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An Exchange-Coupled Complex with Localized High-Spin Fe^{IV} and Fe^{III} Sites of Relevance to Cluster X of *Escherichia coli* Ribonucleotide Reductase

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Intermediates with formally high-valent oxidation states have recently been observed in the reactions of dioxygen with reduced forms of the nonheme diiron enzymes methane monooxygenase (MMO)[1−3] and ribonucleotide reductase (RNR).[1,4,5] MMO compound **Q** is proposed to be a coupled $Fe^{IV}Fe^{IV}$ species with a diamagnetic ground state.[3b] RNR cluster **X**, on the other hand, is an $S = {}^1/_2$ species with a formal $Fe^{III}Fe^{IV}$ oxidation state but has been described as a coupled system consisting of two high-spin Fe^{III} plus a ligand radical.[5] There are two recent examples of well-characterized $Fe^{III}Fe^{IV}$ species, both with $S = {}^3/_2$ ground states. Wieghardt et al. have reported a (μ-nitrido)diiron(III,-IV) complex with $S = {}^3/_2$ arising from an $S = {}^5/_2$ Fe^{III} antiferromagnetically coupled to an $S = 1$ Fe^{IV}.[6] We have characterized the bis(μ-oxo)diiron(III,IV) complex $[Fe_2(O)_2(TPA)_2]^{3+}$ (**1**), where TPA = tris(2-pyridylmethyl)amine, whose equivalent iron sites have led us to suggest that **1** is a valence-delocalized Fe^{III} ($S = {}^1/_2$)−Fe^{IV} ($S = 1$) pair.[7] Complex **1** is particularly relevant to MMO and RNR because it is derived from the reaction of a diiron(III) complex with H_2O_2, a reaction equivalent to that of a diiron(II) complex with O_2. The introduction of a 6-methyl substituent on one of the pyridines of the TPA ligand still allows us to obtain a diiron(III,IV) complex. As reported here, this minor variation in ligand structure gives rise to an $S = {}^1/_2$ complex with EPR properties strikingly similar to those of RNR cluster **X**.[5a−c] However, Mössbauer data show this synthetic $S = {}^1/_2$ complex to be a novel exchange-coupled pair with localized *high-spin* Fe^{IV} and Fe^{III} sites.

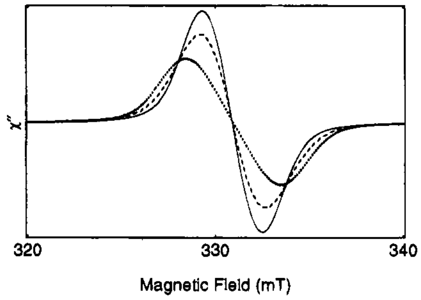

Figure 1. X-band EPR spectra of **3** with all isotopes in natural abundance (—), enriched to >90% in ^{57}Fe (···), and enriched with ^{17}O (from 53% enriched $H_2{}^{17}O$, - - -). Spectra are normalized to equal spin concentrations. Conditions: 0.2 mM **3** in acetonitrile/17 mM $NaClO_4$, $T = 20$ K, 0.2 mW, 1.0 mT modulation.

Treatment of the diiron(III) complex $[Fe_2(\mu\text{-}O)(6\text{-}Me\text{-}TPA)_2(OH)(H_2O)](ClO_4)_3$[8] (**2**), [6-Me-TPA = *N*-(6-methyl-2-pyridylmethyl)-*N*,*N*-bis(2-pyridylmethyl)amine] with H_2O_2 at −40 °C in CH_3CN affords a transient brown species ($\lambda_{max} \approx 350$ nm, $\epsilon \approx 8000$ M^{-1} cm^{-1}), **3**, whose electrospray ionization mass spectra reveal a prominent negative ion cluster at *m/z* 1148, consistent with $\{[Fe_2(O)_2(6\text{-}Me\text{-}TPA)_2](ClO_4)_4\}^-$. This formulation is analogous to **1**, suggesting that we can obtain, with minor variations of the parent TPA ligand, a family of complexes with an $[Fe_2O_2]^{3+}$ core. However, in contrast to the $S = {}^3/_2$ complex **1**, **3** exhibits a nearly isotropic $S = {}^1/_2$ EPR signal at $g = 2.00$ (Figure 1); interestingly, this signal exhibits ^{57}Fe and ^{17}O (from $H_2{}^{17}O$) hyperfine broadening, showing that it is associated with the Fe_2O_2 core.

