Active-Site Modeling

A Functional Model of the Cytochrome *c* Oxidase Active Site: Unique Conversion of a Heme–μ-peroxo–Cu^{II} Intermediate into Heme–superoxo/Cu^{I}**

Jin-Gang Liu, Yoshinori Naruta, and Fumito Tani*

Cytochrome *c* oxidase (C*c*O), the terminal enzyme of the respiratory chain, catalyzes the four-electron reduction of molecular oxygen to water at a low overpotential without the release of the toxic reactive intermediates superoxide and peroxide.[1] The active site of the enzyme at which dioxygen reduction takes place is composed of heme a_3/Cu_B dinuclear center. Heme a_3 is ligated axially by a histidinyl imidazole, and Cu_B is coordinated with tridentate chelation by three histidine ligands, one of which (His240) is linked to a tyrosine residue (Tyr244) by a covalent bond (in the bovine enzyme sequence).[2] The function of this unprecedented Tyr–His cross-link has provoked considerable interest, and it has been proposed to function either as an electron and proton donor to dioxygen bound to heme a_3 or to provide a means to fix Cu_B in an optimal configuration at an optimal distance from heme a_3 during the catalytic reduction of O_2.[3]

The reduction of dioxygen by C*c*O has been proposed to proceed via various intermediates; oxyheme (a heme–superoxide complex) and oxoferryl intermediates have been observed spectroscopically. [1c] A peroxide species $[Fe^{III}–O_2–Cu^{II}]^+$ has been proposed as a possible intermediate in the catalytic cycle, but has yet to be observed in the enzyme.[4]

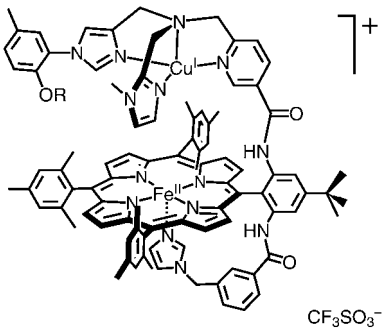
[*] Dr. J.-G. Liu, Prof. Dr. Y. Naruta, Dr. F. Tani
Institute for Materials Chemistry and Engineering
Kyushu University
Higashi-ku, Fukuoka, 812-8581 (Japan)
Fax: (+81) 92-642-2715
E-mail: naruta@ms.ifoc.kyushu-u.ac.jp

[**] This work was supported financially by the Grant-in-Aid for Scientific Research on Priority Areas (#11228207 and #16033247) from the MEXT, and for Scientific Research (A) (#14204073) from the JSPS. This research was supported in part by the Takeda Science Foundation and the P&P project, Green Chemistry, of Kyushu University. J.-G.L. gratefully acknowledges the JSPS for providing a research fellowship for foreign researchers.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

  DOI: 10.1002/ange.200462582

Thus, the exact role of the Cu_B center in the binding and activation of dioxygen are not yet well defined. The construction of functional biomimetic molecules that closely resemble the native enzyme active site is crucial for the elucidation of the C*c*O catalytic mechanism. Considerable progress has been made in the creation of heme/copper model compounds that provide further understanding of the enigmatic heme a_3/Cu_B dinuclear center in C*c*O.[5] Few of the previously reported synthetic models, however, bear a mimic of the Tyr–His cross-link, a feature which appears to play an important role in the mechanism of dioxygen reduction catalyzed by C*c*O.[6–8] Recently, a heme-containing C*c*O-active-site model was reported which lacks the axial imidazole ligand and contains covalently appended copper-chelating groups and a cross-linked Tyr–His mimic. It reacts with dioxygen to form a stable peroxide species at low temperature.[9] Toward the construction of closer structural analogues of the C*c*O active site, a fully integrated model $[(L^{N4\text{-}OH})Cu^{I}/Fe^{II}(TMPIm)]$ (**1a**) was prepared. We report



1a: R = H
1b: R = MOM

herein spectroscopic evidence that the formation of a heme–μ-peroxo–Cu^{II} species is followed by conversion into a heme–superoxo/Cu^{I} intermediate during the oxygenation reaction with this new model compound. The unique conversion of the transiently formed μ-peroxide into the corresponding superoxide species revealed by this synthetic model may provide some clarification of the ill-defined mechanism of dioxygen reduction by C*c*O.

