J|A|C|S
COMMUNICATIONS

Published on Web 02/11/2009

Mononuclear Copper(II)−Superoxo Complexes that Mimic the Structure and Reactivity of the Active Centers of PHM and DβM

Atsushi Kunishita,[‡] Minoru Kubo,[§] Hideki Sugimoto,[‡] Takashi Ogura,[§] Kazunobu Sato,[‡]
Takeji Takui,*[,‡] and Shinobu Itoh*[,†,‡,‖]

Department of Material and Life Science, Graduate School of Engineering, Osaka University, 2-1 Yamada-oka, Suita, Osaka 565-0871, Japan, Department of Chemistry, Graduate School of Science, Osaka City University, 3-3-138 Sugimoto, Sumiyoshi-ku, Osaka 558-8585, Japan, Research Institute of Picobiology, Graduate School of Life Science, University of Hyogo, 3-2-1 Kouto, Kamigori-cho, Ako-gun, Hyogo 678-1297, Japan, and Institute for Molecular Science, National Institutes of Natural Sciences, 38 Nishigo-Naka, Myodaiji, Okazaki 444-8585, Japan

Received December 4, 2008; E-mail: shinobu@mls.eng.osaka-u.ac.jp; takui@sci.osaka-cu.ac.jp

Mononuclear copper(II)−superoxo species have frequently been invoked as key reactive intermediates in many biological and industrial catalytic oxidation processes.[1−4] For instance, peptidylglycine α-hydroxylating monooxygense (PHM) and dopamine β-monooxygenase (DβM) have recently been suggested to involve such an active oxygen species in aliphatic substrate hydroxylation reactions.[5−10] The X-ray structure of oxy-PHM reported by Amzel and co-workers[11] clearly shows that the Cu_B site exhibits a four-coordinate structure with a distorted tetrahedral geometry that includes an end-on superoxide ligand ($O_2^{\bullet -}$), as indicated in Figure 1A. Recently, a great effort has been made to develop model compounds of the mononuclear copper(II)−superoxo intermediate in order to gain insight into the dioxygen activation mechanism at the mononuclear copper reaction centers of PHM and DβH.[12−20] However, the reported copper(II) end-on superoxo complexes prepared by using tripodal tetradentate ligands exhibit a five-coordinate copper(II) center with a trigonal bipyramidal geometry, which is different from the tetrahedral geometries of the enzyme active sites (Figure 1A).[11] Moreover, the reported superoxo complexes exhibit no hydroxylation reactivity toward aliphatic substrates.[21] In this study, we have succeeded in developing new copper(II) end-on superoxo complexes $\mathbf{2^X{-}OO^\bullet}$ that mimic both the structure and reactivity of the mononuclear copper active sites of the enzymes (Scheme 1).

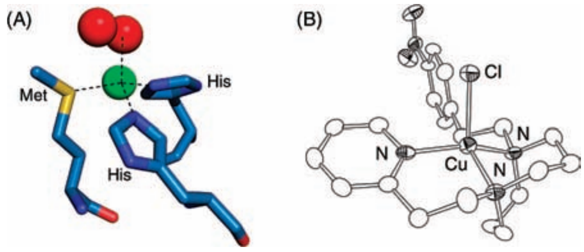
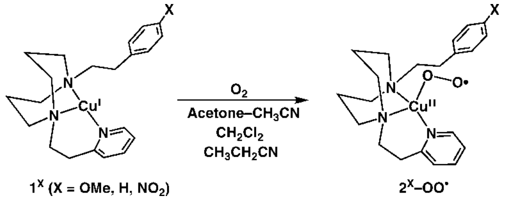


Figure 1. (A) Structure of the Cu_B active center of oxy-PHM.[11] (B) Crystal structure of $\mathbf{2^{NO_2}{-}Cl}$, with thermal ellipsoids showing 50% probability. The counteranion and the hydrogen atoms have been omitted for clarity.

The starting copper(I) complexes $\mathbf{1^X}$ supported by ligands L^X [1-(2-*p*-X-phenethyl)-5-(2-pyridin-2-ylethyl)-1,5-diazacyclooctane; X = OCH_3, H, NO_2] were prepared by mixing the ligand and $[Cu^I(CH_3CN)_4](PF_6)$ in acetonitrile. The Cu(I) complexes $\mathbf{1^X}$ exhibit a three-coordinate T-shaped structure with the N_3 donor set provided by the ligand, and no solvent coordination occurs (Figures S1−S3 in the Supporting Information). Copper(II) complexes of L^X were also prepared in order to gain insight into their structures. Figure 1B shows the X-ray structure of $[Cu^{II}(L^{NO_2})(Cl)]](PF_6)$ ($\mathbf{2^{NO_2}{-}Cl}$), where the four-coordinate copper(II) center with a N_3Cl donor set adopts a distorted tetrahedral geometry. $[Cu^{II}(L^{NO_2})(OAc)](PF_6)$ ($\mathbf{2^{NO_2}{-}OAc}$) also exhibits a distorted tetrahedral geometry, where the acetate anion acts as a monodentate ligand (Figure S4). These results clearly demonstrate that copper(II) complexes supported by the tridentate ligands L^X favor a four-coordinate structure with a tetrahedral geometry.