The 150 K Mössbauer spectrum of a sample of ^{57}Fe-enriched **2** treated with H_2O_2 at −40 °C shows two discernible quadrupole doublets (Figure 2A). The majority doublet has an isomer shift, $\delta = 0.48$ mm/s, typical of a high-spin Fe^{III} center, while the minority doublet has $\delta = 0.04$ mm/s, a value indicative of an Fe^{IV} site. Thus, a fraction of the Fe^{III} originally in **2** has been oxidized to a higher valent state.

Figure 2B,C shows low-temperature spectra recorded in parallel fields of 2.5 and 8.0 T. The data show that 53% of the iron in the sample belongs to the diamagnetic $Fe^{III}Fe^{III}$ complex **2**. The remainder is a paramagnetic species that contributes two distinct subspectra of equal proportion. The magnetic splittings of one site decrease with increasing applied field, implying that its magnetic hyperfine tensor, **A**, has negative components, whereas the second site has positive *A*-values. These observations are indicative of an exchange-coupled pair for which the two spins are aligned parallel and antiparallel relative to the $S = {}^1/_2$ system spin. The intensities of the absorption bands of the 0.05 T spectra (not shown) were observed to depend on the direction of the applied field relative to the γ-rays, proving that the paramagnetic species (47% of Fe) must be associated with the observed $S = {}^1/_2$ EPR signal.[9] EPR quantitation of the sample[10] affords 0.54(6) spins per starting dimer **2**. This agrees well with 0.47(3) spins predicted from the Mössbauer data. Taken together, the two methods yield 1.15 spins/**3**.

We have fitted the Mössbauer spectra of **3** with an $S = {}^1/_2$

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(8) Complex **2** was synthesized according to the published procedure[7] for $[Fe_2(\mu\text{-}O)(TPA)_2(OH)(H_2O)](ClO_4)_3$, with 6-Me-TPA replacing TPA. Anal. Calcd for $[Fe_2(\mu\text{-}O)(6\text{-}Me\text{-}TPA)_2(OH)(H_2O)](ClO_4)_3{\cdot}H_2O$ ($C_{38}H_{45}Cl_3Fe_2N_8O_{16}$): C, 41.96; H, 4.17; N, 10.30. Found: C, 41.86; H, 4.22; N, 10.12. ESI-MS: *m/z* = 1167.0, $\{[Fe_2(\mu\text{-}O)(6\text{-}Me\text{-}TPA)_2(OH)(H_2O)](ClO_4)_4\}^-$. The spectroscopic properties of **2** support the (μ-oxo)diiron(III) formulation: UV−vis in CH_3CN, 326 nm (8300 M^{-1} cm^{-1}) and 370 nm (7300 M^{-1} cm^{-1}) (oxo-to-iron(III) charge transfer); Mössbauer, $\delta = 0.48$ mm/s and $\Delta E_Q = 1.60$ mm/s; EPR silent.

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(10) Samples were prepared at −40 °C and divided into an EPR tube and a Mössbauer cuvette, both precooled to −40 °C and quickly frozen in liquid N_2.

