+/D)^a$	$K_q$ ( $M^{-1}$ ) in the presence of									
			<b>1</b>			<b>2a</b>			<b>2b</b>		<b>2c</b>	
			none <sup>b</sup>	$[Mg^{2+}]^c$	$[Zn^{2+}]^c$	none <sup>b</sup>	$[Mg^{2+}]^c$	$[Zn^{2+}]^c$	none <sup>b</sup>	$[Mg^{2+}]^c$	none <sup>b</sup>	$[Mg^{2+}]^c$
1	MeC <sub>6</sub> H <sub>5</sub>	2.39	<i>d</i>	1.0	1.3	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	
2	<i>p</i> -ClMeC <sub>6</sub> H <sub>4</sub>	2.36	<i>d</i>	3.2	1.9	<i>d</i>	<i>d</i>	0.1	<i>d</i>	<i>d</i>	0.40	
3	<i>p</i> -BrMeC <sub>6</sub> H <sub>4</sub>	2.32	<i>d</i>		4.5	<i>d</i>	<i>d</i>		<i>d</i>	1.5	0.47	
4	<i>m</i> -(Me) <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	2.27	<i>d</i>	11	7.8	<i>d</i>	<i>d</i>	1.1	<i>d</i>	2.5	0.89	
5	<i>o</i> -(Me) <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	2.23	<i>d</i>	12	11	<i>d</i>	0.68	1.3	<i>d</i>	2.9	1.0	
6	<i>p</i> -(Me) <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	2.15	0.48	16	13	<i>d</i>	4.7	7.6	<i>d</i>	7.7	1.5	
7	1,3,5-(Me) <sub>3</sub> C <sub>6</sub> H <sub>3</sub>	2.10	0.32	8.8	7.6	<i>d</i>	4.3	4.3	0.10	4.8	0.1	
8	MeOC <sub>6</sub> H <sub>5</sub>	1.96	16	18	18	0.23	19	12	1.7	11	4.2	
9	1,2,3,4-(Me) <sub>4</sub> C <sub>6</sub> H <sub>2</sub>	1.89	13	17	17	0.36	16	11			4.1	
10	(Me) <sub>5</sub> C <sub>6</sub> H	1.84				3.2	19		14	9.9	2.6	
11	<i>m</i> -Me(MeO)C <sub>6</sub> H <sub>4</sub>	1.80	22	21	23	3.2	19	17	12	11	5.2	
12	<i>p</i> -Me(MeO)C <sub>6</sub> H <sub>4</sub>	1.71				26	27		22	15	6.7	
13	<i>m</i> -(MeO) <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	1.70	26	27	29	21	31	23	21	14	7.6	
14	<i>p</i> -(MeO) <sub>2</sub> C <sub>6</sub> H <sub>4</sub>	1.50	48	34	36	34	32	36	36	20	4.3	

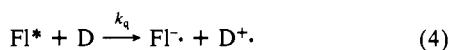
<sup>a</sup>vs. SCE (V), taken from ref 29. <sup>b</sup>In the absence of metal ion. <sup>c</sup>0.10 M. <sup>d</sup>Too small to be determined accurately.



**Figure 5.** Plots of the logarithm of the quenching rate constant  $k_q$  vs. the oxidation peak potential of quenchers  $E^p(D^+/D)$  for the fluorescence quenching of flavin analogues (**1**, **2a-c**) by methyl- and methoxy-substituted benzenes in the absence (○) and presence of 0.10 M  $Mg^{2+}$  ion (●) or  $Zn^{2+}$  ion (◐) in MeCN. Numbers refer to the quenchers in Table III. The solid lines are drawn by the simulation based on eq 6-8. The arrows indicate the  $E^p(D^+/D)$  values when  $\Delta G_{23} = 0$ , see text.

as listed in Table II, suggesting that the metal ion interacts with only the C<sup>2</sup>-carbonyl group.

**Fluorescence Quenching of Flavin Analogue-Metal Ion Complexes.** The fluorescence of a flavin analogue ( $\Phi_f = 0.41$  for **1**) is quenched by electron-transfer reactions with electron donors D (methyl- and methoxy-substituted benzenes) in MeCN (eq 4).<sup>28</sup>



Then, the effect of the metal ion ( $Mg^{2+}$  or  $Zn^{2+}$  ion) on the oxidizing ability of the singlet-excited states of flavin analogues has been examined by comparing the quenching rate constants  $k_q$  in the presence of the metal ion with those in its absence.

