Formation and Characterization of a High-Spin Heme-Copper Dioxygen (Peroxo) Complex

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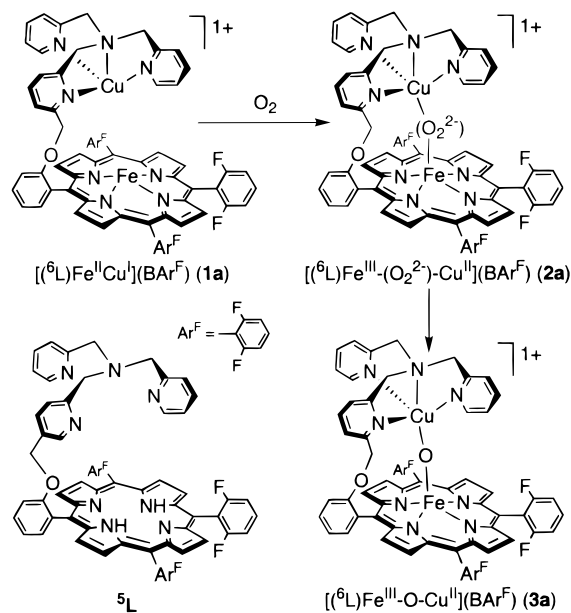Amina S. Woods,¶ Robert J. Cotter,¶ and Kenneth D. Karlin*,§

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In this report, we describe new dioxygen reactivity of reduced heme-copper complexes employing the tethered tetraarylporphyrinate ligands ^{5}L and ^{6}L.[1] Notably, peroxo complex [(^{6}L)Fe^{III}−(O$_2^{2-}$)−Cu^{II}](BAr^F) (**2a**),[5] formed from the reaction of [(^{6}L)Fe^{II}-Cu^I](BAr^F) (**1a**) with O$_2$ (Scheme 1), has physical properties and reactivity which contrast greatly with those of the few other heme-copper peroxo complexes described in the literature.[6−8] These studies are part of our general program aimed at elucidating fundamental aspects of O$_2$-interactions with heme-copper centers,[4,8−12] how copper influences (P)Fe^{II}/O$_2$ chemistry, and how hemes influence copper(I)−dioxygen reactivity patterns. Insights obtained may pertain to heme-copper oxidase (e.g., cytochrome *c* oxidase)[13,14] active-site O$_2$-binding and reduction, or other oxidative processes involving dioxygen.

Reduced Fe^{II}···Cu^I heme-copper compounds [(^{6}L)Fe^{II}Cu^I]-(BAr^F) (**1a**) and [(^{5}L)Fe^{II}Cu^I](BAr^F) (**1b**) (Scheme 1) were

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(1) Recent publications deal with μ-oxo diiron(III) complexes with ^{5}L,[2] and use of both ^{5}L and ^{6}L for μ-oxo Fe^{III}−O−Cu^{II} complexes,[3] and fully reduced heme or heme-copper Fe^{II}···Cu^I compounds.[4.]
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(5) Abbreviations used: BAr^F = B[3,5-(CF$_3$)$_2$C$_6$H$_3$]$_4^-$; P = porphyrinate; THF, tetrahydrofuran; MALDI-TOF-MS, matrix-assisted laser desorption ionization time-of-flight mass spectrometry; F$_8$TPP, tetrakis(2,6-difluorophenyl)porphyrinate; tmpa = tris(2-pyridylmethyl)amine.
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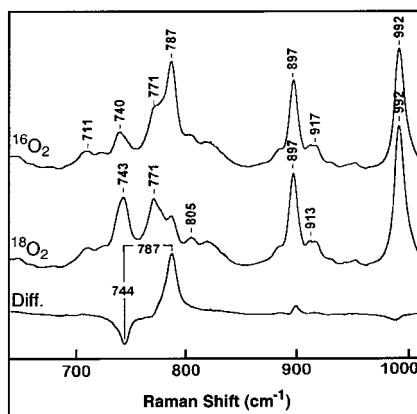
Scheme 1

prepared as previously described.[4] When **1a** reacts with O$_2$ at room temperature in THF, μ-oxo complex [(^{6}L)Fe^{III}−(O)−Cu^{II}]-(BAr^F) (**3a**) forms immediately.[15] However, in MeCN or acetone, prior to forming μ-oxo **3a** {λ_{max} = 438 (Soret), 556 nm}, UV−vis spectroscopy (room temperature) reveals the formation of a new complex {λ_{max} = 418 (Soret), 561, 632 nm} having remarkable thermal stability ($t_{1/2}$(RT) ∼ 60 min). We formulate this O$_2$-adduct as a peroxo complex [(^{6}L)Fe^{III}−(O$_2^{2-}$)−Cu^{II}](BAr^F) (**2a**), based on the following: (1) Dioxygen-uptake measurements {spectrophotometric titration, −40 °C, MeCN} reveal a reaction stoichiometry of one O$_2$ per **1a** complex. (2) MALDI-TOF-MS is consistent with intramolecular adduct formation. [(^{6}L)Fe^{II}Cu^I]$^+$ (**1a**) gives a parent peak at m/z 1159 {(M − BAr^F)$^+$}. This disappears upon oxygenation using ^{16}O$_2$ while a m/z 1191 peak assigned to [(^{6}L)Fe^{III}−(^{16}O$_2^{2-}$)−Cu^{II}]$^+$ (**2a**) appears. The expected increase in mass of 4 is observed when **2a** forms from ^{18}O$_2$, m/z 1195. (3) Resonance Raman spectroscopy of **2a** indicates a peroxo O−O stretching vibration at ν = 787 cm^{-1} {Δ^{16}O$_2$/^{18}O$_2$ = 43 cm^{-1}} (Figure 1).