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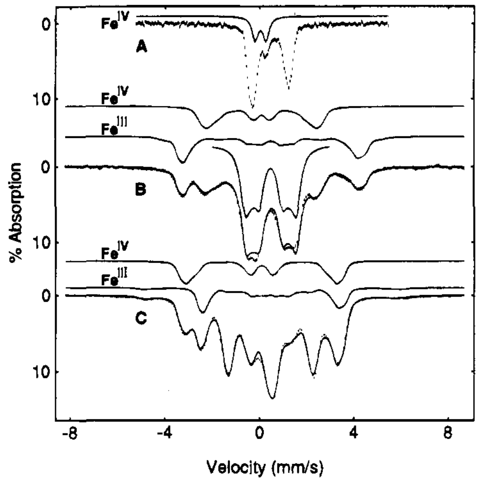

Figure 2. Mössbauer spectra of **3** in acetonitrile. (A) Spectrum recorded at 150 K. To increase the resolution, the line width contribution of the ^{57}Co source was removed by a Fourier transform technique. The doublet assigned to the high-spin Fe^{IV} site is outlined by the solid line. (B and C) Spectra recorded at 1.5 K and 2.5 T (B) and 4.2 K and 8.0 T (C). Solid lines are theoretical curves computed with the parameters of Table 1. Contributions of the Fe^{IV} (23.5% of total Fe) and the Fe^{III} sites (23.5%) are drawn separately above the data. Absorption of diamagnetic **2** (53%, shown separately in B) is included in the theoretical curves.

Table 1. Hyperfine Parameters of Complex **3** at 4.2 K[a]

	A_x (MHz)	A_y (MHz)	A_z (MHz)	a (MHz)[b]	ΔE_Q (mm/s)	η	δ[c] (mm/s)
Fe^{III}	−64.5(20)	−64.5(20)	−64.5(20)	−27.6	1.6(2)	1.0(3)	+0.48(3)
Fe^{IV}	+20(3)	+36.5(15)	+36.5(15)	−23.2[d]	0.5(1)	1.0(3)	+0.08(3)

[a] Derived from a fit of the spectra using the spin Hamiltonian, $\mathscr{H} = 2\beta\mathbf{S}\cdot\mathbf{H} + \sum_i\{\mathbf{S}\cdot\mathbf{A}_i\cdot\mathbf{I}_i - g_n\beta_n\mathbf{H}\cdot\mathbf{I}_i + (eQV_{zz}(i)/12)[3\mathbf{I}_{zi}^2 - 15/4 + \eta(i)(\mathbf{I}_{xi}^2 - \mathbf{I}_{yi}^2)]\}$, where i sums over the two sites. [b] The observed A-tensors are related to the intrinsic a-tensors of the Fe^{III} and Fe^{IV} sites by $\mathbf{A}$(Fe^{III}) = (7/3)$\mathbf{a}$(Fe^{III}) and $\mathbf{A}$(Fe^{IV}) = −(4/3)$\mathbf{a}$(Fe^{IV}).[11] [c] Isomer shift, δ, relative to Fe metal at 298 K. [d] Isotropic part of $\mathbf{a}$(Fe^{IV}).

spin Hamiltonian[11] (see Table 1) using the parameters listed in Table 1. The ΔE_Q and δ of one site are nearly identical to those of diamagnetic **2**, implying that this site is high-spin ($S_1 = {}^5/_2$) Fe^{III}. The isomer shift of the second site, $\delta = 0.08$ mm/s at 4.2 K, is distinctly smaller than that of the Fe^{III} site. While this value for δ is compatible with either a low-spin ($S = {}^1/_2$) Fe^{III} or an Fe^{IV} assignment, only a high-spin ($S_2 = 2$) Fe^{IV} allows one to construct a model that explains the data. The $S = {}^1/_2$ ground state of **3** would thus arise from antiferromagnetic exchange ($H_{ex} = J\mathbf{S}_1\cdot\mathbf{S}_2$, $J > 0$) between a high-spin Fe^{III} and a high-spin Fe^{IV} site.[12] The observed isotropic A-tensor for one site is assigned to Fe^{III} and corresponds to an intrinsic a(Fe^{III}) value of −27.6 MHz, in good agreement with a = −(28−30) MHz reported for Fe^{III} sites in N/O octahedral sites.[14] The A-tensor for the other site is positive and *anisotropic*. The positive **A** values are in agreement with the proposed antiferromagnetic coupling, and the anisotropic (spin-dipolar) contribution is expected for a high-spin Fe^{IV} site. From the temperature dependence of the EPR signal, we estimate the J value for **3** to be >80 cm^{-1}.

