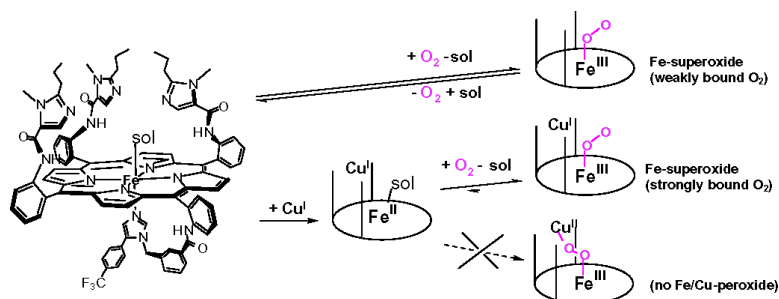


Spectroscopic Evidence for a Heme–Superoxide/Cu(I) Intermediate in a Functional Model of Cytochrome *c* Oxidase

James P. Collman, Christopher J. Sunderland, Katja E. Berg, Michael A. Vance, and Edward I. Solomon

J. Am. Chem. Soc., **2003**, 125 (22), 6648-6649 • DOI: 10.1021/ja034382v • Publication Date (Web): 10 May 2003

Downloaded from <http://pubs.acs.org> on March 29, 2009



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 6 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

[View the Full Text HTML](#)

Spectroscopic Evidence for a Heme–Superoxide/Cu(I) Intermediate in a Functional Model of Cytochrome *c* Oxidase

James P. Collman,* Christopher J. Sunderland, Katja E. Berg, Michael A. Vance, and Edward I. Solomon

Department of Chemistry, Stanford University, Stanford, California 94305-5080

Received January 28, 2003; E-mail: jpc@stanford.edu

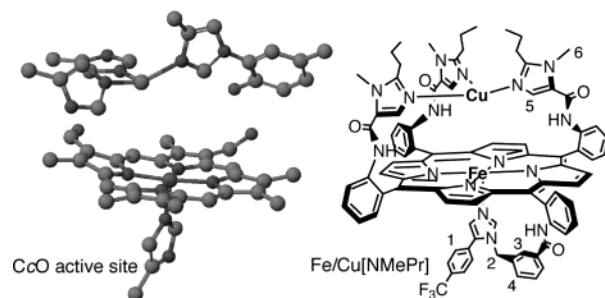
Cytochrome *c* oxidase (CcO) is an example of a heme/copper oxidase that catalyzes the 4e reduction of molecular oxygen to water, a pivotal reaction in aerobic respiration. The catalytic cycle is initiated when dioxygen binds to the reduced active site ($\text{Fe}_{\text{a}_3^{\text{II}}}/\text{Cu}_{\text{B}}^{\text{I}}$) of CcO to form a heme–superoxide complex ($\text{Fe}_{\text{a}_3^{\text{III}}}\text{O}_2^{\bullet-}/\text{Cu}_{\text{B}}^{\text{I}}$).¹ No bioinorganic complex has yet replicated this motif of a heme–superoxide held in proximity to a copper(I) center. Commonly, the reaction of dioxygen with heterobinuclear heme/Cu model complexes is an $\text{Fe}-\text{O}_2-\text{Cu}$ (μ -peroxo) product.^{2–8} Although once proposed to be part of the catalytic cycle of CcO, spectroscopic evidence no longer supports μ -peroxo intermediates in CcO.^{9,10} Thus, existing biomimetic systems may be inappropriate models for elucidating mechanistic details of dioxygen reduction by CcO. Herein we report spectroscopic evidence for the first heterobinuclear biomimetic heme/Cu oxidase model forming a heme–superoxide/ Cu^{I} moiety. These preliminary studies indicate that contrary to other CcO models, reduced heme/Cu centers placed at a distance and in a ligation sphere similar to that in CcO need not form peroxide-bridged complexes. Indeed, a distal metal ion can lead to stabilization of a heme dioxygen complex relative to the Fe-only porphyrin. This suggests Cu_{B} may also have nonredox roles in O_2 binding and reduction.

