reported previously[7] but with some improvement on a linear photodiode array detection of absorption spectra. Details of experimental conditions are described in the figure captions.

Figure 1 shows the absorption spectra of the $^{16}O_2$ (A) and $^{18}O_2$ (B) derivatives, which are represented as difference spectra of the intermediates minus the CO-photodissociated species. The absolute spectra (not shown) indicated that 90% of the enzyme adopted the CO-photodissociated form in the laser beam in the absence of O_2. The two difference spectra in Figure 1 are very much alike and reproduce the previous observation for compound A.[8] Although the peak at 586 nm and the trough at 604 nm resemble those of the Fe^{II}-CO form drawn by a dotted line (C), the broad features of spectra A and B around 530-540 nm are distinct from those of spectrum C. The asymmetry between the 586-nm peak and the 604-nm trough might imply contamination of the subsequent intermediate, because they are more symmetric in the corresponding difference spectrum for the 20-μs transient species reported by Orii[3e] but are asymmetric in the subsequent intermediate. The intensity of the 586-nm band became weaker for lower concentrations of oxygen or for slower flow rates of sample.

Traces A and B in Figure 2 show the RR spectra observed simultaneously with absorption spectra A and B, respectively, shown in Figure 1. These RR spectra are represented as difference spectra, observed minus CO-photodissociated. Since the CO-photodissociated form was measured with the same instrumental conditions at the same time as for the intermediate except for the absence of O_2 in the solution (see caption of Figure 2), any possible contribution from the spectrum of the unphotodissociated form is subtracted in the difference spectra. A Raman band at 569 cm^{-1} in spectrum A is missing in spectrum B, where a new band appears at 540 cm^{-1}. The difference between spectra A and B, delineated by Figure 2C, clearly indicates that the 569-cm^{-1} band for the $^{16}O_2$ derivative is shifted to 540 cm^{-1} for the $^{18}O_2$ derivative. Note that this feature was reproduced for several different preparations of the enzyme and two different excitation wavelengths (425 and 418 nm). The observed isotopic shift (29 cm^{-1}) is slightly larger than the calculated shift for the Fe-O_2 two-body harmonic oscillator (21 cm^{-1}) but is close to the value (26 cm^{-1}) expected when the oxygen is vibrating against the combined body of the iron and its trans ligand (imidazole) and in agreement with the observed isotopic shifts for oxyhemoglobin (oxyHb) (27-28 cm^{-1}).[10a,b] Accordingly, the 569-cm^{-1} band is assigned to the Fe-O_2 stretching vibration [ν(Fe-O_2)]. It was unexpected that the ν(Fe-O_2) frequency of this enzyme would be remarkably close to those of oxyHb (567 cm^{-1}, pH 7.4 at 10 °C,[10a] and 572 cm^{-1}, pH 8.5 at 10 °C[10b]) and oxyMb (569 cm^{-1}, pH 6.8 at 15 °C)[11] and also to that of a model heme *a* imidazole complex (576 cm^{-1}).[12] This may imply that the Fe^{II}-O_2 heme of this enzyme adopts a structure similar to that in oxyHb and oxyMb.

The present ν(Fe-O_2) frequency is different from that (589 cm^{-1}) reported by Varotsis et al.,[6d] who used 10-ns laser pulses. The delay time in this experiment has appreciable distribution but is at most 200 μs, which is the resident time of a molecule in the laser beam. Although this might be a possible origin for the discrepancy, it is noted that the actual time resolution in this experiment strongly depended on the laser power. When we used higher laser power, we could not identify the present intermediate. Whatever the time resolution is, it is important that the species exhibiting the absorption spectrum of compound A gives the ν(Fe-O_2) RR band.

The 569-cm^{-1} RR band of this intermediate was observable upon excitations at 418, 425, and 430 nm but not at 406.7 and 441.6 nm. For oxyHb the ν(Fe-O_2) RR band was reported to be most resonance enhanced at the Soret maximum (413 nm).[13] If the mechanism of intensity enhancement is the same between Hb and this intermediate, the Soret maximum of the $a_3{}^{II}$-O_2 heme would be located around 420-430 nm.

