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

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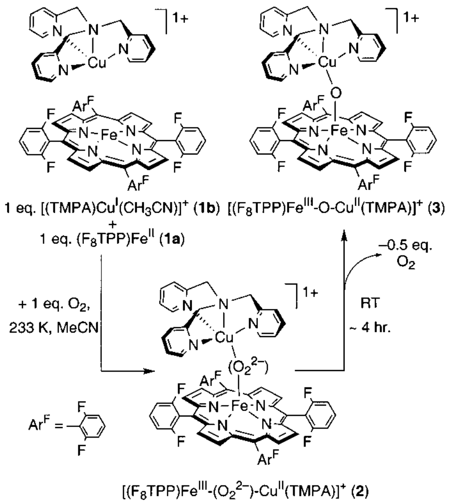
In this report, we describe the formation of a pentacoordinate high-spin heme−peroxo−Cu complex, $[(F_8TPP)Fe^{III}-(O_2^{2-})-Cu^{II}(TMPA)]^+$ (**2**),[1] formed upon addition of O_2 to a 1:1 mixture of the reduced heme complex $(F_8TPP)Fe^{II}$ (**1a**) and copper complex $[(TMPA)Cu^I(CH_3CN)]^+$ (**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.

Scheme 1



Upon addition of dioxygen at −40 °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 °C} revealed an oxygenation stoichiometry of **1a**:**1b**:$\mathbf{O_2}$ = 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 °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 resonances[22] 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 °C), arising from the antiferromagnetic coupling of the $S = 5/2$ high spin ferric heme to the *S*

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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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[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.

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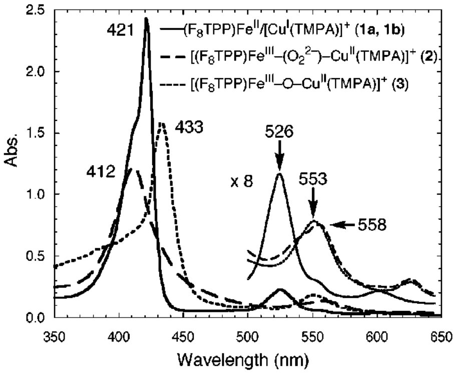

Figure 1. UV-visible spectra of the $(F_8TPP)Fe^{II}$ (**1a**)/$[(TMPA)Cu^I(CH_3CN)](ClO_4)$ (**1b**) oxygenation reaction in MeCN at −40 °C.

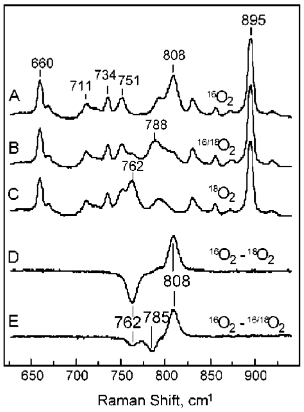

Figure 2. Resonance Raman spectra of $[(F_8TPP)Fe^{III}-(O_2^{2-})-Cu^{II}(TMPA)]^+$ (**2**), formed by oxygenation of $(F_8TPP)Fe^{II}/[(TMPA)Cu^I(CH_3CN)]^+$ (**1a**:**1b** = 1:1) in MeCN at −40 °C using $^{16}O_2$ (A), a scrambled mixed-isotope gas containing 25% $^{16}O_2$, 50% $^{16}O-^{18}O$, and 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.

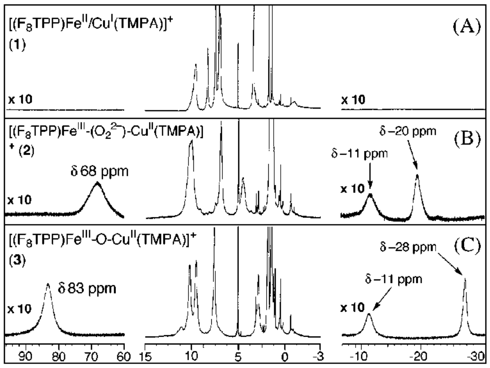

Figure 3. ^1H NMR spectra (400 MHz, CD_3CN, −40 °C) of heme−copper complexes. See text for further explanation.

$= {}^1/_2$ 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 °C, $\lambda_{max} = 433$ (Soret), 553 nm}, with previously assigned downfield 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 $\{\Delta E_Q = 1.14$ mm/s, $\delta = 0.57$ 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 °C) revealed the presence of a heme−superoxo $(F_8TPP)Fe^{III}-(O_2^-)$ intermediate[3,19] $\{\lambda_{max} = 537$ nm$\}$, formed within mixing time (~1 ms) prior to formation of the heme−peroxo−Cu complex **2** $\{\lambda_{max} = 556$ 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 → 556 nm heme-superoxo to heme−peroxo−Cu transformation can be reasonably described by a first-order rate constant with $\Delta H^\ddagger = 45 \pm 1$ kJ mol^{-1} and $\Delta S^\ddagger = -19 \pm 6$ J mol^{-1} K^{-1} ($k = 0.07$ s^{-1}, − 90 °C, $k = 0.32$ s^{-1}, − 80 °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 $\{t_{1/2} = 1016 \pm 20$ s; MeCN, 22 °C, 0.28 mM$\}$, with concomitant release of 0.40−0.45 equiv of O_2.[28] Given the **1a** + **1b** + O_2 → **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 **2** → **3** + $^1/_2$ 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-η^2:η^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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(27) A detailed full study is in progress.

(28) Dioxygen evolution was confirmed by a quantitative alkaline pyrogallol test for O_2 release.

(29) Non-heme (DuBois, J. L.; Mizoguchi, T. J.; Lippard, S. J. *Coord. Chem. Rev.* **2000**, *200*, 443−485) and heme (Chin, D.-H., LaMar, G. N., Balch, A. L. *J. Am. Chem. Soc.* **1980**, *102*, 4344−4350) peroxo−diiron systems are known to undergo similar peroxo-to-oxo transformations which release O_2.