Typical examples for the effect of  $Mg^{2+}$  ion on the fluorescence quenching of flavin analogues are shown by the Stern-Volmer plots in Figure 4, where it can be seen that with an increase in the  $Mg^{2+}$  concentrations, the quenching constant  $K_q (=k_q\tau; \tau$  is the fluorescence lifetime) obtained from the slope of the Stern-Volmer plot (eq 1) increases; a constant  $K_q$  value was attained at high concentrations of  $Mg^{2+}$  ion (e.g., 0.10 M) where most flavin molecules form the 1:1 complex with  $Mg^{2+}$  ion (eq 2) based on the  $K$  values in Table II. The  $K_q$  values for the fluorescence quenching of **1** and **2a-c** by methyl- and methoxy-substituted benzenes in the absence and presence of 0.10 M  $Mg^{2+}$  ion and those of **1** and **2a** in the presence of 0.10 M  $Zn^{2+}$  ion are listed in Table III, together with the oxidation peak potentials of the quenchers  $E^p(D^+/D)$  in MeCN.<sup>29</sup> The logarithms of the

(28) Traber, R.; Vogelmann, E.; Schreiner, S.; Werner, T.; Kramer, H. E. A. *Photochem. Photobiol.* **1981**, *33*, 41.

(29) Fukuzumi, S.; Kochi, J. K. *J. Am. Chem. Soc.* **1981**, *103*, 7240.

**Table IV.** Oxidation Peak Potentials of Quenchers,  $E^p(D^+/D)$ , in the Absence and Presence of  $Mg^{2+}$  or  $Zn^{2+}$  Ion When the Gibbs Energy Change of the Electron Transfer  $\Delta G_{23} = 0$  and the Change in Reduction Potentials of the Singlet-Excited States of Flavin Analogues,  $\Delta E^0(FI^*/FI^-)$ , by the Complex Formation with Metal Ion<sup>a</sup>

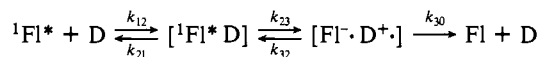
flavin analogue	$E^p(D^+/D)$ (V) in the presence of			$\Delta E^0(FI^*/FI^-)$ , V
	none <sup>b</sup>	$[Mg^{2+}]^c$	$[Zn^{2+}]^c$	
<b>1</b>	2.04	2.36	2.36	0.32
<b>2a</b>	1.85	2.18	2.18	0.33
<b>2b</b>	1.96	2.30		0.34
<b>2c</b>	2.03	2.35		0.32

<sup>a</sup> See text (eq 9). <sup>b</sup> In the absence of metal ion. <sup>c</sup> 0.10 M.

quenching rate constants  $k_q$  ( $= K_q\tau^{-1}$ ) obtained from  $K_q$  and  $\tau$  in the absence and presence of 0.10 M  $Mg^{2+}$  or  $Zn^{2+}$  ion (see Experimental Section) are plotted against the oxidation peak potentials of the donors  $E^p(D^+/D)$  as shown by the open and closed or half-closed circles, respectively, in Figure 5. The log  $k_q$  values of each flavin analogue in the presence of 0.10 M  $Mg^{2+}$  or  $Zn^{2+}$  ion are much larger than those in its absence in the range smaller than the diffusion rate constant (Figure 5). Such an increase of the  $k_q$  value cannot be attributed to a salt effect since the addition of 0.10 M  $NaClO_4$  or  $n-Bu_4NClO_4$  to the FI-D system has caused essentially no effect on the quenching rate constant. Thus, the oxidizing ability of the singlet-excited states of flavin analogues is significantly enhanced by the complex formation with  $Mg^{2+}$  or  $Zn^{2+}$  ion (eq 2).

