Inorg. Chem. **1990**, *29*, 653–659

Contribution from the Department of Applied Chemistry,
Faculty of Engineering, Osaka University, Suita, Osaka 565, Japan

Efficient Catalytic Systems for Electron Transfer from an NADH Model Compound to Dioxygen

Shunichi Fukuzumi,*[,1a] Seiji Mochizuki, and Toshio Tanaka[1b]

Received November 28, 1988

Two-electron reduction of dioxygen with an NADH model compound, 10-methylacridan (AcrH$_2$), occurs efficiently in the presence of metalloporphyrins and perchloric acid in acetonitrile at 298 K. Overall four-electron reduction of dioxygen by AcrH$_2$ has also been made possible by adding iodide ion to the metalloporphyrin-catalyzed system. The catalytic mechanisms are discussed on the basis of detailed kinetics and the kinetic isotope effects for the overall CoTPP$^+$-catalyzed reduction of dioxygen by AcrH$_2$ as well as for each half catalytic cycle, the oxidation of AcrH$_2$ by CoTPP$^+$ and the reduction of dioxygen by CoTPP in the presence of HClO$_4$.

Reduced nicotinamide adenine dinucleotide (NADH) plays a vital role as the electron source in the respiratory chain, where the four-electron reduction of dioxygen by NADH is accompanied by a large free energy change that is coupled to the generation of the H$^+$ electrochemical gradient ($\Delta\mu_{H^+}$).[2] This protonic energy is utilized for ATP synthesis, ion translocation, and protein importation to satisfy the energy requirement of the cell.[3] The NADH plays another vital role as the electron source in the respiratory burst in polymorphonuclear neutrophils (PMN), in which a number of membrane stimulants enhance the two-electron (or one-electron) reduction of dioxygen to produce H_2O_2 (or O_2^-) in order to combat infections.[4] In the two-electron and four-electron reduction to dioxygen by NADH in the mitochondria and the PMN plasma membrane, respectively, iron porphyrins are known to be important components of the electron transport systems.[2-4]

On the other hand, metalloporphyrin-catalyzed reduction of dioxygen has been extensively studied in the electrochemical system in conjunction with the search for an inexpensive cathode material for dioxygen fuel cell,[5] and four-electron reduction of dioxygen to water has been achieved by using various dimeric metalloporphyrins[6] as well as monomeric indium porphyrins.[7] However, no efficient catalytic electron-transfer system has so far been reported for two-electron or four-electron reduction of dioxygen by NADH model compounds,[8] although electrochemical oxidation of NADH has been studied extensively.[9]

We report herein efficient electron-transfer systems for both the two-electron and overall four-electron reduction of dioxygen by an NADH model compound using metalloporphyrins as catalysts in the presence of perchloric acid (HClO$_4$) in acetonitrile (MeCN).[10] 10-Methylacridan (AcrH$_2$) was used as an acid-stable NADH model compound,[11] since common NADH model compounds such as 1-benzyl-1,4-dihydronicotinamide are known to decompose in the presence of an acid.[12] Mechanisms of the metalloporphyrin-catalyzed reduction of dioxygen by AcrH$_2$ are discussed on the basis of detailed kinetic studies on the overall catalytic reactions as well as on each half catalytic cycle, the reduction of a metalloporphyrins by AcrH$_2$ and the oxidation of the reduced metalloporphyrin by dioxygen in the presence of HClO$_4$.

Experimental Section

Materials. 10-Methylacridan (AcrH$_2$) was prepared from 10-methylacridinium iodide (AcrH$^+$I$^-$) by the reduction with NaBH$_4$ in methanol and purified by recrystallization from ethanol.[13] The dideuterated analogue, [9,9'-^{2}H$_2$]-10-methylacridan (AcrD$_2$), was prepared from 10-methylacridone by the reduction with LiAlD$_4$,[14] which was purchased from Aldrich. (Tetraphenylporphinato)iron(III) chloride (FTPPCl) was synthesized and purified according to the literature.[15] Cobalt(II) tetraphenylporphyrin (CoTPP) was prepared as given in the literature.[16] The CoTPP was oxidized by dioxygen in the presence of

---

(1) (a) To whom correspondence should be addressed. (b) Current address: Department of Applied Physics and Chemistry, Fukui Institute of Technology, Fukui 910, Japan.

(2) (a) Alberts, B.; Bray, D.; Lewis, J.; Raff, M.; Roberts, K.; Watson, J. D. *Molecular Biology of the Cell*; Garland Publishing Inc.: New York, 1983. (b) Capaldi, R. A., Ed. *Membrane Proteins in Energy Transduction*; Dekker: New York, 1979.

(3) (a) Hatefi, Y. *Annu. Rev. Biochem.* **1985**, *54*, 1015. (b) Hassinen, I. E. *Biochim. Biophys. Acta* **1986**, *853*, 135.

(4) (a) Badwey, J. A.; Karnovsky, M. L. *Annu. Rev. Biochem.* **1980**, *49*, 695. (b) Gimenez-Gallego, G.; Benavides, J.; Garcia, M. L.; Valdivieso, F. *Biochemistry* **1980**, *19*, 4834. (c) Light, D. R.; Walsh, C.; O'Callaghan, A. M.; Goetzl, E. J.; Tauber, A. I. *Biochemistry* **1981**, *20*, 1468. (d) Gabig, T. G.; Babior, B. M. *J. Biol. Chem.* **1979**, *254*, 9070. (e) Ohno, Y. I.; Hirai, K. I.; Kanoh, T.; Uchino, H.; Ogawa, K. *Blood* **1982**, *60*, 253. (f) Doussiere, J.; Vignais, P. V. *Biochemistry* **1985**, *24*, 7231.

(5) (a) Jahnke, H.; Schonborn, M.; Zimmerman, G. *Top. Curr. Chem.* **1976**, *61*, 133. (b) Buttry, D. A.; Anson, F. C. *J. Am. Chem. Soc.* **1984**, *106*, 59. (c) Shigehara, K.; Anson, F. C. *J. Phys. Chem.* **1982**, *86*, 2776. (d) Forshey, P. A.; Kuwana, T.; Kobayashi, N.; Osa, T. *Adv. Chem. Ser.* **1982**, *No. 201*, 601. (e) Ozer, D.; Parash, R.; Broitman, F.; Mor, U.; Bettelheim, A. *J. Chem. Soc., Faraday Trans. 1* **1984**, *80*, 1139. (f) Bettelheim, A.; White, B. A.; Raybuck, S. A.; Murray, R. W. *Inorg. Chem.* **1987**, *26*, 1009. (g) Bettelheim, A.; Ozer, D.; Parash, R. *J. Chem. Soc., Faraday Trans. 1* **1983**, *79*, 1555.

(6) (a) Collman, J. P.; Denisevich, P.; Konai, Y.; Marrocco, M.; Koval, C.; Anson, F. C. *J. Am. Chem. Soc.* **1980**, *102*, 6027. (b) Durand, R. R.; Bencosme, C. S.; Collman, J. P.; Anson, F. C. *Ibid.* **1983**, *105*, 2710. (c) Liu, H. Y.; Weaver, M. J.; Wang, C.-B.; Chang, C. K. *J. Electroanal. Chem. Interfacial Electrochem.* **1983**, *145*, 439. (d) Chang, C. K.; Liu, H. Y.; Abdalmuhdi, I. *J. Am. Chem. Soc.* **1984**, *106*, 2725. (e) Liu, H.-Y.; Abdalmuhdi, I.; Chang, C. K.; Anson, F. C. *J. Phys. Chem.* **1985**, *89*, 665. (f) Collman, J. P.; Hendricks, N. H.; Kim, K.; Bencosme, C. S. *J. Chem. Soc., Chem. Commun.* **1987**, 1537.

(7) Collman, J. P.; Kim, K. *J. Am. Chem. Soc.* **1986**, *108*, 7847.

