Diiron(II) μ-Aqua Bis(μ-carboxylato) Models of Reduced Dinuclear Non-Heme Iron Sites in Proteins

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Received June 24, 1992

The chemistry of the (μ-oxo)diiron(III) core is well developed and continues to be studied because of the growing awareness of the existence of this unit in metalloproteins.[1] The biologically relevant forms of hemerythrin (Hr), the most thoroughly characterized Fe-O-Fe-containing protein, are deoxyHr and oxyHr, which contain diiron(II) and peroxy diiron(III) units, respectively.[2] The high-spin iron(III) ions of oxyHr and the inactive metHr forms are linked by, and antiferromagnetically coupled through, two carboxylates and an oxo ligand, which is hydrogen bonded to the hydroperoxide in oxyHr.[3] In deoxyHr the two high-spin iron(II) ions are antiferromagnetically coupled through a proposed hydroxide in addition to the carboxylates. A bridging water has been proposed to account for a switch to the ferromagnetic coupling observed in an azide bound form of deoxyHr.[4] Excellent model compounds for the oxo-bridged diiron(III) site in metHr[5] and hydroxide-bridged diiron(II) deoxyHr have been prepared,[6] but no water-bridged diiron complexes have been prepared, although they are known for other divalent transition metals[7] and non-molecular solids.[8] Other less well characterized proteins for which diiron(II) models are relevant are ribonucleotide reductase (RRB2)[9] and methane monooxygenase (MMO).[10]

We report here the preparation and characterization of $[Fe_2(H_2O)(O_2CR)_4(tmen)_2]$ [R = CH_3 (**1**) and C_6H_5 (**2**)],[11] the first μ-aqua-bridged diiron(II) model complexes of reduced non-heme diiron proteins. Compound **1** is prepared by reaction of $Fe(O_2CCH_3)_2 \cdot 4H_2O$ with 1 equiv of tmen in acetonitrile under an inert atmosphere. Volume reduction affords large colorless crystals in 53% yield,[12] which are suitable for X-ray crystallographic study.[13] The IR absorption bands between 2400 and 2000 cm^{-1} for a Nujol mull of **1** are consistent with the strongly hydrogen bonded bridging water in a dimeric complex as was observed for analogous Ni(II) and Co(II) complexes.[7] However, a room temperature ^1H NMR spectrum of **1** shows only three broad paramagnetically shifted resonances,[14a] which are concentration and solvent dependent, indicative of fluxional behavior and possible dissociation. Complex **2** was prepared from an acetonitrile solution containing a 1:1:2:2:0.5 ratio of tmen, $Fe(CF_3SO_3)_2 \cdot 2CH_3CN$, C_6H_5COOH, Et_3N, and H_2O in 90% yield. The room temperature ^1H NMR spectrum of **2** in $CDCl_3$ and C_6D_6 shows two sets of equal intensity, concentration independent, aromatic resonances,[14b] consistent with terminal and bridging benzoate ligands of a dimeric complex. However, only one set of resonances for **2** is observed

