Formation and Spectroscopic Characterization of the PeroxoFe^{III}-Cu^{II} Complex. A Modeling Reaction of the Heme-Cu Site in Cytochrome *c* Oxidase

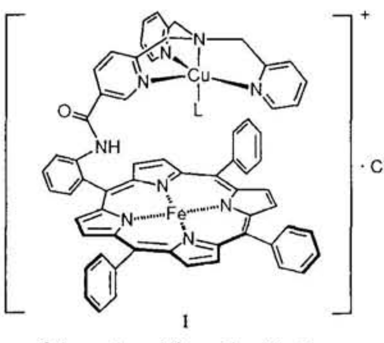
Takao Sasaki, Nobuhumi Nakamura, and Yoshinori Naruta*
Institute for Fundamental Research of Organic Chemistry, Kyushu University, Hakozaki, Fukuoka 812

(Received January 22, 1998; CL-980052)

Formation of a unique peroxo complex Fe^{III}TPP(O_2)-Cu^{II}TPA **2** was observed in the oxygenated reaction of Fe^{II}TPP-Cu^{I}TPA **1** with O_2 at ambient temperature. This complex was characterized by electronic absorption, ESI-MS, resonance Raman, and ESR spectroscopies.

Dioxygen reaction with reduced transition metal complexes is important on the respects of O_2 binding and activation in biological systems.[1] In recent years, a number of studies have appeared on this topic. Among these, the dinuclear metal complexes of Fe and Cu have widely been used in order to determine the structure and reactivity of their intermediates. Beside these *homo*dinuclear metal complexes, a *hetero*dinuclear one is attractive in both its O_2 coordination mode and the relation to the active site of cytochrome *c* oxidase (CcO),[2] where the iron porphyrin (heme a_3) and one copper ion (Cu_B) cooperatively reduce O_2 to water under continuous electron/proton flux.[3] In spite of extensive efforts to identify the intermediates during O_2 reduction in CcO, only a part of them could be assigned by time-resolved resonance Raman spectroscopy.[4] The structures of the most interesting precursor to give the Fe^{IV}=O species subsequently and the resting state are still the subject under discussion. Several heme Fe(or Co)-Cu systems have been proposed as active site models of CcO[5] and notable characterization of the intermediate O_2 complex has been done. We report herein the novel preparation of a stable $Fe^{III}(O_2)$-Cu^{II} complex at ambient temperature and the spectroscopic characterization of its O_2 binding mode.

We synthesized the tetraphenylporphyrin (TPP) with tris(2-pyridylmethyl)-amine (TPA)[6] as a model ligand of the reaction center in cytochrome *c* oxidase. The two ligands were connected by an amide linkage to take somewhat restricted conformation. In the stepwise metallation of the free base, this ligand was lead to the reduced complex Fe^{II}TPP-Cu^{I}TPA (**1**).[7]



1

The reaction of the reduced form **1** with O_2 was measured by UV-vis spectroscopy in CH_3CN at 25 ℃ in the absence of nitrogen base. The spectrum of **1** shows absorptions at λ_{max} (ε, $M^{-1}cm^{-1}$) = 428 (Soret, 104000), 535 (6670), 563 (4630), and 609 (1790) nm. When O_2 was added to the solution, a new compound **2** was detected by the UV-vis spectral change to λ_{max} (ε, $M^{-1}cm^{-1}$) = 419 (85400) , 560 (7890) nm. This compound shows considerable stability at ambient temperature ($t_{1/2} \approx 100$ min). The blue shift of the Soret band is characteristic to the formation of heme Fe-O_2 complexes.[8]

To observe the reaction intermediate directly, this oxygenation reaction was monitored by an electrospray ionization (ESI) mass spectrometer in CH_3CN at 25 ℃ (Figure 1).[9] It shows a unique ion cluster at m/z = 1094 assignable to a monocation $[Fe^{III}TPP$-$Cu^{II}TPA(O_2)]^+$ from the mass number and its isotope pattern (Figure 1, a). Upon using $^{18}O_2$, the spectrum shows an ion cluster at m/z = 1098 which is four mass units larger than with $^{16}O_2$ (Figure 1, b). These results clearly indicate that the oxygenation reaction occurs in the ratio Fe^{II}-Cu^{I}:O_2 = 1:1 and does not give any other oxygenated products in an intermolecular fashion.