(8) It has been reported that two-electron reduction of dioxygen by NADH is catalyzed by a number of transition-metal complexes but the catalytic activity is very low: Vol'pin, M. E.; Novodarova, G. N.; Kolosova, E. M.; Guzhova, N. V.; Kononenko, A. A.; Lejkin, Y. N. *Inorg. Chim. Acta* **1981**, *50*, 21. It has also been reported that an NADH model compound activates dioxygen in the presence of FMN (flavin mononucleotide) and MnTPPS (tetrakis(4-sulfonatophenyl)porphyrin–Mn complex) to give epoxides from olefins: Tabushi, I.; Kodera, M. *J. Am. Chem. Soc.* **1986**, *108*, 1101.

(9) (a) Gorton, L. *J. Chem. Soc., Faraday Trans. 1* **1986**, *82*, 1245. (b) Albery, W. J.; Bartlett, P. N. *J. Chem. Soc., Chem. Commun.* **1984**, 234. (c) Jaegfeldt, H.; Kuwana, T.; Johansson, G. *J. Am. Chem. Soc.* **1983**, *105*, 1805. (d) Natsue, T.; Kato, T.; Akiba, U.; Osa, T. *Chem. Lett.* **1986**, 843. (e) Samec, Z.; Elving, P. J. *J. Electroanal. Chem. Interfacial Electrochem.* **1983**, *144*, 217. (f) Degrand, C.; Miller, L. L. *J. Am. Chem. Soc.* **1980**, *102*, 5278.

(10) A preliminary report has appeared: Fukuzumi, S.; Mochizuki, S.; Tanaka, T. *J. Chem. Soc., Chem. Commun.* **1989**, 391.

(11) (a) Fukuzumi, S.; Ishikawa, M.; Tanaka, T. *J. Chem. Soc., Chem. Commun.* **1985**, 1069. (b) Fukuzumi, S.; Ishikawa, M.; Tanaka, T. *Tetrahedron* **1986**, *42*, 1021.

(12) (a) Johnston, C. C.; Gardner, J. L.; Suelter, C. H.; Metzler, D. E. *Biochemistry* **1963**, *2*, 689. (b) Kim, C. S. Y.; Chaykin, S. *Ibid.* **1968**, *7*, 2339.

(13) (a) Roberts, R. M. G.; Ostović, D.; Kreevoy, M. M. *Faraday Discuss. Chem. Soc.* **1982**, *74*, 257. (b) Colter, A. K.; Saito, G.; Sharom, F. J. *Can. J. Chem.* **1977**, *55*, 2741.

(14) (a) Powell, M. F.; Bruice, T. C. *J. Am. Chem. Soc.* **1983**, *105*, 7139. (b) Ostović, D.; Roberts, R. M. G.; Kreevoy, M. M. *Ibid.* **1983**, *105*, 7629.

(15) (a) Aldler, A. D.; Longo, F. R.; Kampas, F.; Kim, J. *J. Inorg. Nucl. Chem.* **1970**, *32*, 2443. (b) Fleischer, E. B.; Palmer, J. M.; Srivastava, T. S.; Chatterjee, A. *J. Am. Chem. Soc.* **1971**, *93*, 3162. (c) Jones, R. D.; Summerville, D. A.; Basolo, F. *Ibid.* **1978**, *100*, 4416.

(16) (a) Shirazi, A.; Goff, H. M. *Inorg. Chem.* **1982**, *21*, 3420. (b) Aldler, A. D.; Longo, F. R.; Varadi, V. *Inorg. Synth.* **1976**, *16*, 213.

0020-1669/90/1329-0653$02.50/0

HCl in methanol to obtain (tetraphenylporphinato)cobalt(III) chloride (CoTPPCl),[17] which was purified by recrystallization from methanol. The perchlorate salts of $FeTPP^+$ and $CoTPP^+$ were obtained by the metathesis of the corresponding chloride salts with $AgClO_4$ and recrystallized from toluene.[18] Perchloric acid (70%) was obtained from Wako Pure Chemicals. Reagent grade acetonitrile was purified by the successive distillation (four times) over P_2O_5 before use.

Spectral Measurements. Electronic absorption spectra were recorded with a Union SM-401 spectrophotometer with a quartz cell (1-mm or 1-cm i.d.), which was placed in a thermostated compartment at 298 K. The stoichiometry of the catalytic reduction of dioxygen by $AcrH_2$ in the presence of $CoTPP^+$ and $HClO_4$ in MeCN was determined by measuring the absorbances due to $AcrH^+$ with various initial concentrations of $AcrH_2$ and a fixed concentration of dioxygen. The amount of I_2 formed in the catalytic oxidation of I^- by dioxygen in the presence of $CoTPP^+$ and $HClO_4$ was determined by measuring the visible spectrum of I_3^- (λ_{max} = 361 nm, $\epsilon = 2.50 \times 10^4$ M^{-1} cm^{-1}), which was converted quantitatively from I_2 by treating the diluted MeCN solution (×100) of the product I_2 with a large excess amount of NaI.[19] The amount of H_2O_2 formed in the catalytic two-electron reduction of dioxygen by $AcrH_2$ in the presence of $CoTPP^+$ and $HClO_4$ was determined by the standard method (titration by iodide ion) according to the procedure described elsewhere.[19]

The concentration of dioxygen in air-saturated MeCN containing the same concentration of $HClO_4$ as employed in determining the stoichiometry of the catalytic reduction of dioxygen was determined as 2.6×10^{-3} M from the photooxidation of $AcrH_2$ by dioxygen in the presence of $HClO_4$ in MeCN as described elsewhere.[20,21] The dioxygen concentration in air-saturated MeCN determined in this manner was in good agreement with those in aprotic solvents such as alkanes, acetone, alcohol, and carbon tetrachloride.[22]

Kinetic Measurements. Acetonitrile solutions containing various concentrations of dioxygen were prepared by saturating the MeCN solution with a stream of mixture gas (dioxygen and nitrogen) of various partial pressures of dioxygen. The various partial pressures of dioxygen were attained by controlling the flow rates of dioxygen and nitrogen with gas regulators. Rates of the oxidation of $AcrH_2$ at 298 K were monitored by the rise of the absorption band due to $AcrH^+$ (λ_{max} = 358 nm, $\epsilon = 1.8 \times 10^4$ M^{-1} cm^{-1}) by using the conventional spectrophotometer. Kinetic measurements for the fast reactions with half-lives <10 s were performed by using a Union RA-103 stopped-flow spectrophotometer. The stopped-flow measurements were employed for the oxidation of I^- by $CoTPP^+$ in MeCN at 298 K under deaerated conditions by monitoring the rise and decay of the absorption bands at 412 and 434 nm due to the formation of CoTPP and the decay of $CoTPP^+$, respectively. All the kinetic measurements were carried out under pseudo-first-order conditions where the concentrations of dioxygen and $HClO_4$ were maintained at >10-fold excess of the concentration of reductant, which is also maintained at >10-fold excess of the catalyst concentration whenever possible. Pseudo-first-order rate constnats $k^{(1)}$ were determined by least-squares curve fit by use of a Union System 77 microcomputer. The pseudo-first-order plots were linear for 3 or more half-lives with the correlation coefficient $\rho > 0.999$.

