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# Dioxygen Reactivity of Reduced Heme and Heme−Copper Complexes Utilizing Tetraarylporphyrinates Tethered with Both a Pyridyl Axial Ligand and *N,N*-Bis[2-(2-pyridyl)ethyl]amine Chelate

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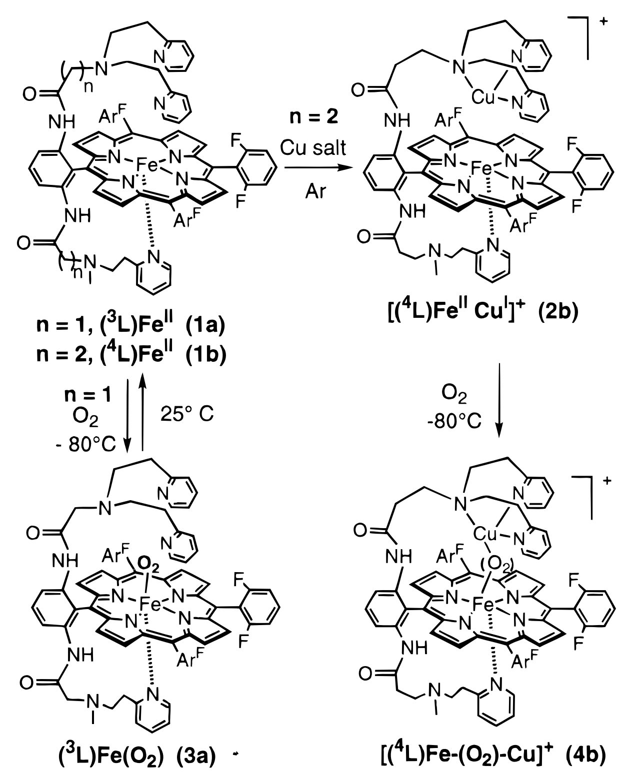
Our continuing interest in developing reactivity models for the heme $a_3$−Cu$_\mathrm{B}$ O$_2$-binding, O$_2$-reduction, and proton pumping site in heme−copper oxidases[1,2] (e.g., cytochrome *c* oxidase (C*c*O))[2−5] includes investigation of reactions of dioxygen with (porphyrinate)Fe$^{\mathrm{II}}$ and (L$^{\mathrm{Cu}}$)Cu$^{\mathrm{I}}$ complexes.[6−9] Thus, (F$_8$TPP)Fe$^{\mathrm{II}}$/[(L$^{\mathrm{Cu}}$)Cu$^{\mathrm{I}}$]$^+$/O$_2$ reactions lead to O−O reductive cleavage and generation of $\mu$-oxo complexes [(F$_8$TPP)Fe$^{\mathrm{III}}$−O−Cu$^{\mathrm{II}}$(L$^{\mathrm{Cu}}$)]$^+$,[10] when employing pyridyl-alkylamine copper-ligand donors, either an N$_4$ tetradentate L$^{\mathrm{Cu}}$ = TMPA or tridentate chelate L$^{\mathrm{Cu}}$ = R-PY2.[9,10] A developing approach in our laboratories[6,14−16] and others[17−21] is to utilize heterobinucleating ligands for such heme/Cu/O$_2$-reactivity studies. We recently showed that reduced compounds [($^n$**L**)Fe$^{\mathrm{II}}$...Cu$^{\mathrm{I}}$]$^+$, where $^n$**L** possess a TMPA moiety covalently tethered to a tetraarylporphyrin periphery, react with O$_2$ giving analogous $\mu$-oxo complexes [($^n$**L**)Fe$^{\mathrm{III}}$−O−Cu$^{\mathrm{II}}$]$^+$.[13,16] Here, we describe oxygenation chemistry using $^3$**L** and $^4$**L** (Scheme 1), with new features: (1) a PY2[10] tridentate chelate is built in, to match the number and type of N-ligands observed in C*c*O.[3−5] (2) while the copper ligands possess the PY2 unit, taking advantage of previously established Cu$^{\mathrm{I}}$/O$_2$ chemistry,[22−24] (3) a heme axial pyridyl ligand is tethered to the porphryin periphery distal to the PY2 Cu ligand, mimicking the C*c*O arrangement,[3−5] and (4) $^3$**L** and $^4$**L** possess different linkers to the PY2 and pyridine base; such model compound variations provide a way to probe the effects of subtle changes in metal environment, analogous to protein enforced active-site geometric relationships.[25]

