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COMMUNICATIONS

Published on Web 05/10/2003

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

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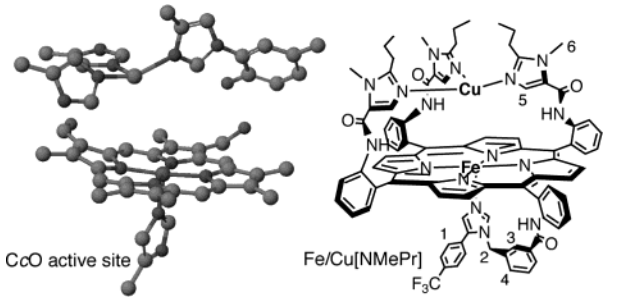
Cytochrome *c* oxidase (C*c*O) is an example of a heme/copper oxidase that catalyzes the 4e reduction of molecular oxygen to water, a pivotal reaction in aerobic respiration. The catalytic cycle is initiated when dioxygen binds to the reduced active site (Fe_{a3}^{II}/Cu_B^I) of C*c*O to form a heme−superoxide complex ($Fe_{a3}^{III}O_2^{\bullet-}$/$Cu_B^I$).[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 Fe−O_2−Cu (μ-peroxo) product.[2−8] Although once proposed to be part of the catalytic cycle of C*c*O, spectroscopic evidence no longer supports μ-peroxo intermediates in C*c*O.[9,10] Thus, existing biomimetic systems may be inappropriate models for elucidating mechanistic details of dioxygen reduction by C*c*O. 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 C*c*O models, reduced heme/Cu centers placed at a distance and in a ligation sphere similar to that in C*c*O 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.

Fe[NMePr] and Fe/Cu[NMePr]$^+$ were synthesized as previously reported.[11] A combination of ^{1}H 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 Fe/Cu[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 [Fe/CuNMePr]$^+$:O_2 binding stoichiometry. Formation of the Fe/Cu·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 M^{-1} cm^{-1}), while the Q-band at 538 nm shifts to 542 nm with a small increase in intensity.

Exposure of Fe/Cu[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 ^{1}H 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 Fe/Cu[NMePr]$^+$·O_2 is assigned as the CO adduct, FeCO/Cu[NMePr]$^+$.[11] Since the affinity of Fe/Cu[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 C*c*O with the Model Complex Fe/Cu[NMePr]$^+$ [a]



[a] Numbers refer to ^{1}H resonances in Figure 1.

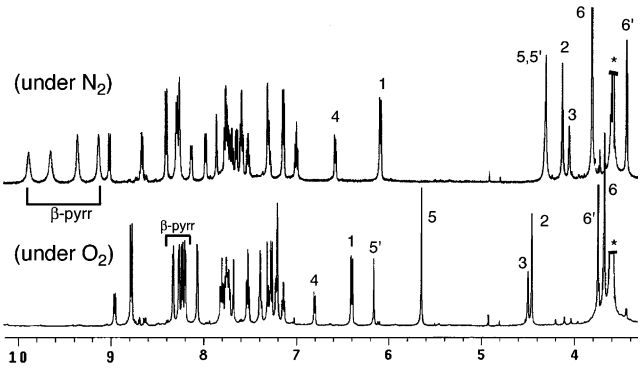


Figure 1. NMR spectra of Fe/Cu[NMePr]$^+$ in 5% d_3-MeCN/d_8-THF before (upper) and after (lower) exposure to 1 atm O_2 at 22 °C. Full assignments are in Supporting Information and ref 11.

followed by ^{19}F NMR spectroscopy, as the CF_3 marker resonance in Fe/Cu[NMePr]$^+$ (δ_{CF3} −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) $Fe^{III}O_2^-$ 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) ν_{O-O} region (~800 cm^{-1}).[13] However, an oxygen isotope sensitive band was observed at 570/544 cm^{-1} ($^{16}O_2$/$^{18}O_2$) (Figure 2). Oxymyoglobin,[14] oxy-picket-fence porphyrin[15] (Im[T_{piv}PP]FeO_2) and oxy-C*c*O,[16] all well-characterized examples of heme−superoxide complexes, manifest Fe−$^{16}O_2$ stretches at 569, 572, and 571 cm^{-1} respectively.[17] More importantly, upon oxygenation of the copper-free complex Fe[NMePr], an oxygen isotope sensitive band appears at 575/554 cm^{-1}, indicating formation of

10.1021/ja034382v CCC: $25.00

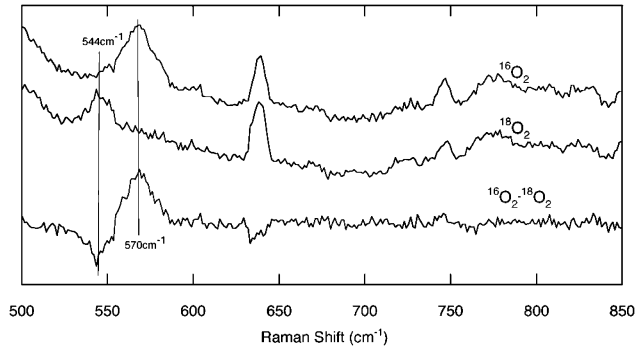


Figure 2. Resonance Raman (22 °C) of Fe/Cu[NMePr]$^+$ after oygenation, with $^{16}O_2$ or $^{18}O_2$ and the difference spectra.

the expected heme−superoxide. The ν_{Fe-O} value of oxy-Fe/Cu[NMePr]$^+$ is therefore consistent with O_2 as a superoxide ligand in this metalloporphyrin.

Further evidence congruent with the FeO_2/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 CoO_2/Cu[NMePr]$^+$, ν_{O-O} and ν_{Co-O} are observable at 1148/1073 cm^{-1} ($^{16}O_2/^{18}O_2$) and 528/504 cm^{-1} ($^{16}O_2/^{18}O_2$), respectively.[12] The ν_{O-O} frequency is characteristic of a cobalt-superoxide, as is the ν_{Co-O} value. The Co−O stretch is ∼46 cm^{-1} lower than in FeO_2/Cu[NMePr]$^+$, typical for Co vs Fe (Por)M-superoxide ν_{M-O} stretches (cf ν_{Fe-O}/ν_{Co-O}, 568/516 cm^{-1}, for (Im)M[T_{piv}PP]O_2).

Thus, we conclude that the dioxygen adduct formed with this heme/Cu system is an imidazole ligated (six-coordinate) heme−superoxide, FeO_2/Cu[NMePr]$^+$, an analogue for oxy-C*c*O.

FeO_2/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 FeO_2/Cu[NMePr]$^+$ is a thermodynamically stable but kinetically labile O_2 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_{pyrr}: 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 O_2), 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 O_2 head-gas.

Clearly there is qualitative difference in the dioxygen affinity of these complexes: FeO_2/Cu[NMePr]$^+$ is stable at room temperature under vacuum, Fe[NMePr]O_2 dissociates without an O_2 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 Co-porphyrins 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 CD_2Cl_2, Fe[NMePr]O_2 decomposes too rapidly for NMR characterization, whereas FeO_2/Cu[NMePr]$^+$ has a lifetime of several hours.

Previous studies have demonstrated that these heme/Cu catalysts are functional (electrocatalytically reducing O_2 to H_2O 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−O_2 and Cu^I centers of FeO_2/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 C*c*O 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 decompositional stabilization of a heme/Cu dioxygen adduct may be insights into some of the reactivity-tuning roles of Cu_B^I early in the catalytic cycle of C*c*O. 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 C*c*O 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.

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JA034382V