Results

Two-Electron Reduction of Dioxygen with $AcrH_2$, Catalyzed by Metalloporphyrins in the Presence of $HClO_4$. An NADH model compound, 10-methylacridan ($AcrH_2$), is stable toward dioxygen in MeCN at 298 K. In the presence of $HClO_4$, however, sluggish oxidation of $AcrH_2$ by dioxygen proceeds in the dark.[23] When a catalytic amount of $FeTPP^+$ is added to the MeCN solution of $AcrH_2$ in the presence of $HClO_4$, $AcrH_2$ (λ_{max} = 285 nm) is readily oxidized by dioxygen to yield $AcrH^+$ (λ_{max} = 358 nm), as shown in Figure 1. The concentration of $FeTPP^+$ (λ_{max} = 399 nm) decreases gradually with reaction time, accompanied by demetalation of $FeTPP^+$ to give H_3TPP^+ (λ_{max} = 436 nm). In the absence of $HClO_4$, no catalytic oxidation of $AcrH_2$ by dioxygen is observed, but instead $FeTPP^+$ is converted to the μ-oxo dimer $(FeTPP)_2O$, following the reduction of $FeTPP^+$ by $AcrH_2$.[24]

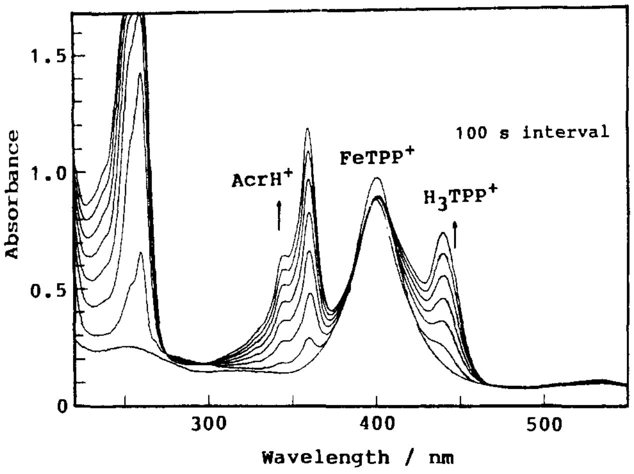


Figure 1. Oxidation of $AcrH_2$ (1.0×10^{-3} M) with dioxygen (2.6×10^{-3} M), catalyzed by $FeTPP^+$ (1.0×10^{-4} M) in the presence of $HClO_4$ (2.0×10^{-2} M) in MeCN.

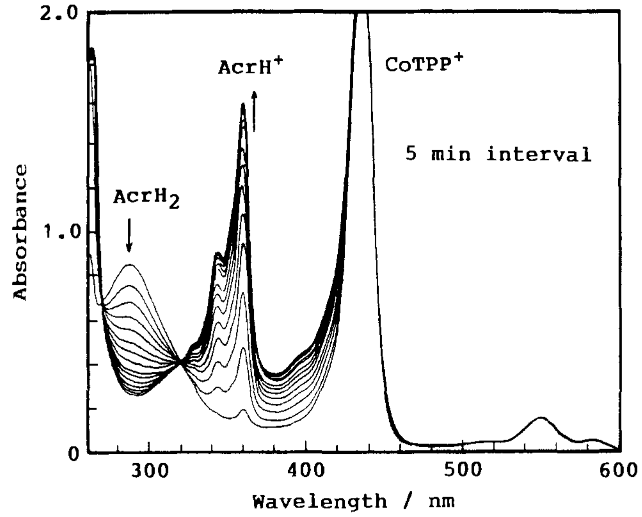
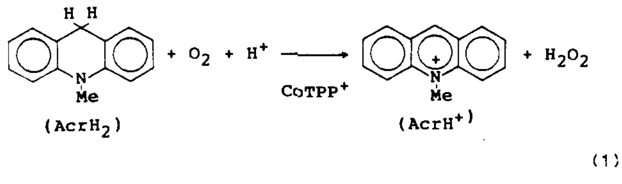


Figure 2. Oxidation of $AcrH_2$ (8.0×10^{-4} M) with dioxygen (2.6×10^{-3} M), catalyzed by $CoTPP^+$ (8.0×10^{-5} M) in the presence of $HClO_4$ (2.0×10^{-3} M) in MeCN.

When $FeTPP^+$ is replaced by $CoTPP^+$, $AcrH_2$ is also readily oxidized by dioxygen catalytically, as shown in Figure 2, where the concentration of $CoTPP^+$ (λ_{max} = 434 nm) remains constant with reaction time. Thus, in the case of $CoTPP^+$, no appreciable demetalation occurs during the reaction. The same result is obtained by using CoTPP instead of $CoTPP^+$ as the starting catalyst, since CoTPP is readily converted to $CoTPP^+$. The stoichiometry of the oxidation of $AcrH_2$ with dioxygen, catalyzed by $CoTPP^+$ or CoTPP in the presence of $HClO_4$, is given by eq 1, which is confirmed by the detection of the equivalent amounts



(1)

---

(17) Sakurai, T.; Yamamoto, K.; Naito, H.; Nakamoto, N. *Bull. Chem. Soc. Jpn.* **1976**, *49*, 3042.

(18) Reed, C. A.; Mashiko, T.; Bently, S. P.; Kastner, M. E.; Scheidt, W. R.; Spartalian, K.; Lang, G. *J. Am. Chem. Soc.* **1979**, *101*, 2948.

(19) (a) Mair, R. D.; Graupner, A. J. *Anal. Chem.* **1964**, *36*, 194. (b) Fukuzumi, S.; Kuroda, S.; Tanaka, T. *J. Am. Chem. Soc.* **1985**, *107*, 3020.

(20) Fukuzumi, S.; Mochizuki, S.; Tanaka, T. *Inorg. Chem.* **1989**, *28*, 2459.

(21) Fukuzumi, S.; Tanaka, T. In *Photoinduced Electron Transfer*; Fox, M. A., Chanon, M., Eds.; Elsevier: Amsterdam, 1988; Chapter 4-10, pp 578-636.

(22) Battino, R., Ed. *Solubility Data Series, Oxygen and Ozone*; Pergamon Press: Oxford, 1981; Vol. 7.

(23) Under irradiation of light λ = 300 nm, which excites the absorption band of $AcrH_2$, the efficient photooxidation of $AcrH_2$ by dioxygen proceeds via photoinduced electron-transfer radical chain reactions; see ref 21.

(24) The use of other NADH model compounds, 1-(X-benzyl)-1,4-dihydronicotinamide (X-BNAH: X = 4-MeO, 4-Me, H, 4-Cl, 2,4-Cl_2), also results in the formation of $(FeTPP)_2O$: Fukuzumi, S.; Kondo, Y.; Mochizuki, S.; Tanaka, T. *J. Chem. Soc., Perkin Trans.* **1989**, 1753.

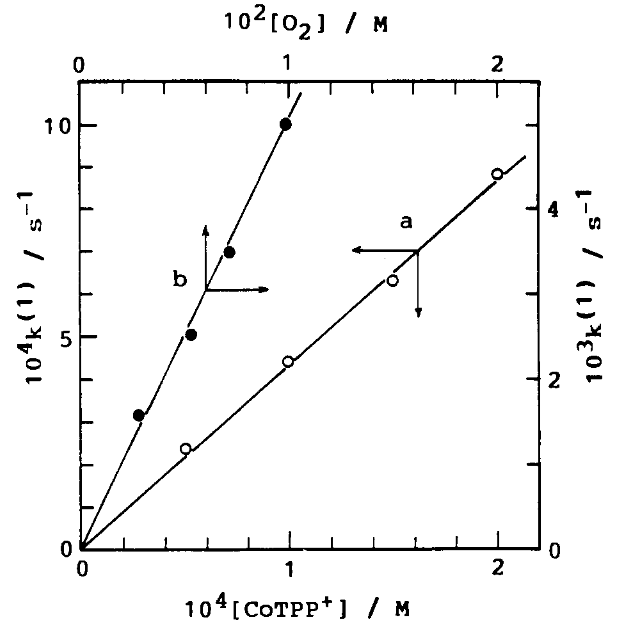


Figure 3. Plots of pseudo-first-order rate constants $k^{(1)}$ vs (a) the $CoTPP^+$ concentration (○) for the oxidation of $AcrH_2$ (1.0×10^{-3} M) with dioxygen (2.6×10^{-3} M), catalyzed by $CoTPP^+$ in the presence of $HClO_4$ (2.0×10^{-3} M), and (b) the O_2 concentration (●) for the oxidation of $AcrH_2$ (1.0×10^{-3} M) with dioxygen, catalyzed by $CoTPP^+$ (1.0×10^{-4} M) in the presence of $HClO_4$ (2.0×10^{-2} M) in MeCN at 298 K.

