Selective formation of a stable μ-peroxo ferric heme-Cu^{II} complex from the corresponding μ-oxo Fe^{III}–Cu^{II} species with hydrogen peroxide†

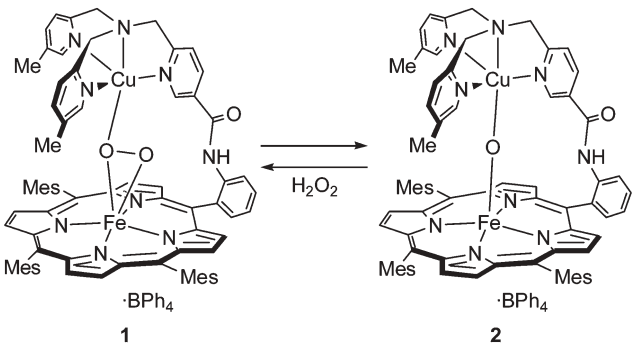
Takefumi Chishiro, Yuichi Shimazaki, Fumito Tani and Yoshinori Naruta*

Received (in Cambridge, UK) 1st September 2004, Accepted 4th October 2004
First published as an Advance Article on the web 12th January 2005
DOI: 10.1039/b413275k

An oxo-bridged ferric heme-copper(II) complex, obtained by thermal transformation of the corresponding peroxo-bridged complex, was reacted with an equimolar amount of H_2O_2 to regenerate the μ-peroxo complex by a ligand exchange from oxo to peroxo, without the formation of a ferryl-oxo species or heme degradation as are observed in general ferric heme-H_2O_2 reactions.

In the catalytic dioxygen reduction of heme-copper oxidases including cytochrome *c* oxidase (C*c*O),[1] ferric heme-peroxo species are considered as important transients for the formation of a ferryl oxo intermediate from the initially formed heme-dioxygen adduct.[2] However, it is still unclear whether the peroxo form is a ferric heme-hydroperoxo, heme-peroxo or heme-μ-peroxo-Cu^{2+} species.[3] The synthetic model studies of C*c*O have indicated either the presence of a heme-hydroperoxo species during the electrochemical O_2 reduction,[4] or the formation of μ-peroxo heme-copper dinuclear complexes.[5,6] We have investigated the reaction of O_2 with the heme-linked copper dinuclear complex [(TMP)Fe^{II}–(5MeTPA)Cu^{I}]$^+$ as a model of C*c*O, and recently reported the isolation and structure of a remarkably stable μ-η^2 : η^1-peroxo complex, [(TMP)Fe^{III}–(O_2)–(5MeTPA)Cu^{II}]BPh_4 (**1**).[7] Therefore, we expected complex **1** to be generated by the reaction of hydrogen peroxide with the oxidized Fe^{III}–Cu^{II} complex of the same ligand as reported for many non-heme iron[8] and copper[9] complexes, although it is generally difficult to prepare stable heme-peroxo species by the reaction of ferric heme and H_2O_2.[2,10–13] In this report, we have performed the reaction of hydrogen peroxide with the corresponding μ-oxo heme-copper complex, [(TMP)Fe^{III}–(O)–(5MeTPA)Cu^{II}]BPh_4 (**2**), and confirmed the formation of **1** spectroscopically. Since **2** was obtained from **1**, the conversion of **2** to **1** could be regarded as the regeneration of the μ-peroxo heme-copper complex (Scheme 1).

The precursor μ-oxo complex **2** was prepared by the thermal transformation of the μ-peroxo complex **1** in CH_3CN at room temperature for several months, and was isolated by recrystallization from acetone as **2**·2(acetone) in 80% yield.‡ The UV–visible spectrum of **2** showed a Soret band at 442 nm, which was significantly red-shifted from that of **1** (420 nm), with new absorption bands at 560 and 596 nm (Fig. 1, dotted line). The ESI mass spectrum of **2** exhibited a peak at m/z = 1232 that is 16 amu smaller than that of the [(TMP)Fe^{III}–(O_2)–(5MeTPA)Cu^{II}]$^+$ cation in **1** (m/z = 1248).[5] By isotopic substitution, [(TMP)Fe^{III}–($^{18}O_2$)–(5MeTPA)Cu^{II}]BPh_4 in dry CH_3CN gave the ^{18}O-labeled complex **2** (m/z = 1234), indicating that the oxygen atom of the peroxo ligand was incorporated in **2**.[5] The oxo ligand is easily exchangable by water oxygen, since the treatment of the solution of ^{16}O-labeled **2** with $H_2{}^{18}O$ rapidly generated ^{18}O-labeled **2**, as confirmed by mass spectra. The crystal structure of **2**§ revealed that an oxo ligand bound to both iron and copper ions, and the Fe–O–Cu moiety had a near-linear structure (see ESI†), as is expected from those of previously reported μ-oxo heme-copper complexes.[5,14] The M–O (M = Fe, Cu) distances in **2** (Fe–O = 1.758(3) Å and



Scheme 1 Interconversion between **1** and **2**.