**Scheme 1**



Syntheses of $^3$**L** and $^4$**L**,[26] metalation (FeCl$_2$), air oxidation, and column chromatography yield porphyrinate−iron(III) complexes with an empty PY2 tether, ($^{3/4}$**L**)Fe$^{\mathrm{III}}$−OH ($\lambda_{\mathrm{max}}$ = 413−415 nm; $\delta_{\mathrm{pyrrole}}$ = ∼80 ppm (room temperature)),[26] with spectrosopic properties paralleling those of (F$_8$TPP)Fe$^{\mathrm{III}}$−OH.[12]

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(10) Abbreviations used: F$_8$TPP, tetrakis(2,6-difluorophenyl)porphyrinate; TMPA, tris(2-pyridylmethyl)amine; R-PY2, *N,N*-bis[2-(2-pyridyl)ethyl]-R-amine; BArF = B[3,5-(CF$_3$)$_2$C$_6$H$_3$]$_4^-$.
(11) These same $\mu$-oxo complexes can also be synthesized from acid−base self-assembly procedures.[9,12,13]
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(25) As a function of the variable tether arm in $^n$**L**, [($^n$**L**)Fe$^{\mathrm{III}}$−O−Cu$^{\mathrm{II}}$]$^+$ exhibit dramatic differences in structure (i.e., near-linear vs severely bent) and $\mu$-oxo basicity.[13]
(26) See Supporting Information.

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Subsequent reduction ($Na_2S_2O_4$(aq)/$CH_2Cl_2$) affords iron(II) species ($^{3/4}$**L**)Fe$^{II}$, **1a** and **1b**, respectively, both characterized by UV−vis absorptions at 434 (Soret), 556, and 530 (sh) nm in $CH_2Cl_2$ solvent, consistent with high-spin ($S$ = 2) pentacoordination.[27] Supporting $^1$H NMR spectroscopic observations[28a] are four downfield $\beta$-pyrrolic signals ($\delta$ 49−54 ppm, in $CD_2Cl_2$, $C_6D_6$, and $(CD_3)_2CO$) plus additional paramagnetically shifted resonances ($CD_2Cl_2$: **1a**, 32.34, 25.77, 24.66, −3.53, −11.35 ppm; **1b**, 24.92, 23.87, 19.2, 17.73, 24.43, −2.93, −12.19 ppm) due to *one* coordinated axial pyridine tether arm.[28b]

($^3$**L**)Fe$^{II}$ (**1a**) binds $O_2$ reversibly (Scheme 1),[29] as monitored by UV−vis and $^1$H NMR spectroscopies. In THF, toluene, or $CH_2Cl_2$, solutions bubbled with $O_2$ at −80 °C change and give rise to a low-temperature stable species **3a**, with 421 (Soret) and 540 nm features (Figure 1). Complex **3a** is formulated as ($^3$**L**)-Fe−$O_2$, a low-spin six-coordinated hemoglobin/myoglobin model compound, since a spectrophotometric titration[26] shows that Fe/$O_2$ = 1:1 (−80 °C, THF), and it gives a diamagnetic ($S$ = 0) $^1$H NMR spectrum. Vacuum/purge followed by oxygenation cycles allows facile interconversion of ($^3$**L**)Fe$^{II}$ (**1a**) and ($^3$**L**)Fe−$O_2$ (**3a**), and even simple warming of **3a** to room temperature gives back the spectrum ($\lambda_{max}$ = 434 nm) of **1a**.[26] Monitoring by $^1$H NMR shows that downfield pyrrole resonances for **1a** begin to appear when solutions of **3a** are warmed to ca. −30 °C. The analogous complex ($^4$**L**)Fe$^{II}$ (**1b**) also reacts to give an $O_2$ adduct with spectral features similar to those of **3a**. However, $O_2$ binding is *not* reversible, again demonstrating how subtle changes in ligand architecture can significantly alter the observed chemistry.[25]