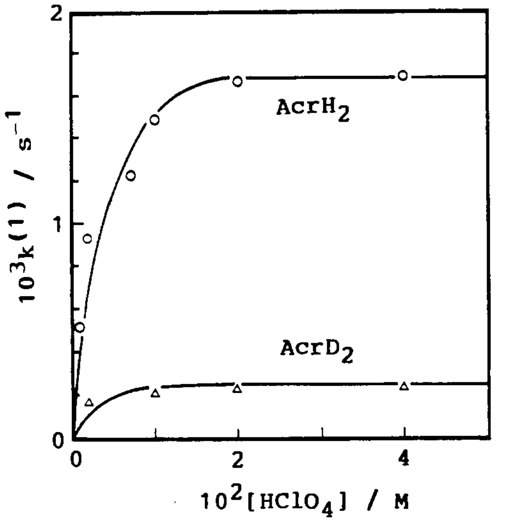


Figure 4. Plots of pseudo-first-order rate constants $k^{(1)}$ vs the $HClO_4$ concentration for the oxidation of 2.0×10^{-4} M $AcrH_2$ (○) and $AcrD_2$ (△) with dioxygen (2.6×10^{-3} M), catalyzed by $CoTPP^+$ (2.0×10^{-4} M) in the presence of $HClO_4$ in MeCN at 298 K.

of $AcrH^+$ and H_2O_2 formed in the reaction (see Experimental Section). This system is subjected to the detailed kinetic analysis as follows.

Rates of the catalytic oxidation of $AcrH_2$ with an excess amount of dioxygen and $HClO_4$ in the presence of a catalytic amount of $CoTPP^+$ obey pseudo-first-order kinetics. The pseudo-first-order rate constant ($k^{(1)}/s^{-1}$) increases linearly with an increase in the $CoTPP^+$ concentration, as shown in Figure 3a, which confirms that $CoTPP^+$ acts as a catalyst in the oxidation of $AcrH_2$ by dioxygen in the presence of $HClO_4$ in MeCN. The $k^{(1)}$ value increases linearly also with an increase in the dioxygen concentration (Figure 3b). The dependence of $k^{(1)}$ on the $HClO_4$ concentration at fixed concentrations of dioxygen and $CoTPP^+$ is shown in Figure 4, where the $k^{(1)}$ value increases with an increase in the $HClO_4$ concentration to reach a constant value. When $AcrH_2$ is replaced by the 9,9′-dideuterated analogue ($AcrD_2$), the $k^{(1)}$ values of $AcrD_2$ are much smaller than the corresponding values of $AcrH_2$ (Figure 4), demonstrating the presence of a large primary kinetic isotope effect, k_H/k_D.[25] The k_H/k_D value is constant with the change in the $HClO_4$ concentration ($k_H/k_D = 7.1 \pm 0.6$).

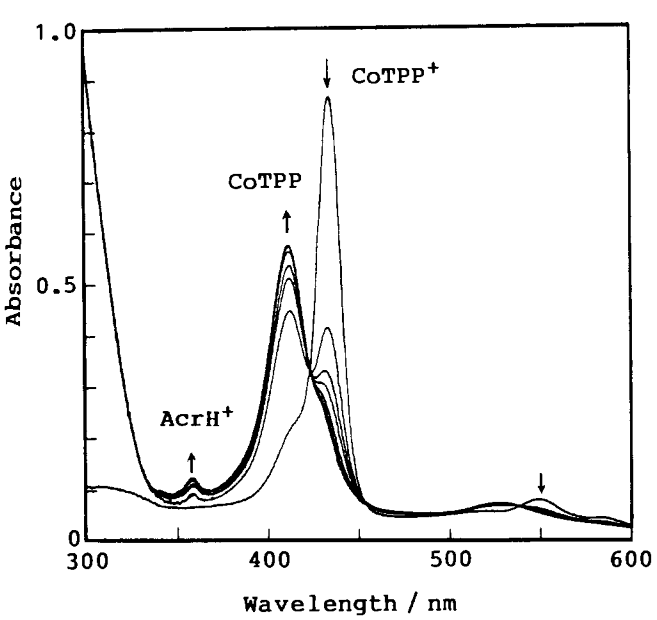


Figure 5. Oxidation of $AcrH_2$ (1.0×10^{-3} M) by $CoTPP^+$ (5.0×10^{-5} M) in the absence of dioxygen in MeCN.

Table I. Rate Constants (k_{obs}) for the Oxidation of $AcrH_2$ and $AcrD_2$ by $CoTPP^+$ in the Absence and Presence of $HClO_4$ in MeCN at 298 K

$[HClO_4]$, M	$k_{obs}(AcrH_2)$,[a] M^{-1} s^{-1}	$k_{obs}(AcrD_2)$,[a] M^{-1} s^{-1}	k_{obs}^H/k_{obs}^D
0	5.7	1.3	4.4
1.0×10^{-4}	4.9	1.1	4.5
1.0×10^{-3}	2.6×10^{-1}	5.9×10^{-2}	4.4
1.0×10^{-2}	*b*	*b*	

[a] The experimental errors are within ±5%. [b] Too slow to be determined accurately.

Oxidation of $AcrH_2$ by $CoTPP^+$ in the Absence of Dioxygen. In the absence of dioxygen, $AcrH_2$ is readily oxidized by $CoTPP^+$ to yield $AcrH^+$ and CoTPP ($\lambda_{max} = 412$ nm), as shown in Figure 5. The stoichiometry of the reaction is given by eq 2. The rate

$$AcrH_2 + 2CoTPP^+ \rightarrow AcrH^+ + H^+ + 2CoTPP \qquad (2)$$

of formation of CoTPP obeys pseudo-first-order kinetics under the conditions that the concentrations of $AcrH_2$ are maintained at >10-fold excess over that of $CoTPP^+$. The pseudo-first-order rate constant was proportional to the $AcrH_2$ concentration (eq 3). When $HClO_4$ is added to the $AcrH_2$–$CoTPP^+$ system, the

$$d[CoTPP]/dt = k_{obs}[AcrH_2][CoTPP^+] \qquad (3)$$

second-order rate constant k_{obs} decreases with an increase in the $HClO_4$ concentration, as shown in Table I. Such a decrease in the rate constant is accompanied by the decrease of the absorbance of $AcrH_2$ due to the protonation equilibrium in MeCN (eq 4).[11,26]

$$AcrH_2 + H^+ \overset{K}{\rightleftharpoons} AcrH_3^+ \qquad (4)$$

The reducing power of $AcrH_2$ is known to be decreased significantly by the protonation,[11,26] and thereby only free $AcrH_2$ may be active for the reduction of $CoTPP^+$. This may be the reason that the k_{obs} value decreases with an increase of the $HClO_4$ concentration (Table I).

---

(25) By the assumption that the secondary kinetic isotope effect is unity, the observed k_{obs} ratios of $AcrH_2$ to $AcrD_2$ corresponds to the primary kinetic isotope effect k_{obs}^H/k_{obs}^D.

(26) Fukuzumi, S.; Kuroda, S.; Goto, T.; Ishikawa, K.; Tanaka, T. *J. Chem. Soc., Perkin Trans. 2* **1989**, 1047.

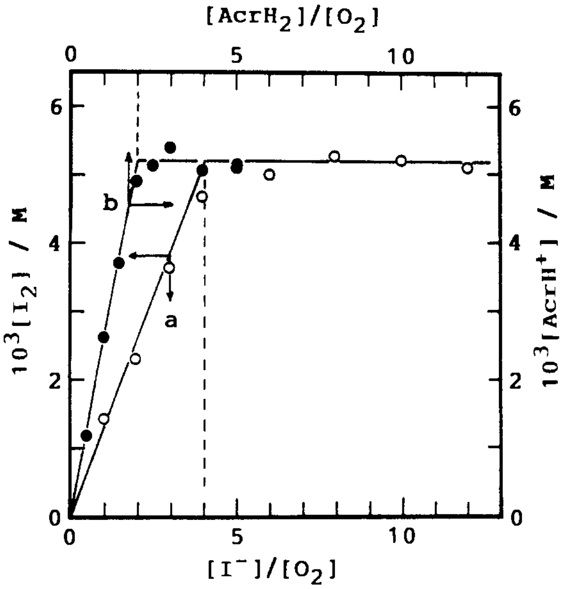


Figure 6. (a) Plot of the I_2 concentration formed in the oxidation of I^- with dioxygen, catalyzed by $CoTPP^+$ (2.5×10^{-5} M), vs the ratio of the I^- concentration to the initial concentration of dioxygen (2.6×10^{-3} M) in the presence of $HClO_4$ (0.10 M) in MeCN. (b) Plot of the $AcrH^+$ concentration formed in the oxidation of $AcrH_2$ with dioxygen, catalyzed by $CoTPP^+$ (2.5×10^{-5} M) in the presence of $HClO_4$ (1.0×10^{-2} M) and NaI in MeCN, vs the ratio of the $AcrH_2$ concentration to the initial concentration of dioxygen (2.6×10^{-3} M).