The oxygenation reaction of **1a** was monitored by UV/Vis spectroscopy. Exposure of **1a** to O_2 in CH_3CN/THF (20%) at −70 °C leads to distinctive spectral changes with clear isosbestic points, as shown in Figure 1. The Soret band shifts from 426 nm ($\varepsilon = 88\,500\ M^{-1}cm^{-1}$) to 429 nm ($\varepsilon = 81\,600\ M^{-1}cm^{-1}$) with a slight decrease in intensity, whereas the Q band at 533 nm ($\varepsilon = 12\,000\ M^{-1}cm^{-1}$) disappears, and a new band at 538 nm ($\varepsilon = 9300\ M^{-1}cm^{-1}$) develops. This species formed at low temperature, intermediate **A**, is unstable ($t_{1/2} =$ 0.5 h, −70 °C), and its gradual transformation into intermediate **B** is marked by a shift in the Soret band at 429 nm to 425 nm, whereas the Q band shifts from 538 nm to 550 nm (Figure 2). Intermediate **B** is observable in the temperature range of −70 to −30 °C, and its UV/Vis spectral characteristics resemble those of the copper-free heme–superoxide species, observed with the same system under similar experimental conditions.

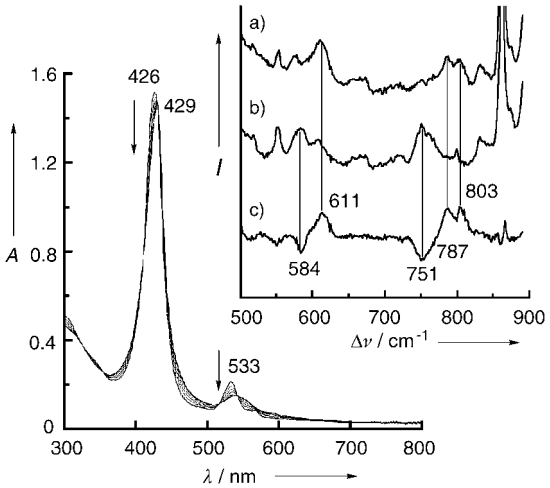


Figure 1. UV/Vis spectral changes of **1a** upon exposure to dioxygen in 20% CH_3CN/THF at −70 °C. Spectral interval is 1 min. Inset: resonance Raman spectra of intermediate **A** formed from a) $^{16}O_2$ and b) $^{18}O_2$; c) difference spectra of a) minus b); conditions: 20% CH_3CN/THF, −70 °C, excitation at 413 nm, 20 mW.

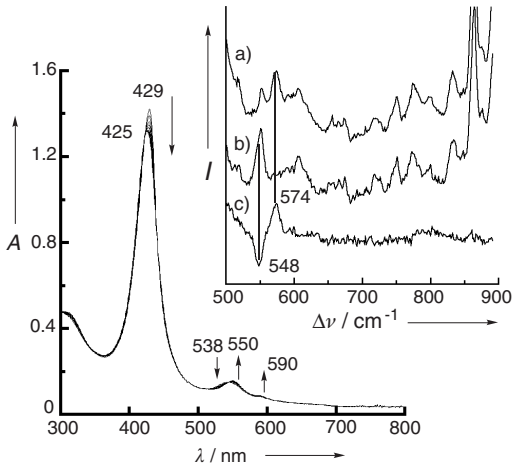


Figure 2. Absorption spectral changes of intermediate **A** in the course of decay in 20% CH_3CN/THF at −70 °C. Spectral interval is 20 min. Inset: resonance Raman spectra of intermediate **B** derived from a) $^{16}O_2$ and b) $^{18}O_2$; c) difference spectra of a) minus b); conditions: 20% CH_3CN/THF, −35 °C, excitation at 413 nm, 20 mW.