Addition of a cuprous salt, $[Cu(CH_3CN)_4]$(BArF), to complexes **1a** and **1b** gives the desired Fe$^{II}$/Cu$^{I}$ complexes [($^3$**L**)Fe$^{II}$Cu$^{I}$]$^+$ (**2a**) and [($^4$**L**)Fe$^{II}$Cu$^{I}$]$^+$ (**2b**) (Scheme 1) having UV−vis absorptions (434 (Soret), 556, 530 (sh) nm) similar to those of **1a** and **1b** at room temperature. In (weakly) coordinating solvents such as MeCN and THF,[30] cooling results in the reversible splitting of the Soret band (∼ 433 and 429 nm, Figure 1), whereas no change is observed in toluene. This behavior is tentatively explained by a competition between the coordination of the tethered pyridine axial base versus the solvent, the latter being favored at low temperature. $^1$H NMR spectra of **2a**,**b** are very similar to those of **1a**,**b**, consistent with a high-spin behavior and coordination to the tethered pyridine axial ligand.[32]

Both **2a** and **2b** react with $O_2$ at −80 °C (THF or EtCN solvents) to give dioxygen adducts **4a** and **4b**, with new UV−vis features at 418 (Soret) and 536 nm, Figure 1. Formation of **4a**,**b** is irreversible, on the basis of the lack of displacement of bound $O_2$ following argon purges or application of a vacuum. The $^4$**L**-containing $O_2$ adduct is formulated as [($^4$**L**)Fe−$O_2$−Cu]$^+$ (**4b**), a peroxo level Fe$^{III}$−Cu$^{II}$ species:[33] (i) the stoichiometry of $O_2$ binding (spectrophotometric titration)[26] is **2b**/$O_2$ = 1:1, (ii) a $^1$H NMR spectrum of **4b** at −80 °C in THF-$d_8$ exhibits diamagnetic behavior, analogous to the Fe/Cu $O_2$ adducts of Collman and co-workers;[33,34] and (iii) complex [($^4$**L**)Fe−$O_2$−Cu]$^+$ (**4b**) is EPR-silent (THF, 84 K), consistent with a strongly coupled peroxo-bridged species. An iron-only $O_2$ adduct (pyridyl)-(porphyrinate)Fe−$O_2$ is ruled out, since this would leave an unreacted Cu(I)−PY2 moiety; [(MePY2)Cu$^{I}$($CH_3CN$)](BArF) is known to react with $O_2$ to give a peroxo dicopper(II) complex with intense 360 nm absorption,[22] which is not observed here.

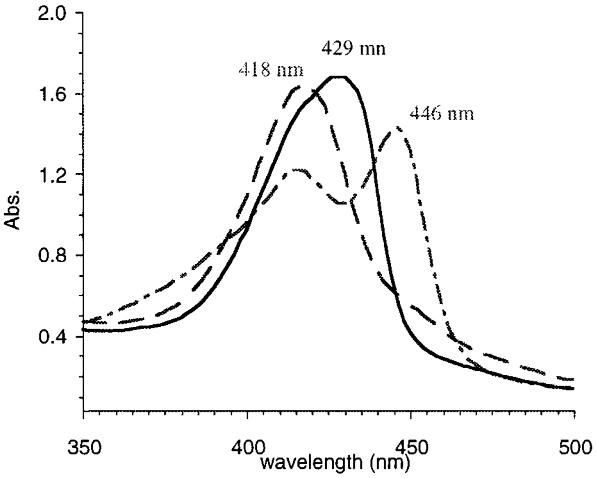

**Figure 1.** [($^4$**L**)Fe$^{II}$Cu$^{I}$]$^+$ (**2b**, $\lambda_{max}$ 429 nm, −80 °C in EtCN) reacts with $O_2$ giving [($^4$**L**)Fe−$O_2$−Cu]$^+$ (**4b**, $\lambda_{max}$ 418 nm). Rapid warming to room-temperature affords products which include $\mu$-oxo complex [($^4$**L**)Fe$^{III}$−O−Cu$^{II}$]$^+$ ($\lambda_{max}$ 446 nm). See text.