We have also been able to obtain ^{1}H and ^{2}H NMR[16] spectra of [(^{6}L)Fe^{III}−(O$_2^{2-}$)−Cu^{II}](BAr^F) (**2a**) (Figure 2), revealing the first case of a paramagnetic high-spin heme-copper dioxygen adduct. In acetone solvent at −80 °C, oxygenation of the high-spin [(^{6}L)Fe^{II}Cu^I](BAr^F) (**1a**) ($\delta_{pyrrole}$ = 80−90 ppm, m, br)[4] leads to further downfield shifting of the pyrrole resonances for **2a** (Figure 2b: $\delta_{pyrrole}$ = 92 ppm, s, br), consistent with a high-spin porphyrinate−iron(III) center. Upfield shifted peaks at ca. −33 (not shown) and −78 ppm are also observed in **2a**,[17] and these ^{1}H NMR features are very similar (but with distinctive shifts) to the overall pattern of downfield pyrrole resonances *and* upfield shifted Cu−ligand peaks observed for μ-oxo complexes (P)Fe^{III}−

(14) Protein X-ray structure references: (a) Yoshikawa, S.; Shinzawa-Itoh, K.; Nakashima, R.; Yaono, R.; Yamashita, E.; Inoue, N.; Yao, M.; Jei-Fei, M.; Libeu, C. P.; Mizushima, T.; Yamaguchi, H.; Tomizaki, T.; Tsukihara, T. *Science* **1998,** *280,* 1723−1729. (b) Ostermeier, C.; Harrenga, A.; Ermler, U.; Michel, H. *Proc. Natl. Acad. Sci. U.S.A.* **1997,** *94,* 10547−10553.
(15) The reaction stoichiometry is thought to be 2[(^{6}L)Fe^{II}Cu^I]$^+$:O$_2$, based on analogy to an untethered analogue, [(F$_8$TPP)Fe−O−Cu(tmpa)]$^{2+}$. Isotope labeling shows that the O-atom in **3a** is derived from dioxygen.[4,11]
(16) Pyrrole resonance assignments for **1a**, **2a**, **3a** were confirmed by also examining ^{2}H NMR spectra of a deuterated analogue of **1a**.
(17) In MeCN solvent, ^{1}H,^{2}H NMR spectra at −40 °C also gave downfield pyrrole resonances and upfield shifted Cu−ligand peaks for **2a**.

Figure 1. Resonance Raman spectra of $[(^6L)Fe^{III}-(O_2^{2-})-Cu^{II}]^+$ (**2a**), formed by oxygenation of $[(^6L)Fe^{II}Cu^I](BAr^F)$ (**1a**) using $^{16}O_2$, $^{18}O_2$, and the difference spectrum (MeCN, RT; 413 nm excitation).

Figure 2. ^1H NMR spectra of heme-copper complexes, -80 °C in $(CD_3)_2$-CO, at 400 MHz. See text for further explanation.

$O-Cu^{II}(L)$,[3,18] including $[(^6L)Fe^{III}-O-Cu^{II}](BAr^F)$ (**3a**) (Figure 2c); these are diagnostic of an $S = 2$ spin system arising from the antiferromagnetic coupling of the $S = 5/2$ heme center to an $S = 1/2$ copper(II) moiety, through a bridging peroxo (in **2a**) or oxo ligand (in **3a**).[18] Species **2a** is EPR silent (MeCN, 88 K), also consistent with antiferromagnetic coupling. The high-spin nature of the heme in **2a** complements the findings of Collman and co-workers,[6] who's several examples of heme-copper–oxygen (peroxo) adducts are all low-spin diamagnetic, probably attributed to the presence of strong axial base ligands (i.e., imidazolyl or pyridyl). We suggest that the peroxo ligand in **2a** is bridging in either a side-on[19] or end-on fashion; the high-spin nature of **2a** is explained by either the lack of or weak axial solvent ligation.
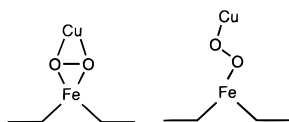
Benchtop UV–vis monitoring (-80 °C) of the $[(^6L)Fe^{II}Cu^I]$-(BAr^F) (**1a**) oxygenation reaction in acetone reveals the formation of a new short-lived species, $\lambda_{max} = 416$ (Soret), 539, 618 nm, which then converts to the peroxo complex $[(^6L)Fe^{III}-(O_2^{2-})-Cu^{II}](BAr^F)$ (**2a**). Further insights are obtained from low-temperature stopped-flow kinetic measurements: (i) Both the $\lambda_{max} = 538$ nm intermediate and the final thermally stable $\lambda_{max} = 561$ nm peroxo complex **2a** are produced (independently) within the mixing time ($\sim$1 ms), with their degree of formation being $[O_2]$