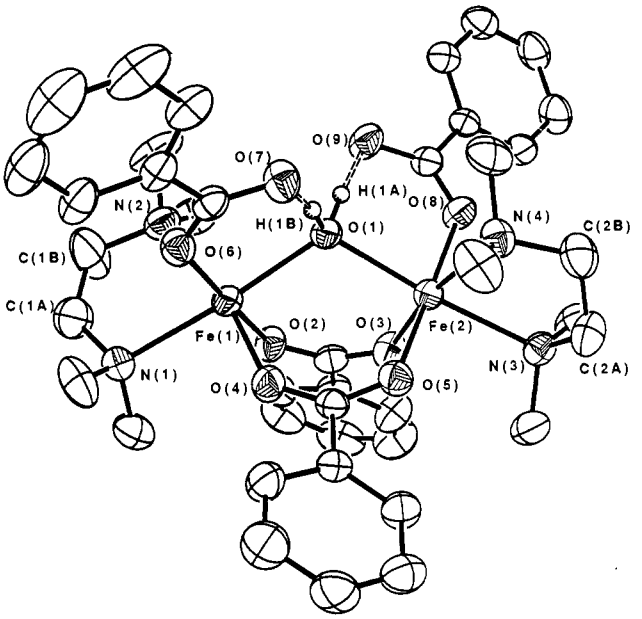

Figure 1. Structure of $[Fe_2(H_2O)(O_2CC_6H_5)_4(tmen)_2]$ (**2**) showing 50% probability thermal ellipsoids and atom-labeling scheme. Hydrogen atoms other than those on the bridging water are omitted for clarity. Selected interatomic distances (Å) and angles (deg) for **1**: Fe···Fe, 3.653 (2); Fe(1)-O(1), 2.188 (4); Fe(2)-O(1), 2.173 (4); Fe(1)-O(2), 2.057 (5); Fe(2)-O(3), 2.117 (5); Fe(1)-O(4), 2.133 (5); Fe(2)-O(5), 2.048 (5); Fe(1)-O(6), 2.090 (5); Fe(2)-O(8), 2.073 (5); Fe(1)-N(1), 2.265 (5); Fe(1)-N(2), 2.268 (5); Fe(2)-N(3), 2.351 (6); Fe(2)-N(4), 2.256 (6); Fe-O-Fe, 113.8 (2). For **2** Fe···Fe, 3.620 (2); Fe(1)-O(1), 2.171 (4); Fe(2)-O(1), 2.176 (4); Fe(1)-O(2), 2.075 (4); Fe(2)-O(3), 2.074 (3); Fe(1)-O(4), 2.084 (5); Fe(2)-O(5), 2.083 (3); Fe(1)-O(6), 2.143 (4); Fe(2)-O(8), 2.110 (3); Fe(1)-N(1), 2.266 (4); Fe(1)-N(2), 2.298 (4); Fe(2)-N(3), 2.362 (4); Fe(2)-N(4), 2.293 (4); Fe-O-Fe, 112.8 (1).

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(11) Abbreviations: tmen, *N,N,N',N'*-tetramethyl-1,2-diaminoethane; Me_3TACN, 1,4,7-trimethyl-1,4,7-triazacyclononane; BPMP, the anion of 2,6-bis[[bis(2-pyridylmethyl)amino]methyl]-4-methylphenol; HPTB, *N,N,N',N'*-tetrakis(2-benzimidazolylmethyl)-2-hydroxy-1,3-diaminopropane; BIPhMe, bis(1-methylimidazol-2-yl)phenylmethoxymethane.

