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Dioxygen Reactivity of Mononuclear Heme and Copper Components Yielding A High-Spin Heme–Peroxo–Cu Complex

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In this report, we describe the formation of a pentacoordinate high-spin heme–peroxo–Cu complex, $[(F_8TPP)Fe^{III}-(O_2^{2-})-Cu^{II}(TMPA)]^+$ (**2**),¹ formed upon addition of O_2 to a 1:1 mixture of the reduced heme complex $(F_8TPP)Fe^{II}$ (**1a**) and copper complex $[(TMPA)Cu^I(CH_3CN)]^+$ (**1b**) [Scheme 1]. Remarkably, mixing of dioxygen with Fe and Cu mononuclear components^{2–5} leads to the heterobinuclear complex **2**, in preference to homobinuclear μ -peroxo or μ -oxo heme-only^{3,6} or copper-only products.^{4,5,7} The present system provides significant new electronic, structural, and mechanistic advances into the dioxygen chemistry of heme–Cu systems. Insights obtained may have relevance to heme–copper oxidase O_2 -binding and reduction,^{8–11} other chemistries or biochemistries involving dioxygen-binding to metals,^{8,12,13} O_2 -activation (e.g., cytochrome P-450 monooxygenase,¹⁴ copper complex O_2 -reactivity,^{5,15,16} and Cu monooxygenases^{15,17,18}), and O–O reductive cleavage.

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(1) Abbreviations used: F_8TPP = tetrakis(2,6-difluorophenyl)porphyrinate; $TMPA$ = tris(2-pyridylmethyl)amine; P = porphyrinate; MALDI-TOF-MS = Matrix Assisted Laser Desorption Ionization Time Of Flight Mass Spectrometry.

(2) The dioxygen chemistry of $(F_8TPP)Fe^{II}$ and $[(TMPA)Cu^I(MeCN)]^+$ have been previously studied. See refs 3–5.

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(7) $(F_8TPP)Fe^{III}-(O_2^{2-})-Fe^{III}(F_8TPP)$ ($\delta_{pyrrole} = 17.5$ ppm, $\lambda_{max} = 414$ (Soret), 536 nm; CH_2Cl_2 , 193 K),³ $(F_8TPP)Fe^{III}-O-Fe^{III}(F_8TPP)$ ($\delta_{pyrrole} = 13.1$ ppm, $\lambda_{max} = 400$ (Soret), 561 nm; CH_2Cl_2 , 193 K),⁶ and $[(TMPA)Cu^{II}-(O_2^{2-})-Cu^{II}(TMPA)]^{2+}$ ($\lambda_{max} = 525$ nm)⁴ products are not observed by UV–visible or NMR spectroscopies.

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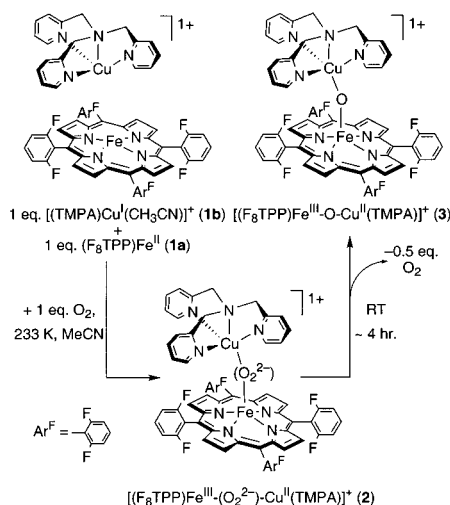
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Scheme 1



Upon addition of dioxygen at $-40^\circ C$ in MeCN solvent to an equimolar solution of $(F_8TPP)Fe^{II}$ (**1a**)^{3,19} and $[(TMPA)Cu^I(CH_3CN)](ClO_4)$ (**1b**)⁴ {reduced spectrum: $\lambda_{max} = 414$ (sh), 421 (Soret), 526 nm}, UV–visible spectroscopy (Figure 1) reveals the formation of a new species { $\lambda_{max} = 412$ (Soret), 558 nm}

We formulate this O_2 -adduct as the peroxo complex $[(F_8TPP)-Fe^{III}-(O_2^{2-})-Cu^{II}(TMPA)]^+$ (**2**) based upon the following: (1) The resonance Raman spectrum of **2** (Figure 2A) presents a peroxo O–O stretching vibration at 808 cm^{-1} that downshifts by 46 cm^{-1} with ^{18}O -labeled dioxygen (Figure 2B). In the scrambled isotope experiment, the ^{16}O – ^{18}O stretch corresponds to a single component at 785 cm^{-1} (Figure 2C), and indicates that the peroxide species is bound in a symmetric fashion. (2) MALDI-TOF-MS of **2** (formed in MeCN solvent) gives a parent peak at m/z 1239 $\{(M - ClO_4^- + MeCN)^+\}$ when $^{16}O_2$ is employed.²⁰ The expected increase in mass of 4 is observed when **2** forms from $^{18}O_2$, m/z 1243. (3) Dioxygen-uptake measurements {spectrophotometric titration: MeCN, $-40^\circ C$ } revealed an oxygenation stoichiometry of **1a:1b:O₂** = 1:1:1.