Scheme 1



The reaction of $\mathbf{1^X}$ and dioxygen was then examined at a low temperature (−85 °C). As a typical example, Figure 2A shows spectral changes that occur during the reaction between $\mathbf{1^H}$ and O_2 in 9:1 acetone−CH_3CN, where characteristic absorption bands at 397 nm (ε = 4200 M^{-1} cm^{-1}), 570 nm (ε = 850 M^{-1} cm^{-1}), and 705 nm (ε = 1150 M^{-1} cm^{-1}) readily appear. Thus, the color of the solution changed from yellow to dark-green during the course

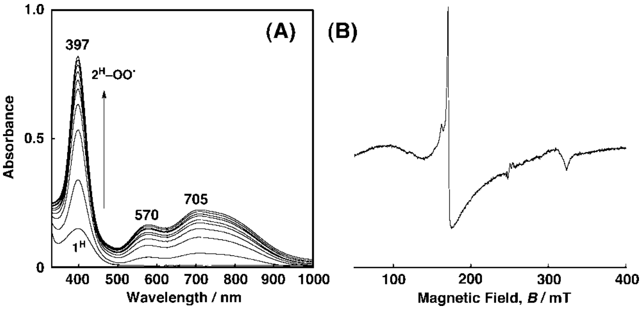


Figure 2. (A) Spectral changes during the reaction of $\mathbf{1^H}$ (0.2 mM) and O_2 in 9:1 acetone−CH_3CN at −85 °C. (B) X-band fine-structure ESR spectrum of $\mathbf{2^H{-}OO^\bullet}$ observed in the microwave parallel-excitation mode ($\mathbf{B}_0$ // $\mathbf{B}_1$) in 9:1 acetone−CH_3CN at 3 K. The microwave frequency used was 9.37007 GHz. The forbidden half-field transitions observed at $g \approx 4$ in the conventional perpendicular mode appear as allowed ones with strong intensities.

[†] Osaka University.
[‡] Osaka City University.
[§] University of Hyogo.
[‖] National Institutes of Natural Sciences.

10.1021/ja809464e CCC: $40.75 © 2009 American Chemical Society

of the reaction. The final spectrum was hardly affected by changing the solvent to CH_2Cl_2 or propionitrile (Figures S7 and S8), indicating that no solvent coordination to the metal ion took place. Moreover, similar spectral changes were obtained in the reactions of O_2 with $\mathbf{1}^{\mathbf{OCH_3}}$ and $\mathbf{1}^{\mathbf{NO_2}}$ (Figures S9 and S10). The final spectrum in Figure 2A is rather similar to those of copper(II) end-on (η^1) superoxo complexes reported to date[17] but is significantly different from that of the reported copper(II) side-on (η^2) superoxo complex.[12] The dark-green species generated using $^{16}O_2$ showed isotope-sensitive resonance Raman bands at 1033 and 457 cm^{-1} when excited with 406.7 nm laser light. These bands shifted to 968 and 442 cm^{-1}, respectively, when $^{18}O_2$ was used instead of $^{16}O_2$ (Figure S11). The peak positions and associated isotope shifts are similar to those of the reported Cu(II)−superoxo complexes and can be assigned to the O−O and Cu−O stretching vibrations, respectively.[16,22] The same Raman bands were obtained in other solvent systems (Figures S12 and S13), also confirming the absence of solvent coordination to the metal center. All of these UV−vis and resonance Raman results indicate that the generated dark-green species are the four-coordinate mononuclear copper(II) end-on superoxo complexes $\mathbf{2^X{-}OO^{\bullet}}$. In support of this, the O_2/copper ion stoichiometry was found to be 1:1 by manometry in a preparative-scale reaction.[23] Although the detailed geometries of $\mathbf{2^X{-}OO^{\bullet}}$ are not clear at present, they may have distorted tetrahedral geometries analogous to those of $\mathbf{2^{NO_2}{-}Cl}$ and $\mathbf{2^{NO_2}{-}OAc}$ (see Figure 1B).