By applying the general scheme for the fluorescence quenching by electron transfer in MeCN to the present system (Scheme I),<sup>22</sup>

#### Scheme I



the observed quenching rate constant  $k_q$  (eq 4) may be expressed by eq 5, which is rewritten as eq 6,<sup>22</sup> where  $\Delta G_{23}^*$  and  $\Delta G_{23}$  are

$$k_q = \frac{k_{12}}{1 + (k_{21}/k_{30})((k_{30}/k_{23}) + (k_{32}k_{23}))} \quad (5)$$

$$k_q = \frac{2.0 \times 10^{10}}{1 + 0.25[\exp(\Delta G_{23}^*/RT) + \exp(\Delta G_{23}/RT)]} \quad (6)$$

the activation Gibbs energy and the Gibbs energy change of the actual electron-transfer process,  $[{}^1FI^*D] \rightarrow [FI^{\cdot-}D^{\cdot+}]$ , respectively. Then, the  $k_q$  value can be calculated as a function of  $\Delta G_{23}$  by using eq 6 and the Rehm-Weller Gibbs energy relationship (eq 7),<sup>22</sup>

$$\Delta G_{23}^* = (\Delta G_{23}/2) + [(\Delta G_0^*)^2 + (\Delta G_{23}/2)^2]^{1/2} \quad (7)$$

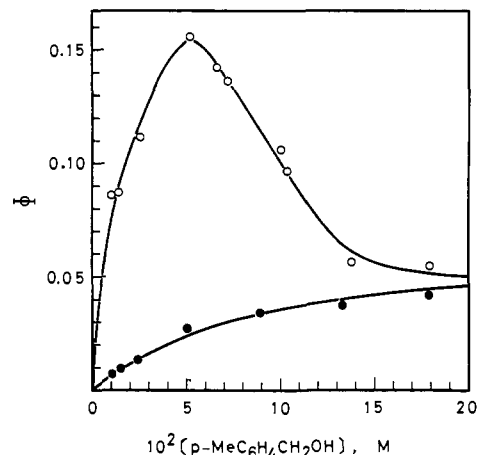
where  $\Delta G_0^*$  is the activation Gibbs energy when  $\Delta G_{23} = 0$ ; the  $\Delta G_0^*$  value is generally taken as 2.4 kcal mol<sup>-1</sup>.<sup>22,30</sup> On the other hand,  $\Delta G_{23}$  is related to the difference between the oxidation peak potential of the donor  $E^p(D^+/D)$  and the reduction potential of the singlet-excited state of the flavin analogue  $E^0(FI^*/FI^-)$  as expressed by eq 8, where  $C$  is a constant which corresponds to

$$\Delta G_{23} = F[E^p(D^+/D) - E^0(FI^*/FI^-) + C] + w_p \quad (8)$$

the difference between  $E^0(D^+/D)$  and  $E^p(D^+/D)$ ,<sup>31</sup>  $w_p$  is the work term required to bring the products  $FI^{\cdot-}$  and  $D^{\cdot+}$  together, and  $w_r$  is the corresponding work term for the reactants which has been neglected. Thus, the curve obtained for log  $k_q$  vs.  $\Delta G_{23}$  can be fitted with each plot of log  $k_q$  vs.  $E^p(D^+/D)$  by sliding the curve of log  $k_q$  vs.  $\Delta G_{23}$  appropriately in the direction of the abscissa  $E^p(D^+/D)$  which is parallel to  $\Delta G_{23}$  (eq 8), as shown by the solid lines in Figure 5. Each arrow in Figure 5 indicates the  $E^p(D^+/D)_0$

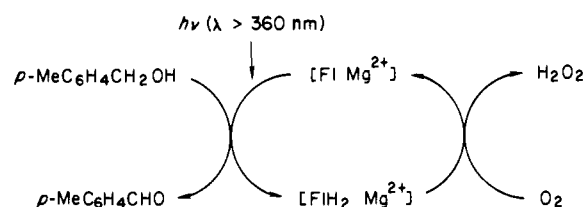
(30) Ebersson, L. *Adv. Phys. Org. Chem.* **1982**, *18*, 79.

(31) The oxidation peak potentials  $E^p(D^+/D)$  in the irreversible system determined by cyclic voltammetry at a constant sweep rate have been shown to be parallel with the standard redox potentials  $E^0(D^+/D)$ , see: Howell, J. O.; Goncalves, J. M.; Amatore, C.; Klasinc, L.; Wightman, R. M.; Kochi, J. K. *J. Am. Chem. Soc.* **1984**, *106*, 3968.



**Figure 6.** Plots of quantum yields ( $\Phi$ ) vs. the concentration of *p*-methylbenzyl alcohol for the photochemical reaction of a flavin analogue 1- $Mg^{2+}$  ion complex with *p*-methylbenzyl alcohol under a degassed condition (O) and an atmospheric pressure of oxygen (●) in MeCN.