Further characterization of $[(F_8TPP)Fe^{III}-(O_2^{2-})-Cu^{II}(TMPA)]^+$ (**2**) comes from NMR spectroscopy.²¹ In MeCN at $-40^\circ C$, the $(F_8TPP)Fe^{II}/[(TMPA)Cu^I(CH_3CN)]^+$ system (**1a:1b** = 1:1) has a single pyrrole resonance at δ 10 ppm (Figure 3A), consistent with a low spin ($S = 0$) system {Evans NMR method, $\mu_B = 0$ }. Oxygenation of the **1a/1b** mixture leads to a downfield shifting of the pyrrole resonances²² for **2** (Figure 3B: $\delta_{pyrrole} = 68$ ppm, s, br), with upfield shifted pyridyl peak resonances also observed at -11 and -20 ppm. An overall $S = 2$ spin state for **2** is assigned (Evans method, $\mu_B = 5.1$, $-40^\circ C$), arising from the antiferromagnetic coupling of the $S = 5/2$ high spin ferric heme to the S

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(20) Coordination of the MeCN to the iron is inconsistent with the pentacoordinate high-spin configuration deduced from the resonance Raman signature of the F_8TPP ligand in **2**. Coordination to the copper center is under investigation.

(21) All pyrrole resonances have been confirmed by 2H NMR on complexes made with pyrrole-deuterated F_8TPPH_2 ligand.

(22) Consistent with a high-spin porphyrinate–iron(III) center.

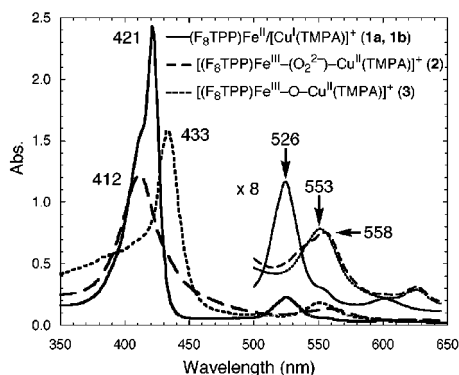


Figure 1. UV-visible spectra of the $(F_8TPP)Fe^{II}/[(TMPA)Cu^I(CH_3CN)](ClO_4)$ (**1a, 1b**) oxygenation reaction in MeCN at $-40\text{ }^\circ\text{C}$.

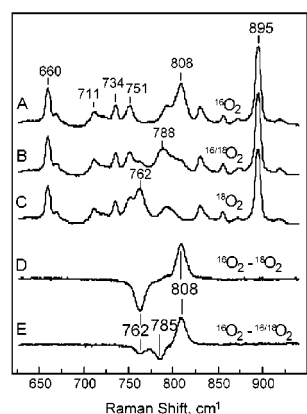


Figure 2. Resonance Raman spectra of $[(F_8TPP)Fe^{III}-(O_2^{2-})-Cu^{II}(TMPA)]^+$ (**2**), formed by oxygenation of $(F_8TPP)Fe^{II}/[(TMPA)Cu^I(CH_3CN)]^+$ (**1a:1b** = 1:1) in MeCN at $-40\text{ }^\circ\text{C}$ using $^{16}O_2$ (A), a scrambled mixed-isotope gas containing 25% $^{16}O_2$, 50% $^{16}O-^{18}O$, and 25% $^{18}O_2$ (B), and a pure $^{18}O_2$ gas (C). All spectra were obtained at room temperature with a 413 nm excitation in MeCN solvent. The difference spectra A minus C, and A minus B are also shown as traces D and E, respectively.

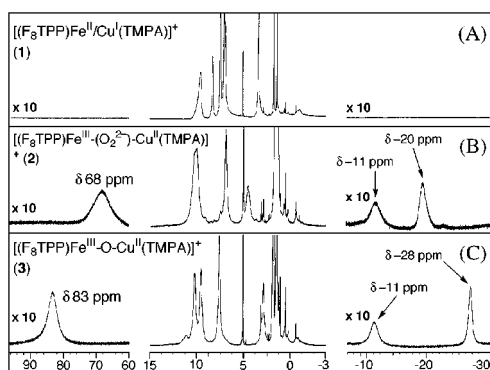


Figure 3. 1H NMR spectra (400 MHz, CD_3CN , $-40\text{ }^\circ\text{C}$) of heme-copper complexes. See text for further explanation.