$\text{Fe}[\text{NMePr}]$ and $\text{Fe}/\text{Cu}[\text{NMePr}]^+$ were synthesized as previously reported.¹¹ A combination of ^1H and ^{19}F NMR, EPR, and IR spectroscopies indicates that the representation in Chart 1 is a reasonable depiction of the structure in solution, with the Fe coordinated by the proximal imidazole, the distal tris-imidazole N-donors directed toward the molecular cavity, and the copper of $\text{Fe}/\text{Cu}[\text{NMePr}]^+$ in a tris-imidazole environment in both the Cu^{I} and Cu^{II} oxidation states.¹¹

Spectrophotometric titration of the Fe/Cu complex with O_2 indicates a 1:1 $[\text{Fe}/\text{Cu}[\text{NMePr}]^+:\text{O}_2$ binding stoichiometry. Formation of the $\text{Fe}/\text{Cu}:\text{O}_2$ adduct is rapid (within the cuvette mixing time) and is marked by a shift in the Soret band at 424 nm to 422 nm which also diminishes in intensity (ϵ 1.8×10^5 to 1.0×10^5 $\text{M}^{-1} \text{cm}^{-1}$), while the Q-band at 538 nm shifts to 542 nm with a small increase in intensity.

Exposure of $\text{Fe}/\text{Cu}[\text{NMePr}]^+$ (in 5% d_3 -MeCN: d_8 -THF) to dioxygen rapidly (<15 s) leads to a single major diamagnetic species as evidenced by the ^1H NMR spectroscopy (Figure 1). The sample is EPR silent. All resonances were assigned by H–H COSY and NOE experiments and support the proposition that the oxygenated adduct possesses the expected mirror-plane symmetry on the NMR time scale. The minor impurity observed in the NMR spectra of $\text{Fe}/\text{Cu}[\text{NMePr}]^+:\text{O}_2$ is assigned as the CO adduct, $\text{FeCO}/\text{Cu}[\text{NMePr}]^+$.¹¹ Since the affinity of $\text{Fe}/\text{Cu}[\text{NMePr}]^+$ for CO is very high (essentially irreversible), trace CO adduct formation can be attributed to contamination of the Fe/Cu system during synthesis. Indeed, this minor impurity is observed in the NMR spectrum of the Fe/Cu system prior to oxygenation. Oxygenation was also

Chart 1. Structural Comparison of the Active Site of CcO with the Model Complex $\text{Fe}/\text{Cu}[\text{NMePr}]^+$ ^a



^a Numbers refer to ^1H resonances in Figure 1.

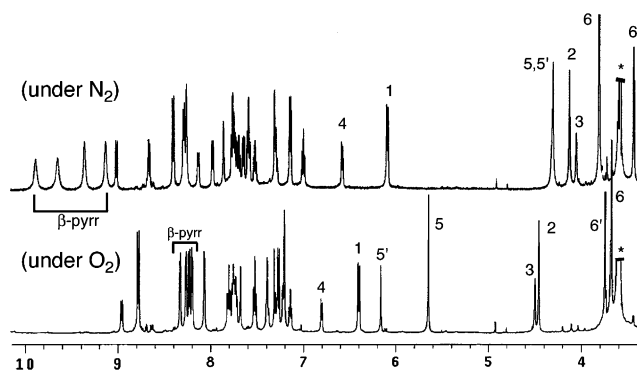


Figure 1. NMR spectra of $\text{Fe}/\text{Cu}[\text{NMePr}]^+$ in 5% d_3 -MeCN/ d_8 -THF before (upper) and after (lower) exposure to 1 atm O_2 at 22 °C. Full assignments are in Supporting Information and ref 11.

followed by ^{19}F NMR spectroscopy, as the CF_3 marker resonance in $\text{Fe}/\text{Cu}[\text{NMePr}]^+$ (δ_{CF_3} –63.4 ppm) shifts upon O_2 addition to –65.8 ppm which upon slow decomposition (hours), becomes a broad signal at –65.1 ppm.