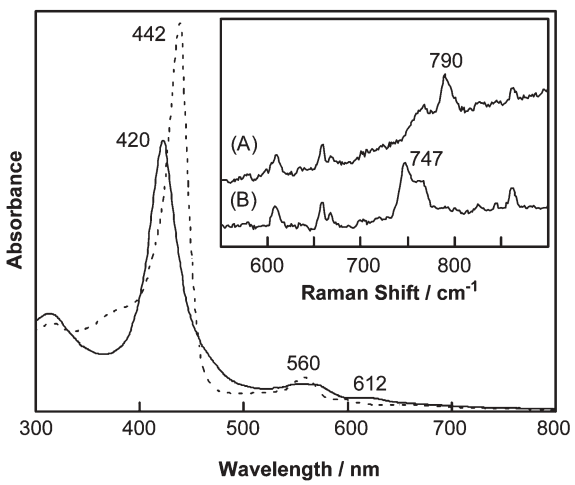


Fig. 1 UV–vis spectra (CH_3CN, room temperature) of [(TMP)Fe^{III}–(O)–(5MeTPA)Cu^{II}]BPh_4 (**2**, dotted line) and the resulting species after the addition of H_2O_2 to **2** (solid line). Inset: resonance Raman spectra of **1** (CH_3CN, −25 °C, λ_{ex} = 413.1 nm) formed by the reaction of **2** with (A) $H_2{}^{16}O_2$ and (B) $H_2{}^{18}O_2$.

† Electronic supplementary information (ESI) available: ORTEP view of [(TMP)Fe^{III}–(O)–(5MeTPA)Cu^{II}]$^+$ in crystals of **2**·3(acetone) drawn with the thermal ellipsoids at the 50% probability level and ESI mass spectra. See http://www.rsc.org/suppdata/cc/b4/b413275k/

*naruta@ms.ifoc.kyushu-u.ac.jp

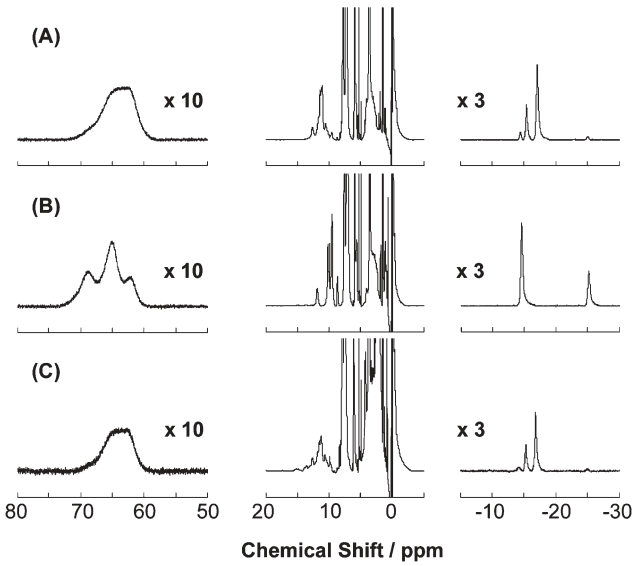


Fig. 2 ^{1}H NMR spectra (20 °C, CD_2Cl_2) of (A) **1**, (B) **2** and (C) a 1 : 1 mixture of **2** and H_2O_2.

Cu–O = 1.856(3) Å, respectively) were significantly shorter than those in **1** (Fe–O = 2.031(4) and 1.890(6) Å, Cu–O = 1.915(5) Å), indicating the stronger interaction of metal ions with the oxo ligand than that with each oxygen atom of the peroxo ligand. The ^{1}H NMR spectrum (CD_2Cl_2, room temperature) of **2**, showing signals for pyridine protons at $\delta = -25.1$ and -14.5 ppm and those of pyrrole protons centered at $\delta = 65.1$ ppm (Fig. 2), was similar to those of previously reported μ-oxo heme-(TMPA)copper¶ complexes.[5,15] An overall $S = 2$ spin state for **2** was assigned (Evans NMR method, $\mu_{eff} = 4.8\ \mu_B$) arising from an antiferromagnetic interaction between the copper(II) and high-spin iron(III) ions through the bridging oxo ligand.