(12) Anal. Calcd for **1**, $C_{20}H_{46}Fe_2N_4O_9$: C, 40.15; H, 7.75; N, 9.36. Found: C, 39.62; H, 7.48; N, 9.39. Anal. Calcd for **2**, $C_{40}H_{54}Fe_2N_4O_9$: C, 56.75; H, 6.43; N, 6.62. Found: C, 56.75; H, 6.37; N, 6.59. For **2** in $CHCl_3$, λ_{max} = 326 nm, ϵ = 730 M^{-1} cm^{-1} sh at 368 nm and λ_{max} = 1026 nm.
(13) X-ray analysis for **1**: orthorhombic, *Pbca*, with a = 12.06 (1) Å, b = 15.808 (6) Å, c = 31.21 (1) Å, V = 5949 (7) Å^3, ρ_{calcd} = 1.336 g cm^{-3}, Z = 8. With use of 2689 unique reflections out to 2θ = 45° Mo Kα, collected at -90° on a single-crystal X-ray diffractometer, the structure was solved by Patterson methods and refined with anisotropic thermal parameters to an R index of 5.75% (R_w = 6.8%). For **2**: triclinic, $P\bar{1}$, with a = 10.859 (6) Å, b = 11.915 (6) Å, c = 17.25 (1) Å, α = 105.74 (4)°, β = 91.50 (5)°, γ = 92.74 (5)°, V = 2108 (2) Å^3, ρ_{calcd} = 1.33 g cm^{-3}, Z = 2. With use of 3853 unique reflections out to 2θ = 45° collected at 21° on a single-crystal X-ray diffractometer, the structure was solved by Patterson methods and refined with anisotropic thermal parameters to an R index of 4.4% (R_w = 5.2%).
(14) (a) $CDCl_3$ solutions of **1** at 21 °C: broad resonances at 77, 46, and 23 ppm downfield of TMS. At -10 °C the solution spectrum is consistent with a dimeric structure: two relatively sharp resonances at 52 and 26 ppm (bridging and terminal O_2CCH_3) and broader resonances of tmen at 94, 87, 76, and 49 ppm. (b) In $CDCl_3$: *m*-H at 10.7 and 8.0 ppm. In C_6D_6: *o*-H at 1.51 and 3.50, *m*-H at 7.80 and 10.37, and *p*-H at 2.21 and 3.84 ppm. (c) In CD_3OD: *o*-H at 8.47, *m*-H at 9.12, and *p*-H at 6.00; $(CH_3)_4C_2H_4$ at 81 and 73 ppm.

0002-7863/92/1514-8741$03.00/0

in CD_3OD and DMSO-d_6 solution, consistent with dissociation (eq 1).

$$Fe_2(H_2O)(O_2CR)_4(tmen)_2 \rightarrow 2Fe(O_2CR)_2(tmen) + H_2O \quad (1)$$

The solid-state structures of **1** and **2** were confirmed by single-crystal X-ray determinations (**2** is shown in Figure 1 and **1** in Figure S-I in the supplementary material). Each complex consists of a μ-aqua bis(μ-O_2CR) diiron core with additional terminal carboxylate ligands coordinated to each Fe and arranged such that the uncoordinated oxygens O(7) and O(9) are involved in strong hydrogen bonding with the bridging water [O(1)···O(7), 2.57 Å in **1** and 2.54 Å in **2**; O(1)···O(9), 2.58 Å in **1** and 2.57 Å in **2**]. This results in a twisting of the bridging water oxygen away from a tetrahedral geometry as reflected in Fe–O–H angles [in **2**: Fe(1)–O(1)–H(1A), 126.3 (3)°; Fe(2)–O(1)–H(1B), 118.4 (2)°; Fe(1)–O(1)–H(1B), 97.0 (2)°; Fe(2)–O(1)–H(1A), 95.6 (2)°; but H(1A)–O(1)–H(1B), 108.7 (3)°]. The hydrogen bonds in **2** are symmetric [O(1)–H(1A), 1.08 Å; O(1)–H(1B), 0.98 Å], but in **1** they are asymmetric [O(1)–H(1A), 0.85 Å; O(1)–H(1B), 1.37 Å]. Fe–O bond lengths for oxygens trans to the terminal carboxylates are considerably shorter than those trans to the nitrogen donors in **1**, but not in **2**. The Fe···Fe separations and the Fe–(μ-O) bonds are considerably larger than those found in diiron(II) complexes with bridging OH^-, $[Fe_2(OH)(O_2CCH_3)_2(Me_3TACN)_2]^+$ (3.32 Å),[6] OR^-, $[Fe_2BPMP(O_2CCH_2CH_3)_2]^+$ (3.348 Å),[15] $[Fe_2(N\text{-}Et\text{-}HPTB)(O_2CPh)_2]^{2+}$ (3.473 Å),[16] or O,OCHO$^-$, $[Fe_2(BIPhMe)_2(HCO_2)_4]$ (3.585 Å),[17] which is consistent with a neutral bridging ligand.

