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Synthetic Models of the Deoxy and Oxy Forms of the Non-Heme Dioxygen-Binding Protein Hemerythrin

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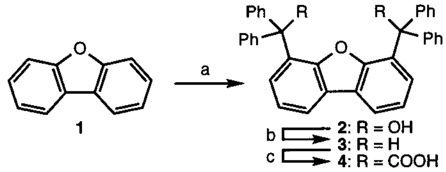
The three prototypical reversible dioxygen-binding proteins comprise hemoglobin (Hb), hemocyanin (Hc), and hemerythrin (Hr) and are distinguished by the presence of active-site heme-iron, dicopper, and non-heme diiron centers, respectively.[1] Pioneering work[2] has shown that an appropriately designed synthetic iron porphyrin[3] can function as a fully operational Hb mimic without the need for a protein matrix. The μ-η^2:η^2-peroxodicopper(II) active center of oxyHc has similarly been modeled.[4,5] Despite the extensive body of published material in the area of synthetic non-heme diiron chemistry,[6−9] small molecule complexes that reproduce the functionally relevant active-site structures and properties of the deoxy and oxy forms of Hr have yet to be reported.

DeoxyHr contains a (μ-hydroxo)bis(μ-carboxylato)diiron(II) core to which are ligated five imidazole groups of histidine residues, affording one 5- and one 6-coordinate iron atom.[10] Dioxygen binding occurs at the coordinatively unsaturated iron(II) center in coupled electron−proton transfer chemistry that yields a terminally bound hydroperoxide ligand hydrogen bonded to the bridging oxygen atom of the resulting (μ-oxo)bis(μ-carboxylato)diiron(III) core.[10] From recent studies of the protein[11] and from synthetic work,[12] the task of mimicking the functional chemistry of Hr outside of the protein environment would appear to be formidable. In the present paper we describe our recent efforts to meet this challenge through the synthesis of an asymmetric (μ-hydroxo)bis(μ-carboxylato)diiron(II) complex which, upon exposure to dioxygen in the presence of *N*-methylimidazole (*N*-MeIm), generates a species exhibiting spectroscopic properties that closely match those of oxyHr.

To facilitate the assembly and stabilization of the desired bis(μ-carboxylato)diiron Hr model complexes, a new dinucleating dicarboxylate ligand, dibenzofuran-4,6-bis(diphenylacetate) (Ph_4DBA), was designed. Computer modeling of this ligand revealed the carboxylate groups to have the ability to orient themselves orthogonal to one another for bridging two metals in a syn−syn bidentate[13] mode. Multigram quantities of the free acid of Ph_4DBA were readily obtained from dibenzofuran (**1**) in three steps in ~40% overall yield (Scheme 1). Double deprotonation of **1**[14] followed by trapping with benzophenone yielded, upon treatment with acid, diol **2**. Subsequent reduction of **2** with triethylsilane/boron trifluoride[15] led to nearly quantitative isolation of compound **3**. Addition of carbon dioxide to the dianion of **3**[16] followed by an acidic workup generated diacid **4**. The crude product, washed with water and Et_2O, proved pure enough by NMR spectroscopy[17] for use in subsequent metalation chemistry.

Scheme 1



(a) *n*-BuLi/TMEDA/hexane, Ph_2CO, HCl(*aq*), 60%
(b) Et_3SiH/CH_2Cl_2, $BF_3{\cdot}Et_2O$, Na_2CO_3(*aq*), 98%
(c) *n*-BuLi/THF, CO_2(*g*), HCl(*aq*), 60%

The neutral, air-sensitive diiron(II) compound [Fe_2(μ-OH)(μ-Ph_4DBA)$(TMEDA)_2$(OTf)] (**5**), where TMEDA = *N,N,N′,N′*-tetramethylethylenediamine and OTf = triflate, was assembled in THF/CH_3CN solution at room temperature by mixing diacid **4**, Et_3N, $Fe(OTf)_2{\cdot}2CH_3CN$,[18] TMEDA, and H_2O in a 1:3:2:2:1.5 molar ratio. Recrystallization of the crude product from CH_2Cl_2/Et_2O led to analytically pure material and afforded X-ray quality crystals sufficient to characterize **5** as a (μ-hydroxo)bis(μ-carboxylato)diiron(II) complex with two bidentate TMEDA ligands, one per metal center, and one terminally bound triflate anion, cis to the bridging hydroxide, completing the coordination spheres of one 5- and one 6-coordinate iron.[19] The 77 K zero-field Mössbauer spectrum of the resulting asymmetric complex **5** (Figure S1) displayed a very broad doublet ($\Gamma \approx 0.70$ mm s^{-1}) that was fit as two overlapping quadrupole doublets ($\delta_1 = 1.04$, $\Delta E_{Q1} = 2.81$; $\delta_2 = 1.33$, $\Delta E_{Q2} = 2.81$ mm s^{-1}), reflecting the inequivalence of the two iron centers. These isomer shift and quadrupole splitting parameters are indicative of high-spin Fe(II) and, when averaged, are identical to those reported for deoxyHr ($\delta = 1.19$, $\Delta E_Q = 2.81$ mm s^{-1}).[20]

