Formation of Superoxide Ion via One-Electron Transfer from Electron Donors to Singlet Oxygen

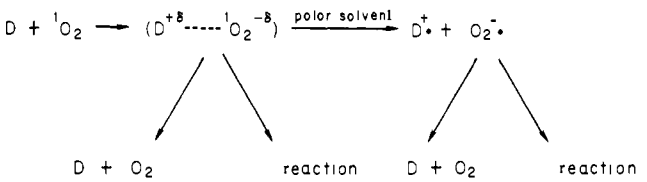
Isao Saito,*† Teruo Matsuura,† and Kenzo Inoue*‡

Contribution from the Department of Synthetic Chemistry, Faculty of Engineering, Kyoto University, Kyoto 606, Japan, and the Department of Industrial Chemistry, Ehime University, Ehime 790, Japan. *Received September 22, 1982*

Abstract: The formation of superoxide ion ($O_2^{-}\cdot$) via one-electron transfer from substituted *N,N*-dimethylanilines to singlet oxygen ($^1O_2$) was examined in phosphate buffer by employing a water-soluble oxygen source and a combination of *p*-nitrotetrazolium blue (NBT) and superoxide dismutase (SOD) as a detecting reagent for $O_2^{-}\cdot$. When a solution of an electron-donor-substituted *N,N*-dimethylaniline, NBT, and 3-(1,4-epidioxy-4-methyl-1,4-dihydro-1-naphthyl)propionic acid in phosphate buffer at pH 7.5 was incubated at 35 °C, NBT was reduced to formazan. The inhibition of the reduction of NBT to formazan by SOD indicated the formation of $O_2^{-}\cdot$. Control experiments demonstrated that $O_2^{-}\cdot$ is produced by a direct reaction between $^1O_2$ and the amine. Both the yield of $O_2^{-}\cdot$ and the quenching rate constants of $^1O_2$ are well correlated with the oxidation potentials of these amines. Tetramethyl-*p*-phenylenediamine having a quenching rate constant close to a diffusion-controlled limit is the most effective for the generation of $O_2^{-}\cdot$. A plot of the log of the quenching rate constant of $^1O_2$ by the amines against the calculated free-energy change (ΔG) for full electron transfer showed a linear relationship with a slope of -0.19 ± 0.05 mol/kcal. The correlation of the log of the relative yield of $O_2^{-}\cdot$ against ΔG strongly supports the electron-transfer mechanism for the formation of $O_2^{-}\cdot$. It was demonstrated that an electron-transfer reaction giving rise to $O_2^{-}\cdot$ is only possible for aromatic amines with oxidation potentials less than ~0.5 V vs. SCE in aqueous media.

The one-electron-transfer process giving rise to a substrate radical cation and superoxide ion ($O_2^{-}\cdot$) pair has been suggested to play an important role in the primary step of the oxidation of highly electron-rich substrates with molecular oxygen.[1] In principle, singlet molecular oxygen ($^1O_2$) could be more susceptible to one-electron transfer with electron donors (D) than triplet oxygen does, since excitation of molecular oxygen to $^1O_2$ results in an increase in the one-electron reduction potential by 1 eV. In fact, one-electron-transfer mechanisms have been postulated in the interaction between $^1O_2$ and various types of electron donors. These examples include (1) [2 + 2] cycloadditions of $^1O_2$ to enamines first proposed by Foote et al.,[2] (2) reactions with phenols,[3] sulfides,[4] and azines,[5] and (3) quenching of $^1O_2$ by phenols,[3a] sulfides,[4] amines,[6] and azide anion.[7] It has also been suggested that mitochondorial components such as NADH and reduced cytochrome *c* are capable of undergoing one-electron transfer with $^1O_2$ giving rise to $O_2^{-}\cdot$, while remaining unaffected by triplet oxygen.[8] However, direct evidence for the formation of superoxide ion ($O_2^{-}\cdot$) had not been obtained in either the reaction or the quenching process.

We have recently reported the chemical evidence for the formation of $O_2^{-}\cdot$ in the reaction between $^1O_2$ and *N,N*-dimethyl-*p*-anisidine in aqueous media.[9] In the meantime, Peters and Rodgers[8b] have detected the $O_2^{-}\cdot$ formation in the reaction of NADH with $^1O_2$ by monitoring benzoquinone reduction in a laser flash experiment. More recently, Manring and Foote[10] have reported the detection of cation radical in the reaction between photochemically generated $^1O_2$ and tetramethyl-*p*-phenylenediamine (TMPD) in aqueous media. On the other hand, Schaap et al.[11] have reported that [2 + 2] cycloaddition of $^1O_2$ to a series of enol ethers does not proceed via a one-electron-transfer mechanism. Manring and Foote[10] also reported that the formation of $TMPD^{+}\cdot$ from $^1O_2$ is not observed in any solvent other than aqueous medium. Thus the one-electron transfer giving rise to a radical cation–$O_2^{-}\cdot$ pair seems to highly depend on the ease of ionization of substrates and the solvation of the resulting ions as generally recognized in many other electron-transfer reactions.[12] In polar aqueous media, particularly in biological system, the one-electron-transfer process may be expected to play an important role in the interaction of $^1O_2$ with electron-rich substrates.



The dye-sensitized photooxidation of aliphatic and aromatic amines has been extensively investigated for many years.[6,13] Amines are also well-known as $^1O_2$ quenchers, with the quenching efficiency being in the order tertiary > secondary > primary amines.[6,13] Based on a correlation between quenching rates and ionization potentials, Ogryzlo and Tang[6a] have first proposed that the quenching of $^1O_2$ by alkylamines may proceed via a charge-

† Kyoto University.
‡ Ehime University.

(1) For examples, see: (a) Adam, W. *Adv. Heterocycl. Chem.* **1977**, *21*, 437. (b) Krebs, A.; Schmalstieg, H.; Jarchow, O.; Klaska, K.-H. *Tetrahedron Lett.* **1980**, *21*, 3171. (c) Meijer, E. W.; Wymberg, H. *Ibid.* **1981**, *22*, 785. (d) Eberlein, G.; Bruce, T. C. *J. Am. Chem. Soc.* **1982**, *104*, 1449 and references therein.
(2) (a) Foote, C. S.; Dzakapasu, A. A.; Liu, J. W.-P. *Tetrahedron Lett.* **1975**, 1247. (b) Foote, C. S.; Liu, J. W.-P. *Ibid.* **1968**, 3267.
(3) (a) Thomas, M. J.; Foote, C. S. *Photochem. Photobiol.* **1978**, *24*, 683. (b) Matsuura, T.; Yoshimura, N.; Nishinaga, A.; Saito, I. *Tetrahedron* **1972**, *28*, 4933.
(4) (a) Foote, C. S.; Peters, J. W. *J. Am. Chem. Soc.* **1971**, *93*, 3795. (b) Kacher, M. L.; Foote, C. S. *Photochem. Photobiol.* **1979**, *29*, 765. (c) Gu, C.-L.; Foote, C. S.; Kacher, M. L. *J. Am. Chem. Soc.* **1981**, *103*, 5949. (d) Jongsma, S. J.; Cornelisse, J. *Tetrahedron Lett.* **1981**, *22*, 2919.
(5) Landis, M. E.; Madoux, D. C. *J. Am. Chem. Soc.* **1979**, *101*, 5106.
(6) (a) Ogryzlo, E. A.; Tang, C. W. *J. Am. Chem. Soc.* **1970**, *92*, 5034. (b) Furukawa, K.; Ogryzlo, E. A. *J. Photochem.* **1972**, *1*, 163. (c) Young, R. H.; Martin, R. L. *J. Am. Chem. Soc.* **1972**, *94*, 5183. (d) Monroe, B. M. *J. Phys. Chem.* **1977**, *81*, 1861. (e) Young, R. H.; Martin, R. L.; Feriozi, D.; Brewer, D.; Kayser, R. *Photochem. Photobiol.* **1973**, *17*, 233. (f) Young, R. H.; Brewer, D.; Kayser, R.; Martin, R.; Feriozi, D.; Keller, R. A. *Can. J. Chem.* **1974**, *52*, 2889.
(7) Harbour, J. R.; Issler, S. L. *J. Am. Chem. Soc.* **1982**, *104*, 903.
(8) (a) Peters, G.; Rodgers, M. A. J. *Biochem. Biophys. Res. Commun.* **1980**, *96*, 770. (b) Peters, G.; Rodgers, M. A. J. *Biochem. Biophys. Acta* **1981**, 637.
(9) Saito, I.; Matsuura, T.; Inoue, K. *J. Am. Chem. Soc.* **1981**, *103*, 188.
(10) Manring, L. E.; Foote, C. S. *J. Phys. Chem.* **1982**, *86*, 1257.
(11) Zaklika, K. A.; Kaskar, B.; Schaap, A. P. *J. Am. Chem. Soc.* **1980**, *102*, 386.
(12) (a) Davidson, R. S. *Mol. Assoc.* **1979**, *1*, 215. (b) Lewis, F. D. *Acc. Chem. Res.* **1979**, *12*, 152.
(13) For reviews, see: Bellus, D. *Adv. Photochem.* **1979**, *11*, 105.