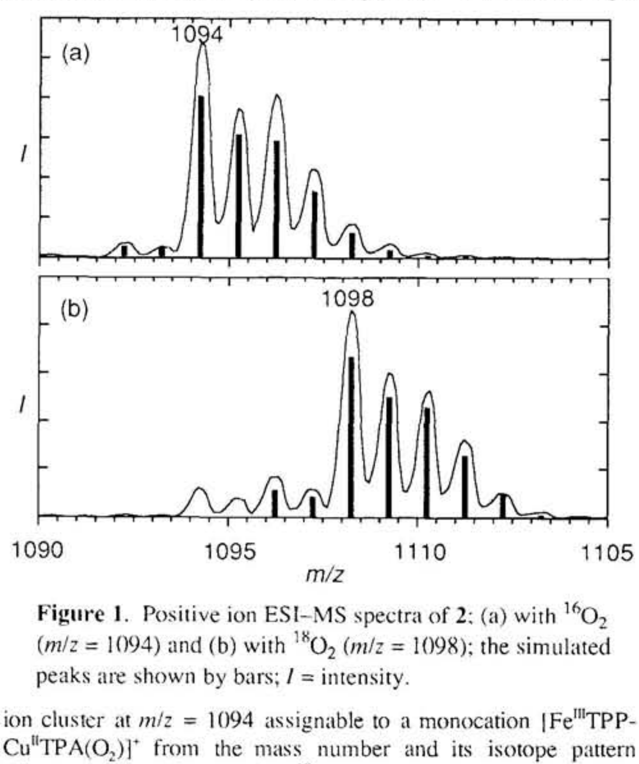


Figure 1. Positive ion ESI–MS spectra of **2**: (a) with $^{16}O_2$ (m/z = 1094) and (b) with $^{18}O_2$ (m/z = 1098); the simulated peaks are shown by bars; I = intensity.

Resonance Raman spectra of **2** were observed in CH_3CN at 25 ℃ in order to characterize the binding mode of the peroxo dianion (Figure 2).[10] The oxygenation of **1** with $^{16}O_2$ indicated an oxygen-sensitive Raman band at 803 cm^{-1} which shifted to 759 cm^{-1} with use of $^{18}O_2$ ($\Delta ^{16}O_2/^{18}O_2$ = 44 cm^{-1}). On using $^{16}O^{18}O$ isotopically mixed gas ($^{16}O_2$:$^{16}O^{18}O$:$^{18}O_2$=30.5:39.0:30.5), the appearance of a new band at 781 cm^{-1} indicates the presence of a O-O bond and these isotope shifts are in good agreement with the calculated ones. The value of this ν(O-O) band (803 cm^{-1}) is in the range of the 2e$^-$ reduced peroxide level.[3] The Cu_2^{II} and Fe_2^{III} complexes with end-on or side-on peroxide ligands have values in this region, e.g. $\{[Cu(TMPA)]_2(O_2)\}^{2+}$ (834 cm^{-1}, end-on),[11] and

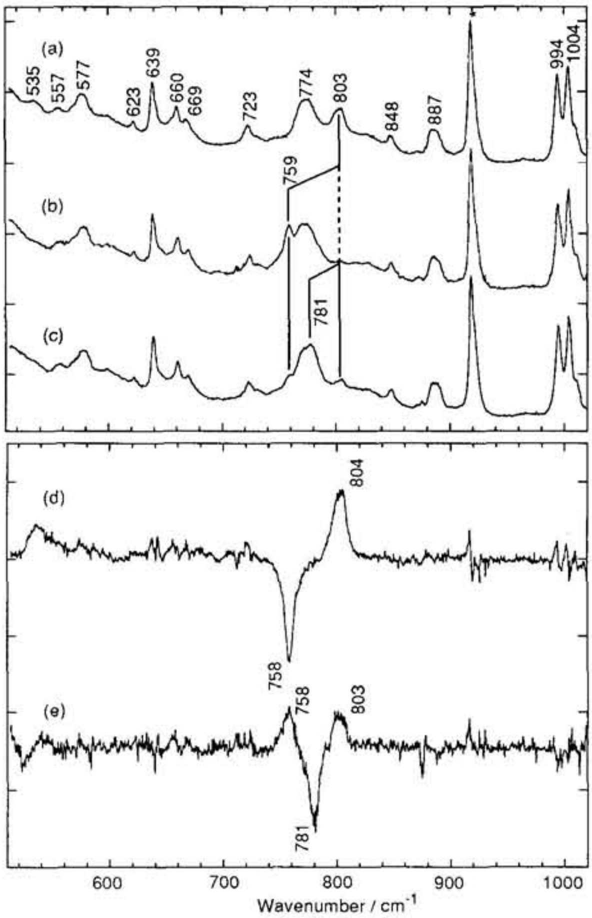
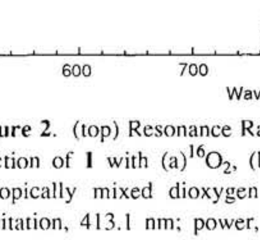
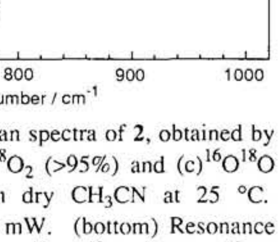