When the triflate ligand in **5** was replaced in a metathesis reaction with the noncoordinating tetraphenylborate (BPh_4) anion, [Fe_2(μ-OH)(μ-Ph_4DBA)$(TMEDA)_2$(CH_3CN)]BPh_4 (**6**) was obtained following recrystallization of the crude product from $CH_2Cl_2/CH_3CN/Et_2O$. An X-ray crystal structure analysis of **6** showed that the $\{Fe_2(\mu\text{-OH})(\mu\text{-}Ph_4DBA)(TMEDA)_2\}^+$ unit of **5** was preserved and that, in place of the coordinated triflate in **5**, was an acetonitrile molecule (Figure 1). The metric parameters of the dimetallic core of **6** are very similar to those of other crystallographically characterized (μ-hydroxo)bis(μ-carboxylato)diiron(II) complexes.[21,22] The Fe−O_{bridge} bond distances range from 1.946(2) to 2.043(2) Å, and the Fe−O_{bridge}−Fe bond angle averages to 105.65(8)° for the two chemically equivalent molecules of **6** in the crystallographic asymmetric unit. The difference in coordination number of the two iron atoms in **6** is

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(19) A complete structure determination was hampered by severe disorder in the TMEDA ligands and lattice solvent molecules. Anal. Calcd for $C_{53}H_{59}N_4O_9F_3SFe_2$ C, 58.04; H, 5.42; N, 5.11. Found C, 57.89; H, 5.33; N, 5.11.
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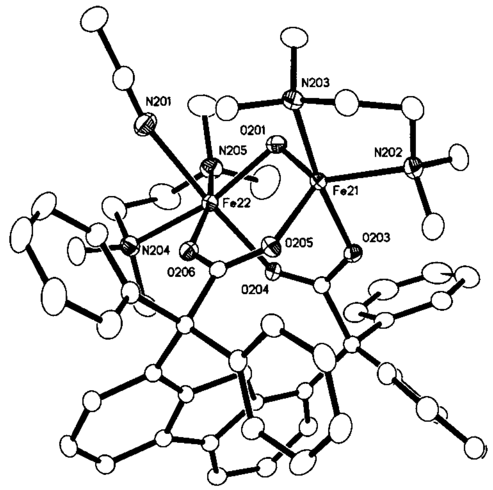

Figure 1. ORTEP diagram of [$Fe_2(\mu\text{-}OH)(\mu\text{-}Ph_4DBA)(TMEDA)_2(CH_3CN)]^+$ showing 30% probability thermal ellipsoids for all nonhydrogen atoms. Selected interatomic distances (Å): Fe21···Fe22, 3.1638(5); Fe21−O201, 1.959(2); Fe21−O203, 2.102(2); Fe21−O205, 2.040(2); Fe21−N202, 2.172(2); Fe21−N203, 2.254(2); Fe22−O201, 2.026(2); Fe22−O204, 2.143(2); Fe22−O206, 2.140(2); Fe22−N201, 2.336(2); Fe22−N204, 2.219(2); Fe22−N205, 2.282(2). The Fe21−O201−Fe22 bond angle is 105.12(8)°.

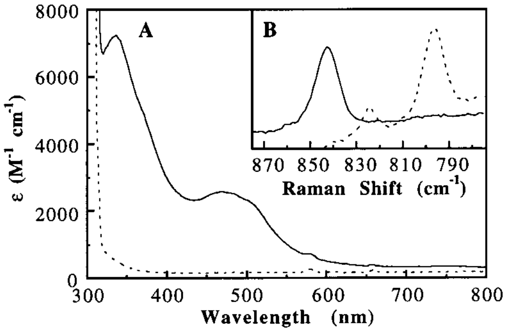

Figure 2. [$Fe_2(\mu\text{-}OH)(\mu\text{-}Ph_4DBA)(TMEDA)_2(OTf)$] (**5**) in CH_2Cl_2 containing 3 equiv of *N*-MeIm at −78 °C. (A) Electronic absorption spectra before (- - -) and after (—) addition of dioxygen. (B) Resonance Raman spectra (excited at 514.5 nm) after oxygenation with $^{16}O_2$ (—) and $^{18}O_2$ (- - -).

reflected by the systematically shorter bond lengths for the 5-coordinate, distorted trigonal bipyramidal Fe21 compared to the 6-coordinate, pseudooctahedral Fe22 (Figure 1).

