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Hydroperoxo−Copper(II) Complex Stabilized by N_3S-Type Ligand Having a Phenyl Thioether

Masahito Kodera,*,† Toshio Kita,† Izumi Miura,†
Noritoshi Nakayama,† Tomohisa Kawata,† Koji Kano,† and
Shun Hirota‡

Department of Molecular Science and Technology
Doshisha University, Kyotanabe
Kyoto 610-0321, Japan
Department of Chemistry
Graduate School of Science, Nagoya University
Chikusa-ku, Nagoya 464-01, Japan

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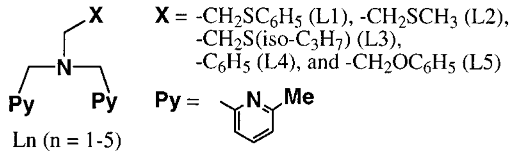
The hydroperoxo−copper(II) (CuO_2H) complex is a key intermediate in O_2-activation by dopamine β-monooxygenase (DBM), which has two copper ions (Cu_H and Cu_M) at a distance larger than 4 Å, and the O_2 molecule is activated at the Cu_M site to hydroxylate the benzylic position of dopamine.[1] The reduced form of DBM has a $Cu_H^I(His)_3 \cdots Cu_M^I(His)_2X(Met)$-type of structure.[2] In the oxidized form of peptidylglycine α-hydroxylating monooxygenase (PHM), structurally and functionally similar to DBM, the sulfur atom of the Met coordinates to the Cu_M^{II} ion (Cu−S, 2.68 Å).[3] Since the Met is essential for enzymatic activity of PHM,[3c] it might participate, through coordination to the Cu_M, in the formation and activation of the CuO_2H intermediate.

It is known that N_3S- and N_2S_2-type ligands having methyl thioethers stabilize Cu(I) complexes by coordination of the sulfur donors which may prevent O_2-binding, and Cu(II) complexes of N_3S-type ligands react with H_2O_2 to only oxygenate the ligands to the sulfoxide and sulfone derivatives.[4] Here, we report the synthesis and characterization of the CuO_2H complex of N_3S-type ligand having a phenyl thioether (PhS-ether), 2-bis(6-methyl-2-pyridylmethyl)amino-1-(phenylthio)ethane (L1), and that the PhS-ether specifically stabilizes the CuO_2H complex. This is the first example for a CuO_2H complex of a N_3S-type ligand having a thioether and may give some spectral and mechanistic information for the O_2-activation by DBM and PHM.

The ligand L1 forms Cu(II) complexes $[Cu(L1)Cl](ClO_4)$ (**1**) and $[Cu(L1)(OH)]_2(ClO_4)_2$ (**2**), and the other N_3S-, N_3-, and N_3O-type ligands (L*n*, $n = 2-5$) shown in Scheme 1 form similar complexes. The mononuclear and di-μ-hydroxo-bridged dinuclear structures of **1** and **2** are revealed by X-ray analysis[5] (Figure 1 and Supporting Information) where the Cu(II) ion in **1** takes distorted square-pyramidal geometry on the basis of τ value[6] 0.39. The PhS-sulfur in **1** weakly coordinates to Cu(II) ion with the Cu−S bond length 2.6035(3) Å, similar to 2.68 Å in the oxidized PHM.[3] The $S-Cu^{II}$ LMCT band of **1** in MeCN appears at 410 nm, clearly showing that the PhS-sulfur coordinates to Cu(II) ion in solution.[7] The highly polarizable sulfur donor in **1** stabilizes the low-valence state of the Cu ion, as shown by the Cu^I/Cu^{II} redox potentials, 0.124, 0.106, and 0.101 V versus SCE, for **1**, $[Cu(L4)Cl](ClO_4)$, and $[Cu(L5)Cl](ClO_4)$, respectively.[8]