Resonance Raman spectroscopy was used to probe the binding of dioxygen to **1a** under the same experimental conditions used for UV/Vis spectroscopic measurements. Raman spectra of intermediate **A** exhibit two groups of isotope-sensitive bands, one at 787, 803 cm^{-1} ($^{16}O_2$)/751 cm^{-1} ($^{18}O_2$), and the other at 611 cm^{-1} ($^{16}O_2$)/584 cm^{-1} ($^{18}O_2$) (Figure 1, inset). Bands at both 787 and 803 cm^{-1} are assigned to the O–O stretch ($\nu_{O\text{–}O}$) vibration. The appearance of two bands near 800 cm^{-1} with $^{16}O_2$ may be caused by vibrational coupling between the bound O_2 molecule and the internal vibration mode of the porphyrin (799 cm^{-1}), which produces the spectral split.[10] In the case of $^{18}O_2$, however, the two original bands disappear, and only one band at 751 cm^{-1} is observed. This observation rules out the existence of two

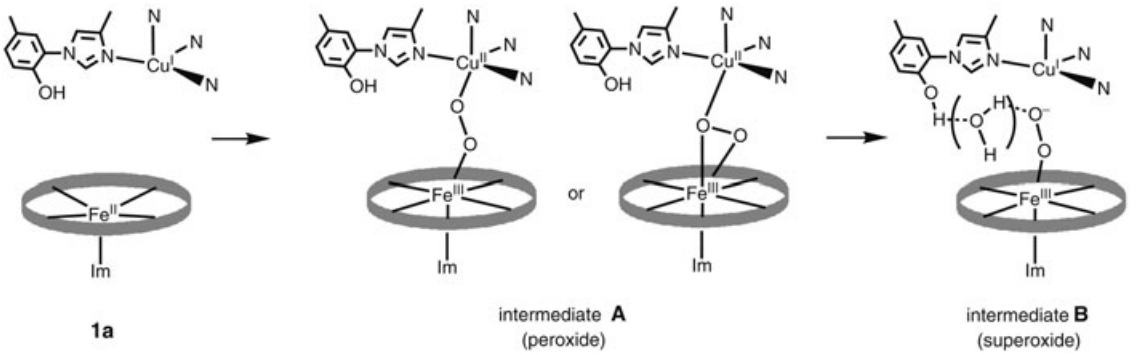
potentially bound O_2 species. The observed ν_{O-O} value is comparable to those of our previously reported peroxide compounds[9,11] (for example, $[(TMP)Fe^{III}-O_2-Cu^{II}(5\text{-}MeTPA)]^+$: 790 cm^{-1} ($^{16}O_2$)/746 cm^{-1} ($^{18}O_2$)) and is also similar to those of the reported dioxygen adducts in the peroxy state (TMP-5-MeTPA = *N*-{2-[10,15,20-tris(2,4,6-trimethylphenyl)porphyrin-5-yl]phenyl}-6-{[bis(5-methylpyridin-2-ylmethyl)amino]methyl}nicotinamide).[5] The band at 611 cm^{-1} is assigned to the ν_{Fe-O_2} mode of a peroxo species. The value of the ν_{Fe-O_2} band observed herein is quite similar to those reported for peroxo–myoglobin[12a] (617 cm^{-1}) and low-spin iron–peroxide model compounds (617–632 cm^{-1}).[12b,c] Thus, the Raman bands for the O–O stretch and the Fe–O stretch are observed simultaneously for the initially formed intermediate **A**.

Raman spectra of intermediate **B**, however, do not exhibit an oxygen-isotope-sensitive band in the region near 800 cm^{-1} or above. Instead, an isotope-sensitive band is observed at 574 cm^{-1} ($^{16}O_2$)/548 cm^{-1} ($^{18}O_2$) (Figure 2, inset). The observed isotopic frequency shift ($\Delta\nu_{(^{16}O_2/^{18}O_2)} = 26$ cm^{-1}) matches well with the expected value for the ν_{Fe-O_2} mode in the diatomic harmonic approximation. Oxymyoglobin[13] (570 cm^{-1}), oxyC*c*O[14] (572 cm^{-1}), the reported C*c*O model compound[15] $Fe-O_2$/[Cu(NMePr)]$^+$ (570 cm^{-1}), and all well-characterized examples of heme–superoxide complexes manifest similar $Fe-O_2$ stretch vibrations.[16] Accordingly, the Raman band at 574 cm^{-1} is assigned to the $Fe-O_2$ stretch vibration of a superoxide species. More importantly, upon oxygenation of the copper-free complex with the same system, an oxygen-isotope-sensitive band appears at 576 cm^{-1} ($^{16}O_2$)/549 cm^{-1} ($^{18}O_2$), which indicates formation of the expected heme–superoxide species and is consistent with the UV/Vis spectroscopic results. Furthermore, the oxidation marker band (ν_4) of the porphyrin in resonance Raman exhibits a clear frequency shift from 1357 cm^{-1} for the reduced Fe^{II}–heme unit of **1a** to 1365 cm^{-1} for the Fe^{III}–heme moiety of the superoxide-like intermediate **B**, via 1361 cm^{-1} for the initially formed peroxide intermediate **A**. The ν_4 band, which can be clearly identified without interference from other modes, is sensitive to the extent of π back-donation from iron to the π* antibonding orbitals of porphyrin.[17] Consequently, when the π back-donation decreases, an increased frequency of ν_4 is observed. The spin-state marker band (ν_2) of the porphyrin ring appears at $\approx$1563 cm^{-1} for both intermediates **A** and **B**, consistent with the presence of a six-coordinated low-spin ferric heme. By comparison with these values, the ν_2 band appears at 1552 cm^{-1} for the high-spin dioxygen–heme/Cu adduct with the parent model compound,[9] which lacks the axially bound imidazole.