Addition of an equimolar amount of H_2O_2 to the CH_3CN solution of **2** at room temperature rapidly caused the absorption spectral change; the 442, 560 and 595 nm bands corresponding to **2** were changed to 420, 557 and 612 nm bands, respectively. The resulting spectrum is identical to that of **1** (Fig. 1, solid line). The ESI-MS of the resulting solution showed a new peak at $m/z = 1248$ corresponding to the $[(TMP)Fe^{III}–(^{16}O_2)–(5MeTPA)Cu^{II}]^+$ cation, and by using $H_2{}^{18}O_2$, an $m/z = 1252$ peak from the $[(TMP)Fe^{III}–(^{18}O_2)–(5MeTPA)Cu^{II}]^+$ cation was observed (see ESI†). The resonance Raman spectrum (Fig. 1, inset) of this species is also similar to that of **1** in solution: a Raman band which was assigned to the O–O stretching vibration was observed ($\nu(^{16}O–^{16}O) = 790\ cm^{-1}$) and shifted on isotopic substitution with $H_2{}^{18}O_2$ ($\nu(^{18}O–^{18}O) = 747\ cm^{-1}$).[7] In the ^{1}H NMR spectrum, the addition of H_2O_2 to **2** led to significant shifting of the signals of pyridine protons ($\delta = -17.1$ and -15.4 ppm) with a slight shift of the pyrrole protons ($\delta = 63.5$ ppm).[5,15] These resonances were identical to those of **1** (Fig. 2). The spin state was also assigned as $S = 2$ (Evans method, $\mu_{eff} = 5.0\ \mu_B$), indicating the antiferromagnetic coupling between the copper(II) ion and the high-spin iron(III) ion, mediated by the bridging peroxo ligand. Based on these results, we conclude that complex **1** is generated by the reaction of complex **2** with H_2O_2 (Scheme 1). Although ^{16}O-bridged complex **2** was used in the ^{18}O-labeled experiments, neither the mass peak ($m/z = 1250$) nor the Raman band corresponding to the scrambled peroxide species ($\nu(^{16}O–^{18}O) = 769\ cm^{-1}$) was observed. These observations indicate that both oxygen atoms of the peroxo ligand originate from hydrogen peroxide, not from the oxo ligand in **2**. Thus, this reaction can be considered as a ligand exchange.

In general, the addition of H_2O_2 to a ferric heme generates the heme-hydroperoxo adduct, which is observed only as a transient species with very low stability[10] and followed by the rapid formation of a ferryl oxo species[2,10–12] and/or the heme degradation reaction.[13] In contrast, the reaction of **2** with H_2O_2 showed neither the generation of such ferryl oxo species nor heme degradation, because the resulting peroxo-bridged species **1** is highly stable. The peroxo complex **1** prepared in this manner again gradually transformed to the μ-oxo complex **2**, indicating the reversible interconversion between **1** and **2** (Scheme 1).

In summary, we obtained the oxo-bridged heme-copper complex **2** by the thermal transformation of **1**. Complex **2** reacted with hydrogen peroxide to regenerate the μ-peroxo complex **1** by ligand exchange from oxo to peroxo, confirmed by spectroscopic methods. This is the first example of a stable peroxo species generated by H_2O_2 and a ferric heme complex. This result indicates that our (TMP)Fe–(5MeTPA)Cu complex prefers the stable peroxo-bridged form. Further investigations are in progress in our laboratory.

We are thankful for financial support from Grants-in-Aid for Science Research on Priority Areas (#15036254) from MEXT and for Scientific Research (A) (#14204073) from JSPS, Takeda Science Foundation, and P&P project, Green Chemistry, of Kyushu University.

Takefumi Chishiro, Yuichi Shimazaki, Fumito Tani and Yoshinori Naruta*
Institute for Materials Chemistry and Engineering, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan.
E-mail: naruta@ms.ifoc.kyushu-u.ac.jp; Fax: +81-92-642-2715; Tel: +81-92-642-2731

Notes and references

‡ Elemental analysis for **2**·2(acetone). Found (%): C 74.38, H 6.03, N 7.71; Calcd for $C_{104}H_{99}O_4N_9FeCuB$ (%): C 74.84, H 5.98, N 7.55.

§ Crystal data for **2**·3(acetone): $C_{107}H_{105}O_5N_9FeCuB$, reddish-purple crystals with size 0.08 × 0.14 × 0.27 mm, triclinic, $a = 13.035(6)$, $b = 14.360(6)$, $c = 25.42(1)$ Å, $\alpha = 75.13(3)$, $\beta = 89.45(5)$, $\gamma = 83.78(4)°$, $V = 4570(3)$ Å^3, space group $P\bar{1}$, $Z = 2$, $F(000) = 1820$, $d_{calc} = 1.255\ g\ cm^{-3}$, μ(Mo–Kα) = 0.451 mm^{-1}. The data were collected ($6.0 \leqslant 2\theta \leqslant 55.0°$) at −140 °C on a Rigaku RAXIS imaging plate area detector with graphite monochromated Mo–Kα ($\lambda = 0.71075$ Å) radiation; 42538 reflections collected, 20528 used ($R_{int} = 0.119$) and 1118 parameters. $R1 = 0.080$ for 8721 $I > 2\sigma(I)$ data, $wR2 = 0.168$ for all data. An additional acetone molecule may be lost *in vacuo* before the elemental analysis. The final difference Fourier map did not show any significant features except in the proximity of the metal (iron and copper) sites. CCDC 248588. See http://www.rsc.org/suppdata/cc/b4/b413275k/ for crystallographic data in .cif or other electronic format.

