

Heme/Cu/O₂ Reactivity: Change in Fe^{III}–(O₂²⁻)–Cu^{II} Unit Peroxo Binding Geometry Effected by Tridentate Copper Chelation

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Biomimetic studies employing well-characterized small-molecule heme/Cu complexes have been aimed at shedding light on fundamental aspects of dioxygen chemistry of porphyrinate–iron(II)–copper(I) assemblies.^{1,2} Such investigations are inspired by the presence of a heme a₃–Cu_B binuclear active site in heme–copper oxidases (such as cytochrome *c* oxidase) which catalyze the four-electron/four-proton reduction of O₂.^{1,3–5} The first spectroscopically observable intermediate is a heme–O₂ adduct (a Fe^{III}–O₂⁻ moiety) which undergoes a reductive O–O cleavage assisted by electron transfer (ET) from Cu^I_B. Calculations suggest ET may proceed through an iron(III)–(hydro)peroxo moiety (which may interact with Cu^{II}_B) where O–O cleavage leads to subsequent Cu^{II}_B binding of a water/hydroxide O atom derived from O₂.^{1,6}

In this report, we describe a system which mimicks the initial O₂ binding to the heme, followed by interaction with the neighboring copper(I) ion to give a heme–peroxo–copper product. The tridentate copper chelate employed effects considerable O–O bond weakening of the O₂-derived peroxo ligand, and a new μ-η²:η² (side-on to both heme and copper) structural type forms.

The binucleating ligand ²L was metalated, and the resulting complex was reduced, forming [(²L)Fe^{II}Cu^I]⁺ (**1**).⁷ Laboratory benchtop reactions with dioxygen at –80 °C in CH₂Cl₂/6%EtCN demonstrate the immediate transformation of **1** (λ_{max}, 426, 530 nm)⁸ to [(²L)Fe^{III}–(O₂²⁻)–Cu^{II}]⁺ (**3**) (λ_{max}, 419, 488, 544, 575 nm),⁸ formulated as a heme–peroxo–copper complex, Scheme 1. ¹H NMR spectroscopy (–90 °C, acetone-*d*₆) reveals that **3** has the distinctive characteristics of an *S* = 2 system, with the high-spin iron(III) center strongly magnetically coupled to Cu^{II} through a peroxo bridge.^{9,10} Consistent with this are pyrrole resonances at 110 ppm, and diagnostic^{9,10} upfield and downfield copper-ligand hydrogen resonances (e.g., at –31, –27, –11, –8, 29, 37, and 39 ppm), characteristic of the *S* = 2 spin system.¹¹

Resonance Raman spectroscopic investigation of **3** confirms the peroxo assignment and rules out the presence of ferryl–oxo (Fe=O) and Fe–O–Cu entities. A single O–O stretching frequency is observed at 747 cm⁻¹ (Δ(¹⁸O₂) = –40 cm⁻¹) (Figure 1).^{11,12} In a ¹⁶O–¹⁸O mixed isotope experiment (Figure 1) a single band is observed at 730 cm⁻¹, between the ¹⁶O–¹⁶O and ¹⁸O–¹⁸O stretching bands. The very low ν(O–O) and the absence of a splitting of the 730 cm⁻¹ band are indicative of a symmetrical binding of the peroxide group in a side-on μ-η²:η² geometry; see also further discussion below.

Formation of **3** from reaction of **1** with O₂ in CH₂Cl₂/6%EtCN was monitored by low-temperature UV–vis stopped-flow spectroscopy.⁷ Rapid generation of an intermediate, formulated as