Further evidence comes from EPR spectroscopic experiments. Frozen samples of both intermediates **A** and **B** are EPR silent. The EPR-silent character of the former is ascribed to the strong antiferromagnetic coupling between the Cu^{II} and Fe^{III} ions through a peroxo (O_2^{2-}) bridge as observed in other examples.[5,9,11] It also rules out the presence of a side-on η^2-peroxo–heme/Cu^I or Cu^{II} adduct, because the side-on η^2-peroxo–heme moiety produces a strong EPR marker signal at $g = 4.2$.[18] In the case of intermediate **B** it may be understood that the Cu^I complex sits just above, or is directed away from the superoxide $Fe^{III}-O_2^-$ moiety without direct contact between the two metal ions. Therefore we conclude that the dioxygen adduct intermediate **A**, initially formed from the heme/Cu model compound **1a**, is an imidazole-ligated low-spin heme–μ-peroxo–Cu^{II} species (μ-1,2 or μ-η^2:η^1), which is subsequently transformed into a more stable superoxide intermediate **B**.

The preferred formation of a heme–superoxo/Cu^I intermediate derived from its precursor, heme–peroxo–Cu^{II}, rather than from the direct oxygenation of **1a** tempts us to postulate the scenario of dioxygen binding to this heme/Cu model compound. Exposure of the reduced Fe^{II}/Cu^I form to dioxygen may initially result in a transient end-on $Cu^{II}-O_2^-$ species en route to the heme–μ-peroxo–Cu^{II} intermediate.[19] The μ-peroxo intermediate thus formed is quite unstable owing to the electron-push effect of the axial imidazole,[20] and as a result, the bound peroxide breaks away from the cupric ion to form the thermodynamically more stable heme–superoxo/Cu^I intermediate. The redox couples of the two metal centers integrated in **1a** (heme–superoxo/heme–peroxo and $(L^{N4\text{-}OH})Cu^I/Cu^{II}$) are likely to be responsible for this unique transformation. When the potential of the former couple is more negative than that of the latter, the conversion of heme–peroxo–Cu^{II} into heme–superoxo/Cu^I becomes thermodynamically allowed.[21] The nearby cross-linked cresolyl OH group could facilitate the conversion of the newly formed peroxide into the superoxide by hydrogen-bonding stabilization, possibly by water molecules (Scheme 1).[22]

Notably, a similar bridged peroxo complex is also generated through the oxygenation of an analogous model compound **1b**, in which the cross-linked phenolic hydroxy



Scheme 1. Dioxygen binding and subsequent transformation proposed for the model compound **1a**. Im = imidazole group.

function is protected by a methoxymethyl (MOM) group. Its O–O stretch (ν_{O-O}) mode appears at 788 and 804 cm^{-1} ($^{16}O_2$)/752 cm^{-1} ($^{18}O_2$), and the ν_{Fe-O} stretch mode appears at 617 cm^{-1} ($^{16}O_2$)/589 cm^{-1} ($^{18}O_2$). All oxygen-sensitive Raman bands of the peroxide formed with **1b** shift slightly to higher wavenumbers relative to those of **1a**. The UV/Vis spectroscopic features of the MOM-protected peroxide in solution are quite similar to those of the peroxide initially generated (intermediate **A**) from **1a**; the thermal decay of the MOM-protected peroxide, however, gives a heme–μ-oxo–Cu^{II} species, and quite unlike that of the superoxide species associated with **1a**.