The corresponding k_{obs} values of $AcrD_2$ were also determined as listed in Table I. The k_H/k_D value is constant with the change in the $HClO_4$ concentration, $k_H/k_D = 4.4 \pm 0.4$. It should be noted that the k_H/k_D value in the absence of dioxygen (Table I) is significantly smaller than the corresponding value ($k_H/k_D = 7.1 \pm 0.6$) observed in the presence of dioxygen (Figure 4).

Overall Four-Electron Reduction of Dioxygen with $AcrH_2$, Catalyzed by $CoTPP^+$ in the Presence of $HClO_4$ and Iodide Ion. Iodide ion is stable toward dioxygen in the absence and presence of $HClO_4$ in MeCN. When a catalytic amount of $CoTPP^+$ is added to an MeCN solution of iodide ion in the presence of dioxygen and $HClO_4$, the iodide ion is oxidized by dioxygen catalytically to yield iodine. The stoichiometry of the formation of iodine is determined by the spectral titration (see Experimental Section) shown in Figure 6a and is given by eq 5. Thus, $CoTPP^+$ catalyzes efficiently also the overall four-electron reduction of dioxygen by iodide ion in the presence of $HClO_4$ to yield water.

$$4I^- + O_2 + 4H^+ \xrightarrow[CoTPP^+]{} 2I_2 + 2H_2O \qquad (5)$$

The reduction of $CoTPP^+$ by iodide ion occurs rapidly in the absence of dioxygen in MeCN. Rates of the reduction of $CoTPP^+$ by an excess amount of iodide ion also obey pseudo-first-order kinetics. The pseudo-first-order rate constant $k^{(1)}$ increases with an increase in the I^- concentration, as shown in Figure 7, where a linear correlation between $k^{(1)}$ and $[I^-]$ is obtained with an intercept.

The catalytic four-electron reduction of dioxygen by iodide ion to water (eq 5) can be combined successfully with the two-electron reduction of dioxygen by $AcrH_2$ (eq 1) in order to achieve the overall four-electron reduction of dioxygen by $AcrH_2$. Thus, when iodide ion is added to the catalytic system of the two-electron reduction of dioxygen (eq 1), the stoichiometry is changed to the overall four-electron reduction of dioxygen by $AcrH_2$, as shown in the spectral titration (Figure 6b) (eq 6). The concentration of $CoTPP^+$ remained constant and no appreciable amount of CoTPP has been observed during the reaction.

$$2AcrH_2 + O_2 + 2H^+ \xrightarrow[CoTPP^+/I^-]{} 2AcrH^+ + 2H_2O \qquad (6)$$

Rates of the four-electron reduction of dioxygen by $AcrH_2$ were determined under the pseudo-first-order conditions in which the concentrations of dioxygen, $HClO_4$, and H_2O[27] were maintained at large excess over the concentrations of $AcrH_2$ and $CoTPP^+$. Dependences of the pseudo-first-order rate constants $k^{(1)}$ of both $AcrH_2$ and $AcrD_2$ on the I^- concentration are shown in Figure 8, where both rate constants $k^{(1)}$ exhibits a maximal dependence on the I^- concentration at 1×10^{-4} M. It should be noted that essentially the same results are obtained when I^- is replaced by I_2. The primary kinetic isotope effect k_H/k_D remains constant ($k_H/k_D = 2.4 \pm 0.2$) with the change in the I^- concentration (Figure 9). On the other hand, $k^{(1)}$ shows a linear correlation with the $HClO_4$ concentration in MeCN containing H_2O (1.1 M),[28] as shown in Figure 9, where the intercept corresponds to an acid-independent term in $k^{(1)}$.

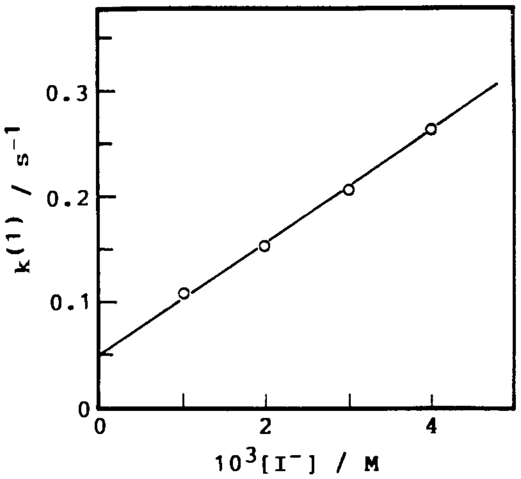


Figure 7. Plot of pseudo-first-order rate constant $k^{(1)}$ vs the I^- concentration for the reduction of $CoTPP^+$ by I^- in MeCN.

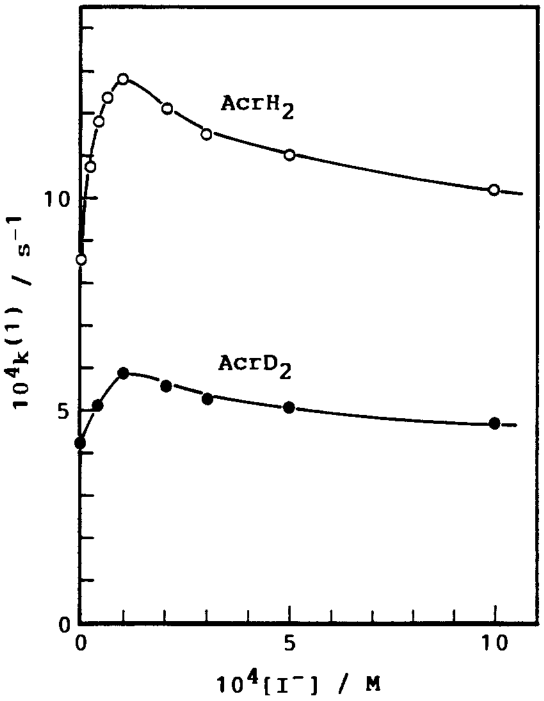


Figure 8. Plots of pseudo-first-order rate constants $k^{(1)}$ vs the I^- concentration for the four-electron reduction of dioxygen (2.6×10^{-3} M) with 1.0×10^{-3} M $AcrH_2$ and $AcrD_2$, catalyzed by $CoTPP^+$ (1.0×10^{-4} M) in the presence of $HClO_4$ (1.0×10^{-2} M) and I^- in MeCN containing H_2O (1.1 M) at 298 K.

---

[27] The rates were determined in the presence of an excess amount of H_2O (1.1 M), since the rates without addition of H_2O would be affected by H_2O that was produced in the four-electron reduction of dioxygen by $AcrH_2$ because of the change of the protonation equilibrium (eq 4) with resultant increase of the H_2O concentration.

[28] In the presence of H_2O (1.1 M), little protonation of $AcrH_2$ occurs in MeCN, and thereby only the catalytic effect of $HClO_4$ is observed in Figure 9.