#### Scheme II



value when  $\Delta G_{23} = 0$ . From eq 8,  $E^p(D^+/D)_0$  is related to  $E^0(FI^*/FI^-)$  by eq 9. Then, according to eq 9, the positive shift

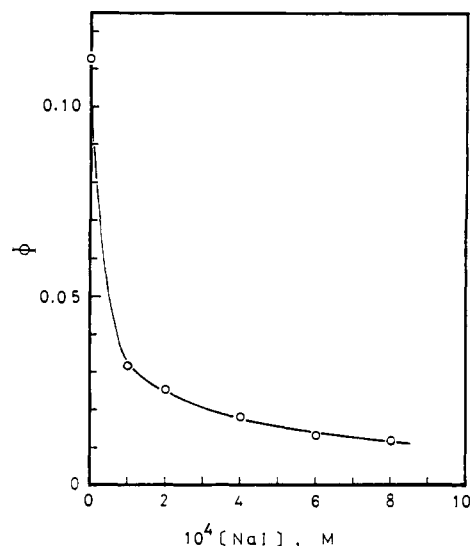
$$E^p(D^+/D)_0 = E^0(FI^*/FI^-) - C - w_p/F \quad (9)$$

of the  $E^p(D^+/D)_0$  value in the presence of 0.10 M  $Mg^{2+}$  or  $Zn^{2+}$  ion relative to that in its absence as indicated by two arrows in each plot in Figure 5 is equal to the corresponding shift of  $E^0(FI^*/FI^-)$ , since the other terms in eq 9 may be invariant in the presence of the metal ion.<sup>32</sup> Thus, the increase of the oxidizing ability of the singlet-excited states of flavin analogues by the complex formation with  $Mg^{2+}$  or  $Zn^{2+}$  ion can be evaluated quantitatively as the positive shifts of  $E^0(FI^*/FI^-)$ , i.e.,  $\Delta E^0(FI^*/FI^-)$ . The  $\Delta E^0(FI^*/FI^-)$  values listed in Table IV are approximately constant ( $0.33 \pm 0.01$  V) for different flavin analogue-metal ion complexes.

Such large positive shifts of  $E^0(FI^*/FI^-)$  in flavin analogue-metal ion complexes (Table IV) suggest that the photocatalytic oxidation of *p*-methylbenzyl alcohol (Figure 1) may proceed via a flavin analogue-metal ion complex, e.g.,  $[FI Mg^{2+}]$  acting as a photocatalyst in which the metal ion increases the oxidizing ability of the excited state of a flavin analogue to make it possible to oxidize *p*-methylbenzyl alcohol to the corresponding aldehyde as shown in Scheme II. In fact, the quenching constant of a flavin analogue 1- $Mg^{2+}$  ion complex by *p*-methylbenzyl alcohol ( $K_q = 10$  M<sup>-1</sup>) was much larger than that of a free flavin **1** ( $< 0.1$  M<sup>-1</sup>).

**Kinetics and Mechanism.** The quantum yields ( $\Phi$ ) for the photochemical reactions of a flavin analogue **1** with *p*-methylbenzyl alcohol in the presence of 0.10 M  $Mg^{2+}$  ion under a degassed condition and an atmospheric pressure of oxygen are shown in Figure 6, as a function of the concentration of *p*-methylbenzyl alcohol. The  $\Phi$  value under a degassed condition increases with an increase in the alcohol concentration to reach a maximum ( $\Phi_{max} = 0.16$  at  $5 \times 10^{-2}$  M) and then decreases to approach a constant value. On the other hand, the  $\Phi$  value under an atmospheric pressure of oxygen, which is much smaller than that under a degassed condition in the region of low concentrations of *p*-

(32) The redox potentials of benzene derivatives used in this study may not be changed in the presence of  $Mg^{2+}$  or  $Zn^{2+}$  ion since no interaction between benzene derivatives and  $Mg^{2+}$  ion has been observed.