Warming of these low-temperature stable $O_2$ adducts **4a** and **4b** (with excess $O_2$ removed in vacuo) shows interesting differences. For [($^4$**L**)Fe−$O_2$−Cu]$^+$ (**4b**), the major product (>70%) is the $\mu$-oxo complex formulated as [($^4$**L**)Fe$^{III}$−O−Cu$^{II}$]$^+$, possessing the distinctive features {(i) red-shifted Soret band (446 nm) (Figure 2), (ii) $^1$H NMR $\delta_{pyrrole}$ = ∼70 ppm (room temperature), (iii) upfield-shifted Cu−ligand resonances} observed in established [(porphyrinate)Fe$^{III}$−O−Cu$^{II}$]$^+$ complexes,[13,31] including the closely related [($F_8$TPP)Fe$^{III}$−O−Cu$^{II}$(MePY2)]$^+$ .[9,35] By contrast, no $\mu$-oxo final species is ever observed from the $O_2$ adduct of $^3$**L**, **4a**;[36] features associated with ($^3$**L**)Fe$^{III}$−OH (vide supra) are observed and are tentatively assigned to a product [($^3$**L**)-Fe$^{III}$−OH•••Cu$^{II}$]$^{n+}$.

In summary, a new class of binucleating ligands[37] for use in heme−Cu complex chemical studies has been synthesized. These should be useful for more in-depth $O_2$ reactivity studies, having a tridentate ligand for copper with its own previously established Cu$^{I}$/$O_2$ chemistry. The initial studies reported here, with reversible heme/$O_2$ binding, formation of a heme/Cu/$O_2$ adduct [($^4$**L**)Fe−$O_2$−Cu]$^+$ (**4b**), and observation of ligand architecture influences on reactivity, validate the design of ligands and approach. Further characterization and studies are in progress.

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**Supporting Information Available:** Figure S1, with UV−vis spectra illustrating reversible $O_2$-binding for iron-only complex **1a**. Synthesis and characterization of ligands and complexes. This material is available free of charge via the Internet at http://pubs.acs.org.

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(28) (a) $^{19}$F NMR spectra of **1a** and **1b** show four resonances between −108 and −110 ppm (THF; relative to $CF_3C_6H_5$) in a 1:1:2:2 ratio, consistent with the low symmetry of these ligands. (b) Support for this assignment comes from study of an analogue heme with a ligand similar to $^3$**L** which has the axial pyridine tether arm, but otherwise has only a nitro *o*-phenyl substituent instead of the whole PY2 tether arm. In this case, very similarly located extra $^1$H NMR peaks are observed for a high-spin Fe(II) complex.

(29) See: Collman, J. P.; Fu, L. *Acc. Chem. Res.* **1999**, *32*, 455−463.

(30) In solvents such as THF and MeCN, ($F_8$TPP)Fe$^{II}$ is high-spin. In noncoordinating solvents (e.g., $CD_2Cl_2$, $C_7D_8$), it is four-coordinate and intermediate-spin ($S$ = 1).[8,31]

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(32) Full characterization will be given elsewhere.

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(35) The minor product contains the ($^4$**L**)Fe$^{III}$−OH moiety, $\lambda_{max}$ = 414 nm (Figure 2), $\delta_{pyrrole}$ = ∼80 ppm (room temperature).

(36) A $\mu$-oxo product is not observed even in an acid−base synthesis, by addition of a Cu(II) salt and base to ($^3$**L**)Fe$^{III}$−OH.

(37) For purposes of comparison, we have also synthesized analogues of $^3$**L** and $^4$**L** which lack the pyridine containing arm axial base ligand.