Note Added in Proof. After submission of this paper, the reported ν(Fe-O_2) frequency by Varotsis et al.[6d] was corrected from 589 to 571 cm^{-1},[14a] in agreement with the present results. On the other hand, the ν(Fe-O_2) RR band for the mixed-valence cytochrome oxidase was found at 568 cm^{-1},[14b] which is also very close to the present observation.

Acknowledgment. This study was carried out partly under the NIBB Cooperative Research Program for the Okazaki Large Spectrograph (89-522) and supported partly by a Grant-in-Aid for Encouragement of Young Scientists (62780230) to T.O. and for Scientific Research in Priority Area (63635005) to T.K. from the Ministry of Education, Science, and Culture, Japan.

(10) (a) Brunner, H. *Naturwissenschaften* **1974**, *61*, 129. (b) Nagai, K.; Kitagawa, T.; Morimoto, H. *J. Mol. Biol.* **1980**, *136*, 271-289.
(11) Van Wart, H. E.; Zimmer, J. *J. Biol. Chem.* **1985**, *260*, 8372-8377.
(12) Oertling, W. A.; Kean, R. T.; Wever, R.; Babcock, G. T. *J. Am. Chem. Soc.*, in press.
(13) Walters, M. A.; Spiro, T. G. *Biochemistry* **1982**, *21*, 6989-6995.
(14) (a) Varotsis, C.; Woodruff, W. H.; Babcock, G. T. *J. Am. Chem. Soc.* **1990**, *112*, 1297. (b) Han, S.; Ching, Y.; Rousseau, D. L. *Biochemistry* **1990**, *29*, 1380-1384.

10-Methylacridine Dimer Acting as a Unique Two-Electron Donor in the One-Electron Reduction of Triphenylmethyl Cation

Shunichi Fukuzumi,*[1a] Toshiaki Kitano, and Masashi Ishikawa[1b]

Department of Applied Chemistry
Faculty of Engineering, Osaka University
Suita, Osaka 565, Japan
Received March 19, 1990

Most organic reductants (DH) are known as two-electron donors, and the two-electron oxidation is normally accompanied by the removal of a proton (eq 1).[2,3] On the other hand, or-

$$\mathrm{DH} \xrightarrow{-e} \mathrm{DH}^{+}\cdot \xrightarrow{H^{+}} \mathrm{D}\cdot \xrightarrow{-e} \mathrm{D}^{+} \qquad (1)$$

ganometallic compounds containing metal-metal bonds such as permethylated catenates of group 4B elements, Me_3M-$M'Me_3$ (M, M' = Sn, Ge, Sn), are known to act as pure two-electron donors by successive one-electron oxidation which involves cleavage of the metal-metal bond following the initial one-electron oxidation (eq 2).[4-6] However, no organic reductant acting as a pure

$$\mathrm{M\text{-}M'} \xrightarrow{-e} \mathrm{M\text{-}M'^{+}}\cdot \xrightarrow{M^{+}} \mathrm{M'}\cdot \xrightarrow{-e} \mathrm{M'^{+}} \qquad (2)$$

two-electron donor without removal of a proton has so far been reported, since oxidative C-C bond cleavage is generally believed to be extremely difficult compared to that of C-H as well as metal-metal bonds.[7,8]