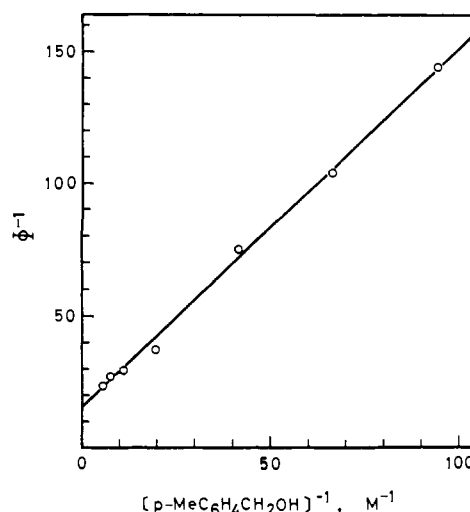
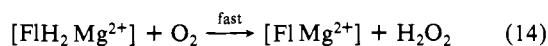
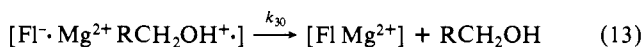
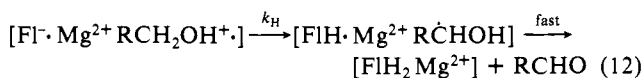
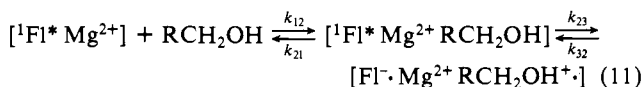
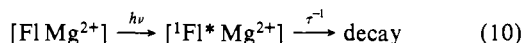


**Figure 7.** Effect of a triplet quencher NaI on the quantum yields ( $\Phi$ ) for the photochemical reaction of a flavin analogue **1**-Mg<sup>2+</sup> ion complex with *p*-methylbenzyl alcohol under a degassed condition in MeCN.

methylbenzyl alcohol ( $<5 \times 10^{-2}$  M), increases with an increase in the alcohol concentration to approach a constant value in the absence of oxygen in high alcohol concentrations ( $>0.14$  M) (Figure 6). Since oxygen is a well-known triplet quencher,<sup>6</sup> the photochemical reaction in the absence of oxygen may proceed mainly via the triplet excited state of **1**-Mg<sup>2+</sup> ion complex which is quenched by oxygen to decrease the quantum yield in the presence of oxygen. Indeed, the addition of iodide ion (NaI) which is another well-known triplet quencher of the flavin system<sup>6b,33</sup> decreases the quantum yield of the photochemical reaction of **1** with *p*-methylbenzyl alcohol in the absence of oxygen by depopulating the triplet state [<sup>3</sup>Fl\* Mg<sup>2+</sup>] to approach the  $\Phi$  value in the presence of oxygen under otherwise the same conditions, as shown in Figure 7. It was confirmed that the same concentration of NaI as used for the triplet quenching (Figure 7) has hardly affected the fluorescence of **1**-Mg<sup>2+</sup> ion complex, and thereby, the singlet population is not sufficiently affected by the low concentration of NaI. The reason why the  $\Phi$  value under a degassed condition decreases in the region of high concentrations of *p*-methylbenzyl alcohol ( $>5.0 \times 10^{-2}$  M in Figure 6) is not clear at present.

The quantum yield  $\Phi_d$  of photodegradation of **1**-Mg<sup>2+</sup> ion complex in the absence of *p*-methylbenzyl alcohol under a degassed condition was  $6.2 \times 10^{-4}$  which is negligibly small compared with the  $\Phi_d$  value in the presence of *p*-methylbenzyl alcohol under otherwise the same conditions as those in Figure 6. Moreover, the  $\Phi_d$  value of **1**-Mg<sup>2+</sup> ion complex is much smaller than that of a free flavin analogue **1** ( $\Phi_d = 1.6 \times 10^{-2}$ ). Thus, Mg<sup>2+</sup> ion not only increases the oxidizing ability of the excited states of flavin analogues (Table IV) but also stabilizes the flavin analogues against irradiation of the visible light to prevent the photodegradation by forming the complexes (eq 2).

The dependence of quantum yield on the concentration of *p*-methylbenzyl alcohol in the presence of oxygen (Figure 6) can be explained by the following reaction mechanism (eq 10–14),



**Figure 8.** Plot of  $\Phi^{-1}$  vs.  $[\textit{p}\text{-MeC}_6\text{H}_4\text{CH}_2\text{OH}]^{-1}$  for the photooxidation of *p*-methylbenzyl alcohol by oxygen, catalyzed by a flavin analogue **1**-Mg<sup>2+</sup> ion complex in MeCN.