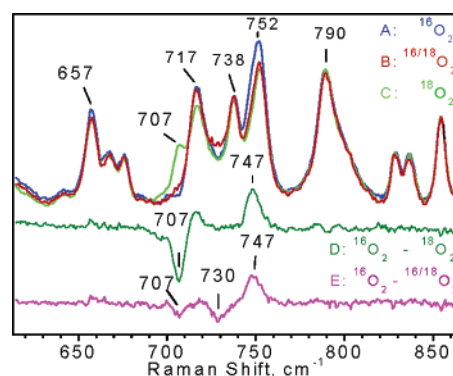
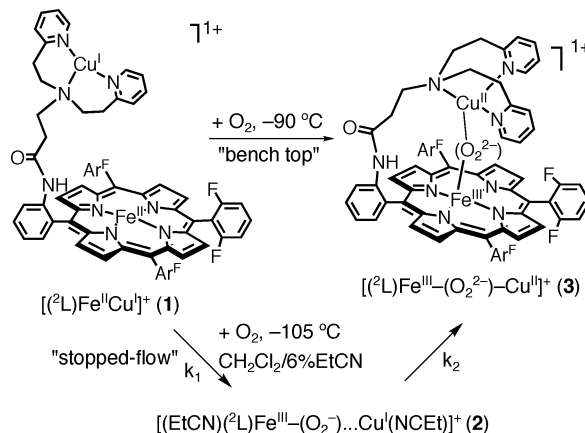


Figure 1. Resonance Raman spectra of **3**, formed by the oxygenation of **1** with ¹⁶O₂ (A), a scrambled isotope gas containing 25% ¹⁶O₂, 50% ¹⁶O–¹⁸O, and 25% ¹⁸O₂ (B), and ¹⁸O₂ (C). Difference spectra are also shown. All spectra were obtained at 90 K with 413 nm excitation.¹⁰

Scheme 1



superoxo complex [(²L)Fe^{III}–(O₂⁻)...Cu^I(NCEt)]⁺ (**2**) (λ_{max} = 544 nm), is observed at –105 °C (Scheme 1, *k*₁ = 5.23 ± 0.09 × 10⁴ M⁻¹ s⁻¹). The UV–vis spectrum of the closely related dioxygen adduct [(F₈)Fe^{III}(O₂⁻)]¹³ in CH₂Cl₂/6%EtCN is essentially the same¹⁰ [and ν(O–O) = 1178 cm⁻¹ in THF].¹⁰ Our description of **2** is further supported by the knowledge that copper(I) complexes with the bis(2-(2-pyridyl)ethyl)amine moiety (as found here in ²L) are known to *not* react with O₂ in nitrile solvents.¹⁰ Intramolecular reaction of copper(I) with the superoxo moiety to produce peroxo complex **3** occurs with *k*₂ = 2.74 ± 0.04 × 10¹ s⁻¹ at –105 °C.

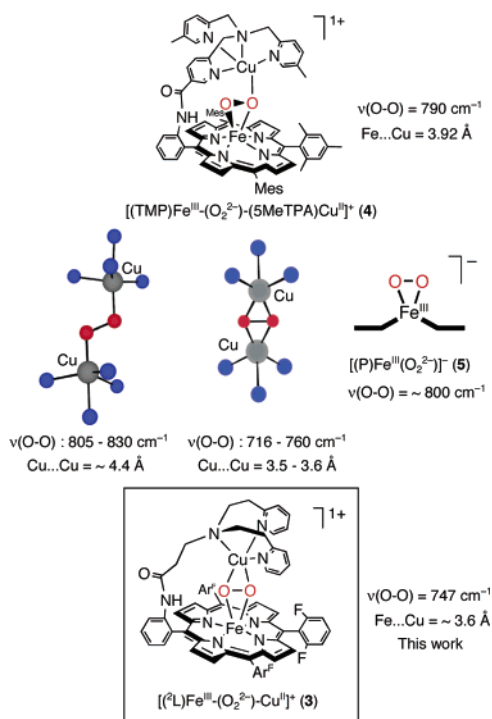
X-ray absorption spectroscopic studies also support a side-on/side-on peroxo-ligand binding structure in [(²L)Fe^{III}–(O₂²⁻)–Cu^{II}]⁺ (**3**).⁷ Copper K-edge EXAFS is most consistent with a five-coordinate metal center with two N scatterers at 2.028(7) Å, 2 O scatterers at 1.898(7) Å (as expected for Cu^{II}–O_{peroxo} distances),¹²