0002-7863/83/1505-3200$01.50/0 © 1983 American Chemical Society

transfer intermediate. Gollnick and Lindner[14] observed that the photooxygenation rate of amines is also a function of their ionization potentials and proposed that both quenching and reaction of $^1O_2$ with amines pass through the common intermediates. Young et al.[6e] have also demonstrated that $^1O_2$ suffers physical quenching by a series of substituted *N,N*-dimethylanilines through partial charge-transfer intermediates in methanol, and they showed that the quenching rate should be more than 100 times greater than any possible oxidation of these amines in the same solvent. In the present paper, we report the detail of our results on the $O_2^{-}\cdot$ formation via one-electron transfer from substituted *N,N*-dimethylanilines to chemically generated $^1O_2$ in aqueous media. The yields of $O_2^{-}\cdot$ determined by the assay utilizing *p*-nitrotetrazolium blue (NBT) and superoxide dismutase (SOD) are compared to the calculated free energy change (ΔG) for full electron transfer as well as to the quenching rate constants. We will also discuss the mechanism of the quenching of $^1O_2$ by these amines.

Experimental Section

Ultraviolet spectra were recorded with a Shimazu UV-200 spectrophotometer. Proton magnetic spectra were recorded with a Varian T-60 spectrometer and carbon-13 magnetic resonance spectra were recorded with a Varian FT-80A spectrometer. High-performance liquid chromatography (HPLC) was performed on a Waters ALC/GPC 240 equipped with Nucleosil $5C_{18}$ column or with a radial pack A. The cyclic voltamagram was recorded with a Yanagimoto P-1000 in 0.04 M Britten–Robinson buffer (pH 7.5). All potentials in volts are referred to the saturated calomel reference electrode (SCE).

Superoxide dismutase (SOD, type I, 3400 units), ferricytochrome *c* (type III), and catalase (2400 units) were purchased from Sigma. *p*-Nitrotetrazolium blue (NBT), *N,N*-dimethyltoluidines, *p*-chloro-*N,N*-dimethylaniline, and *N,N*-dimethylaniline were purchased from Nakarai Chemicals. Other substituted *N,N*-dimethylanilines were prepared from the corresponding anilines according to the literature procedure.[15] Other chemicals were commerically available and used without further purification. Heat-denatured SOD was obtained by boiling SOD in water for 10 min. Doubly distilled water was used in all cases.

Preparation of 3-(1,4-Epidoxy-4-methyl-1,4-dihydro-1-naphthyl)-propionic Acid (1). A solution of 3-(4-methyl-1-naphthyl)propionic acid **2**[16] (2.1 g, 1 mM) in the presence of methylene blue (4×10^{-5} M) in dichloromethane–methanol (8:5) was irradiated with a 500-W tungsten–bromine lamp at 0 °C under oxygen bubbling for 8 h. After removal of the solvent at 0 °C, the residue was purified by rapid column chromatography (silica gel, precooled $CHCl_3$) to give pale yellow solids which contain **1** and a small amount of **2** (less than 10%). Further purification by column chromatography gave almost pure **1** (1.9 g): KI titration 96%; ^1H NMR ($CDCl_3$) δ 1.9 (s, 3 H, Me), 2.70–2.85 (m, 4 H, $-CH_2CH_2-$), 6.7 (s, 2 H, $-CH=CH-$), 7.2–7.4 (m, 4 H, aromatic H), 9.2 (br s, 1 H, COOH); ^{13}C NMR ($CDCl_3$–CD_3OD) δ 14.8, 24.2, 27.2, 77.8, 79.3, 119.3, 119.4, 125.8, 125.9, 136.0, 139.2, 139.6, 140.2, 174.5.

Generation of $^1O_2$ from Thermolysis of 1. The generation of $^1O_2$ from the thermolysis of **1** was confirmed by the following two methods.[17,18] (i) A solution containing imidazole (7.2 mM), nitrosodimethylaniline (0.04 mM) and **1** (1.7 mM) in 0.26 M phosphate buffer (pH 7.0) was kept under stirring at 35 ± 0.2 °C. The disappearance of nitrosodimethylaniline was monitored at 438 nm by UV spectroscopy.[17] (ii) The reaction with α-lipoic acid (5.9 mM) in $NaHCO_3$-saturated aqueous solution was monitored at 330 nm by UV spectroscopy in the presence of **1** (2 mM).[18] The yield of $^1O_2$ from the thermolysis of **1** was determined by the reaction of diphenylisobenzofuran (DPBF) (0.4 mM) with **1** (0.13 mM) in methanol. The disappearance of DPBF was measured at 410 nm by UV spectroscopy.

Determination of Activation Parameters. Solutions of **1** (each 0.17 mM) in phosphate buffer (pH 7.0) were shaken in a thermostat-maintained bath (±0.1 °C) at 40, 35, 30, and 26 °C. The decomposition of **1** was monitored by the appearance of 298-nm absorption of parent naphthalene **2** by UV spectroscopy. Activation energies were calculated from Arrhenius plots of the rate constants obtained from the first-order plots for the decomposition of **1**.

Detection of $O_2^{-}\cdot$. The amount of $O_2^{-}\cdot$ was determined from the formazan formation–time curves by using SOD.[19] The formazan formation–time curves were made by the preparation of solutions, each containing **1** (2.5 mM), *N,N*-dimethyl-*p*-anisidine (DMA, 1 mM), and NBT (1 mM) in 0.26 M phosphate buffer at appropriate pH in the presence or absence of SOD (140 units) in an Erlenmeyer flask with a jointed stopper. After flushing with N_2 the sample solutions were incubated at 35 ± 0.1 °C under rigorous shaking. After a fixed time of incubation a constant volume of DMF was added to dissolve formazan. The amount of formazan formation was measured at 560 nm by UV spectroscopy. The same procedure was used for the detection of $O_2^{-}\cdot$ in the reaction of other amines. The reduction of ferricytochrome *c* (0.05 mM) as a detector for $O_2^{-}\cdot$ was also used under the same conditions. Since ferricytochrome *c* contains a small amount of reduced form, the absorbance of ferrocytochrome *c* formed in the reaction was corrected by substracting the initial absorbance from the observed one.

Control Experiments for Generation of $O_2^{-}\cdot$. Paired solutions containing **1** (2.5 mM), DMA (1 mM), and NBT (1 mM) with and without SOD (140 units) in 0.26 M phosphate buffer (pH 7.5) were prepared as described above. Appropriate amounts of additives were then added to the reaction systems prior to incubation. In case of N_2O, N_2O gas was passed through the reaction mixture for 1 min. After incubation at 35 °C, a constant volume of DMF was added and the amount of formazan was measured by absorption at 560 nm in each case. The yield of $O_2^{-}\cdot$ was calculated from the difference of the absorbance in the absence and presence of SOD.