where R denotes *p*-MeC<sub>6</sub>H<sub>4</sub>CH<sub>2</sub>. The excitation of [FlMg<sup>2+</sup>] gives the singlet-excited state [<sup>1</sup>Fl\* Mg<sup>2+</sup>] which decays by radiative and nonradiative pathways (eq 10). The singlet-excited state [<sup>1</sup>Fl\* Mg<sup>2+</sup>] can be quenched by electron-transfer reaction with *p*-methylbenzyl alcohol (eq 11). The triplet state [<sup>3</sup>Fl\* Mg<sup>2+</sup>] formed by the intersystem crossing from [<sup>1</sup>Fl\* Mg<sup>2+</sup>] may also be quenched by *p*-methylbenzyl alcohol under a degassed condition. In the presence of oxygen, however, such a pathway via [<sup>3</sup>Fl\* Mg<sup>2+</sup>] may be inhibited. The reversible electron transfer (eq 11) may be followed by the irreversible proton transfer from RCH<sub>2</sub>OH<sup>+</sup> to Fl<sup>•</sup> in the radical ion pair [Fl<sup>•</sup>Mg<sup>2+</sup>RCH<sub>2</sub>OH<sup>+</sup>] (eq 12), which may compete with the back-electron-transfer process (eq 13). The subsequent reaction following the proton transfer to give the final products [FlH<sub>2</sub>Mg<sup>2+</sup>]<sup>34</sup> and RCHO is known to be fast.<sup>35</sup> Then, the flavin analogue-Mg<sup>2+</sup> ion complex [FlMg<sup>2+</sup>] may be regenerated by the reaction of the reduced flavin complex [FlH<sub>2</sub>Mg<sup>2+</sup>] with oxygen, yielding hydrogen peroxide as well (eq 14).<sup>36</sup>

By applying the steady-state approximation to the reactive species [<sup>1</sup>Fl\* Mg<sup>2+</sup>], [<sup>1</sup>Fl\* Mg<sup>2+</sup>RCH<sub>2</sub>OH], [Fl<sup>•</sup>Mg<sup>2+</sup>RCH<sub>2</sub>OH<sup>+</sup>], and [FlH<sup>•</sup>Mg<sup>2+</sup>RCHOH] in the pathways in eq 10–14, the dependence of  $\Phi$  on [RCH<sub>2</sub>OH] can be derived as given by eq 15, which is rewritten as eq 16. The validity of eq 16 is

$$\Phi = k_{12}k_{23}k_H\tau / ((k_H + k_{30}) \times (k_{21} + k_{23}) + k_{21}k_{32} + k_{12}k_{23}(k_H + k_{30})\tau[\text{RCH}_2\text{OH}]) \quad (15)$$

$$\frac{1}{\Phi} = \frac{k_H + k_{30}}{k_H} + \frac{(k_H + k_{30})(k_{21} + k_{23}) + k_{21}k_{23}}{k_{12}k_{23}k_H\tau[\text{RCH}_2\text{OH}]} \quad (16)$$

confirmed by the plot of  $1/\Phi$  against  $1/[\text{RCH}_2\text{OH}]$  which gives a linear correlation as shown in Figure 8. From eq 15 or 16, the limiting quantum yield  $\Phi_\infty$  is given by eq 17. Then, the ratio of

$$\Phi_\infty = k_H / (k_H + k_{30}) \quad (17)$$

$k_H$  to  $k_{30}$  is evaluated as 0.071 with eq 17 and the  $\Phi_\infty$  value which is obtained from the intercept in Figure 8. According to eq 16,

(33) (a) Song, P.-S.; Moore, T. A. *J. Am. Chem. Soc.* **1968**, *90*, 6507. (b) Song, P.-S.; Metzler, P. E. *Photochem. Photobiol.* **1967**, *6*, 691.

(34) Yano, Y.; Sakaguchi, T.; Nakazato, M. *J. Chem. Soc., Perkin Trans. 2* **1984**, 595.

(35) (a) Ahmad, R.; Wu, Z.; Armstrong, D. A. *Biochemistry* **1983**, *22*, 1806. (b) Ahmad, R.; Armstrong, D. A. *Ibid.* **1982**, *21*, 5445. (c) Bruce, T. C.; Taulane, J. P. *J. Am. Chem. Soc.* **1976**, *98*, 7769.