Figure 2. (top) Resonance Raman spectra of **2**, obtained by reaction of **1** with (a)$^{16}O_2$, (b)$^{18}O_2$ (>95%) and (c)$^{16}O^{18}O$ isotopically mixed dioxygen in dry CH_3CN at 25 °C. Excitation, 413.1 nm; power, 5 mW. (bottom) Resonance Raman difference spectra of **2**, (d)$^{16}O_2$–$^{18}O_2$ and (e)($^{16}O_2$+ $^{18}O_2$)/2–$^{16}O^{18}O$ mixed gas. Each resource datum was normalized with the largest peak at 918 cm^{-1} arising from the solvent (*).

$[CuHB(3,5\text{-}ipr_2pz)_3]_2(O_2)$ (741 cm^{-1}, side-on).[12] The values of ν(O-O) in this region also have been reported in the peroxide anions bind to a single metal, $[Cu_2(XYL\text{-}O\text{-})(O_2)]^+$(803 cm^{-1}, end-on)[13] and $[FeTPP(O_2)]^-$(804 cm^{-1}, side-on).[14, 15] These results suggest that **2** can take either of the two O_2 coordination modes between the end-on (**2a**) and the side-on peroxide (**2b**). The degree of the magnetic interaction between the two paramagnetic metals is remarkably different in these two structures.[16] The silent ESR spectrum of **2** in a frozen solution (CH_3CN, 6K) indicates the presence of the strong magnetic coupling between the two metals.



2a



2b

All these results support that **2** is a peroxide complex (**2a**) with end-on μ-1,2 geometry. The heme a_3-Cu_B site of cytochrome *c* oxidase at its resting state shows silent ESR spectrum interestingly.[17] The reason of this antiferromagnetic interaction, however, has not been in agreement between the model studies[18] and the X-ray crystal analysis.[19] Therefore, in consideration of the stability of **2a** at ambient temperature, the peroxide bridged structure is one of the most likely candidates of the resting form.

References and Notes

1 Recent reviews in this topic: E. I. Solomon, U. M. Sundaram, and T. E. Machonkin, *Chem. Rev.*, **96**, 2563 (1996); B. J. Wallar and J. D. Lipscomb, *Chem. Rev.*, **96**, 2625 (1996); M. Ito, K. Fujisawa, N. Kitajima, and Y. Moro-oka in "Oxygenases and Model Systems," ed by T. Funabiki, Kluwer Academic, Dordrecht (1997), p. 345.

2 a) T. Tsukihara, H. Aoyama, E. Yamashita, T. Tomizaki, H. Yamaguchi, K. Shinzawa-Itoh, R. Nakashima, R. Yaono, and S. Yoshikawa, *Science*, **269**, 1069 (1995); T. Tsukihara, H. Aoyama, E. Yamashita, T. Tomizaki, H. Yamaguchi, K. Shinzawa-Itoh, R. Nakashima, R. Yaono, and S. Yoshikawa, *Science*, **272**, 1136 (1996). b) S. Iwata, C. Ostermeier, B. Ludwig, and H. Michel, *Nature*, **376**, 660 (1995).

3 Recent reviews, see; a) S. Ferguson-Miller and G. T. Babcock, *Chem. Rev.*, **96**, 2889 (1996) and references cited therein. b) T. Kitagawa and T. Ogura, *Prog. Inorg. Chem.*, **45**, 431 (1997).

4 a) T. Ogura, S. Hirota, D. A. Proshlyakov, K. Shinzawa-Itoh, S. Yoshikawa, and T. Kitagawa, *J. Am. Chem. Soc.*, **118**, 5443 (1996). b) C. A. Varotsis, and G. T. Babcock, *J. Am. Chem. Soc.*, **117**, 11260 (1995).