Determination of Quenching Rate Constants. The total quenching rate constants ($k_r + k_q$) for the reaction of $^1O_2$ with various substituted *N,N*-dimethylanilines were determined by Young's technique.[20] Solutions containing **1** (4.5×10^{-4} M), DPBF (5×10^{-5} M) and varying amounts of amine (0–~40 mM) in MeOH–H_2O (1:1) were shaken at 35 °C. The disappearance of DPBF was monitored at 411 nm by UV spectroscopy.

Measurement of Oxidation Potentials. Half-wave potentials of the amines were measured by cyclic voltammetry in 40 mM Britten–Robinson buffer (pH 7.5) in a cell containing a glassy carbon electrode, a Pt auxiliary electrode, and a SCE reference electrode at 25 ± 0.1 °C. a sample solution was bubbled with purified N_2 to remove dissolved oxygen for 20 min prior to analysis. N_2 was passed through the solution during analysis. The oxidation potentials of the amines were also measured by the methods of differential dropping mercury electrode under the same conditions. In all cases, half-peak oxidation potentials were employed as the half-wave oxidation potentials.

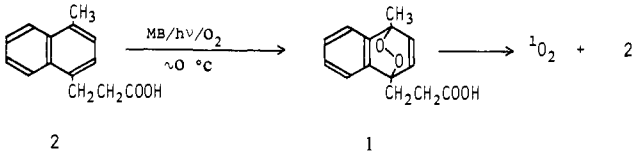
Reaction of DMA with $^1O_2$. The reaction of DMA with $^1O_2$ in the presence and absence of NBT was monitored by HPLC (eluent, MeOH–H_2O 85:15) with naphthalene as an internal standard. Solutions containing **1** (2.5 mM), DMA (1 mM) with and without NBT (1 or 5 mM) in 0.26 M phosphate buffer (pH 7.5) was shaken at 35 °C as described above. After addition of a constant volume of DMF and a standard solution, the disappearance of DMA was measured by HPLC. The peak areas were integrated with a Shimazu CA-1 autointegrator.

Results and Discussion

One of the most effective methods for detecting short-lived $O_2^{-}\cdot$ is the assay utilizing superoxide dismutase (SOD), owing to its highly specific and rapid ($k = 2 \times 10^9$ M^{-1} s^{-1})[19] reaction with $O_2^{-}\cdot$. In our attempts to determine the amount of $O_2^{-}\cdot$ formed in the reaction between *N,N*-dimethylanilines and $^1O_2$ in aqueous system, we needed a suitable chemical $^1O_2$ source. A water-soluble compound that can generate $^1O_2$ efficiently under mild conditions (up to 40 °C) and give no significant effect on the SOD activity is highly desirable. Photochemical methods can be used as $^1O_2$ sources only with special precaution, since irradiation of dye sensitizers in the presence of oxygen and electron donors may often produce $O_2^{-}\cdot$ with the intervention of photoexcited dyes[21] and the activity of SOD would probably be influenced by illumination. Various types of chemical $^1O_2$ sources have thus far been reported.[22] Among them 1,4-endoperoxides derived from substituted

(14) Gollnick, K.; Lindner, J. H. E. *Tetrahedron Lett.* **1973**, 1903.
(15) Zweig, A.; Laucaster, J. E.; Neglia, M. T.; Jura, W. H. *J. Am. Chem. Soc.* **1964**, *86*, 4130.
(16) Fieser, L. F.; Gates, M. D., Jr. *J. Am. Chem. Soc.* **1940**, *62*, 2335.
(17) Kraljic, I.; Mohsni, S. El. *Photochem. Photobiol.* **1978**, *28*, 577.
(18) Schaap, A. P.; Thayer, A. L.; Faler, G. R.; Goda, K.; Kimura, T. *J. Am. Chem. Soc.* **1974**, *96*, 4025.
(19) (a) McCord, J. M.; Fridovich, I. *J. Biol. Chem.* **1969**, *244*, 6049. (b) Klug, D.; Rabani, J.; Fridovich, I. *Ibid.* **1972**, *247*, 4839.
(20) Young, R. H. Wehrly, K.; Martin, R. L. *J. Am. Chem. Soc.* **1971**, *93*, 5774.
(21) (a) Usui, Y.; Koizumi, M. *Bull. Chem. Soc. Jpn.* **1967**, *40*, 440. (b) Beauchamp, C.; Fridovich, I. *Anal. Biochem.* **1971**, *44*, 276. (c) Srinivasan, V. S.; Podolski, D.; Westrick, N. J.; Neckers, D. C. *J. Am. Chem. Soc.* **1978**, *100*, 6513. (d) Cox, G. S.; Whitten, D. G. *Chem. Phys. Lett.* **1980**, *67*, 511. (e) Steichen, D. S. Foote, C. S. *J. Am. Chem. Soc.* **1981**, *103*, 1855 and references therein.

Scheme I



2 1

naphthalenes are known to release $^1O_2$ under very mild conditions.[23] In addition, the half-lives for the decomposition naphthalene endoperoxides to release $^1O_2$ are controlled by introducing alkyl substituents in appropriate positions of the naphthalene ring.[23] Thus we have chosen 3-(4-methyl-1-naphthyl)propionic acid (**2**) as a parent naphthalene. Methylene blue (MB) sensitized photooxygenation of **2** in dichloromethane–methanol at 0 °C followed by rapid column chromatography gave the corresponding naphthalene 1,4-endoperoxide **1** in 80% yield. The endoperoxide **1** can be handled without difficulty at temperatures below 15 °C. The confirmation of the generation of $^1O_2$ from **1** was made by the reaction either with α-lipoic acid[18] or with a mixture of imidazole and nitrosodimethylaniline,[17] an assay for detecting $^1O_2$ in water. For example, the absorbance of α-lipoic acid at 330 nm in the presence of **1** decreased with increasing reaction time at 35 °C, indicating the liberation of $^1O_2$ from **1**. Warming the solution of **1** at 35 °C produced parent naphthalenes **2** with liberation of $^1O_2$, and the efficiency for the $^1O_2$ generation was estimated to be more than 82% by trapping reaction using DPBF in methanol. The rate constants for the decomposition of **1** at the temperature ranging from 25 to 40 °C were determined in phosphate buffer at pH 7.0. The first-order rate constants were 4.9×10^{-4} s^{-1} at 35 °C and 3.1×10^{-4} s^{-1} at 30 °C, which correspond to the activation energy (E_a) of 17 kcal/mol. The endoperoxide **1** was soluble up to 10 mM in 0.26 M phosphate buffer at pH 7.5. Thus the endoperoxide **1** may be used as a convenient, mechanistically less complicated, $^1O_2$ source for the singlet oxygen reactions of biological systems in aqueous system under mild conditions. The usefulness of **1** as a $^1O_2$ source has already been demonstrated in several systems.[24]

Formation of Superoxide Ion from Singlet Oxygen. The quenching of $^1O_2$ with aliphatic or aromatic amines has been proposed to proceed via a charge-transfer-like complex.[6] Spin inversion within a charge-transfer complex would result in physical quenching of $^1O_2$, whereas chemical reaction within the complex may yield intermediate(s) for the oxidation products as proposed by Gollnick.[14] However, in polar aqueous solvent with amines of low oxidation potentials, the charge-transfer-like complex would be expected to dissociate to amine radical cation and O_2^-. The radical cation–O_2^-· pair thus formed would be extremely short lived because of the rapid reverse electron transfer and the possible chemical reaction within a cage. In our attempts to detect such short-lived O_2^-· in the reactioin of substituted *N,N*-dimethylanilines with $^1O_2$ in aqueous medium, a combination of *p*-nitrotetrazolium blue (NBT) and SOD was used as a detecting reagent.[25] In some cases ferricytochrome *c* was also used in place of NBT.[26] NBT[27] and ferricytochrome *c*[26] react with O_2^-· with the rate constants of 6×10^4 and 5×10^5 M^{-1} s^{-1}, respectively.