Complexes **1** and **3** both have [Fe$_2$(O)$_2$L$_2$]$^{3+}$ formulations, but the precise structure of **3** is not established. Whether **3** retains the bis(μ-oxo)diiron core found for **1**[15] or adopts an isomeric structure wherein one of the oxo bridges becomes a terminal oxo ligand does not affect the principal conclusion, namely that **3** is an antiferromagnetically coupled system with *localized* high-spin Fe^{III}−high-spin Fe^{IV} sites. In contrast, **1** is best described as a *valence delocalized* low-spin Fe^{III}−low-spin Fe^{IV} species. The difference in metal spin states in the two complexes is ascribed to the presence of one 6-methyl substituent to the TPA ligand in **3**. This methyl group introduces steric effects that prevent formation of the shorter metal−ligand bonds required for a low-spin configuration and give rise to the high-spin configurations found in **3**.[16]

The introduction of ^{57}Fe or ^{17}O into **3** results in broadening of its $S = {}^1/_2$ EPR signal, similar to that observed with RNR cluster **X**. Although **X** is described as a coupled diiron(III)−ligand radical species,[5] the existence of **3** with similar EPR properties suggests that an Fe^{III}Fe^{IV} model should also be considered in describing the electronic structure of **X**. Indeed, recent ^{57}Fe ENDOR results[17] indicate that one of the Fe sites of **X** has an anisotropic A-tensor similar to that of the Fe^{IV} site of **3** and, therefore, some Fe^{IV} character, suggesting that **3** can serve as a valuable model for **X**. We now have synthesized and characterized two distinct diiron(III,IV) complexes with an Fe$_2$O$_2$ core that relate to the intermediates of MMO and RNR. These results suggest that the Fe$_2$O$_2$ unit may play a significant role in the high-valent chemistry of nonheme diiron enzymes.

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(12) Because high-spin d^4 is complementary to high-spin d^6, the data can be analyzed with the expressions developed for [Fe$_2$S$_2$]$^+$ clusters.[11] The observed g-values are obtained from $\mathbf{g}_i = (7/3)\mathbf{g}_i$(Fe^{III}) − (4/3)$\mathbf{g}_i$(Fe^{IV}). Since $\mathbf{g}$(Fe^{III}) is isotropic, the Fe^{IV} site must have a g-value close to g = 2.0. This would be true if $10Dq/\lambda \geq 200$ for the Fe^{IV} site, where $\lambda \approx 100$ cm^{-1} is the spin−orbit coupling constant and $10Dq$ is the octahedral splitting. A ratio of ≥200 is not unreasonable; using the f and g functions of Jørgensen,[13] we estimate $10Dq \geq 20\,000$ cm^{-1}.

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(15) We note that 6-Me$_3$-TPA, a ligand for which all three pyridine rings have 6-methyl substituents, can form [Fe$_2$(μ-O)$_2$(6-Me$_3$-TPA)$_2$](ClO$_4$)$_2$, the first crystallographically characterized example of an Fe$_2$(μ-O)$_2$ core. Zang, Y.; Dong, Y.; Que, L., Jr.; Kauffmann, K.; Münck, E. *J. Am. Chem. Soc.* **1995**, *117*, 1169−1170.

(16) In support of this notion, we have found that [Fe^{III}(TPA)(acac)](ClO$_4$)$_2$ is a low-spin complex, while [Fe^{III}(6-Me-TPA)(acac)](ClO$_4$)$_2$ is a high-spin complex (acac = 2,4-pentanedione monoanion). Zang, Y.; Dong, Y.; Kim, J.; Que, L., Jr., results to be published.

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