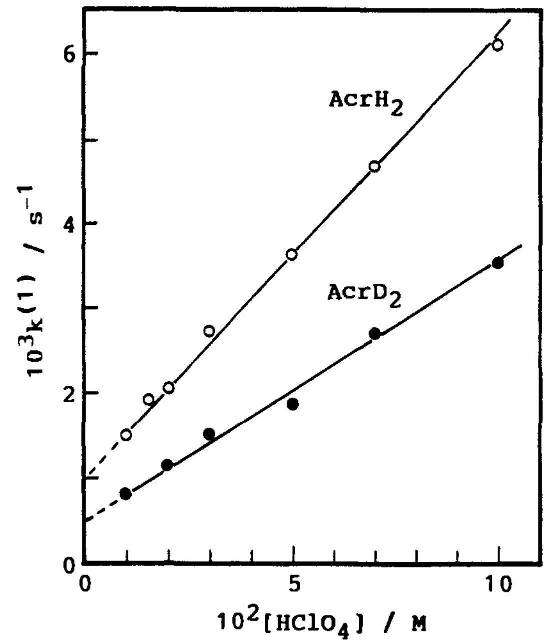
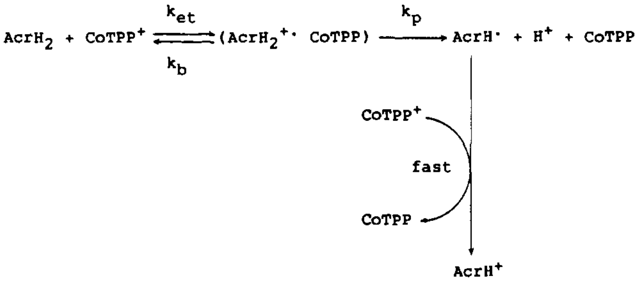


Figure 9. Plots of $k^{(1)}$ vs the HClO$_4$ concentration for the four-electron reduction of dioxygen (2.6×10^{-3} M) with AcrH$_2$ and AcrD$_2$ (1.0×10^{-3} M), catalyzed by CoTPP$^+$ (1.0×10^{-4} M) in the presence of HClO$_4$ and I$^-$ (5.0×10^{-5} M) in MeCN containing H$_2$O (1.1 M) at 298 K.

Scheme I



Iodine formed in the overall four-electron reduction of dioxygen by iodide ion (eq 6) is readily reduced by AcrH$_2$ to regenerate iodide ion in MeCN (eq 7). Rates of the oxidation of AcrH$_2$

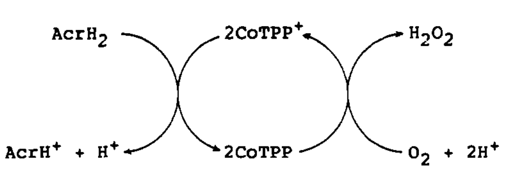
$$\mathrm{AcrH_2 + I_2 \xrightarrow{k_{ox}} AcrH^+ + H^+ + 2I^-} \qquad (7)$$

by iodine also obey pseudo-first-order kinetics in the presence of water in MeCN when the protonation of AcrH$_2$ hardly occurs in the presence of HClO$_4$.[11,26] The second-order rate constants k_{ox} were obtained as 15 and 4.2 M^{-1} s^{-1} for the oxidation of AcrH$_2$ and AcrD$_2$, respectively, by I$_2$ in the presence of 1.0×10^{-3} M in MeCN containing 1.1 M H$_2$O.[29]

Discussion

Mechanism of the Oxidation of AcrH$_2$ by CoTPP$^+$. The electron transfer from AcrH$_2$ to CoTPP$^+$ is endothermic, on the basis of the redox potentials of AcrH$_2$ ($E_{ox}° = 0.80$ V vs SCE)[30] and CoTPP$^+$ ($E_{red}° = 0.35$ V vs SCE).[20] However, once AcrH$_2^{•+}$ produced in the electron transfer is deprotonated to give AcrH$^•$, the electron transfer from AcrH$^•$ ($E_{ox}° = -0.43$ V vs SCE)[30] to CoTPP$^+$ is highly exothermic. Thus, two-electron oxidation of AcrH$_2$ by 2 equiv of CoTPP$^+$ may proceed as shown in Scheme I, where the endothermic electron transfer from AcrH$_2$ to CoTPP$^+$ is followed by the deprotonation of AcrH$_2^{•+}$, and the subsequent facile electron transfer from AcrH$^•$ to CoTPP$^+$ makes the overall

Scheme II



reaction exothermic. According to Scheme I, the rate of formation of CoTPP may be given by eq 8, where k_{et} is the rate constant

$$d[\mathrm{CoTPP}]/dt = 2k_{et}k_p[\mathrm{AcrH_2}][\mathrm{CoTPP^+}]/(k_b + k_p) \qquad (8)$$

of electron transfer from AcrH$_2$ to CoTPP$^+$, k_p is the rate constant of deprotonation of AcrH$_2^{•+}$, and k_b is the rate constant of back electron transfer from CoTPP to AcrH$_2^{•+}$. In the presence of HClO$_4$, AcrH$_2$ is in protonation equilibrium (eq 4) when the second-order rate constant k_{obs} is given by eq 9, where K is the

$$k_{obs} = \frac{2k_{et}k_p}{(k_b + k_p)(1 + K[\mathrm{H^+}])} \qquad (9)$$

protonation equilibrium constant (eq 4). Under the conditions that $K[\mathrm{H^+}] \gg 1$,[11] the k_{obs} value is expected to decrease with an increase in the HClO$_4$ concentration, in accordance with the experimental results in Table I. The observed kinetic isotope effect ($k_{obs}{}^{\mathrm{H}}/k_{obs}{}^{\mathrm{D}} = 4.4 \pm 0.4$) may be caused by the deprotonation of AcrH$_2^{•+}$ in Scheme I.

A more quantitative discussion on Scheme I can be achieved by comparing the observed rates to those predicted by the Marcus theory for the rates of outer-sphere electron-transfer reactions.[31] The Marcus relation for the rate constant of the electron transfer from the reductant (1) to the oxidant (2), k_{12}, is given by eq 10,[31]

$$k_{12} = (k_{11}k_{22}K_{12}f)^{1/2} \qquad (10)$$

where k_{11} and k_{22} are the rate constants of the corresponding self-exchanges between AcrH$_2$ and AcrH$_2^{•+}$ and K_{12} is the equilibrium constant for the electron transfer from AcrH$_2$ to CoTPP$^+$. The K_{12} value is obtained from the $E_{ox}°$ value of AcrH$_2$[30] and the $E_{\mathrm{r}}\mathrm{e}_{\mathrm{d}}°$ value of CoTPP$^+$[20] by using eq 11.[32] The

$$\log K_{12} = (F/-2.3RT)(E_{ox}° - E_{red}°) \qquad (11)$$

$$\log f = (\log K_{12})^2/[4 \log (k_{11}k_{22}/Z^2)] \qquad (12)$$

parameter f in eq 10 is given by eq 12,[31] in which Z, the frequency factor, is taken to be 10^{11} M^{-1} s^{-1}.[31] Thus, the rate constant of outer-sphere electron transfer from AcrH$_2$ to CoTPP$^+$ (k_{et}) is calculated as 6.9 M^{-1} s^{-1} from the one-electron oxidation potential of AcrH$_2$ ($E_{ox}° = 0.80$ V vs SCE)[30] and the self-exchange rate constant ($k_{11} = 1.7 \times 10^9$ M^{-1} s^{-1}),[30] together with the one-electron reduction potential of CoTPP$^+$ ($E_{red}° = 0.35$ V vs SCE)[20] and the self-exchange rate constant ($k_{22} = 20$ M^{-1} s^{-1}),[33] as noted above by using eqs 10–12. According to eq 9, the observed rate constant in the absence of HClO$_4$ (6.3 M^{-1} s^{-1}) corresponds to $2k_{et}k_p/(k_b + k_p)$, and thus $k_{obs} < 2k_{et}$. This relation is consistent with the calculation described above.