The heme–superoxide intermediate **B** from **1a** is stable below −30 °C and does not revert to the deoxy state even under vacuum. On the other hand, the process of dioxygen binding to the corresponding distal copper-free complex in the same ligand system is reversible, and the oxy species reverts to its deoxy form when argon is bubbled through the solution, a vacuum is applied, or the temperature is increased. The evident difference between these two complexes may suggest that a copper complex in the distal site avoids the superoxide-releasing autoxidation of the heme–superoxide intermediate, thereby enhancing the binding of dioxygen to iron, possibly in cooperation with hydrogen bonding with the phenolic OH group.

In summary, a novel heme/Cu C*c*O model has been prepared in which the heme iron center is axially ligated by a proximal imidazole group and the copper is bound to a cross-linked *N*-(2-hydroxyphenyl)imidazole ligand; these moieties represent two important residues around the heme a_3/Cu_B as observed in the enzyme active site. Spectroscopic evidence demonstrates the unique transformation of the heme–μ-peroxo–Cu^{II} intermediate into the heme–superoxo/Cu^{I} species in the course of the oxygenation reaction at low temperature. The superoxide intermediate is thermodynamically more stable than its precursor in the presence of a trace amount of water. With this heme/Cu model compound, initial binding of the copper ion to dioxygen en route to the heme–μ-peroxo–Cu^{II} species is proposed. These results imply that during the process of dioxygen binding and activation as catalyzed by this model compound, the copper center not only plays a role as a redox center but also stabilizes the heme–superoxide intermediate; the phenolic hydroxy group plays a dominant role in the generation of the corresponding superoxide intermediate. This synthetic model study also suggests that after one-electron reduction of the oxyheme unit in C*c*O under physiological conditions, O–O bond cleavage could occur through a heme–hydroperoxy rather than a heme–peroxo–Cu species.

Experimental Section

The UV/Vis electronic spectra were recorded on a Hamamatsu PMA-11 CCD spectrophotometer with a D_2/W_2 light source. O_2 gas previously dried with molecular sieves was introduced with an O_2 line to a degassed solution of **1a** or **1b** (1.0×10^{-4} mol L^{-1}) in 20% CH_3CN/THF in a 0.2-cm quartz cuvette at −70 °C.

Resonance Raman spectra were obtained on a SpectraPro-300i spectrometer (Acton Research) with a 2400-groove grating, a Beamlok 2060 Kr ion laser (Spectra-Physics), a holographic super-notch filter (Kaiser Optical Systems), and a LN-1100PB CCD detector (Princeton Instruments) cooled with liquid N_2. Spectra were collected in spinning cells (2.0-cm diameter, 1500 rpm) at −70 °C for intermediate **A** and −35 °C for intermediate **B**, at an excitation wavelength $\lambda = 413.1$ nm (20 mW), 90° scattering geometry, and 5-min data accumulation. Peak frequencies were calibrated relative to indene and CCl_4 standards (accurate to ±1 cm^{-1}). During each Raman experiment, UV/Vis spectra were collected simultaneously.

Received: November 11, 2004
Published online: February 18, 2005

Keywords: copper · heme proteins · iron · metalloenzymes · oxidoreductases

[1] a) S. Ferguson-Miller, G. T. Babcock, *Chem. Rev.* **1996**, *96*, 2889; b) G. T. Babcock, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 12971; c) T. Kitagawa, *J. Inorg. Biochem.* **2000**, *82*, 9.

[2] a) S. Yoshikawa, K. Shinzawa-Itoh, R. Nakashima, R. Yaono, E. Yamashita, N. Inoue, M. Yao, M. J. Fei, C. P. Libeu, T. Mizushima, H. Yamaguchi, T. Tomizaki, T. Tsukihara, *Science* **1998**, *280*, 1723; b) C. Ostermeier, A. Harrenga, U. Ermler, H. Michel, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 10547.