(1) (a) To whom correspondence should be addressed at Osaka University. (b) Current address: Laboratory of Organic Chemistry, Osaka Women's University, Sakai, Osaka 590, Japan.
(2) In highly acidic media some organic reductants act as one-electron donors when the radical cations are stable; see: (a) Bard, A. J.; Ledwith, A.; Shine, H. J. *Adv. Phys. Org. Chem.* **1976**, *13*, 155. (b) Howell, J. O.; Concalves, J. M.; Amatore, C.; Klasinc, L.; Wightman, R. M.; Kochi, J. K. *J. Am. Chem. Soc.* **1984**, *106*, 3968.
(3) For an extensive review of radical cation chemistry, see: Bauld, N. L. *Tetrahedron* **1989**, *45*, 5307.
(4) Kochi, J. K. *Organometallic Mechanism and Catalysis*; Academic Press: New York, 1978.
(5) Fukuzumi, S.; Kitano, T.; Mochida, K. *J. Am. Chem. Soc.* **1990**, *112*, 3246.
(6) One-electron reduction of aromatic ketones by a mixture of magnesium iodide and magnesium has been known for a long time; see: Gomberg, M.; Bachmann, W. E. *J. Am. Chem. Soc.* **1927**, *49*, 236, 2584.
(7) A few examples of photoinduced oxidative cleavage of C-C bonds which results in complex redox reactions have recently been reported; see: (a) Wayner, D. D. M.; Gravelle, L. *Tetrahedron Lett.* **1988**, *29*, 431. (b) Arnold, D. R.; Fahie, B. J.; Lamont, L. J.; Wierzchowski, J.; Young, K. M. *Can. J. Chem.* **1987**, *65*, 2734.
(8) (a) Griller, D.; Simões, J. A. M.; Mulder, P.; Sim, B. A.; Wayner, D. D. M. *J. Am. Chem. Soc.* **1989**, *111*, 7872. (b) Bordwell, F. G. *Acc. Chem. Res.* **1988**, *21*, 456.

0002-7863/90/1512-5631$02.50/0

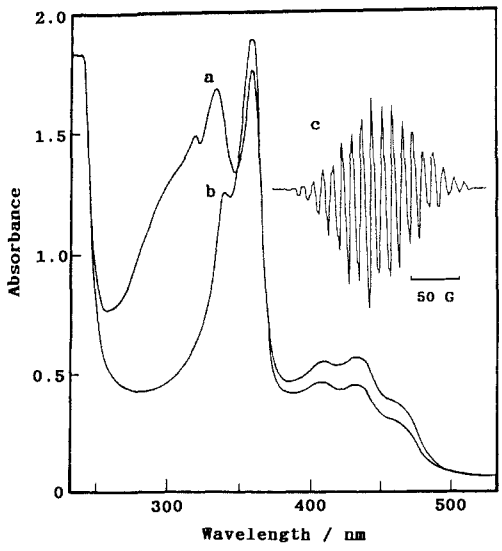


Figure 1. (a) Electronic absorption spectrum observed in the reaction of $(AcrH)_2$ (5.0×10^{-5} M) with Ph_3C^+ (1.0×10^{-4} M) in deaerated MeCN and (b) that after introducing dioxygen to the solution. (c) ESR spectrum observed in the reaction of $(AcrH)_2$ (1.5×10^{-3} M) with Ph_3C^+ (1.5×10^{-3} M) in deaerated MeCN.

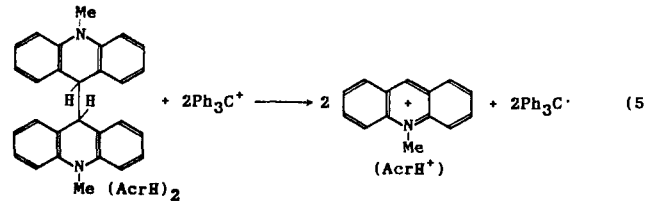
We report herein the unique donor property of an NAD dimer analogue,[9-11] 10,10′-dimethyl-9,9′-biacridine [$(AcrH)_2$],[12,13] acting as a pure two-electron donor in the one-electron reduction of triphenylmethyl cation (Ph_3C^+) compared to the corresponding monomer, 9,10-dihydro-10-methylacridine ($AcrH_2$), acting as a normal hydride (two-electron and one-proton) donor in the two-electron reduction of Ph_3C^+.