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(19) Side-on bound peroxo ferric complexes $[(P)Fe^{III}(O_2^{2-})]^-$ are known to be high spin with out-of-plane Fe(III).[20]

and temperature dependent: the 538 nm intermediate is favored at lower temperatures, while the 561 nm peroxo product is favored at higher *T*, (ii) the Cu(I) in **1a** does *not* act independently of the heme, since neither Cu–O_2 (superoxo) nor Cu–O_2–Cu (peroxo) adducts appear to form,[21] and (iii) the 538 nm intermediate converts to the 561 nm peroxo complex **2a** in a first-order reaction, $\Delta H^{\ddagger} = 37.4 \pm 0.4$ kJ/mol and $\Delta S^{\ddagger} = -28.7 \pm 2.3$ J/(mol·K) (-94 to -60 °C). Our preliminary analysis suggests a kinetic model where two forms (in equilibrium) of $[(^6L)Fe^{II}Cu^I]$-(BAr^F) (**1a**) exist. Complex **1a** favored at higher *T* reacts with O_2 to directly give $[(^6L)Fe^{III}-(O_2^{2-})-Cu^{II}](BAr^F)$ (**2a**) ($\lambda_{max} = 561$ nm), while a form of **1a** preferred at low *T* reacts to give the 538 nm intermediate, which then isomerizes to **2a**. We conjecture that the 538 nm intermediate is either an O_2-adduct (e.g., heme-O_2) of **1a** possessing a Cu^I(tmpa) moiety with modified O_2-reactivity,[23] or it is already a heteronuclear heme-Cu/O_2 (peroxo) adduct. This study provides the first kinetic insights obtained for O_2-reactivity with a synthetic heme-copper complex.

As heme-copper peroxo species are relatively new types of O_2-adducts, their behavior in reactions with various reagents is of considerable interest. Preliminary observations indicate $[(^6L)$-$Fe^{III}-(O_2^{2-})-Cu^{II}](BAr^F)$ (**2a**) reacts with two or more equivalents of cobaltacene (as a reductant) to cleanly give μ-oxo $[(^6L)Fe^{III}-O-Cu^{II}](BAr^F)$ (**3a**). This behavior again differs with Collman's low-spin (hence, easier to reduce) heme-copper peroxo compounds,[25] which undergo complete reduction (to the $Fe^{II}\cdots Cu^I$ species) with this reagent.[26]

Strong contrasts also exist in the O_2-reactivity of $[(^6L)Fe^{II}Cu^I]^+$ (**1a**) versus $[(^5L)Fe^{II}Cu^I]^+$ (**1b**). Complex **1b** forms a peroxo-level O_2-adduct $\{\nu_{(O-O)} = 809$ cm^{-1}; $\Delta^{16}O_2/^{18}O_2 = 53$ cm^{-1}; THF solvent$\}$, but the stoichiometry of reaction differs $\{$**1b**: $O_2 = 2{:}1\}$, and ^1H NMR spectroscopy (MeCN) indicates that the product is diamagnetic and *not* paramagnetic. Thus, as also seen for their μ-oxo complexes,[3] the ligand architecture dramatically influences the observed $^6L/^5L$ heme-copper peroxo chemistry.

In conclusion, binucleating ligands such as ^6L result in new patterns of heme-copper–dioxygen reactivity, here in particular the generation of the first high-spin heme-Cu peroxo species.

Supporting Information Available: Eyring kinetic plot (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(21) This conclusion is based on past Cu^I(tmpa)/O_2 kinetic studies.[22]
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(23) One possibility (being investigated) is that a pyridyl arm from the Cu-tmpa moiety ligates the heme-iron providing for formation of a pyridyl–Fe–O_2 species (with known $\sim$538 nm absorption),[9,24] while at the same time signficantly reducing the reactivity of the Cu^I-tmpa moiety toward O_2.
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(26) Collman's peroxo complex formed by O_2-reaction with an analogue porphyrinate–Co^{II}–Cu^I binuclear complex also undergoes full reduction with cobaltacene; Collman, J. P.; Fu, L.; Herrmann, P. C.; Zhang, X. *Science* **1997**, *275*, 949–951.