Typically, diamagnetism of oxygenated heme/Cu complexes is attributed to formation of Cu^{II} antiferromagnetically coupled to the Fe^{III} center via a bridging O_2^{2-} unit.^{2,6} Our experience with the Co-porphyrins of this ligand,¹² led us to believe that an alternate O_2 adduct, a picket-bound Cu^{I} above the (magnetically coupled) $\text{Fe}^{\text{III}}\text{O}_2^{\bullet-}$ superoxide may be a viable formulation.

Resonance Raman spectroscopy (1 mM in CH_2Cl_2 , λ_{ex} 407 nm, 20 mW) failed to detect an oxygen isotope sensitive band in the (Por)M–O–O–M'(L) $\nu_{\text{O}-\text{O}}$ region (~ 800 cm^{-1}).¹³ However, an oxygen isotope sensitive band was observed at 570/544 cm^{-1} ($^{16}\text{O}_2/^{18}\text{O}_2$) (Figure 2). Oxymyoglobin,¹⁴ oxy-picket-fence porphyrin¹⁵ ($\text{Im}[\text{T}_{\text{piv}}\text{PP}]\text{FeO}_2$) and oxy-CcO,¹⁶ all well-characterized examples of heme–superoxide complexes, manifest $\text{Fe}-^{16}\text{O}_2$ stretches at 569, 572, and 571 cm^{-1} respectively.¹⁷ More importantly, upon oxygenation of the copper-free complex $\text{Fe}[\text{NMePr}]$, an oxygen isotope sensitive band appears at 575/554 cm^{-1} , indicating formation of

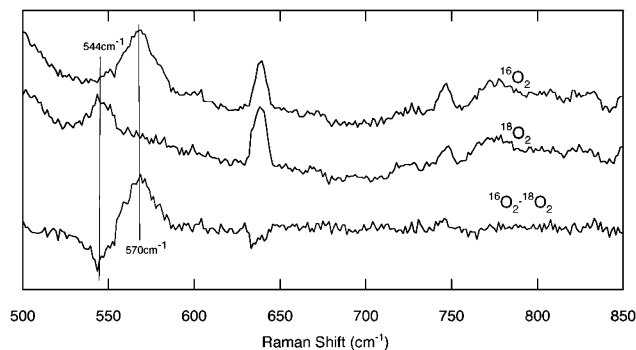


Figure 2. Resonance Raman (22 °C) of Fe/Cu[NMePr]⁺ after oxygenation, with ¹⁶O₂ or ¹⁸O₂ and the difference spectra.

the expected heme-superoxide. The $\nu_{\text{Fe-O}}$ value of oxy-Fe/Cu[NMePr]⁺ is therefore consistent with O₂ as a superoxide ligand in this metalloporphyrin.

Further evidence congruent with the FeO₂/Cu[NMePr]⁺ (superoxide) formulation evolves from a consideration of the corresponding Co porphyrin analogues, where superoxides are known to be more stable and resonance enhancement of both M–O and O–O occurs.¹² In CoO₂/Cu[NMePr]⁺, $\nu_{\text{O-O}}$ and $\nu_{\text{Co-O}}$ are observable at 1148/1073 cm⁻¹ (¹⁶O₂/¹⁸O₂) and 528/504 cm⁻¹ (¹⁶O₂/¹⁸O₂), respectively.¹² The $\nu_{\text{O-O}}$ frequency is characteristic of a cobalt-superoxide, as is the $\nu_{\text{Co-O}}$ value. The Co–O stretch is ~46 cm⁻¹ lower than in FeO₂/Cu[NMePr]⁺, typical for Co vs Fe (Por)M-superoxide $\nu_{\text{M-O}}$ stretches (cf $\nu_{\text{Fe-O}}/\nu_{\text{Co-O}}$, 568/516 cm⁻¹, for (Im)M[T_{piv}PP]O₂).