Scheme 1



Addition of 2.5 equiv of H_2O_2 to a solution of **2** in MeCN at −40 °C generated a dark green species **3** that exhibits an intense absorption band at 357 nm (4300 M^{-1} cm^{-1}) and a d−d band at 600 nm (140 M^{-1} cm^{-1}) (Scheme 2 and Figure 2). The former band is close to the $HO_2^- - Cu^{II}$ LMCT bands at 380 nm (890 M^{-1} cm^{-1}) of a five-coordinate CuO_2H complex of bis(6-pivalamide-2-pyridylmethyl)(2-pyridylmethyl)amine (bppa)[9] and at 395 nm (7000 M^{-1} cm^{-1}) of a five-coordinate Cu_2O_2H complex of 2,6-bis[bis[2-(2-pyridyl)ethyl]amino]phenolate (XYL-O^-),[10] but far from that at 604 nm (1180 M^{-1} cm^{-1}) of a four-coordinate CuO_2H complex of sterically hindered hydrotrispyrazolyl borate ligand $[HB(3\text{-}^tBu\text{-}5\text{-}^iPrpz)_3]$.[11] The bands at 357 and 600 nm of **3** are tentatively assigned to the $HO_2^- - Cu^{II}$ LMCT and d−d transitions, respectively. The ESR spectrum of a frozen solution of **3** ($g_{\|} = 2.24$, $g_{\perp} = 2.06$, $A_{\|} = 168$ G) in MeCN at 77 K,[12] similar to that of **1** ($g_{\|} = 2.25$, $g_{\perp} = 2.09$, $A_{\|} = 101$ G), is characteristic of a square-pyramidal mononuclear Cu(II) complex.[13] **3** shows clear hyperfine splitting with relatively large $A_{\|}$ value, different from the broad ESR spectrum of the four-coordinate CuO_2H complex of $HB(3\text{-}^tBu\text{-}5\text{-}^iPrpz)_3$.[11] The reso-

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* To whom correspondence should be addressed. Dr. Masahito Kodera. Department of Molecular Science and Technology, Faculty of Engineering, DoshishaUniversity, Kyotanabe, Kyoto 610-0321, Japan. Telephone number: +81-774-65-6652. Fax number: +81-774-65-6848. E-mail address: mkodera@mail.doshisha.ac.jp.

† Doshisha University.

‡ Nagoya University.

(1) (a) Stewart, L. C.; Klinman, J. P. *Annu. Rev. Biochem.* **1988**, *57*, 551. (b) Blackburn, N. J.; Pettingill, T. M.; Seagraves, K. S.; Shigeta, R. T. *J. Biol. Chem.* **1990**, *265*, 15383. (c) Pettingill, T. M.; Strange, R. W.; Blackburn, N. J. *J. Biol. Chem.* **1991**, *266*, 16996. (d) Blackburn, N. J. In *Bioinorganic Chemistry of Copper*; Karlin, K. D., Tyeclar, Z., Eds.; Chapman & Hall: New York, 1993; pp 164−183. (e) Tian, G.; Berry, J. A.; Klinman, J. P. *Biochemistry* **1994**, *33*, 226. (f) Klinman, J. P. *Chem. Rev.* **1996**, *96*, 2541. (g) Blackburn, N. J.; Rhames, F. C.; Ralle, M. Jaron, S. *J. Biol. Inorg. Chem.* **2000**, *5*, 341.

(2) (a) Blackburn, N. J.; Hasnain, S. S.; Pettingill, T. M.; Strange, R. W. *J. Biol. Chem.* **1991**, *266*, 23120. (b) Reedy, B. J.; Blackburn, N. J. *J. Am. Chem. Soc.* **1994**, *116*, 1924.

(3) (a) Merkler, D. J.; Kulathila, R.; Consalvo, A. P.; Young, S. D.; Ash, D. E. *Biochemistry* **1992**, *31*, 7282. (b) Prigge, S. T.; Kolhekar, A. S.; Eipper, B. A.; Mains, R. E.; Amzel, L. M. *Science* **1997**, *278*, 1300. (c) Eipper, B. A.; Quon, A. S. W.; Mains, R. E.; Boswell, J. S.; Blackburn, N. J. *Biochemistry* **1995**, *34*, 2857.