When an anerobic solution of **1**, *N,N*-dimethyl-*p*-anisidine (DMA), and NBT (1 mM) in 0.2 M phosphate buffer at pH 7.5 was shaken at 35 °C, NBT was reduced to formazan which was detectable by its characteristic UV absorption at 560 nm. The formation of formazan increased with increasing incubation time (Figure 1). The O_2^-· inhibiting enzyme, SOD, substantially inhibited the formazan formation, whether it was added during or prior to the reaction, whereas the addition of heat-denaturated SOD did not retard the formazan formation. As shown in Figure 2, the extent of the inhibition of NBT reduction by SOD increased with increasing units of added SOD and approached a maximum inhibition at 140 units/mg of SOD. The value (60%) for the maximum inhibition implies that the other 40% of the total NBT reduction arises from uncharacterized O_2^-· independent reaction(s). Since NBT can be reduced by electron-donating species other than O_2^-· the background NBT reduction in the presence of SOD (Figure 1, curve c) was substracted from the total NBT reduction as frequently employed in the quantification of O_2^-· using NBT.[19,28] From the known stoichiometry[28,29] of the NBT reduction by O_2^-· and the amount of the NBT reduction inhibited by SOD, the yield

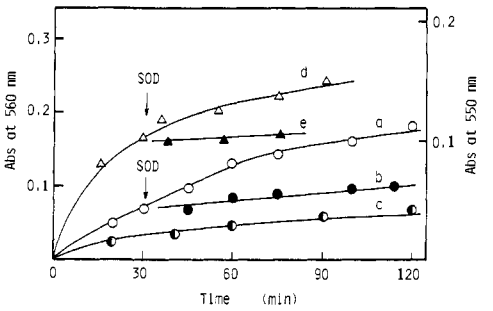


Figure 1. Reduction of NBT and ferricytochrome *c* during incubation of the endoperoxide **1** and DMA in the presence and absence of SOD in phosphate buffer (pH 7.5) at 35 °C. Curve (a) ○, (b) ●, and (c) ◐ for reduction of NBT (left scale); curve (d) △ and (e) ▲ for reduction of ferricytochrome *c* (right scale). SOD (140 units) was added at the point indicated by arrow (b,e) or prior to the incubation (c). [**1**] = 2 mM; [DMA] = 1 mM; [NBT] = 1 mM; [ferricytochrome *c*] = 0.05 mM.

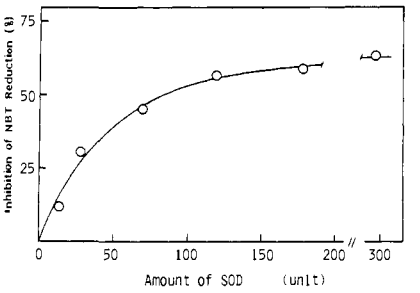
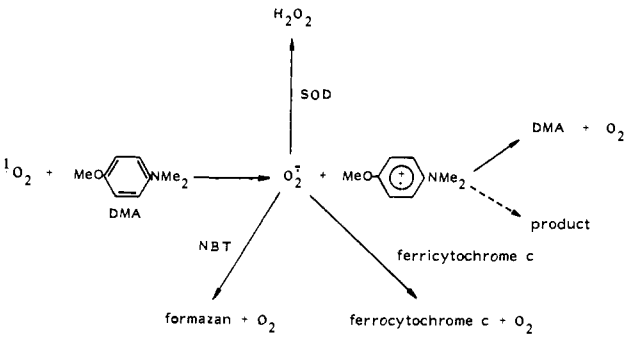


Figure 2. Inhibition of the reduction of NBT by SOD for the reaction of **1** with DMA in phosphate buffer (pH 7.5) at 35 °C for 2 h. [**1**] = 2.5 mM; [DMA] = 1 mM; [NBT] = 1 mM.

Scheme II



(22) (a) Denny, R. W.; Nickon, A. In "Organic Reaction"; Wiley: New York, 1973; Vol. 20, p 133. (b) Murray, R. W. "Singlet Oxygen"; Wasserman, H. H., Murray, R. W., Eds.; Academic Press: New York, 1979; p 59.
(23) (a) Saito, I.; Matsuura, T., ref 22b; p 511. (b) Wasserman, H. H. Larsen, D. L. *J. Chem. Soc., Chem. Commun.* **1972**, 253. (c) Saito, I.; Nagata, W.; Matsuura, T. *Tetrahedron Lett.* **1981**, *22*, 4231.
(24) (a) Inoue, K.; Matsuura, T.; Saito, I. *Photochem. Photobiol.* **1982**, *35*, 133. (b) Inoue, K.; Matsuura, T.; Saito, I. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 2101.
(25) Lee-Ruff, E. *Chem. Soc. Rev.* **1977**, *6*, 195.
(26) Butler, J.; Jayson, G. G.; Swallow, A. J. *Biochim. Biophys. Acta* **1975**, *408*, 215.
(27) Bielski, B. H. J.; Richter, H. W. *J. Am. Chem. Soc.* **1977**, *99*, 3019.
(28) Rapp, U.; Adams, W. C.; Miller, R. W. *Can. J. Biochem.* **1973**, *51*, 159.
(29) Recently, Bielski et al.[30] reported that the reaction of NBT with O_2^-· is very complex. In the present experiments we calculated the amount of O_2^-· by using the stoichiometry in which 1 mol of NBT is reduced by 4 mol of O_2^-· to form 1 mol of formazan.[27,28]

Table I. Effect of Various Additives on the Formation of Superoxide Ion in the Reaction of *N,N*-Dimethyl-*p*-anisidine with Singlet Oxygen in Phosphate Buffer (pH 7.5) at 35 °C (2 h)

system	additive	superoxide ion,[a] ×10² mM	yield,[b] %
1, DMA, NBT[h]		1.02	1.1
		1.70[c]	1.8
		0[d]	0
	NaN_3 15 mM)	0.35	0.37
	i-PrOH (2 M)	0.97	1.04
	N_2O[e]	0.83	0.89
	catalase[f]	1.09	1.18
DMA, NBT	O_2 bubbling[g]	0.25	0.26
	H_2O_2 (2.5 mM)	0	0
1, NBT	H_2O_2 (2.5 mM)	0	0

[a] Calculated from the difference of the formazan formation between the absence and the presence of SOD. [b] Yield based on DMA initially used. [c] In $NaHCO_3$ saturated aqueous solution (pH 8.3). [d] At 18 °C. [e] See the Experimental Section. [f] 2400 units/mg. [g] For 4-h reaction. [h] [1] = 2 mM; [DMA] = 1 mM; [NBT] = 1 mM.

of the $O_2^{-}\cdot$ trapped by NBT is calculated to be 1.1% on the basis of DMA initially used after 2-h reaction with 1 mM of NBT under the specific conditions. When a higher concentration of NBT (3 mM) was used under otherwise identical conditions, the yield of $O_2^{-}\cdot$ increased up to 1.9%. However, further addition of NBT no more increased the yield of $O_2^{-}\cdot$. The rate of $O_2^{-}\cdot$ is also known to be pH dependent with the longer lifetime at higher pH,[31] whereas the activity of SOD was reported to be invariable over the pH range (pH 4.8–9.5).[19b] Since the dismutation of $O_2^{-}\cdot$ competes with the reaction with NBT, the yield of $O_2^{-}\cdot$ would become higher at increasing pH. As expected, the yield of $O_2^{-}\cdot$ at 2-h reaction with 1 mM of NBT increases as follows: 0.2% at pH 7.0, 1.1% at pH 7.5, and 1.7% at pH 8.0. Similar results have been obtained when ferricytochrome *c* was used as a $O_2^{-}\cdot$ detecting reagent in place of NBT, where the formation of ferricytochrome *c* was monitored by its UV absorption at 550 nm (Figure 1, curves d and e).