The fine-structure ESR spectrum of $\mathbf{2^H{-}OO^{\bullet}}$ (Figure 2B) in an acetone−CH_3CN glass at 3 K was acquired in the parallel microwave-excitation mode, in which weak ESR-forbidden transitions in the conventional perpendicular mode become allowed, giving evidence for the triplet state in a straightforward manner. As Figure 2B clearly shows, the half-field absorption signal with a salient feature of g anisotropy was observed, illustrating that $\mathbf{2^H{-}OO^{\bullet}}$ is a spin triplet in the ground state.[24] From the observed fine-structure spectrum, the principal g values and zero-field splitting parameter were estimated to be $(g_1, g_2, g_3) = (2.125, 2.033, 2.015)$ and $|D'| \geq 13.7$ mT, respectively. The estimated distance between the two unpaired electron spins was ∼2.73 Å, assuming a localized model for the triplet state. The spin−spin distance is nearly identical to the distance between the copper and the distal oxygen atom of the end-on superoxide ligand in the enzyme active site (2.78 Å, Figure 1A).[11] Such a good agreement between the estimated spin−spin distance and the $Cu{-}O_{distal}$ distance in the enzymatic system further supports the end-on binding mode of $O_2^{\bullet -}$ in our model system. A complete fine-structure ESR spectral simulation and sophisticated quantum-chemical calculations for all of the magnetic tensors, to interpret the experimentally derived parameters, are underway.

Notably, $\mathbf{2^X{-}OO^{\bullet}}$ gradually decomposed even at low temperatures, obeying first-order kinetics ($k_{\mathrm{OMe}} = 7.8 \times 10^{-4}$ s^{-1}, $k_{\mathrm{H}} = 2.5 \times 10^{-4}$ s^{-1}, and $k_{\mathrm{NO_2}} = 1.3 \times 10^{-4}$ s^{-1}); the kinetic isotope effect $k_{\mathrm{H}}{}^{\mathrm{H}}/k_{\mathrm{H}}{}^{\mathrm{D}}$ was found to be 4.1 at −60 °C using $\mathrm{L^H}$-d_4 with a perdeuterated phenethyl sidearm. ^{1}H NMR analysis of the modified ligand obtained by the ordinary workup treatment of the reaction mixture using NH_4OH demonstrated that the phenethyl sidearm was ∼30% hydroxylated based on the starting material. Furthermore, an isotope-labeling experiment using $^{18}O_2$ confirmed that the origin of the oxygen atom incorporated into the product was molecular oxygen. In summary, this study presents mononuclear copper(II)−superoxo complexes $\mathbf{2^X{-}OO^{\bullet}}$ having triplet ($S = 1$) ground states that mimic both the structure (tetrahedral geometry with an end-on-bound $O_2^{\bullet -}$) and reactivity (aliphatic hydroxylation) of PHM and DβM. Further studies on the reaction mechanism of the aliphatic hydroxylation process are in progress.

Acknowledgment. This work was financially supported in part by Grants-in-Aid for Scientific Research on Priority Area (19020058, 20036044, and 20037057 for S.I.) and the Global Center of Excellence (GCOE) Program ("Picobiology: Life Science at Atomic Level" to T.O.) from MEXT, Japan, and by a Grant-in-Aid for Scientific Research (20350082 to S.I.) from JSPS, Japan. The authors also thank Asahi Glass Foundation for financial support and JSPS for a Research Fellowship (to A.K.).