Compounds **5** and **6** represent the first synthetic diiron complexes that reproduce not only the (μ-hydroxo)bis(μ-carboxylato)diiron(II) core structure of deoxyHr but also the coordination numbers at each iron(II) center, thereby affording an open site for potential binding of dioxygen. When dioxygen was bubbled for ~30 s into a colorless solution of **5** in CH_2Cl_2 containing 3 equiv of *N*-MeIm at −78 °C, the solution turned red-orange. Optical absorptions at 336 and ~470 nm reached their maximum intensity ($\epsilon_{336} \approx 7300$; $\epsilon_{470} \approx 2600$ M^{-1} cm^{-1}; $\epsilon_{336}/\epsilon_{470} = 2.8$) after approximately 5 min (Figure 2A). Under these conditions, formation of this initial species **7** was irreversible, and the product was not stable indefinitely, the intensity of the visible bands slowly diminishing over the course of several hours. The UV−vis spectrum of **7** is remarkably similar to that of oxyHr,[23] which exhibits bands centered at 500 and 330 nm with $\epsilon_{330} = 6800$, $\epsilon_{500} = 2200$ M^{-1} cm^{-1}, and $\epsilon_{330}/\epsilon_{500} = 3.1$. The 500-nm band of oxyHr has been attributed to a hydroperoxide-to-Fe(III) charge-transfer transition,[24] which is considerably higher in energy than the peroxo-to-Fe(III) charge-transfer transitions ($\lambda_{max} \approx 600$ nm) of μ-1,2-peroxodiiron(III) complexes.[25] The 470-nm band of **7** is, therefore, tentatively assigned to the ligand-to-metal charge-transfer transition(s) of a terminally bound hydroperoxide−iron(III) unit.

Direct evidence for a dioxygen-bound moiety in **7** was obtained through resonance Raman spectroscopy. Excitation at 514.5 nm into the low-energy side of the 470-nm band of **7** prepared with $^{16}O_2$ yielded a resonance-enhanced Raman band at 843 cm^{-1} (Figure 2B), which falls within the range expected for the O−O stretch of a metal-bound peroxide unit.[26] This assignment was confirmed by preparing a sample of **7** using $^{18}O_2$, wherein the O−O stretch was observed at 797 cm^{-1} (Figure 2B), a value consistent with that expected for a largely uncoupled harmonic oscillator. These O−O stretching frequencies match those of oxyHr,[27] thereby indicating that O_2 binds to both **7** and oxyHr in a very similar manner, probably as a terminal hydroperoxide.

Frozen solution (THF, 77 K) Mössbauer spectra of both **5** plus 3 equiv of *N*-MeIm and of **7** indicate retention of dinuclear structures. Fits of the data to quadrupole doublets revealed parameters for the solution of **5** + 3*N*-MeIm ($\delta = 1.23$, $\Delta E_Q = 2.85$ mm s^{-1}) very close to the values for **5** in the solid state (vide supra). Addition of dioxygen to form **7** shifted δ to ~0.50 mm s^{-1} and ΔE_Q to ~1.42 mm s^{-1}, values which compare favorably to those for oxyHr ($\delta_{av} \approx 0.50$, $\Delta E_{Qav} \approx 1.48$ mm s^{-1})[20] and support the presence of a (μ-oxo)diiron(III) unit.[6] Hydrogen bonding of this putative hydroperoxide ligand to the μ-oxo atom in a manner similar to that proposed for oxyHr[28] remains to be established.

In summary, we have synthesized a new dinucleating dicarboxylate ligand that has allowed for the preparation and characterization of novel iron complexes having functionally relevant structural and spectroscopic features of deoxy- and oxyHr. Two-metal oxidation[12] is neither a thermodynamically nor kinetically hindered process in Hr, and we have devised a synthetic system that mimics this chemistry. Efforts to characterize further the putative hydroperoxide adduct **7** and to understand its formation and decomposition chemistry are currently in progress.

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Supporting Information Available: Details of the syntheses, X-ray crystallographic study, and physical characterization of **5**−**7** (34 pages, print/PDF). See any current masthead page for ordering and Internet access instructions. See any current masthead page for ordering information and Web access instructions.

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