Thus, we conclude that the dioxygen adduct formed with this heme/Cu system is an imidazole ligated (six-coordinate) heme-superoxide, FeO₂/Cu[NMePr]⁺, an analogue for oxy-CcO.

FeO₂/Cu[NMePr]⁺ is stable for several hours at room temperature. The oxygenated complex does not revert to deoxy Fe/Cu[NMePr]⁺ under vacuum (three freeze/pump/thaw cycles), although exposure to a head-gas of CO rapidly produces FeCO/Cu[NMePr]⁺. This indicates FeO₂/Cu[NMePr]⁺ is a thermodynamically stable but kinetically labile O₂ adduct.

Without distal copper, Fe[NMePr] behaves as expected for an imidazole-ligated heme. In coordinating solvents the complex is a six-coordinate and low-spin complex (λ_{max} 428 nm, δ_{pyr} : 8.5–8.6 ppm). By contrast to the Fe/Cu complex, Fe[NMePr] does not form an observable (by NMR) dioxygen complex at room temperature (1 atm O₂), although binding can be driven to completion at low temperature (–60 °C). In coordinating solvents Fe[NMePr] binds oxygen reversibly: The low-temperature oxy- species reverts to deoxy-Fe[NMePr] by warming to room temperature or upon removal (at –60 °C) of the O₂ head-gas.

Clearly there is qualitative difference in the dioxygen affinity of these complexes: FeO₂/Cu[NMePr]⁺ is stable at room temperature under vacuum, Fe[NMePr]O₂ dissociates without an O₂ head-gas and requires low temperature for complete binding. Thus, it is apparent that Cu^I in the distal site strongly enhances dioxygen binding to iron, paralleling behavior recently described for the Coporphyrins of this ligand system.¹² In the Co analogues, H-bonding from the terminal oxygen of the bound superoxide to the picket amide NH's was found to be stronger in Co/Cu[NMePr]⁺ than in Co[NMePr], and a similar contribution may also be in effect for Fe/Cu[NMePr]⁺. The Fe/Cu superoxide is also more stable to degradation than is the Fe-only superoxide. For example, at room temperature in CD₂Cl₂, Fe[NMePr]O₂ decomposes too rapidly for

NMR characterization, whereas FeO₂/Cu[NMePr]⁺ has a lifetime of several hours.

Previous studies have demonstrated that these heme/Cu catalysts are functional (electrocatalytically reducing O₂ to H₂O under physiologically relevant conditions, with minimal leakage of partially reduced oxygen species).¹⁸ Those studies did not find a direct role for Cu in O–O bond activation. We now have spectroscopic evidence that the Fe–O₂ and Cu^I centers of FeO₂/Cu[NMePr] are not covalently interacting, in contrast to the usual finding that binuclear heme/Cu complexes form Fe^{III}/Cu^{II} μ -peroxo complexes.^{2–8} Few dioxygen complexes of heme/Cu CcO models are stable at room temperature, especially when coordinated with an axial nitrogen base. These results indicate that stabilization of partially reduced dioxygen intermediates of a heme/Cu complex does not require μ -peroxo formation. These observations of nonredox contributions from copper to the thermodynamic and decomposition stabilization of a heme/Cu dioxygen adduct may be insights into some of the reactivity-tuning roles of Cu^I early in the catalytic cycle of CcO. We are currently investigating the stereoelectronic characteristics of Fe/Cu[NMePr]⁺ that leads it to so closely mimic the reactivity of the Fe/Cu site of CcO with respect to its dioxygen binding and reduction chemistry.