(36) (a) Massey, V.; Palmer, G.; Ballou, D. In "Flavins and Flavoproteins"; Kamin, H., Ed.; University Park Press: Baltimore, MD, 1971; p 349. (b) Massey, V.; Palmer, G.; Ballou, D. In "Oxidases and Related Redox Systems"; King, T. E.; Mason, H. S.; Morrison, M., Eds.; University Park Press: Baltimore, MD, 1973; p 25. (c) Kemal, C.; Chan, T. W.; Bruce, T. C. *J. Am. Chem. Soc.* **1977**, *99*, 7272. (d) Eberlein, G.; Bruce, T. C. *Ibid.* **1983**, *105*, 6685.

the ratio of the intercept to slope ( $i/s$ ) in Figure 8 is given by eq 18, where  $\alpha = (1 + k_H/k_{30})^{-1} \simeq 1$ , since  $k_H/k_{30} \ll 1$ . Then, by

$$\frac{i}{s} = \frac{k_{12}\tau}{1 + (k_{21}/k_{30})((k_{30}/k_{23}) + (k_{32}/k_{23})\alpha)} \quad (18)$$

comparing eq 5 with eq 18, the quenching constant  $K_q (=k_q\tau)$  is obtained as 11 from the  $i/s$  value in Figure 8. This value agrees well with the  $K_q$  value of the fluorescence quenching of **1**-Mg<sup>2+</sup> ion complex by *p*-methylbenzyl alcohol described in the previous section ( $K_q = 10$ ). Such an agreement strongly supports the reaction mechanism (eq 10-14) where the photocatalytic oxidation

of *p*-methylbenzyl alcohol proceeds mainly via the singlet-excited state of a flavin analogue-Mg<sup>2+</sup> ion complex as the reactive catalyst.

Registry No. **1**, 35804-39-8; **2a**, 69083-37-0; **2b**, 76902-97-1; **2c**, 91028-36-3; Mg(ClO<sub>4</sub>)<sub>2</sub>, 10034-81-8; Zn(ClO<sub>4</sub>)<sub>2</sub>, 13637-61-1; MeC<sub>6</sub>H<sub>5</sub>, 108-88-3; *p*-ClMeC<sub>6</sub>H<sub>4</sub>, 106-43-4; *p*-BrMeC<sub>6</sub>H<sub>4</sub>, 106-38-7; *m*-(Me)<sub>2</sub>C<sub>6</sub>H<sub>4</sub>, 108-38-3; *o*-(Me)<sub>2</sub>C<sub>6</sub>H<sub>4</sub>, 95-47-6; *p*-(Me)<sub>2</sub>C<sub>6</sub>H<sub>4</sub>, 106-42-3; 1,3,5-(Me)<sub>3</sub>C<sub>6</sub>H<sub>3</sub>, 108-67-8; MeOC<sub>6</sub>H<sub>5</sub>, 100-66-3; 1,2,3,4-(Me)<sub>4</sub>C<sub>6</sub>H<sub>2</sub>, 488-23-3; (Me)<sub>3</sub>C<sub>6</sub>H<sub>3</sub>, 700-12-9; *m*-Me(MeO)C<sub>6</sub>H<sub>4</sub>, 100-84-5; *p*-Me(MeO)C<sub>6</sub>H<sub>4</sub>, 104-93-8; *m*-(MeO)<sub>2</sub>C<sub>6</sub>H<sub>4</sub>, 151-10-0; *p*-(MeO)<sub>2</sub>C<sub>6</sub>H<sub>4</sub>, 150-78-7; *p*-methylbenzyl alcohol, 589-18-4; *p*-methylbenzaldehyde, 104-87-0; hydrogen peroxide, 7722-84-1.

## Polymer Films on Electrodes. 17. The Application of Simultaneous Electrochemical and Electron Spin Resonance Techniques for the Study of Two Viologen-Based Chemically Modified Electrodes

John G. Gaudiello, Pushpito K. Ghosh, and Allen J. Bard\*

Contribution from the Department of Chemistry, The University of Texas at Austin, Austin, Texas 78712. Received June 22, 1984