Supporting Information Available: Experimental details for the synthetic procedures, results of structure determinations (CIF), and additional spectroscopic and kinetic data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Itoh, S. *Curr. Opin. Chem. Biol.* **2006**, *10*, 115–122.
(2) Mirica, L. M.; Ottenwaelder, X.; Stack, T. D. P. *Chem. Rev.* **2004**, *104*, 1013–1046.
(3) Lewis, E. A.; Tolman, W. B. *Chem. Rev.* **2004**, *104*, 1047–1076.
(4) Rolff, M.; Tuczek, F. *Angew. Chem., Int. Ed.* **2008**, *47*, 2344–2347.
(5) Klinman, J. P. *Chem. Rev.* **1996**, *96*, 2541–2561.
(6) Francisco, W. A.; Wille, G.; Smith, A. J.; Merkler, D. J.; Klinman, J. P. *J. Am. Chem. Soc.* **2004**, *126*, 13168–13169.
(7) Chen, P.; Solomon, E. I. *J. Am. Chem. Soc.* **2004**, *126*, 4991–5000.
(8) Yoshizawa, K.; Kihara, N.; Kamachi, T.; Shiota, Y. *Inorg. Chem.* **2006**, *45*, 3034–3041.
(9) Gherman, B. F.; Heppenr, D. E.; Tolman, W. B.; Cramer, C. J. *J. Biol. Inorg. Chem.* **2006**, *11*, 197–205.
(10) Crespo, A.; Marti, M. A.; Roitberg, A. E.; Amzel, L. M.; Estrin, D. A. *J. Am. Chem. Soc.* **2006**, *128*, 12817–12828.
(11) Prigge, S. T.; Eipper, B. A.; Mains, R. E.; Amzel, L. M. *Science* **2004**, *394*, 836–837.
(12) (a) Fujisawa, K.; Tanaka, M.; Moro-oka, Y.; Kitajima, N. *J. Am. Chem. Soc.* **1994**, *116*, 12079–12080. (b) Chen, P.; Root, D. E.; Campochiano, C.; Fujisawa, K.; Solomon, E. I. *J. Am. Chem. Soc.* **2003**, *125*, 466–474.
(13) (a) Cramer, C. J.; Tolman, W. B. *Acc. Chem. Res.* **2007**, *40*, 601–608. (c) Hong, S.; Huber, S. M.; Gagliardi, L.; Cramer, C. C.; Tolman, W. B. *J. Am. Chem. Soc.* **2007**, *129*, 14190–14192.
(14) (a) Fujii, T.; Yamaguchi, S.; Funahashi, Y.; Ozawa, T.; Tosha, T.; Kitagawa, T.; Masuda, H. *Chem. Commun.* **2006**, 4428–4430. (b) Fujii, T.; Yamaguchi, S.; Hirota, S.; Masuda, H. *Dalton Trans.* **2008**, 164–170.
(15) (a) Izzet, G.; Zeitouny, J.; Akdas-Killig, H.; Frapart, Y.; Ménage, S.; Douziech, B.; Jabin, I.; Le Mest, Y.; Reinaud, O. *J. Am. Chem. Soc.* **2008**, *130*, 9514–9523. (b) Lande, A.; Parisel, O.; Gerard, H.; Moliner, V.; Reinaud, O. *Chem.— Eur. J.* **2008**, *14*, 6465–6473.
(16) Würtele, C.; Gaoutchenova, E.; Harms, K.; Holthausen, M.; Sundermeyer, J.; Schindler, S. *Angew. Chem., Int. Ed.* **2006**, *45*, 3867–3869.
(17) (a) Maiti, D.; Fry, H. C.; Woertink, J. S.; Vance, M. A.; Solomon, E. I.; Karlin, K. D. *J. Am. Chem. Soc.* **2007**, *129*, 264–265. (b) Maiti, D.; Lee, D.; Gaoutchenova, K.; Würtele, C.; Holthausen, M. C.; Sarjeant, A. A. N.; Sundermeyer, J.; Schindler, S.; Karlin, K. D. *Angew. Chem., Int. Ed.* **2008**, *47*, 82–85. (c) Maiti, D.; Lee, D.; Sarjeant, A. A. N.; Pau, M. Y. M.; Gaoutchenova, K.; Sundermeyer, J.; Karlin, K. D. *J. Am. Chem. Soc.* **2008**, *130*, 6700–6701.
(18) Jazdzewski, B. A.; Reynolds, A. M.; Holland, P. L.; Young, V. G., Jr.; Kaderli, S.; Zuberbühler, A. D.; Tolman, W. B. *J. Biol. Inorg. Chem.* **2003**, *8*, 381–393.
(19) Komiyama, K.; Furutachi, H.; Nagatomo, S.; Hashimoto, A.; Hayashi, H.; Fujinami, S.; Suzuki, M.; Kitagawa, T. *Bull. Chem. Soc. Jpn.* **2004**, *77*, 59–72.
(20) Lanci, M. P.; Smirnov, V. V.; Cramer, C. J.; Gauchenova, E. V.; Sundermeyer, J.; Roth, J. P. *J. Am. Chem. Soc.* **2007**, *129*, 14697–14709.
(21) Reinaud and co-workers[15] observed a ligand hydroxylation reaction in the oxygenation of a mononuclear copper(I) complex supported by the calix[6]arene−tren ligand. They suggested that the active oxygen species was a mononuclear copper(II)−superoxo intermediate, but direct detection of such an intermediate was unsuccessful.
(22) The spectrum taken with a mixed-isotope gas containing a 1:2:1 stoichiometric mixture of $^{16}O_2/^{16}O^{18}O/^{18}O_2$ matches well the addition spectrum taken with $^{16}O_2$ and $^{18}O_2$ (Figure S14), as in the case of other end-on superoxo copper(II) complexes.[16,17]
(23) Reversibility of the O_2 binding was demonstrated by the fact that $\mathbf{2^X{-}OO^{\bullet}}$ gradually disappeared to regenerate $\mathbf{1^X}$ when argon gas was flashed onto the solution at −70 °C.
(24) Bencini, A.; Gatteschi, D. *EPR of Exchange Coupled Systems*; Springer-Verlag: New York, 1990; p 167.

JA809464E