= $1/2$ copper(II) through the bridging peroxide ligand.^{23,24} We have previously observed and reported this characteristic pattern of downfield shifted pyrrole resonances and upfield shifted peaks in similar $(P)Fe^{III}-X-Cu^{II}$ ($X = O_2^{2-}, O_2^-$) systems (including **3**, Scheme 1) having $S = 2$ spin states.^{19,23,24} In fact, thermal decomposition of **2** yields μ -oxo complex $[(F_8TPP)Fe^{III}-O-Cu^{II}(TMPA)]^+$ (**3**) {Scheme 1; $\mu_B = 5.1$, Evans method, $-40\text{ }^\circ\text{C}$, $\lambda_{max} = 433$ (Soret), 553 nm }, with previously assigned down-

field shifted pyrrole resonances (δ 83 ppm), and upfield shifted peaks at -11 (pyridyl 5-H) and -28 (pyridyl 3-H) ppm (Figure 3C).^{6,24}

The electronic structure of complex **2** was further probed by Mössbauer spectroscopy (4.2 K, zero field), which shows a sharp quadrupole doublet (Figure S1) with parameters $\{\Delta E_Q = 1.14\text{ mm/s}, \delta = 0.57\text{ mm/s}\}$ typical for high-spin ferric compounds. The isomer shift is significantly larger than those (0.33–0.45 mm/s) observed for high-spin ferric-heme compounds,²⁵ and is consistent with binding of an electron-rich peroxide ligand.²⁶ The magnetic field dependence (data not shown) is also consistent with **2** being an integer spin system.²⁷ Porphyrin skeletal modes in the high-frequency region of the resonance Raman spectra confirm these conclusions, and reveal a pentacoordinate high-spin configuration of the heme iron in the peroxo intermediate **2** (data not shown).

Stopped-flow UV-visible spectroscopy (500–700 nm monitoring, acetone, -94 to $-75\text{ }^\circ\text{C}$) revealed the presence of a heme-superoxo $(F_8TPP)Fe^{III}-(O_2^-)$ intermediate^{3,19} $\{\lambda_{max} = 537\text{ nm}\}$, formed within mixing time ($\sim 1\text{ ms}$) prior to formation of the heme-peroxo-Cu complex **2** $\{\lambda_{max} = 556\text{ nm}\}$, with little or no Cu-only O_2 -adducts observed. The overall kinetics are complicated by 2–3 side reactions with minor absorbance changes, but the main $537 \rightarrow 556\text{ nm}$ heme-superoxo to heme-peroxo-Cu transformation can be reasonably described by a first-order rate constant with $\Delta H^\ddagger = 45 \pm 1\text{ kJ mol}^{-1}$ and $\Delta S^\ddagger = -19 \pm 6\text{ J mol}^{-1}\text{ K}^{-1}$ ($k = 0.07\text{ s}^{-1}$, $-90\text{ }^\circ\text{C}$, $k = 0.32\text{ s}^{-1}$, $-80\text{ }^\circ\text{C}$).

As mentioned, μ -peroxo complex $[(F_8TPP)Fe^{III}-(O_2^{2-})-Cu^{II}(TMPA)]^+$ (**2**) transforms thermally to the μ -oxo complex $[(F_8TPP)Fe^{III}-O-Cu^{II}(TMPA)]^+$ (**3**). We find that this occurs in a slow reaction $\{t_{1/2} = 1016 \pm 20\text{ s}; \text{MeCN}, 22\text{ }^\circ\text{C}, 0.28\text{ mM}\}$, with concomitant release of 0.40–0.45 equiv of O_2 .²⁸ Given the **1a** + **1b** + $O_2 \rightarrow \mathbf{2}$ stoichiometry (vide supra), and that the subsequent decomposition of **2** yielding **3** releases ~ 0.5 equiv O_2 , the fate of all oxygen atoms in the formation and decomposition of **2** is known. The mechanism of O–O reductive cleavage in the transformation $\mathbf{2} \rightarrow \mathbf{3} + 1/2\text{ }O_2$ will be the object of future study.²⁹

In conclusion, complex $[(F_8TPP)Fe^{III}-(O_2^{2-})-Cu^{II}(TMPA)]^+$ (**2**) contains a symmetrically bound peroxide (i.e., most likely μ -1,2 or μ - $\eta^2:\eta^2$) in a high-spin heme–Cu antiferromagnetically coupled $S = 2$ system. The resonance Raman mixed-isotope experiment, the Mössbauer spectroscopic data, the stopped-flow kinetics, and the observation of O_2 evolution in the crudely biomimetic reductive O–O cleavage reaction {i.e., thermal decomposition of **2**} are all significant new advances.

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Supporting Information Available: Mössbauer spectrum (Figure S1) for complex **2** (4.2 K, MeCN) (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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