Mechanism of the Catalytic Two-Electron Reduction of Dioxygen by AcrH$_2$. The simplest reaction scheme of the catalytic two-electron reduction of dioxygen by AcrH$_2$ may be given by Scheme II. The rate-determining step may be the oxidation of AcrH$_2$ by CoTPP$^+$ by AcrH$_2$, since CoTPP formed in the reduction of CoTPP$^+$ by AcrH$_2$ is known to be readily oxidized by O$_2$ in the presence of HClO$_4$ in MeCN to yield CoTPP$^+$ and H$_2$O$_2$.[27,34] However, the dependences of rates of the oxidation

---

(29) The rates were determined in the presence of H$_2$O (1.1 M) in order to prevent the protonation of AcrH$_2$,[11] which would cause the decrease in the rates without adding H$_2$O, since H$^+$ is produced in the oxidation of AcrH$_2$ by I$_2$ (eq 7).

(30) Fukuzumi, S.; Koumitsu, S.; Hironaka, K.; Tanaka, T. *J. Am. Chem. Soc.* **1987**, *109*, 305.

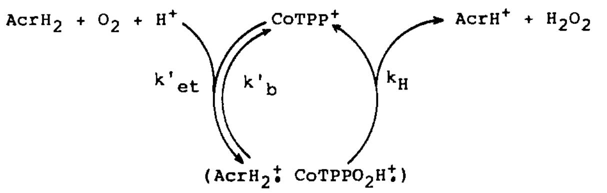
(31) Marcus, R. A. *Annu. Rev. Phys. Chem.* **1964**, *15*, 155.

(32) The work terms are neglected, since in the present case one of the reactants and products is uncharged.

(33) Rohrbach, D. F.; Deutsch, E.; Heineman, W. R.; Pasternack, R. F. *Inorg. Chem.* **1977**, *16*, 2650.

(34) (a) Pavlović, D.; Ašperger, S.; Dokuzović, Z.; Jurišić, B.; Ahmeti, X.; Sertić, M.; Murati, I. *J. Chem. Soc., Dalton Trans.* **1985**, 1095. (b) Pavlović, D.; Ašperger, S.; Domi, B. *Ibid.* **1986**, 2535. (c) Fukuzumi, S.; Mochizuki, S.; Tanaka, T. *Chem. Lett.* **1989**, 27.

Scheme III



for $AcrH_2$ by $CoTPP^+$ on the $HClO_4$ concentration are different in the absence and presence of dioxygen; the rate in the absence of dioxygen decreases with an increase in the $HClO_4$ concentration (Table I), but the rate in the presence of dioxygen increases with an increase in the $HClO_4$ concentration to reach a constant value (Figure 4). Moreover, the kinetic isotope effects (k_{obs}^H/k_{obs}^D) are different in the absence (4.4 ± 0.4) and presence of dioxygen (7.1 ± 0.6). Such differences indicate that the mechanisms of oxidation of $AcrH_2$ by $CoTPP^+$ in the presence of $HClO_4$ are different in the absence and presence of dioxygen and that the acid-catalyzed reduction of O_2 by CoTPP should also be involved in the rate-determining step in the catalytic two-electron reduction of O_2 by $AcrH_2$.

An alternative reaction mechanism may be given by Scheme III, where electron transfer from $AcrH_2$ to $CoTPP^+$ occurs in concert with the reduction of O_2 by CoTPP in the presence of $HClO_4$. In such a case, electron transfer from $AcrH_2$ to $CoTPP^+$ in the presence of O_2 and $HClO_4$ may produce a radical pair ($AcrH_2^{\bullet+}$,$CoTPPO_2H^{\bullet+}$), which may disappear by the facile hydrogen transfer from $AcrH_2^{\bullet+}$ to $CoTPPO_2H^{\bullet+}$ to yield $AcrH^+$ and H_2O_2, accompanied by regeneration of $CoTPP^+$ (Scheme III). The proton transfer from $AcrH_2^{\bullet+}$ to $CoTPPO_2H^{\bullet+}$ may be unlikely to occur, since $CoTPPO_2H^{\bullet+}$ is already protonated. According to Scheme III, the observed pseudo-first-order rate constant (Figures 3 and 4) may be given by eq 13, where k'_{et} is the

$$k^{(1)} = \frac{k'_{et}k_H[H^+][CoTPP^+][O_2]}{(k'_b + k_H)(1 + K[H^+])} \qquad (13)$$

rate constant of the electron transfer from $AcrH_2$ to $CoTPP^+$ in the presence of O_2, k'_b is the rate constant of the back electron transfer, k_H is the rate constant of the hydrogen transfer from $AcrH_2^{\bullet+}$ to $CoTPPO_2H^{\bullet+}$, and K is the protonation equilibrium constant of $AcrH_2$. This equation can explain well the observed kinetics that the catalytic rate is proportional to the concentrations of $CoTPP^+$ and dioxygen and increases with an increase in the $HClO_4$ concentration to reach a constant value (Figures 3 and 4, respectively). According to eq 13, the kinetic isotope effect in the presence of dioxygen in Figure 4 (k_{obs}^H/k_{obs}^D = 7.1 ± 0.6) may be ascribed to that of hydrogen transfer from $AcrH_2^{\bullet+}$ to $CoTPPO_2H^{\bullet+}$. This may be the reason that the observed kinetic isotope effect in the presence of dioxygen is different from that in the absence of dioxygen.

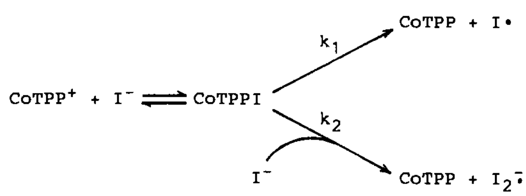
Free $HO_2^\bullet$ radicals which could have escaped from the radical pair ($AcrH_2^{\bullet+}$,$CoTPPO_2H^{\bullet+}$) may not be involved in the $CoTPP^+$-catalyzed two-electron reduction of O_2 by $AcrH_2$, since $HO_2^\bullet$ radicals are known to initiate the radical chain reactions (eqs 14 and 15).[21,35] In such a case, the kinetic formulation would be clearly different from that observed in Figure 3.[36]

$$AcrH_2 + HO_2^\bullet \rightarrow AcrH^\bullet + H_2O_2 \qquad (14)$$

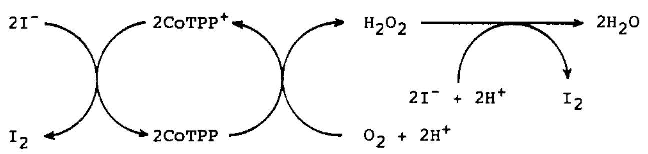
$$AcrH^\bullet + O_2 + H^+ \rightarrow AcrH^+ + HO_2^\bullet \qquad (15)$$

Mechanism of the Overall Four-Electron Reduction of Dioxygen by Iodide Ion. It has long been known that the reduction of transition-metal complexes by iodide ion occurs through the two parallel paths, the reduction by one and two molecules of iodide ion.[37,38] Thus, the reduction of $CoTPP^+$ by iodide ion may be given as shown in Scheme IV, where I^- forms a complex with $CoTPP^+$, followed by the two parallel paths; one is the intramolecular electron transfer from I^- to $CoTPP^+$, and the other is the reduction of CoTPPI by I^- to yield CoTPP and $I_2^{\bullet-}$. The iodine atom formed in the intramolecular electron transfer (k_1) is in rapid equilibrium with I^- to give $I_2^{\bullet-}$ (eq 16).[39] The $I_2^{\bullet-}$ may be oxidized

Scheme IV



Scheme V



$$I^\bullet + I^- \rightleftharpoons I_2^{\bullet-} \qquad (16)$$

$$CoTPP^+ + I_2^{\bullet-} \rightarrow CoTPP + I_2 \qquad (17)$$

further by $CoTPP^+$ (eq 17), since the electron transfer from $I_2^{\bullet-}$ (E_{ox}° = 0.16 V vs SCE)[40] to $CoTPP^+$ is exothermic, on the basis of their redox potentials. According to Scheme IV and eqs 16 and 17, the rate of formation of CoTPP in the reduction of $CoTPP^+$ with a large excess of I^-, when all the $CoTPP^+$ molecules form the complex with I^-, may be given by eq 18. This kinetic

$$d[CoTPP]/dt = 2(k_1 + k_2[I^-])[CoTPP^+] \qquad (18)$$

formulation agrees with the experimental results in Figure 7, where the pseudo-first-order rate constant shows a linear correlation with the I^- concentration, in which the intercept corresponds to $2k_1$.