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Chart 1



and one longer N scatterer at 2.171(12) Å. A well-ordered outer-sphere Fe scatterer is also found at 3.62(1) Å.⁷ This distance is close in line with those known from EXAFS studies of μ - η^2 : η^2 -peroxo dicopper(II) moieties found in synthetically derived complexes,^{14,15} as well as in oxy-hemocyanin, a copper-containing O₂ carrier protein.^{16,17} The iron center K-edge EXAFS fits to either a five- or six-coordinate metal center with four N(pyrrole) scatterers at \sim 2.1 Å and one or two O at \sim 1.9 Å.⁷ Unfortunately, the Cu \cdots Fe vector could not be reliably located at the Fe edge due to the multitude of outer-sphere scattering pathways.¹⁸ An analysis of the preedge feature, which corresponds to an Fe(1s) \rightarrow Fe(3d) transition, also suggests a side-on peroxo coordination to iron. In **3**, this feature at 7113.2(2) eV is 2–4 times weaker and \sim 1.5 eV lower in energy than those found in five-coordinate (P)Fe^{III} ¹³ complexes with nominal C_{4v} symmetry.^{19,20} However, this weak preedge feature resembles that of [(F₈)Fe^{III}(O₂²⁻)]⁻,^{7,13,21} which contains a side-on (η^2) bound peroxo ligand.

Side-on peroxo binding to a heme iron(III) moiety has been recently observed in the crystallographically characterized heme-copper assembly [(TMP)Fe^{III}-(O₂²⁻)-(5MeTPA)Cu^{II}]⁺ (**4**),²² this has an overall μ - η^2 : η^1 -peroxo coordination, with end-on ligation to the copper with a tridentate chelate, Chart 1. However, we formulate **3** as having a new μ - η^2 : η^2 side-on/side-on structure, Chart 1. This is supported by the EXAFS spectroscopic analysis comparisons and the ¹⁶O–¹⁸O resonance Raman data. Peroxo-dicopper(II) structures with μ - η^2 : η^2 ligation occur when using tridentate (or bidentate) nitrogen chelates.¹² They possess considerably diminished O–O stretching frequencies compared to end-on bound μ -1,2-peroxo-dicopper(II) complexes which form with tridentate chelates (Chart 1).^{12,23} Yet, **4** and η^2 -peroxo ferric heme complexes [(P)Fe^{III}-(O₂²⁻)]⁻ (**5**) possess ν (O–O) \approx 800 cm⁻¹ (Chart 1).^{22,24} The significantly reduced O–O stretching frequency and weaker O–O bond in [(²L)Fe^{III}-(O₂²⁻)-Cu^{II}]⁺ (**3**) must be due to the imposed tridentate copper ligand environment, strongly preferring a side-on copper peroxo binding (Chart 1).

In conclusion, a reduced Fe^{II}–Cu^I heme-copper complex, with new heterobinucleating ligand ²L, reacts with dioxygen to give a

heme-superoxo and then an Fe^{III}–(O₂²⁻)–Cu^{II} product. The latter possesses an O–O bond stretching which is 40-to-60 cm⁻¹ lower compared to those of eight other known heme-peroxo-copper complexes possessing tetradentate ligands for copper.^{1,25,26} This result is the consequence of the tridentate copper chelate employed (within ²L), which *strongly* prefers a side-on peroxo ligation and which is well-known to weaken the peroxide O–O bond.²³ While peroxo-bridged complex **3** possesses an Fe \cdots Cu distance (\sim 3.6 Å) that is not likely to occur in heme-copper oxidases, new insights into how reductive O–O cleavage can be facilitated as a function of Fe–peroxo–Cu structure will be derived from future investigations, most likely employing a tridentate chelate for copper.

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Supporting Information Available: Synthetic details, XAS data and analyses, and kinetics Eyring plots. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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