Upon mixing of $(AcrH)_2$ with Ph_3C^+ in deaerated acetonitrile (MeCN) at 298 K, $(AcrH)_2$ is readily oxidized to $AcrH^+$ (λ_{max} = 358 nm), accompanied by the formation of a new absorption band at λ_{max} = 320 nm, which disappears when dioxygen is introduced to the deaerated solution as shown in Figure 1 (parts a and b). The electron spin resonance (ESR) measurement of the deaerated product solution reveals the formation of triphenylmethyl radical (g = 2.0024) as also shown in Figure 1 (part c). The triphenylmethyl radical is stable in MeCN at 298 K,[14] but disappears when dioxygen is introduced to the deaerated solution to yield triphenylmethyl peroxide (eqs 3 and 4).[15] Thus,

$$Ph_3C^\bullet + O_2 \rightarrow Ph_3COO^\bullet \quad (3)$$

$$Ph_3COO^\bullet + Ph_3C^\bullet \rightarrow Ph_3COOCPh_3 \quad (4)$$

$(AcrH)_2$ acts as a two-electron donor in the one-electron reduction of Ph_3C^+ (eq 5).[16] In contrast, the corresponding monomer ($AcrH_2$) is known to act as a hydride donor in the two-electron reduction of Ph_3C^+ (eq 6).[17]



$$AcrH_2 + Ph_3C^+ \rightarrow AcrH^+ + Ph_3CH \quad (6)$$

The rates of formation of $AcrH^+$ in eqs 5 and 6 obey second-order kinetics, showing first-order dependence on each reactant concentration. The second-order rate constants (k_{obsd}) for the reduction of Ph_3C^+ by $(AcrH)_2$ and $AcrH_2$ in deaerated MeCN at 298 K are determined as 5.7×10 and 1.7×10^3 M^{-1} s^{-1}, respectively. The same k_{obsd} value of $AcrH_2$ is obtained in the presence of dioxygen in MeCN at 298 K. The k_{obsd} value of $(AcrH)_2$ is also unaffected by the presence of dioxygen, although the product is changed from $Ph_3C^\bullet$ to $Ph_3COOCPh_3$ as mentioned above. Thus, the rate-determining step in the formation of $AcrH^+$ may be electron transfer from $(AcrH)_2$ to Ph_3C^+ (eq 7), followed by facile cleavage of the C–C bond of $(AcrH)_2^{\bullet+}$ to yield $AcrH^+$ and $AcrH^\bullet$ (eq 8).[18] In fact, no ESR signal has been detected

$$(AcrH)_2 + Ph_3C^+ \rightarrow (AcrH)_2^{\bullet+} + Ph_3C^\bullet \quad (7)$$

$$(AcrH)_2^{\bullet+} \xrightarrow{\text{fast}} AcrH^\bullet + AcrH^+ \quad (8)$$

in the electron-transfer reaction from $(AcrH)_2$ to Fe^{3+} (rapid-mixing technique has been used), indicating that the lifetime of $(AcrH)_2^{\bullet+}$ is shorter than 10 ms.[19] The second electron transfer from $AcrH^\bullet$ to Ph_3C^+ (eq 9) may be much faster than the initial

$$AcrH^\bullet + Ph_3C^+ \rightarrow AcrH^+ + Ph_3C^\bullet \quad (9)$$

electron transfer from $(AcrH)_2$ to Ph_3C^+, since the electron transfer may be highly exothermic, judging from the one electron oxidation potential of $AcrH^\bullet$ (E°_{ox} = −0.43 V vs SCE)[20,21] and the one electron reduction potential of Ph_3C^+ (0.27 V vs SCE).[22] The one electron oxidation potential of $(AcrH)_2$ is determined as 0.23 V (vs SCE) by kinetic analysis of the cyclic voltammograms by applying the reported method in determining the one electron oxidation potential of $AcrH_2$ (E°_{ox} = 0.80 V vs SCE),[20,21] which is more positive than that of $(AcrH)_2$. Thus, electron transfer from $(AcrH)_2$ to Ph_3C^+ is slightly exergonic,[23] while electron transfer from $AcrH_2$ to Ph_3C^+ is highly endergonic. In consequence, $(AcrH)_2$ may prefer to undergo the electron-transfer pathway (eq 7), while $AcrH_2$ can transfer a hydride ion to Ph_3C^+ (eq 6). To our knowledge the unique donor property of $(AcrH)_2$ demonstrated by the present study may be the first example of an organic reductant acting as a pure two-electron donor in the one-electron reduction of a substrate.