In the presence of dioxygen and $HClO_4$, CoTPP formed in the reduction of $CoTPP^+$ by I^- (Scheme IV and eq 17) may be readily oxidized by dioxygen to produce $CoTPPO_2H^{\bullet+}$, as in Scheme III. The $CoTPPO_2H^{\bullet+}$ may be reduced by I^- in the presence of $HClO_4$ to yield $CoTPPO_2H_2^{\bullet+}$. The $CoTPPO_2H^{\bullet+}$ may be further reduced by I^- to yield H_2O. Thus, as shown in Scheme V, $CoTPP^+$ can catalyze the overall four-electron reduction of dioxygen by I^- (eq 5).

Mechanism of the Overall Four-Electron Reduction of Dioxygen by $AcrH_2$. The combination of the $CoTPP^+$-catalyzed oxidation of $AcrH_2$ by dioxygen in the presence of $HClO_4$ (Scheme III) and the catalytic four-electron reduction of dioxygen by I^- (Scheme V) results in the successful overall four-electron reduction of dioxygen by $AcrH_2$ (eq 6). Iodine formed in the four-electron reduction of dioxygen by I^- (Scheme V) is readily reduced by $AcrH_2$ to regenerate I^- (eq 7). Thus, a mechanism of the catalytic overall four-electron reduction of dioxygen with $AcrH_2$ in the presence of $CoTPP^+$, $HClO_4$, and I^- may be given as shown in Scheme VI by combining the reduction of I_2 by $AcrH_2$ (eq 7) with schemes III and V. According to Scheme VI, the pseudo-first-order rate constant for the formation of $AcrH^+$ may be given by eq 19.[41] A maximal dependence of the rate constant on the I^-

$$k^{(1)} = \frac{k'_{et}k_H[H^+][O_2][CoTPP^+]}{k'_b + k_H} + k_{ox}[I_2] \qquad (19)$$

---

(35) Fukuzumi, S.; Ishikawa, M.; Tanaka, T. *J. Chem. Soc., Perkin Trans. 2* **1989**, 1037.

(36) In the case of radical-chain oxidation of $AcrH_2$ with $HO_2^\bullet$, which is produced by the initiation reaction of $AcrH_2$ with $CoTPP^+$ in the presence of O_2 and decays by the bimolecular termination reaction, the observed rate would be proportional to $[AcrH_2]^{3/2}[CoTPP^+]^{1/2}$.[21,35]

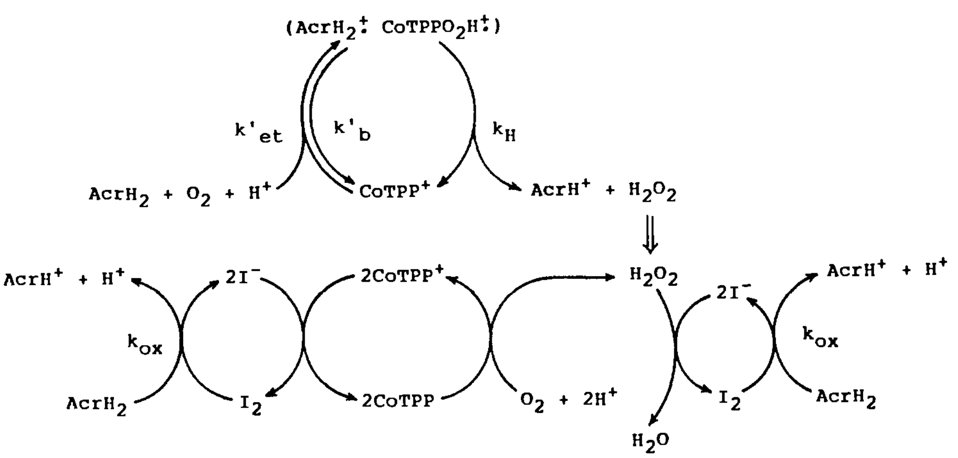
(37) (a) Donnan, F. G.; Rossignnal, R. Le *J. Chem. Soc.* **1903**, 703. (b) Friedman, H. B.; Anderson, B. E. *J. Am. Chem. Soc.* **1939**, *61*, 116. (c) Raycheba, J. M. T.; Margerum, D. W. *Inorg. Chem.* **1981**, *20*, 1441. (d) Tejeda, P. P.; Velasco, J. R.; Burgos, F. S. *J. Chem. Soc., Dalton Trans.* **1983**, 2679.

(38) Nord, G.; Pedersen, B.; Floryan-Løborg, E.; Pagsberg, P. *Inorg. Chem.* **1982**, *21*, 2327.

(39) Nagarijan, V.; Fessenden, R. W. *J. Phys. Chem.* **1985**, *89*, 2330.

(40) Nord, G.; Pedersen, B.; Farver, O. *Inorg. Chem.* **1978**, *17*, 2233.

Scheme VI



concentration in Figure 8 can be well explained by eq 19 as follows. Under the steady-state conditions, the rate of reduction of I_2 by $AcrH_2$ (eq 7) is balanced with the rate of oxidation of I^- by $CoTPP^+$ (eq 16), when the steady-state concentration of I_2 is given by eq 20.[42] According to eq 20, the steady-state concentration

$$[I_2] = k_1[\mathrm{CoTPP}^+]/k_{ox}[\mathrm{AcrH}_2] \qquad (20)$$

of I_2 is independent of the I^- concentration. This may be the reason that the rate of the overall four-electron reduction of dioxygen by $AcrH_2$ does not increase with an increase in the I^- concentration $> 10^{-4}$ M and that the same results are obtained when I^- is replaced by I_2 in Figure 8. The steady-state concentration of I_2, which can be estimated as 1.7×10^{-4} M from the rate constants of k_1 and k_{ox} by using eq 20, agrees with the I^- concentration with which the maximum rate is obtained in Figure 8.[43] The decrease in the rate with a further increase in the I^- concentration (Figure 9) may be caused by the formation of I_3^- by the reaction of I_2 with I^-. Thus, there is an optimal concentration of I^- employed in the catalytic four-electron reduction of dioxygen by $AcrH_2$ in the presence of I^-, $CoTPP^+$, and $HClO_4$. The dependence of the rate on the $HClO_4$ concentration (Figure 9) also agrees with eq 19. In this case, the presence of H_2O (1.1 M) decreases the protonation equilibrium constant (eq 4),[11] and thereby the rate constant increases with an increase in the $HClO_4$ concentration (compare Figure 9 with Figure 4 in which no H_2O is added).[41] The intercept seen in Figure 9 corresponds to the second term in eq 19, which is independent of the acid concentration.

In conclusion, the catalytic four-electron reduction of dioxygen by an NADH model compound ($AcrH_2$) has been achieved by the appropriate combination of two catalytic systems, as shown in Scheme VI, the catalytic two-electron reduction of dioxygen by $AcrH_2$ (Scheme III) and the catalytic four-electron reduction of dioxygen by I^- (Scheme V).

---

(41) The protonation of $AcrH_2$ (eq 4) can be neglected under the experimental conditions in Figures 8 and 9,[28] and thus, the effect is not included in eq 19.

(42) The concentration of I^- employed in Figure 9 is small enough to allow the second term in eq 18 for the formation rate of I_2 $(=(^1/_2)\mathrm{d}[\mathrm{CoTPP}]/\mathrm{d}t)$ to be neglected, $k_2[I^-]$, in deriving eq 20.

(43) The k_1 value is obtained as 2.5×10^{-2} s^{-1} from the intercept in Figure 7. Then, the steady-state concentration of I_2 is evaluated as 1.7×10^{-4} M $(=(2.5 \times 10^{-2})(1.0 \times 10^{-4})/[15(1.0 \times 10^{-3})])$ by using eq 20.