(4) (a) Casella, L.; Gullotti, M.; Bartosek, M.; Pallanza, G.; Laurenti, E. *J. Chem. Soc., Chem. Commun.* **1991**, 1235. (b) Champloy, F.; Benali-Chéif, N.; Bruno, P.; Blain, I.; Pierrot, M.; Réglier, M. *Inorg. Chem.* **1998**, *37*, 3910. (c) Ohta, T.; Tachiyama, T.; Yoshizawa, K.; Yamabe, T.; Uchida, T.; Kitagawa, T. *Inorg. Chem.* **2000**, *39*, 4358.

(5) **1** ($C_{22}H_{25}N_3ScuCl_2O_4$, MW 561.97) crystallized in the triclinic space group *P*1 with $a = 12.718(1)$ Å, $b = 12.897(2)$ Å, $c = 7.7543(9)$ Å, $\alpha = 100.80(1)°$, $\beta = 98.05(1)°$, $\gamma = 76.431(9)°$, $V = 1208.3(3)$ Å^3, $Z = 2$, $R(Rw) = 0.057(0.063)$, GOF = 1.69. **2**·2MeCN·H_2O ($C_{48}H_{60}N_8S_2Cu_2Cl_2O_{11}$, MW 1187.17) crystallized in the monoclinic space group $P2_1/c$ with $a = 11.192(4)$ Å, $b = 19.327(4)$ Å, $c = 12.971(5)$ Å, $\beta = 101.97(3)°$, $V = 2745(1)$ Å^3, $Z = 2$, $R(Rw) = 0.083(0.094)$, GOF = 1.31.

(6) Addison, A. W.; Rao, T. N.; Reedijk, J.; Rijin, J. V.; Verschoor, G. C. *J. Chem. Soc., Dalton Trans.* **1984**, 1349.

(7) The UV−vis spectrum of **1** in MeCN shows three bands at 337 nm (ϵ 1280 M^{-1} cm^{-1}), 410 nm (ϵ 430 M^{-1} cm^{-1}), and 650 nm (ϵ 180 M^{-1} cm^{-1}) with a low-energy shoulder (750 nm, ϵ 140 M^{-1} cm^{-1}), which are respectively assigned to $Cl^- - Cu^{II}$ and $S-Cu^{II}$ LMCTs and d−d transitions, while those of $[Cu(L4)Cl](ClO_4)$ and $[Cu(L5)Cl](ClO_4)$ show two absorption bands at around 340 and 740 nm respectively assigned to $Cl^- - Cu^{II}$ LMCTs and d−d transitions.

(8) The CVs of **1**, $[Cu(L4)Cl](ClO_4)$, and $[Cu(L5)Cl](ClO_4)$ were measured with a glassy carbon, a platinum, and a Ag/Ag^+ (TBAP/MeCN) electrodes as a working, a counter, and a reference electrodes, respectively, with a scan rate of 1×10^2 mV s^{-1}, at a concentration of 1×10^{-3} M in MeCN with 0.1 M tetra-*n*-butylammonium perchlorate (TBAP) under N_2.

(9) Wada, A.; Harata, M.; Hasegawa, K.; Jitsukawa, K.; Masuda, H.; Mukai, M.; Kitagawa, T.; Einaga, H. *Angew. Chem., Int. Ed.* **1998**, *37*, 798.

(10) (a) Karlin, K. D.; Cruse, R. W.; Gultneh, Y. *J. Chem. Soc., Chem. Commun.* **1987**, 599. (b) Karlin, K. D.; Gbosh, P.; Cruse, R. W.; Faroop, A.; Gultneh, Y.; Jacobson, R. R.; Blackburn, N. J.; Strange, R. W.; Zubieta, J. *J. Am. Chem. Soc.* **1988**, *110*, 6769. (c) Root, D. E., Mahroof-Tahir, M.; Karlin, K. D.; Solomon, E. I. *Inorg. Chem.* **1998**, *37*, 4838.

(11) Chen, P.; Fujisawa, K.; Solomon, E. I. *J. Am. Chem. Soc.* **2000**, *122*, 10177.

(12) The ESR spectrum of **2** in MeCN at 77K shows broad signal with $g = 2.11$ because of magnetic interaction operating between the two Cu(II) ions. The broad signal of **2** was quantitatively changed to the sharp rohmbic signals of **3** upon addition of H_2O_2.