**Abstract:** Simultaneous electrochemical and electron spin resonance (SEESR) techniques were used to study the environment and rates of electron transfer in two viologen-based chemically modified electrodes. Electrodes derivatized with *N,N'*-bis-[3-(trimethoxysilyl)propyl]-4,4'-bipyridinium, where the redox sites are covalently anchored to the polymer backbone, exhibit broad and featureless spectra upon reduction, suggesting that the spin sites are restrained and immobile. The ESR signal intensity as a function of the extent of film reduction indicates that the rate on electron transfer (self-exchange) is high,  $>10^6$  M<sup>-1</sup> s<sup>-1</sup>. Electrodes covered with the perfluorosulfonate polymer Nafion in which MV<sup>2+</sup> (MV<sup>2+</sup> = methyl viologen) has been incorporated display ESR spectra upon reduction that are similar to those of MV<sup>•+</sup> in solution, suggesting that the radicals are free to tumble on the ESR time scale. The rates of electron exchange are smaller in the Nafion modified electrodes ( $\leq 8 \times 10^5$  M<sup>-1</sup> s<sup>-1</sup>); this is consistent with physical diffusion of the redox species contributing substantially, if not completely, to charge transport in these films.

This paper concerns the application of electron spin resonance (ESR) spectroscopic techniques to polymer electrodes and a comparison of the behavior of a cation radical (viologen) in two different types of polymer films on an electrode surface: one, where the radical ion is attached to the polymer backbone, and the other, a polyelectrolyte, to which the species is held by electrostatic binding.

Chemically modified electrodes have been characterized by a variety of techniques. Electrochemical methods such as cyclic voltammetry, chronoamperometry, chronopotentiometry, and rotating disk voltammetry have been used to determine the redox, charge transport, and catalytic properties of these electrodes.<sup>1</sup> Spectroscopic techniques, e.g., X-ray photoelectron, Auger, UV-vis, photoacoustic, photothermal, and reflectance spectroscopy, have also provided information about the morphology of the modifying layer.<sup>1</sup> Electron spin resonance spectroscopy has recently been introduced in conjunction with electrochemical techniques to characterize polymer modified electrodes, e.g., in the study tetracyanoquinodimethane (TCNQ)-modified Pt electrodes.<sup>2</sup> Albery et al. have developed an electrochemical cell for ESR studies based on convective diffusion and used it to study polymer films of poly(nitrostyrene) and poly(vinylanthraquinone).<sup>3</sup>

In both previous studies the electrogenerated radical ions were attached to the polymer backbone and the ESR signal was a single line. ESR measurements are particularly attractive for the study of modified electrodes because information concerning the environment and mobility of surface-confined redox couples, as well as some insight into the mechanism of charge transport, can be obtained.

Viologens have been extensively studied in recent years in their role as electron acceptors in photochemical<sup>4,5</sup> and photoelectrochemical<sup>6,7</sup> schemes. The reversibility of the 2+/1+ couple and the large visible spectral changes that accompany the oxidation/reduction process have made the viologens especially useful in modified electrode studies as well.<sup>8-10</sup> Cyclic voltammetric

(3) Albery, W. J.; Compton, R. G.; Jones, C. C. *J. Am. Chem. Soc.* **1984**, *106*, 469-473.

(4) Young, R. C.; Meyer, T. J.; Whitten, D. G. *J. Am. Chem. Soc.* **1975**, *97*, 4781-4782.

(5) (a) Moradpour, A.; Amouyal, E.; Keller, P.; Kagan, H. *Nouv. J. Chim.* **1978**, *2*, 547-549. (b) Kiwi, J.; Gratzel, M. J. *J. Am. Chem. Soc.* **1979**, *101*, 7214-7217.

(6) (a) Bookbinder, D. C.; Bruce, J. A.; Dominey, R. N.; Lewis, N. S.; Wrighton, M. S. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 6280-6289. (b) Dominey, R. N.; Lewis, N. S.; Bruce, J. A.; Bookbinder, D. C.; Wrighton, M. S. *J. Am. Chem. Soc.* **1982**, *104*, 467-482.

(7) (a) Reichman, B.; Fan, F.-R. F.; Bard, A. J. *J. Electrochem. Soc.* **1980**, *127*, 333-338. (b) Ward, M. D.; White, J. R.; Bard, A. J. *J. Am. Chem. Soc.* **1983**, *105*, 27-31.

(1) Murray, R. W. "Electroanalytical Chemistry"; Bard, A. J., Ed.; Dekker: New York, NY, 1984; Vol. 13, p 191 and references therein.

(2) Izelt, G.; Day, R. W.; Kinstle, J. F.; Chambers, J. Q. *J. Phys. Chem.* **1983**, *87*, 4592-4598.