¶ Abbreviation used: TMPA = tris(2-pyridylmethyl)amine.

1 M. M. Pereira, M. Santana and M. Teixeira, *Biochim. Biophys. Acta*, 2001, **1505**, 185.
2 S. Ferguson-Miller and G. T. Babcock, *Chem. Rev.*, 1996, **96**, 2889.
3 M. R. A. Blomberg, P. E. M. Siegbahn, G. T. Babcock and M. Wikström, *J. Am. Chem. Soc.*, 2000, **122**, 12848; M. R. A. Blomberg and P. E. M. Siegbahn, *Inorg. Chem.*, 2003, **42**, 5231.
4 J. P. Collman, R. Boulatov, C. J. Sunderland and L. Fu, *Chem. Rev.*, 2004, **104**, 561; H. Shin, D.-H. Lee, C. Kang and K. D. Karlin, *Electrochim. Acta*, 2003, **48**, 4077.

5 E. Kim, E. E. Chufán, K. Kamaraj and K. D. Karlin, *Chem. Rev.*, 2004, **104**, 1077; E. E. Chufán and K. D. Karlin, *J. Am. Chem. Soc.*, 2003, **125**, 16160.
6 T. Sasaki, N. Nakamura and Y. Naruta, *Chem. Lett.*, 1998, 351; Y. Naruta, T. Sasaki, F. Tani, Y. Tachi, N. Kawato and N. Nakamura, *J. Inorg. Biochem.*, 2001, **83**, 239.
7 T. Chishiro, Y. Shimazaki, F. Tani, Y. Tachi, Y. Naruta, S. Karasawa, S. Hayami and Y. Maeda, *Angew. Chem., Int. Ed.*, 2003, **42**, 2788.
8 M. Costas, M. P. Mehn, M. P. Jensen and L. Que, Jr., *Chem. Rev.*, 2004, **104**, 939.
9 E. A. Lewis and W. B. Tolman, *Chem. Rev.*, 2004, **104**, 1047; L. M. Mirica, X. Ottenwaelder and T. D. P. Stack, *Chem. Rev.*, 2004, **104**, 1013.
10 H. K. Baek and H. E. Van Wart, *Biochemistry*, 1989, **28**, 5714.
11 M. Sono, M. P. Roach, E. D. Oulter and J. H. Dawson, *Chem. Rev.*, 1996, **96**, 2841.
12 D. A. Proshlyakov, T. Ogura, K. Shinzawa-Itoh, S. Yoshikawa and T. Kitagawa, *Biochemistry*, 1996, **35**, 8580.
13 P. R. Ortiz de Montellano and K. Auclair, in *The Porphyrin Handbook*, ed. K. M. Kadish, K. M. Smith and R. Guilard, Academic Press, California, USA, 2003, **vol. 12**, p. 183; P. R. Ortiz de Montellano, *Acc. Chem. Res.*, 1998, **31**, 543; T. N. St. Claire and A. L. Balch, *Inorg. Chem.*, 1999, **38**, 684.
14 A. Nanthakumar, S. Fox, N. N. Murthy and K. D. Karlin, *J. Am. Chem. Soc.*, 1993, **115**, 8513; T. D. Ju, R. A. Ghiladi, D.-H. Lee, G. P. F. van Strijdonck, A. S. Woods, R. J. Cotter, J. V. G. Young and K. D. Karlin, *Inorg. Chem.*, 1999, **38**, 2244.
15 R. A. Ghiladi, T. D. Ju, D.-H. Lee, P. Moënne-Loccoz, S. Kaderli, Y.-M. Neuhold, A. D. Zuberbühler, A. S. Woods, R. J. Cotter and K. D. Karlin, *J. Am. Chem. Soc.*, 1999, **121**, 9885; R. A. Ghiladi, K. R. Hatwell, K. D. Karlin, H.-W. Huang, P. Moënne-Loccoz, C. Krebs, B. H. Huynh, L. A. Marzilli, R. J. Cotter, S. Kaderli and A. D. Zuberbühler, *J. Am. Chem. Soc.*, 2001, **123**, 6183.