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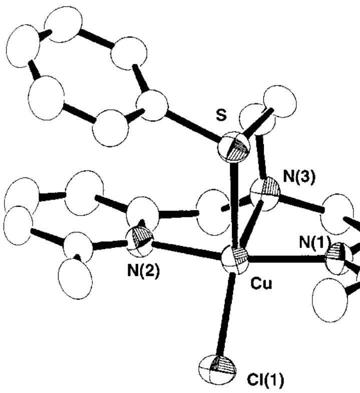
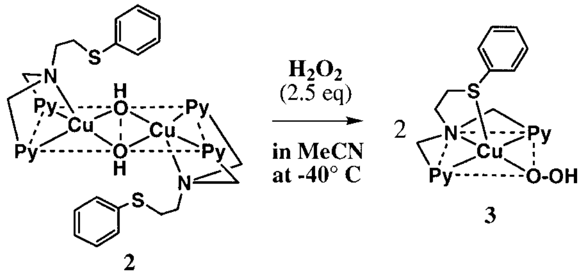


Figure 1. ORTEP view (40% probability) of $[Cu(L1)Cl]^+$ (**1**). Unlabeled atoms (open circles) represent carbon atoms. Selected bond lengths (Å) and angles (deg): Cu−Cl(1), 2.2159(3); Cu−N(1), 2.0277(2); Cu−N(2), 2.0149(2); Cu−N(3), 2.0832(3); Cu−S, 2.6035(3); Cl(1)−Cu−N(1), 96.65(1); Cl(1)−Cu−N(2), 95.87(1); Cl(1)−Cu−N(3), 141.276(7); Cl(1)−Cu−S, 132.221(8); N(1)−Cu−N(2), 164.675(3); N(1)−Cu−N(3), 82.49(1); N(1)−Cu−S, 84.047(9); N(2)−Cu−N(3), 82.22(1); N(2)−Cu−S, 94.015(9); N(3)−Cu−S, 86.37(1).

Scheme 2



nance Raman spectrum obtained with 363.8 nm excitation of **3** in MeCN at −30 °C shows a strong resonance-enhanced Raman band at 881 cm^{-1}, which shifted to 832 cm^{-1} ($\Delta\nu = 49$ cm^{-1}) when **3** was labeled with $H_2{}^{18}O_2$.[14] The $\Delta\nu$ value observed is almost equal to the calculated one at 50 cm^{-1}. These values are in the range of ν_{O-O} vibrations typical of peroxide species. The ν_{O-O} vibration at 881 cm^{-1} of **3** is close to those at 856 cm^{-1} for the CuO_2H complex of bppa,[9] at 843 cm^{-1} for the CuO_2H complex of HB(3-^{t}Bu-5-^{i}Prpz)$_3$,[11] and at 892 cm^{-1} for the Cu_2O_2H complex of (XYL-O$^-$).[10c] These Raman data indicate that the hydroperoxo moiety is bound to the Cu(II) ion and the intense absorption band at 357 nm can be assigned to the $HO_2{}^-{-}Cu^{II}$ LMCT. A relatively weak $S{-}Cu^{II}$ LMCT band might be involved in the wide-based low-energy region of the $HO_2{}^-{-}Cu^{II}$ LMCT band (Figure 2). These data demonstrate that **3** is a mononuclear CuO_2H complex $[Cu(L1)(O_2H)](ClO_4)$ where the PhS-sulfur coordinates to Cu(II) ion.

As shown in Figure 2, relatively high stability of **3** was observed with measurement of the decomposition rate in MeCN at −40 °C. The spontaneous decomposition of **3** monitored at 357 nm obeys good first-order kinetics with $k = 5.9 \times 10^{-5}$ s^{-1} at −40 °C (half-life time $\tau_{1/2} = 3.3$ h). The N_3- and N_3O-type ligands having a phenyl (L4) and a phenoxy (L5) group instead of the PhS-ether in L1 were used to clarify the effect of the PhS-ether on the stabilization of **3**. The CuO_2H complexes $[Cu(Ln)(O_2H)](ClO_4)$ $\{n = 4$ (**4**) and 5 (**5**)$\}$ were generated upon reaction of the di-μ-hydroxo−dicopper(II) complexes of L4 and L5 with H_2O_2 in MeCN at −40 °C, and showed characteristic $HO_2{}^-{-}Cu^{II}$ LMCT bands and ESR spectra similar to those of **3**. The generation and decomposition of **4** and **5** monitored at around 350 nm are shown in Figure 2. The half-life times of **4** and **5** are 18 and 69 s at −40 °C, respectively, and are much shorter than that of **3**, demonstrating that the PhS-ether specifically stabilizes the CuO_2H complex. The highly polarizable PhS-sulfur donor may enhance the covalent character of the Cu−O bond to stabilize **3**.