[3] a) P. E. M. Siegbahn, M. R. A. Blomberg, *Biochim. Biophys. Acta* **2004**, *1655*, 45; b) D. A. Proshlyakov, M. A. Pressler, C. DeMaso, J. F. Leykam, D. L. DeWitt, G. T. Babcock, *Science* **2000**, *290*, 1588; c) E. Pinakoulaki, U. Pfitzner, B. Ludwig, C. Varotsis, *J. Biol. Chem.* **2002**, *277*, 13563.

[4] a) G. T. Babcock, M. Wikstrom, *Nature* **1992**, *356*, 301; b) M. Fabian, G. Palmer, *Biochemistry* **1995**, *34*, 13802; c) D. A. Proshlyakov, M. A. Pressler, G. T. Babcock, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 8020; d) H. Michel, J. Behr, A. Harrenga, A. Kannt, *Annu. Rev. Biophys. Biomol. Struct.* **1998**, *27*, 329; e) T. L. Poulos, H. Li, C. S. Raman, *Curr. Opin. Chem. Biol.* **1999**, *3*, 131.

[5] a) E. Kim, E. E. Chufan, K. Kamaraj, K. D. Karlin, *Chem. Rev.* **2004**, *104*, 1077; b) J. P. Collman, R. Boulatov, C. J. Sunderland, L. Fu, *Chem. Rev.* **2004**, *104*, 561; c) J. P. Collman, R. Boulatov, C. J. Sunderland in *The Porphyrin Handbook*, *Vol. 11* (Eds.: K. M. Kadish, K. M. Smith, R. Guilard), Academic Press, San Diego, **2003**, pp. 1–49.

[6] Cross-linked phenol–imidazole compounds: a) J. P. Collman, Z. Wang, M. Zhong, L. Zeng, *J. Chem. Soc. Perkin Trans. 1* **2000**, 1217; b) K. M. McCauley, J. M. Vrtis, J. Dupont, W. A. van der Donk, *J. Am. Chem. Soc.* **2000**, *122*, 2403; c) J. A. Cappuccio, I. Ayala, G. I. Elliott, I. Szundi, J. Lewis, J. P. Konopelski, B. A. Barry, O. Einarsdottir, *J. Am. Chem. Soc.* **2002**, *124*, 1750; d) M. Aki, T. Ogura, Y. Naruta, T. H. Le, T. Sato, T. Kitagawa, *J. Phys. Chem. A* **2002**, *106*, 3436.

[7] Crosslinked phenol–imidazole copper complexes: K. Kamaraj, E. Kim, B. Galliker, L. N. Zakharov, A. L. Rheingold, A. D. Zuberbuhler, K. D. Karlin, *J. Am. Chem. Soc.* **2003**, *125*, 6028.

[8] Metal-free dinucleating ligands bearing a Tyr244 mimic: a) J. P. Collman, R. A. Decreau, S. Costanzo, *Org. Lett.* **2004**, *6*, 1033; b) J. P. Collman, R. A. Decreau, C. Zhang, *J. Org. Chem.* **2004**, *69*, 3546.

[9] J.-G. Liu, Y. Naruta, F. Tani, T. Chishiro, Y. Tachi, *Chem. Commun.* **2004**, 120.

[10] The vibrational coupling phenomenon in the resonance Raman spectra of dioxygen adducts of heme proteins and other model compounds has been reported previously: L. M. Proniewicz, J. R. Kincaid, *Coord. Chem. Rev.* **1997**, *161*, 81.

[11] a) Y. Naruta, T. Sasaki, F. Tani, Y. Tachi, N. Kawato, N. Nakamura, *J. Inorg. Biochem.* **2001**, *83*, 239; b) T. Chishiro, Y. Shimazaki, F. Tani, Y. Tachi, Y. Naruta, S. Karasawa, S. Hayami,