5 a) A. Nanthakumar, S. Fox, N. N. Murthy, and K. D. Karlin, *J. Am. Chem. Soc.*, **119**, 3898 (1997). b) H. H. Zhang, A. Filipponi, A. Di Cicco, M. J. Scott, R. H. Holm, B. Hedman, and K. O. Hodgson, *J. Am. Chem. Soc.*, **119**, 2470 (1997). c) J. P. Collman, L. Fu, P. C. Herrmann, and X, Zhang, *Science*, **275**, 949 (1997); J. P. Collman, P. C. Herrmann, B. Boitrel, X. Zhang, T. A. Eberspacher, and L. Fu, *J. Am. Chem. Soc.*, **116**, 9783 (1994).

6 The metal free ligand of **1**, $C_{63}H_{47}N_9O$; ^1H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ = 8.92(d, ^3J(H,H) = 8 Hz, 1H, 6-phenyl), 8.87(s, 6H, pyrrole β), 8.83(s, 1H, pyrrole β), 8.82(s, 1H, pyrrole β), 8.21-8.14(9H, *o*-phenyl, 6-pyridyl), 8.07(d, ^3J(H,H) = 8 Hz, 1H, 3-phenyl), 7.89(dd, ^3J(H,H) = 8 Hz, 1H, 5-phenyl), 8.81-7.70(10H, 3-, 4-, and 5-phenyl, 2-pyridyl), 7.55(dd, ^3J(H,H) = 8 Hz, 1H, 4-phenyl), 7.15(dd, ^3J(H,H) = 8 Hz, 2H, 4-pyridyl), 6.98(d, ^3J(H,H) = 8 Hz, 1H, 3-pyridyl), 6.80(dd, ^3J(H,H) = 7 Hz, 2H, 5-pyridyl), 3.43(s, 4H, $-CH_2-$), 3.34(s, 2H, $-CH_2-$), -2.78(s, 2H, pyrrole NH); MS (FAB): m/z : 947 $M+H^+$.

7 The detailed synthetic procedure of Fe^{II}TPP-Cu^{I}TPA will be described elsewhere.

8 G. B. Jameson and J. B. Ibers, in "Bioinorganic Chemistry," ed by I. Bertini, H. B. Gray, S. J. Lippard, and J. S. Valentine, University Science, Mill Valley (1994), Chap. 4, p. 167.

9 In this O_2 reaction, the sample solution was introduced from the cell for UV/vis spectroscopy, the formation of **2** was confirmed by both spectra. ESI-MS spectral data were collected with a PE Sciex API 300 triple quadrapole mass spectrometer (Sciex, Thonhill, ON, Canada).

10 To confirm the progress of the oxygenation reaction, UV-vis absorption spectra were simultaneously monitored by a spectrometer with a photodiode array detector during its resonance Raman measurement.

11 M. J. Baldwin, P. K. Ross, J. E. Pate, Z. Tyeklár, K. D. Karlin, and E. I. Solomon, *J. Am. Chem. Soc.*, **110**, 3690 (1988).

12 N. Kitajima, K. Fujisawa, C. Fujimoto, Y. Moro-oka, S. Hashimoto, T. Kitagawa, K. Toriumi, K. Tatsumi, and A. Nakamura, *J. Am. Chem. Soc.*, **114**, 1227 (1992).

13 J. E. Pate, R. W. Cruse, K. D. Karlin, and E. I. Solomon, *J. Am. Chem. Soc.*, **109**, 2624 (1987).

14 J. N. Burstyn, J. A. Roe, A. R. Miksztal, B. A. Shaevitz, G. Lang, and J. S. Valentine, *J. Am. Chem. Soc.*, **110**, 1382 (1988).

15 Recently, a tailed cavity porphyrin shows ν(O-O) at 802 cm^{-1} with a very close relation. J. P. Collman, *Inorg. Chem.*, **36**, 5145 (1997).

16 Side-on O_2 complexes of hemes show characteristic ESR signals at g = 9.5, 4.2, 1.3. see ref. 20.

17 B. F. Van Gelder and H. Beinert, *Biochim. Biophys. Acta*, **189**, 1 (1969).

18 R. H. Holm, *Pure Appl. Chem.*, **67**, 217 (1995); K. D. Karlin, S. Fox, A. Nanthakumar, N. N. Murthy, N. Wei, H. V. Obias, and C. F. Martens, *Pure Appl. Chem.*, **67**, 289 (1995).

19 Recent X-ray crystal analysis of CcO suggests the presence of a peroxo bridge in the Fe-Cu site, T. Tsukihara and S. Yoshikawa, personal communication.