The Mössbauer spectra confirm the high-spin iron(II) oxidation state.[18] Only one doublet is observed for **2**, but the appearance of two resolvable doublets for **1** indicates that the structural asymmetry is reflected in slightly different electronic environments for the two iron atoms. These values are very similar to a fit of the spectrum of the diiron(II) form of RRB2 from *Escherichia coli*.[19]

Preliminary magnetic susceptibility measurements on powdered samples and frozen solutions of **1** and **2** indicate substantially weaker magnetic coupling between iron atoms than is observed for OH^--bridged **3**[6d] ($J = -13.1$ cm^{-1}) and deoxyhemerythrin ($J = -12$ to -38 cm^{-1} by MCD-ESR,[4] and -15 cm^{-1} by ^{1}H NMR[20]). This decreased coupling in **1** and **2** is consistent with the weakly ferromagnetic behavior found in deoxyHrN_3.[4] Complexes **1** and **2** are EPR silent,[21] in contrast to deoxyHrN_3, which exhibits a low-field EPR signal.[4,22] However, either type of EPR behavior is consistent with an integer-spin ground state.[15b,23,24]

In conclusion, the first examples of diiron(II) complexes containing a bridging water have been prepared. The structure of **2** has been shown by ^{1}H NMR to be maintained in noncoordinating aprotic solvents, but not in protic or strongly coordinating solvents. Protonation of the bridging oxygen results in the longest Fe(II)···Fe(II) and Fe–O_{bridge} distances yet observed in similar tribridged structures. These longer distances are reflected in considerably weaker intramolecular magnetic interactions in complexes **1** and **2** compared to analogous hydroxo-bridged complexes. The magnetic interactions in **1** and **2** resemble the weakly ferromagnetic interactions observed in the diiron(II) forms of deoxyHrN_3, MMO, and RRB2.

Acknowledgment. We thank Dr. N. Ravi and Professor B. H. Huynh for Mössbauer data, R. Reagan, Dr. P. Wang, Dr. S. Nimmala, and Professor E. P. Day for magnetic data, and Dr. M. P. Hendrich for EPR spectra. This research was supported in part by the University Research Committee of Emory University, the donors of the Petroleum Research Fund, administered by the American Chemical Society, and the National Institutes of Health (GM 46506).

Supplementary Material Available: Magnetic susceptibility plots for **2**, an ORTEP drawing of **1**, and tables of atomic positional and thermal parameters and bond lengths and angles of **1** and **2** (23 pages); observed and calculated structure factors for **1** and **2** (10 pages). Ordering information is given on any current masthead page.

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Catalytic Mechanism of Cytochrome P-450: Evidence for a Distal Charge Relay

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Received June 22, 1992

The cytochromes P-450 are responsible for a plethora of critical biotransformations in humans, animals, microbes, insects, and plants.[1-6] They have received considerable attention from mechanistic chemists due to the enzyme's ability to catalytically oxygenate recalcitrant substrates with controlled regiospecificity and stereochemistry. The oxygenase cycle of cytochrome P-450 involves sequential substrate binding, ferric–ferrous reduction of the heme active center, and dioxygen association to form a ternary complex analogous to the oxygenated forms of hemoglobin and myoglobin. Subsequent steps are less well understood, but can be hypothetically visualized as input of a second reducing equivalent, heterolytic scission of the O–O bond releasing water, and formation of a transient metal–oxo complex that is two oxidation equivalents above the ferric resting state, analogous to compound I in the peroxidases.[7] Completion of the oxygenase cycle involves hydrogen abstraction from the substrate and "oxygen rebound"[8,9] with product release, regenerating the ferric resting state.

To date, the only published X-ray structures of a cytochrome P-450 are that of P-450$_{cam}$ (P450101), active in the hydroxylation of camphor in *Pseudomonas*.[10] The crystal structure of cytochrome *c* peroxidase (CCP)[11] provided a strong suggestion as to

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