The following control experiments confirmed that $O_2^{-}\cdot$ is resulted from a direct reaction between $^1O_2$ and DMA (see Table I): (i) Incubation of **1** and NBT without DMA never produced $O_2^{-}\cdot$ under the conditions. (ii) Autoxidation of DMA under oxygen bubbling was negligibly slow, and only a small amount of $O_2^{-}\cdot$ (0.26%) was produced even after prolonged reaction (4 h). (iii) Incubation of the complete system at below 20 °C, where the generation of $^1O_2$ from **1** is completely suppressed, never produced $O_2^{-}\cdot$. (iv) $O_2^{-}\cdot$ was not formed when H_2O_2 was added in place of **1**. (v) Addition of a $^1O_2$ quencher, NaN_3 (15 mM), to the reaction system inhibited 60% of the $O_2^{-}\cdot$ formation. In this context, Harbour and Issler have recently suggested the possibility that $O_2^{-}\cdot$ is formed by a direct reaction of azide anion with $^1O_2$ in the quenching process.[7] However, in an independent experiment utilizing **1** (2 mM), NaN_3 (15 mM), and NBT (1 mM) in phosphate buffer (pH 7.5), the formation of $O_2^{-}\cdot$ could not be observed under our experimental conditions. The possibility that $O_2^{-}\cdot$ is formed by the decomposition of hydroperoxidic products formed via a $^1O_2$-initiated radical chain autoxidation[5,32] of DMA under the aqueous alkaline conditions is ruled out by the following facts: (i) Addition of N_2O or 2-propanol as a free radical scavenger to the reaction system did not affect the $O_2^{-}\cdot$ formation appreciably. (ii) The yield of $O_2^{-}\cdot$ was not changed whether the reaction was conducted under anerobic conditions or under oxygen bubbling (Table I). Furthermore, addition of catalase did not inhibit the $O_2^{-}\cdot$ formation. It has been reported that radical ion intermediates are formed via electron-transfer in the reactions of peroxides with electron donors.[33,34] For example, the reaction of certain endoperoxides with TMPD is presumed to proceed via a charge-transfer-like complex.[34] In the present case, however, a bimolecular reaction between **1** and DMA or NBT was negligible since the rate constant for the decomposition of **1** is not changed by the addition of DMA or NBT. All the results in Table I support the direct involvement of $^1O_2$ in the formation of $O_2^{-}\cdot$.

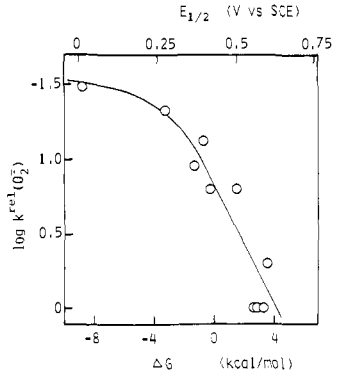


Figure 3. Plot of the logarithm of the relative yield of $O_2^{-}\cdot$ against free energy change for full electron transfer (lower scale) and against half-wave oxidation potentials of amines (upper scale).

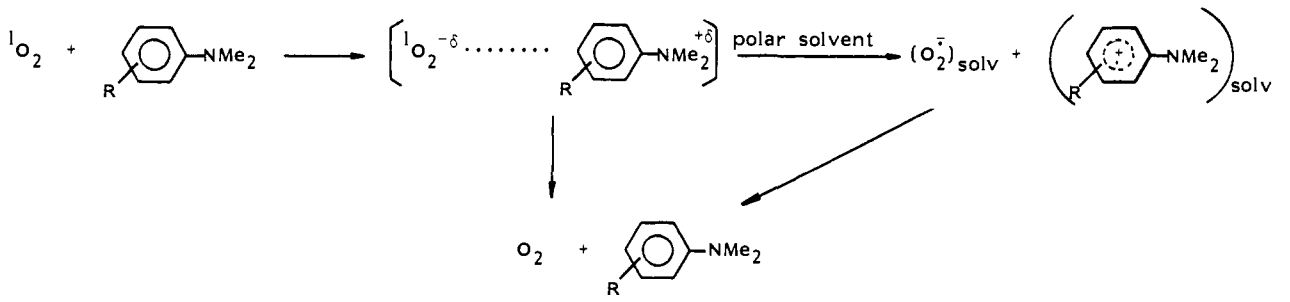
Under the conditions, a 2-h reaction of **1** and DMA resulted in only 3% conversion of DMA while the decomposition of **1** being more than 80% as evidenced by HPLC analysis. The result is consistent with the previous finding[6e] that the quenching rates are 2 orders of magnitude greater than the oxidation rates in the reaction of $^1O_2$ with *N,N*-dimethylanilines in methanol. Thus, it seems highly probable that the major portion of the $O_2^{-}\cdot$ formation results from the quenching process rather than the oxidation reaction.

The formation of $O_2^{-}\cdot$ from the reaction of **1** with other substituted *N,N*-dimethylanilines was next examined under the same conditions. The yields of $O_2^{-}\cdot$ and the oxidation potentials of these amines measured at pH 7.5 are listed in Table III along with the quenching rate constants. The amines having oxidation potentials less than 0.5 V vs. SCE are all effective for the generation of $O_2^{-}\cdot$. Particularly, TMPD is the most effective for the generation of $O_2^{-}\cdot$ (9.2% yield), in accordance with the recent observation[10] that the reaction of $^1O_2$ and TMPD gives $TMPD^{+}\cdot$ via one-electron transfer in aqueous solvent. In contrast, *p*-chloro-*N,N*-dimethylaniline and *N,N*-dimethylaniline, both of which have higher oxidation potentials, did not produce $O_2^{-}\cdot$ under the conditions. Thus the yields of $O_2^{-}\cdot$ appear to correlate with the oxidation potentials of these amines. A plot of the log of the relative yield of $O_2^{-}\cdot$ against the calculated free energy change (ΔG) for full electron transfer is shown in Figure 3. We are not confident whether the amount of $O_2^{-}\cdot$ trapped by NBT accurately corresponds to the rate of the electron transfer, owing to the inherent complex redox reactions involving NBT.[30] Nevertheless, the relationship shown in Figure 3 is remarkably similar to that observed in the quenching of $^1O_2$ by these aromatic amines (vide infra).

All of these results strongly suggest that $O_2^{-}\cdot$ is formed via one-electron transfer from the amines to $^1O_2$ during the quenching process. Manring and Foote[10] reported the formation of $TMPD^{+}\cdot$ in the reaction of $^1O_2$ in aqueous medium but could not observe the cation radical for any other electron-rich aromatic amines, including DMA, under the same conditions. This is probably due to too low concentrations of the cation radical formed to be detected by laser flash spectroscopy in the case of DMA. It is likely that the initially formed charge-transfer-like complex between $^1O_2$ and DMA decay to ground state very rapidly but in competition with the dissociation of free ions in aqueous medium. In

(30) Bielski, B. H. J.; Shive, G. G.; Bajuk, S. *J. Phys. Chem.* **1980**, *84*, 830.
(31) Bielski, B. H. J.; Allen, A. O. *J. Phys. Chem.* **1977**, *81*, 1048.
(32) (a) Tang, R.; Yue, H.; Wolf, J. F.; Mares, F. *J. Am. Chem. Soc.* **1978**, *100*, 5248. (b) Schaap, A. P.; Zaklika, K. A.; Kaskar, B.; Fung, L. W.-M. *Ibid.* **1980**, *102*, 389 and references therein.
(33) Zupancic, J. J.; Horn, K. A.; Schuster, G. B. *J. Am. Chem. Soc.* **1980**, *102*, 5279 and references therein.
(34) Boyd, J. D.; Foote, C. S.; Imagawa, D. K. *J. Am. Chem. Soc.* **1980**, *102*, 3641.