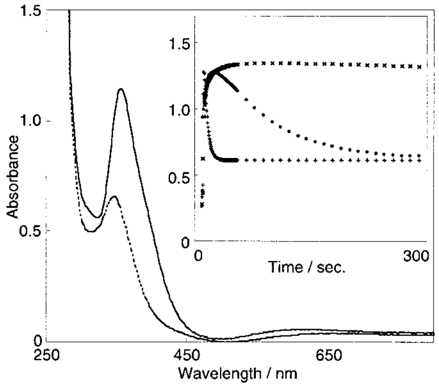


Figure 2. Electronic spectra of **2** (- - -) and **3** (—) in MeCN at −40 °C. The inset shows the time course monitored at 350 nm for generation and decomposition of the CuO_2H complexes, **3** (×), **4** (●), and **5** (+) in MeCN at −40 °C.

We also found that a methyl and an isopropyl thioethers of the N_3S-type ligands (L2 and L3) are not effective to stabilize the CuO_2H complex. Upon addition of H_2O_2 to the di-μ-hydroxo−dicopper(II) complexes of L2 and L3 in MeCN at −40 °C, the CuO_2H complexes were not detected at all, and the alkyl thioethers were converted to the sulfoxides and sulfones, indicating that the alkyl thioethers are directly oxidized by H_2O_2 added. The most significant characteristic of the PhS-ether, compared with the alkyl thioethers, is high stability against oxidation by H_2O_2. That is why the PhS-ether of L1, being not oxidized by H_2O_2, can stabilize the CuO_2H complex through coordination. Although the Met residue at the Cu_M site is an alkyl thioether, it may be protected from oxidation in the active site of DBM and PHM,[15] and furthermore it is not likely that the Met is directly oxidized by free H_2O_2 because the CuO_2H species is formed reductively via O_2-binding. Therefore, we propose that the Met in DBM and PHM, being not oxidized in the catalytic cycle, may be responsible for formation of the CuO_2H intermediate through coordination to the Cu_M ion.

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Supporting Information Available: Tables S1−S8: crystallographic experimental details, final atomic coordinates, thermal parameters, and full bond distances and angles for **1** and **2**, and syntheses and spectroscopic data of L1 and complexes **1** and **2**, and Figures S1-S3: ORTEP view of **2**, ESR, and resonance Raman spectra of **3**. (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) (a) Sugiura, Y. *Inorg. Chem.* **1978**, *17*, 2176. (b) Duggan, M.; Ray, N.; Hathaway, B.; Tomlinson, G.; Brint, P.; Plein, K. *J. Chem. Soc., Dalton Trans.* **1980**, 1342. (c) Karlin, K. D.; Hayes, J. C.; Juen, S.; Hutchinson, J. P.; Zubieta, J. *Inorg. Chem.* **1982**, *21*, 4108.

(14) The resonance Raman spectrum of **3** shows a very weak signal at 569 cm^{-1}, which shifts to 552 cm^{-1} with $^{18}O_2$ labeling. It is difficult to assign the peak to ν_{Cu-O} due to the poor quality. Similar difficulty to observe the ν_{Cu-O} was reported by Root and Solomon with the Cu_2O_2H complex of (XYL-O$^-$) in ref 10c.

(15) Although the Met residue of the DBM and PHM is not oxidized in the catalytic cycle, it is reported that in nitrile hydratase, cysteine thiolate groups bound to iron (or cobalt) ion are oxidized to sulfenate and sulfinate. See for example: Tyler, L. A.; Noveron, J. C.; Olmstead, M. M.; Mascharak, P. K. *Inorg. Chem.* **2000**, *39*, 357.