Acknowledgment. This material is based upon work supported by the NSF under Grant No. CHE-013206. Support from NIH Grant No. 017880 also contributed to this work.

Supporting Information Available: Further experimental data including NMR and Raman data (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- (1) Ferguson-Miller, S.; Babcock, G. T. *Chem. Rev.* **1996**, *96*, 2889.
- (2) Collman, J. P.; Herrmann, P. C.; Boitrel, B.; Zhang, X. M.; Eberspacher, T. A.; Fu, L.; Wang, J. L.; Rousseau, D. L.; Williams, E. R. *J. Am. Chem. Soc.* **1994**, *116*, 9783.
- (3) Collman, J. P.; Fu, L.; Herrmann, P. C.; Zhang, X. M. *Science* **1997**, *275*, 949.
- (4) Collman, J. P.; Fu, L.; Herrmann, P. C.; Wang, Z.; Rapta, M.; Broring, M.; Schwenninger, R.; Boitrel, B. *Angew. Chem., Int. Ed.* **1998**, *37*, 3397.
- (5) Sasaki, T.; Nakamura, N.; Naruta, Y. *Chem. Lett.* **1998**, 351.
- (6) Kopf, M. A.; Karlin, K. D. *Inorg. Chem.* **1999**, *38*, 4922.
- (7) Ghiladi, R. A.; Ju, T. D.; Lee, D. H.; Moenne-Loccoz, P.; Kaderli, S.; Neuhold, Y. M.; Zuberbuhler, A. D.; Woods, A. S.; Cotter, R. J.; Karlin, K. D. *J. Am. Chem. Soc.* **1999**, *121*, 9885.
- (8) Ghiladi, R. A.; Hatwell, K. R.; Karlin, K. D.; Huang, H. W.; Moenne-Loccoz, P.; Krebs, C.; Huynh, B. H.; Marzilli, L. A.; Cotter, R. J.; Kaderli, S.; Zuberbuhler, A. D. *J. Am. Chem. Soc.* **2001**, *123*, 6183.
- (9) Fabian, M.; Wong, W. W.; Gennis, R. B.; Palmer, G. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 13114.
- (10) Proshlyakov, D. A.; Pressler, M. A.; Babcock, G. T. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 8020.
- (11) Collman, J. P.; Sunderland, C. J.; Boulatov, R. *Inorg. Chem.* **2002**, *41*, 2282.
- (12) Collman, J. P.; Berg, K. E.; Sunderland, C. J.; Aukauloo, A.; Vance, M. A.; Solomon, E. I. *Inorg. Chem.* **2002**, *41*, 6583.
- (13) Collman, J. P.; Boulatov, R.; Sunderland, C. J. In *The Porphyrin Handbook*; Kadish, K. M., Smith, K. M., Guilard, R., Eds.; Academic Press: (Boston) 2003; Vol. 11.
- (14) Tsubaki, M.; Nagai, K.; Kitagawa, T. *Biochemistry* **1980**, *19*, 379.
- (15) Burke, J. M.; Kincaid, J. R.; Peters, S.; Gagne, R. R.; Collman, J. P.; Spiro, T. G. *J. Am. Chem. Soc.* **1978**, *100*, 6083.
- (16) Varotsis, C.; Woodruff, W. H.; Babcock, G. T. *J. Biol. Chem.* **1990**, *265*, 11131.
- (17) Heme-superoxides seldom demonstrate enhancement of $\nu_{\text{O-O}}$ with rRaman due to lack of an appropriate excited-state transition and consequently are identified by the Fe–O stretch. By comparison, enhancement of $\nu_{\text{O-O}}$ (~800 cm⁻¹) is a common characteristic of stable Fe/Cu μ -peroxides.
- (18) Boulatov, R.; Collman, J. P.; Shiryayeva, I. M.; Sunderland, C. J. *J. Am. Chem. Soc.* **2002**, *124*, 11923.

JA034382V