Scheme III



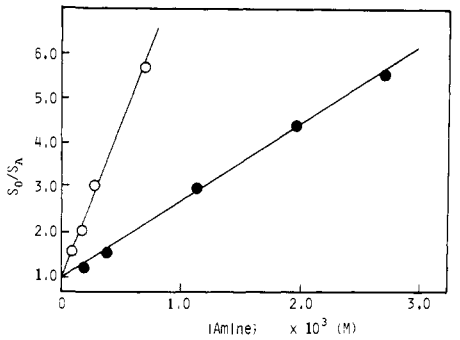


Figure 4. Plots of S_0/S_A vs. amine concentration. (●) for DMA and (○) for TMPD.

case of less electron-rich aromatic amines, the decay rate is so fast that the formation of $O_2^{-}\cdot$ is not observable as in the cases of *N,N*-dimethylaniline and *p*-chloro-*N,N*-dimethylaniline.

Determination of Quenching Rate Constant. We have determined the quenching rate constants of chemically generated $^1O_2$ by these *N,N*-dimethylanilines in 1:1 methanol–water by using the kinetic technique developed by Young et al.[20] DPBF was used as the $^1O_2$ acceptor. In this case, the following kinetic scheme can be written.

$$^1O_2 + \text{amine} \xrightarrow{k_q} O_2 + \text{amine}$$

$$^1O_2 + \text{amine} \xrightarrow{k_r} \text{product}$$

$$^1O_2 + \text{DPBF} \xrightarrow{k_A} \text{product}$$

$$^1O_2 \xrightarrow{k_d} O_2$$

Application of steady-state treatment gives eq 1, where K is the

$$-\frac{d[DPBF]}{dt} = K\left(\frac{k_A[DPBF]}{k_A[DPBF] + k_r[amine] + k_q[amine] + k_d}\right) \quad (1)$$

rate of formation of $^1O_2$. Since the concentration of DPBF is very low, the above equation can be approximated as eq 2. This

$$-\frac{d[DPBF]}{dt} = K\left(\frac{k_A[DPBF]}{k_r[amine] + k_q[amine] + k_d}\right) \quad (2)$$

approximation give linear plots for the log of the disappearance of DPBF vs. time whose slopes are given by eq 3. A plot of S_0/S_A

$$\text{slope} = K\left(\frac{k_A}{k_r[amine] + k_q[amine] + k_d}\right) \quad (3)$$

(slopes in the absence and presence of amine) vs. amine concentration gives a slope of $(k_r + k_q)/k_d$. The overall quenching rate constant $k_Q = (k_r + k_q)$ can be calculated from an average k_d for $^1O_2$ in methanol–water ($k_d = 2.81 \times 10^5\ s^{-1}$).[35] As shown in Figure 4, the plots of S_0/S_A vs. amine concentration gave straight lines with slopes of 6.6×10^3 and 1.6×10^3 M for TMPD and for DMA, respectively. The k_Q values are calculated to be $1.8 \times 10^9\ M^{-1}\ s^{-1}$ for TMPD and $4.5 \times 10^8\ M^{-1}\ s^{-1}$ for DMA. Among these amines, TMPD is the most effective $^1O_2$ quencher and the quenching rate is fairly close to the diffusion-controlled limit in water, $6.4 \times 10^9\ M^{-1}\ s^{-1}$.[36] The quenching rate constants obtained for other amines are summarized in Table II. The observed quenching rates in 1:1 methanol–water are somewhat larger than the reported values obtained in methanol. The solvent effect is consistent with the involvement of a polar transition state in the quenching reaction. A Hammett plot also gave a straight line with σ, giving a ρ value of −1.54, as indicated in Figure 5. This ρ value is in fairly good agreement with the values obtained from the literature, i.e., −1.39[6e] or −1.70[6f] for *N,N*-dimethylanilines

Table II. Rate Constants (k_Q) for the Quenching of Singlet Oxygen by Substituted *N,N*-Dimethylanilines and the Yields of Superoxide Ion

N,N-dimethyl-aniline	k_Q,[a] $\times 10^8$ $M^{-1}\ s^{-1}$	$E_{1/2}$,[b] V vs. SCE	ΔG,[c] kcal/mol	% yield of superoxide ion[d] (rel yield)
p-N(Me)$_2$	18.2 (10.0[e])	0.05	−8.73	9.2 (30.7)
o-N(Me)$_2$	11.0	0.29	−3.20	6.2 (20.7)
3,4-MeO)$_2$	8.8	0.37	−1.35	2.7 (9.0)
m-N(Me)$_2$	7.4	0.40	−0.66	4.0 (13.3)
p-MeO	4.4 (1.8[e])	0.42	−0.20	1.9 (6.3)
o-MeO	3.2	0.50	1.64	1.9 (6.3)
2,4,6-(Me)$_3$	1.5	0.54	2.56	0.3 (1.0)
2,4-(Me)$_2$	1.3	0.57	3.25	0.3 (1.0)
p-Me	1.1 (1.2[e])	0.55	2.80	0.3 (1.0)
m-MeO	0.84 (0.48[e])	0.60	3.95	0.6 (2.0)
H	0.36 (0.73[e])	0.73	6.95	ND[f]
p-Cl	0.36	0.76	7.64	ND[f]

[a] Determined in 1:1 methanol–water. [b] Measured in Britten–Robinson buffer (pH 7.5) at 25 °C. [c] Calculated from eq 4. [d] Obtained from the reaction of **1** (2.5 mM), amine (1 mM), and NBT (3 mM) in phosphate buffer (pH 7.5) at 35 °C for 2 h. [e] Reference 6f. [f] Not detected.

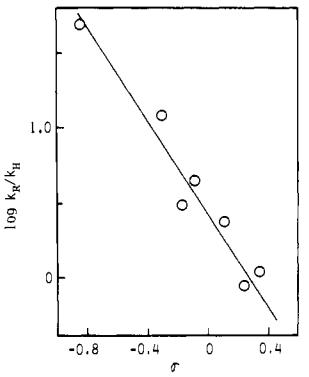


Figure 5. Hammett plot vs. σ for substituted *N,N*-dimethylanilines in the quenching reaction of $^1O_2$.

(35) (a) Merkel, P. B.; Kearns, D. R. *J. Am. Chem. Soc.* **1972**, *94*, 7244. (b) Young, R. H.; Brewer, D.; Keller, R. A. *Ibid.* **1973**, *95*, 375.

(36) Calvert, J. G.; Pitts, J. N., Jr. "Photochemistry"; Wiley: New York, 1966; p 128.

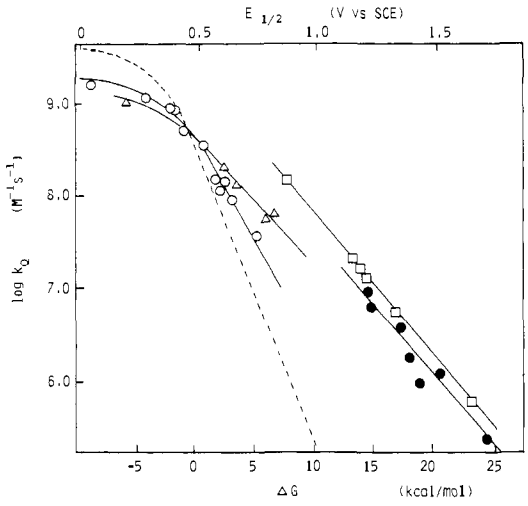


Figure 6. Plots of the logarithm of the quenching rate constants of $^1O_2$ against free energy change for electron transfer (lower scale) and against half-wave oxidation potentials (upper scale); *N,N*-dimethylanilines in methanol–water (○, this work); phenols[3a] (●); methoxybenzenes[4] (□); *N,N*-dimethylanilines in methanol[6e] (△). The reported quenching rate constants are the values in methanol. The broken line represents the expected relationship for full electron transfer reported by Rehm and Weller.[37]

and −1.72 for phenols.[3a] The negative ρ values obtained for the *N,N*-dimethylanilines also suggests a significant charge-transfer character in the intermediate involved in the quenching reaction of $^1O_2$.