Y. Maeda, *Angew. Chem.* **2003**, *115*, 2894; *Angew. Chem. Int. Ed.* **2003**, *42*, 2788.
[12] a) M. Ibrahim, I. G. Denisov, T. M. Makris, J. R. Kincaid, S. G. Sligar, *J. Am. Chem. Soc.* **2003**, *125*, 13714; b) R. Y. N. Ho, G. Roelfes, B. L. Feringa, L. Que, Jr., *J. Am. Chem. Soc.* **1999**, *121*, 264; c) A. J. Simaan, S. Dopner, F. Banse, S. Bourcier, G. Bouchoux, A. Boussac, P. Hildebrandt, J. J. Girerd, *Eur. J. Inorg. Chem.* **2000**, *2000*, 1627.
[13] E. A. Kerr, N.-T. Yu, D. E. Bartnicki, H. Mizukami, *J. Biol. Chem.* **1985**, *260*, 8360.
[14] C. Varotsis, W. H. Woodruff, G. T. Babcock, *J. Biol. Chem.* **1990**, *265*, 11131.
[15] J. P. Collman, C. J. Sunderland, K. E. Berg, M. A. Vance, E. I. Solomon, *J. Am. Chem. Soc.* **2003**, *125*, 6648.
[16] a) K. Nakamoto, I. R. Paeng, T. Kuroi, T. Isobe, H. Oshio, *J. Mol. Struct.* **1988**, *189*, 293; b) J. P. Collman, J. I. Brauman, T. R. Halbert, K. S. Suslick, *Proc. Natl. Acad. Sci. USA* **1976**, *73*, 3333; c) E. A. Kerr, H. C. Mackin, N.-T. Yu, *Biochemistry* **1983**, *22*, 4373; d) M. A. Walters, T. G. Spiro, K. S. Suslick, J. P. Collman, *J. Am. Chem. Soc.* **1980**, *102*, 6857.
[17] a) T. G. Spiro, J. M. Burke, *J. Am. Chem. Soc.* **1976**, *98*, 5482; b) J. M. Burke, J. R. Kincaid, S. Peters, R. R. Gagne, J. P. Collman, T. G. Spiro, *J. Am. Chem. Soc.* **1978**, *100*, 6083.
[18] a) E. E. Chufan, K. D. Karlin, *J. Am. Chem. Soc.* **2003**, *125*, 16160; b) M. Selke, M. F. Sisemore, J. S. Valentine, *J. Am. Chem. Soc.* **1996**, *118*, 2008; c) J. N. Burstyn, J. A. Roe, A. R. Miksztal, B. A. Shaevitz, G. Lang, J. S. Valentine, *J. Am. Chem. Soc.* **1988**, *110*, 1382.
[19] The very fast binding of O_2 to copper complexes with tetradentate tripodal ligands, and dioxygen association with Cu^{I}_{B} prior to binding to heme a_3 during the reaction of O_2 with reduced C*c*O have been proposed: a) K. D. Karlin, S. Kaderli, A. D. Zuberbuhler, *Acc. Chem. Res.* **1997**, *30*, 139; b) R. S. Blackmore, C. Greenwood, Q. H. Gibson, *J. Biol. Chem.* **1991**, *266*, 19245; c) M. Oliveberg, B. G. Malmstrom, *Biochemistry* **1991**, *30*, 7053; d) M. Oliveberg, B. G. Malmstrom, *Biochemistry* **1992**, *31*, 3560; e) M. I. Verkhovsky, J. E. Morgan, M. Wikstrom, *Biochemistry* **1994**, *33*, 3079; f) J. A. Bailey, C. A. James, W. H. Woodruff, *Biochem. Biophys. Res. Commun.* **1996**, *220*, 1055.
[20] a) K. Yamaguchi, Y. Watanabe, I. Morishima, *J. Chem. Soc. Chem. Commun.* **1992**, 1709; b) S.-I. Ozaki, M. P. Roach, T. Matsui, Y. Watanabe, *Acc. Chem. Res.* **2001**, *34*, 818.
[21] For the analogous complexes, the redox potentials of $(TPP)Fe^{II}$–O_2/$(TPP)Fe^{III}$–O_2^{2-} (−0.73 V versus SCE, DMF) and (TMPA)-Cu^{I}/Cu^{II} (−0.61 V versus SCE, DMF) have been reported; TPP = *meso*-tetraphenylporphinate, TMPA = tris(2-pyridylmethyl)amine; see: a) S. E. Jones, G. S. Srivatsa, D. T. Sawyer, T. G. Traylor, T. C. Mincey, *Inorg. Chem.* **1983**, *22*, 3903; b) N. Wei, N. N. Murthy, Q. Chen, J. Zubieta, K. D. Karlin, *Inorg. Chem.* **1994**, *33*, 1953.
[22] Addition of a trace amount of water to the reaction mixture at low temperature greatly enhances the conversion of the peroxy into the superoxy intermediate.