Acknowledgment. This work was supported in part by a grant-in-aid (to S.F.) for scientific research from the Ministry of Education, Science and Culture, Japan.

(9) One-electron reduction of nicotinamide adenine dinucleotide (NAD^+) and related compounds is known to yield the NAD dimer and analogues.[10] Although possible involvement of these dimers in redox enzymatic processes has been noted,[11] little is known about the reactivities of the dimers as compared to the corresponding monomers.

(10) (a) Elving, P. J. *Topics in Bioelectrochemistry and Bioenergetics*; Milazzo, G., Ed.; Wiley: New York, 1976; Vol. 1, p 179. (b) Carelli, V.; Liberatore, F.; Casini, A.; Mondelli, R.; Arnone, A.; Carelli, I.; Rotilio, G.; Mavelli, I. *Bioorg. Chem.* **1980**, *9*, 342.

(11) Czochralska, B.; Szweykowska, M.; Shugar, D. *Arch. Biochem. Biophys.* **1980**, *199*, 497.

(12) The dimer $(AcrH)_2$ was prepared by the one-electron reduction of $AcrH^+$ by hexamethylditin.[5]

(13) The dimer $(AcrH)_2$ is also formed in the reduction of $AcrH^+$ by $Mn(CO)_5^-$; see: Lehmann, R. E.; Bockman, T. M.; Kochi, J. K. *J. Am. Chem. Soc.* **1990**, *112*, 458.

(14) (a) Chu, T. L.; Weissman, S. I. *J. Chem. Phys.* **1954**, *22*, 21. (b) Kraeutler, B.; Jaeger, C. D.; Bard, A. J. *J. Am. Chem. Soc.* **1978**, *100*, 4903.

(15) (a) Davies, A. G. *Organic Peroxides*; Butterworth: London, 1961; p 51. (b) Pryor, W. A. *Free Radicals*; McGraw-Hill: New York, 1966; p 290. (c) Janzen, E. G.; Johnston, F. J.; Ayers, C. L. *J. Am. Chem. Soc.* **1967**, *89*, 1176.

(16) The triphenylmethyl radical is known to be in equilibrium with the dimer; see: Colle, T. H.; Glaspie, P. S.; Lewis, E. S. *J. Org. Chem.* **1978**, *43*, 2722.

(17) Ishikawa, M.; Fukuzumi, S.; Goto, T.; Tanaka, T. *Bull. Chem. Soc. Jpn.* **1989**, *62*, 3754.

(18) No dissociation of the dimer $(AcrH)_2$ to $AcrH^\bullet$ may occur, since $(AcrH)_2$ is stable toward dioxygen, which is known to trap $AcrH^\bullet$ efficiently; see: Fukuzumi, S.; Ishikawa, M.; Tanaka, T. *J. Chem. Soc., Perkin Trans. 2* **1989**, 1037.

(19) By applying the same technique, the ESR signal due to $AcrH_2^{\bullet+}$, the lifetime of which is 80 ms, has been detected: Fukuzumi, S.; Kitano, T., to be published.

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

(21) Similar E°_{ox} values of $AcrH_2$ and AcrH have recently been reported; see: Hapiot, P.; Moiroux, J.; Savéant, J.-M. *J. Am. Chem. Soc.* **1990**, *112*, 1337.

(22) (a) Volz, H.; Lotsch, W. *Tetrahedron Lett.* **1969**, 2275. (b) Breslow, R.; Bahary, W.; Reinmuth, W. *J. Am. Chem. Soc.* **1961**, *83*, 1763. (c) Wasielewski, M. R.; Breslow, R. *J. Am. Chem. Soc.* **1976**, *98*, 4222.

(23) The rather small rate constant (5.7×10 M^{-1} s^{-1}) of $(AcrH)_2$ may be ascribed to the steric effect in the electron-transfer reaction being nonadiabatic, since the intrinsic barrier of electron transfer of Ph_3C^+ is known to be small; see: Lown, J. W. *Proc. Chem. Soc., London* **1963**, 283.