Quenching Mechanism. The free energy change (ΔG) associated with electron-transfer process is represented by the following Weller equation (eq 4)[37] where $E(D/D^+\cdot)$ is the oxidation po-

$$\Delta G = 23.06[E(D/D^+\cdot) - E(A^-\cdot/A)] - e^2/\epsilon\alpha - \Delta E_{0,0} \qquad (4)$$

tential of electron donor, $E(A^-\cdot/A)$ the reduction potential of electron acceptor, $\Delta E_{0,0}$ the excited energy of a reacting molecule. $e^2/\epsilon\alpha$ is the Coulombic attraction term, where ϵ and α denote the dielectric constant of solvent and the radical ion distance, respectively. The ΔG values are calculated by using 0.57 V vs. SCE[38] (pH 7.2) for $E(O_2^-\cdot/O_2)$, 22.5 kcal/mol for $\Delta E_{0,0}$, and 0.53 kcal/mol[39] for $e^2/\epsilon\alpha$. The calculated ΔG values ranging from −8.7 to 7.6 kcal/mol are listed in Table II. In Figure 6 is shown the plot of the log of the total quenching rate (k_Q) vs. ΔG. In the exothermic region (ΔG = −8.7 to ~−3.2 kcal/mol), the total quenching rate (k_Q) is fairly close to a diffusion-controlled limit. In the endothermic region, the plot of the log k_Q vs. ΔG is linear with a slope of −0.19 ± 0.05 mol/kcal. It was reported that the limiting slope of the log of the quenching rate against ΔG is −0.73 mol/kcal in the region where ΔG exceeds 5 kcal/mol.[37] Rehm and Weller[37] also reported that the plot of the log of the rate of fluorescence quenching of benzanthracene and pyrene-4-carboxylic acid by quenchers, including aromatic amines, against ΔG is curved, with a slope of −0.3 ± 0.07 mol/kcal in the endothermic region. If one assumes that the fluorescence quenching of benzanthracene or pyrene-4-carboxylic acid proceed via full electron transfer, comparison of this slope to that for the quenching of $^1O_2$ suggests that the quenching of $^1O_2$ by *N,N*-dimethylanilines in methanol–water has about 60% of the charge transfer expected for full electron transfer. A similar argument has been made by Thomas and Foote[3a] who observed the slope of −0.13 mol/kcal (−3.1 V^{-1}) for $^1O_2$–phenol system in organic solvents and suggested that the phenol reaction has about 44% of the charge-transfer character expected for full electron transfer. If the quenching reaction of $^1O_2$ with these amines is conducted in phosphate buffer at alkaline pH rather than in aqueous methanol, a more significant contribution of the charge-transfer complex would be expected in the transition state. In fact, the formation of $O_2^-\cdot$ was actually observed under such conditions as mentioned earlier. Unfortunately, the quenching rates could not be measured in a buffered aqueous solution because of the insolubility of DPBF in the solvent. It is interesting here to compare this slope to those for phenols,[3a] methoxybenzenes,[41] and *N,N*-dimethylanilines[6e] reported in methanol. As shown in Figure 6, the plots for phenols and methoxybenzenes have a same slope (−0.13 mol/kcal), whereas the slope for *N,N*-dimethylanilines reported by Young et al. is −0.12 mol/kcal in methanol.[3a,6e] Small but similar values of these slopes suggest that all the compounds interact with $^1O_2$ by similar mechanisms. Interestingly, in the experiment utilizing chemically generated $^1O_2$ in aqueous methanol, the slope (−0.19 mol/kcal) for the *N,N*-dimethylanilines is slightly larger than the reported value in pure methanol (−0.12 mol/kcal), suggesting that there is a more significant contribution of charge-transfer character in the quenching process in aqueous solvents, although the slope does not necessarily represent the degree of charge transferred at the reaction transition state as pointed out by Schuster et al.[42]

Mechanism of $O_2^-\cdot$ Formation. It was confirmed that the formation of $O_2^-\cdot$ is resulted from a direct reaction between $^1O_2$ and an electron-rich *N,N*-dimethylaniline in buffered aqueous solutions. Among these amines, TMPD is the most effective for the generation of $O_2^-\cdot$. TMPD quenches $^1O_2$ with the rate constant of 1.8×10^9 M^{-1} s^{-1} in 1:1 methanol–water and is expected to have much larger quenching rate constant in buffered solution. As mentioned earlier, the transition state for the quenching of $^1O_2$ by TMPD has a significant charge-transfer character. In highly polar aqueous solvent, the charge-transfer complex would rapidly dissociate to $TMPD^+\cdot$ and $O_2^-\cdot$. Consistent with these results is the recent report by Manring and Foote[10] that the formation $TMPD^+\cdot$ ($k_r = 3.3 \times 10^9$ M^{-1} s^{-1}) is only detectable in aqueous solvent, suggesting the importance of the solvation of the resulting ion pair. In fact, the reduction of NBT in the reaction of **1** with DMA was negligibly slow in ethanol. With less electron-rich amines such as DMA, the charge-transfer complex dissociates to free ions with only a slower rate compared to the decay rate to ground state. As a result, only a small amount (1–~2%) of $O_2^-\cdot$ can be detected even in aqueous solvent. The correlation of the log k_{rel} ($O_2^-\cdot$) against ΔG shown in Figure 3 is entirely consistent with the electron-transfer mechanism for the formation of $O_2^-\cdot$. The present results demonstrate that one of the major modes of the interaction of $^1O_2$ with electron-rich TMPD is one-electron transfer and that the electron transfer giving rise to $O_2^-\cdot$ is only possible for the amines with oxidation potentials less than ~0.5 V vs. SCE in highly polar aqueous solvents.

In conclusion, the results described here clearly demonstrate that the generation of $O_2^-\cdot$ from the reaction of $^1O_2$ with electron donors via one-electron transfer is indeed a viable process. There is much current interest in uncovering chemical processes which could give rise to $O_2^-\cdot$ in vivo.[43,44] There are a number of electron-rich substrates with oxidation potentials less than ~0.5 V vs. SCE in biological systems such as mitochondrial components.[8c] These substrates may well generate $O_2^-\cdot$ upon interaction with $^1O_2$ by a similar electron-transfer mechanism as suggested by Peters and Rodgers.[8a] Furthermore, the water-soluble endoperoxide **1** may be used as a mechanistically less complicated singlet oxygen source for the singlet oxygen reactions of biological systems in aqueous system.

Acknowledgment. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education of Japan.

(37) Rehm, D.; Weller, A. *Isr. J. Chem.* **1970**, *8*, 259.
(38) (a) Ilau, Y. A.; Meisel, D., Czapski, G. *Isr. J. Chem.* **1974**, *12*, 891. (b) Sawyer, D. T.; Seo, E. T. *Inorg. Chem.* **1977**, *16*, 499. (c) Koppenol, W. H. *Nature* (London) **1976**, *262*, 420. We thank Professor Koppenol for informing us of the references.
(39) The distance of radical ion was assumed to be 7 Å.[40]
(40) Vogelmann, E.; Rauscher, W.; Kramer, H. E. A. *Photochem. Photobiol.* **1979**, *29*, 771.
(41) Saito, I.; Imuta, M.; Matsuura, T. *Tetrahedron* **1972**, *28*, 5307. See also ref 3a.
(42) Scandola, F.; Balzani, V.; Schuster, G. B. *J. Am. Chem. Soc.* **1981**, *103*, 2519.
(43) Michlson, A. M.; McCord, J. M.; Fridorich, I. "Superoxide and Superoxide Dismutases"; Academic Press; New York, 1977.
(44) Sawyer, D. T.; Gibian, M. J. *Tetrahedron* **1979**, *35*, 1471.

K.I. is grateful for financial support by the Japan Society for the Promotion of Science.

Registry No. 1, 76673-35-3; **2**, 76673-34-2; $^1O_2$, 7782-44-7; $O_2^{-\cdot}$, 11062-77-4; NaN_3, 26628-22-8; *i*-PrOH, 67-63-0; N_2O, 10024-97-2; SOD, 9054-89-1; DPBF, 5471-63-6; NBT, 298-83-9; methylene blue, 61-73-4; imidazole, 288-32-4; *p*-nitroso-*N*,*N*-dimethylaniline, 138-89-6; α-lipoic acid, 62-46-4; ferricytochrome *c*, 9007-43-6; catalase, 9001-05-2; *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenyldiamine, 100-22-1; *N*,*N*,*N*′,*N*′-tetramethyl-*o*-phenylenediamine, 704-01-8; 3,4-dimethoxy-*N*,*N*-dimethylaniline, 2748-79-0; *N*,*N*,*N*′,*N*′-tetramethyl-*m*-phenylenediamine, 22440-93-3; *N*,*N*-dimethyl-*p*-methoxyaniline, 701-56-4; *N*,*N*-dimethyl-*o*-methoxyaniline, 700-75-4; *N*,*N*,2,4,6-pentamethylaniline, 13021-15-3; *N*,*N*,2,4-tetramethylaniline, 769-53-9; *N*,*N*,*p*-trimethylaniline, 99-97-8; *m*-methoxy-*N*,*N*-dimethylaniline, 15799-79-8; *N*,*N*-dimethylaniline, 121-69-7; *p*-chloro-*N*,*N*-dimethylaniline, 698-69-1.

Intermolecular Interactions of the C–F Bond: The Crystallographic Environment of Fluorinated Carboxylic Acids and Related Structures

Peter Murray-Rust,[1a] William C. Stallings,[1b] Claire T. Monti, Robert K. Preston, and Jenny P. Glusker*

Contribution from the Institute for Cancer Research, The Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111. *Received July 26, 1982*

Abstract: The structure of the 1:2 complex of a monoethyl ester of (+)-*erythro*-fluorocitrate and (−)-methylbenzylamine was determined by X-ray crystallographic methods and refined to $R = 0.049$. The fluorocitrate portion of the molecule has a similar backbone conformation to that determined in earlier studies by us for two other fluorocitrates, with a gauche arrangement of the F–C–C–OH group. The hydrogen bonding from the carboxyl group to the nitrogen atom of the cation is such that the adjacent fluorine atom also lies near this cation with H··O and H··F distances of 2.00 (2) and 2.29 (1) Å and N–H··O,F angles of 159 and 124°, respectively. Thus, while fluorine does not form a strong hydrogen bond, some interaction appears present. In order to examine the generality of this observation, and also the observation in our earlier paper on rubidium ammonium fluorocitrate that the fluorine took part in the coordination sphere of the metal cation, the Cambridge Crystallographic Data File was searched for similar interactions of C–F bonds. It appears that the C–F bond is capable of significant interactions with alkali metal cations and with proton donors, although these are generally weaker than the corresponding ones involving C–O and C–N groups. The examples found in the data file are discussed in detail.

The biochemistry of fluorine-containing compounds has been studied extensively by Peters,[2] Kun,[3] Walsh[4] and their co-workers. Such studies[5] have mainly centered around the replacement of a C–H bond by a C–F bond and the resulting behavior of the fluorinated substrate analogue in the active site of an enzyme or receptor. Fluorine is small and the C–F bond is slightly shorter than a C–OH bond, but the high electronegativity of fluorine[6] might be expected to cause it to behave differently from hydrogen. For example, it has been shown[4,7] that the electron-withdrawing ability of fluorine is sufficient to cause sodium fluoropyruvate to exist mainly as the *gem*-diol, rather than as the carbonyl form, even in an aqueous environment.

When fluorine is substituted for hydrogen in a C–H bond of a substrate of an enzyme, the resulting behavior of the fluorinated compound in the active site of the enzyme is of interest. Often the fluoro analogue behaves initially as a competitive inhibitor of the enzyme. In some cases this inhibition ultimately becomes irreversible, possibly through the formation of covalent links and/or loss of F^-. Such an inhibition and inactivation can occur in vivo when animals eat plants containing fluoroacetate.[2] Fluoroacetate is converted to fluorocitrate by the enzyme citrate synthase and violently toxic effects result. This toxicity is believed to occur because fluorocitrate, instead of the normal substrate citrate, inhibits and ultimately inactivates the enzyme aconitase.[2,8] This action of a fluorinated substrate is referred to by Peters[2] as a "lethal synthesis" since the fluorocitrate synthesized in vivo is the toxic agent, not the ingested fluoroacetate.

Experiments on the absolute configuration of the biochemically active isomer of fluorocitrate[8-10] have shown that the fluorine atom has been substituted in the area of the citrate molecule that is *not* acted on by the enzyme aconitase (the "aconitase-inactive" end of citrate). As a result of a study of the crystal structure of the rubidium salt containing the active isomer we were able to show that the fluorine atom in fluorocitrate, unlike a carbon-bound hydrogen atom in citrate, takes part in the coordination sphere of the metal. This led us to propose[8] that such metal chelation is the reason that fluorocitrate is a strong inhibitor and inactivator of aconitase, even though the isomer involved has fluorine on the "aconitase-inactive" end of the molecule. We showed that this isomer, when bound to metal with the fluorine atom near the metal, had a free carboxyl group that could project into the active-site area of the enzyme and so could possibly cause inhibition and inactivation. The presence of fluorine (from a C–F bond) in the metal coordination sphere is an integral part of this mechanism, which accounts for the powerful biochemical activity of only one of the four isomers of fluorocitrate.

A general review of the literature on the possible modes of interaction of C–F bonds with other groups has been made. Most effort has been given to the possibility of C–F··H–O bonding, particularly in 2-fluoroethanol, which has been intensively studied.[11-16] This has a gauche conformation in the gas phase with

(1) (a) On leave from the Department of Chemistry, University of Stirling, Stirling, Scotland FK9 4LA. (b) Present address: Biophysics Research Division, Institute of Science and Technology, University of Michigan, Ann Arbor, MI 48105.
(2) Peters, R. A. *Endeavour* **1954**, *90*, 147.
(3) Kun, E. In "Biochemistry Involving Carbon-Fluorine Bonds"; Filler, R., Ed.; American Chemical Society: Washington, D.C., 1976; ACS Symp. Ser. No. 28, Chapter 1, pp 1-22.
(4) Goldstein, J. A.; Cheung, Y.-F.; Marletta, M. A.; Walsh, C. *Biochemistry* **1978**, *17*, 5567.
(5) "Carbon-Fluorine Compounds. Chemistry, Biochemistry Biological Activities. A Ciba Foundation Symposium"; Elsevier-Excerpta Medica-North Holland: Amsterdam, London, New York, 1972.
(6) Pauling, L. *J. Am. Chem. Soc.* **1929**, *51*, 1010.
(7) Hurley, T. J.; Carrell, H. L.; Gupta, R. K.; Schwartz, J.; Glusker, J. P. *Arch. Biochem. Biophys.* **1979**, *193*, 478.
(8) Carrell, H. L.; Glusker, J. P.; Villafranca, J. J.; Mildvan, A. S.; Dummel, R. J.; Kun, E. *Science (Washington, D.C.)* **1970**, *170*, 1412.
(9) Stallings, W. C.; Monti, C. T.; Belvedere, J. F.; Preston, R. K.; Glusker, J. P. *Arch. Biochem. Biophys.* **1980**, *203*, 65.
(10) Marletta, M. A.; Srere, P. A.; Walsh, C. *Biochemistry* **1981**, *20*, 3719.
(11) Griffith, R. C.; Roberts, J. D. *Tetrahedron Lett.* **1974**, 3499.
(12) Hobza, P.; Mulder, F.; Sandorfy, C. *J. Am. Chem. Soc.* **1981**, *103*, 1360.
(13) Curtiss, L. A.; Frurip, D. J.; Blander, M. *J. Am. Chem. Soc.* **1978**, *100*, 79.

0002-7863/83/1505-3206$01.50/0 © 1983 American Chemical Society