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EPR and Mössbauer Spectroscopy, and DFT Analysis of a High-Spin Fe^{IV}-oxo Complex

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Abstract

The kinetically competent oxidant in non-heme iron enzymes is a high-spin Fe^{IV}-oxo species, which are not as well characterized as the intermediate-spin species of heme systems. The present work gives a detailed characterization of the structurally similar [Fe^{IV}H₃buea(O)]⁻, [Fe^{III}H₃buea(O)]²⁻ and [Fe^{III}H₃buea(OH)]⁻ (H₃buea = tris[(*N'*-*tert*-butylureaylato)-*N*-ethylene]aminato) complexes using Mössbauer and dual-frequency/dual-mode electron paramagnetic resonance (EPR) spectroscopies. The [Fe^{IV}H₃buea(O)]⁻ complex has a high-spin (*S* = 2) configuration imposed from the C₃-symmetric ligand. EPR spectroscopy of the [Fe^{IV}H₃buea(O)]⁻ complex is the first documented example of an EPR signal from an Fe^{IV}-oxo complex, demonstrating the ability to detect and quantify Fe^{IV} species with EPR spectroscopy. Quantitative simulations allowed determination of the zero-field parameter, *D* = +4.7 cm⁻¹, and the species concentration. Density functional theory calculations of the zero-field parameter are found to be in agreement with the experimental value and indicate the major contribution to the *D*-value is from spin-orbit coupling of the ground state with an excited *S* = 1 electronic configuration at 1.2 eV. ¹⁷O isotope enrichment experiments allowed a determination of the hyperfine constant ¹⁷⁰*A_z* = 10 MHz for [Fe^{IV}H₃buea(O)]⁻ and ¹⁷⁰*A_y* = 8 MHz, ¹⁷⁰*A_z* = 12 MHz for [Fe^{III}H₃buea(OH)]⁻. The isotropic hyperfine constant (¹⁷⁰*A_{iso}* = -16.8 MHz) was derived from the experimental value to allow a quantitative determination of the spin polarization (*ρ_p* = 0.56) of the oxo *p*-orbitals of the Fe-oxo bond in [Fe^{IV}H₃buea(O)]⁻. This is the first experimental determination for non-heme complexes, and indicates significant covalency in the Fe-oxo bond. High-field Mössbauer spectroscopy gave an ⁵⁷Fe *A_{dip}* tensor of (+5.6, +5.3, -10.9) MHz and *A_{iso}* = -25.9 MHz for the [Fe^{IV}H₃buea(O)]⁻ complex, and DFT calculations are in agreement with the nuclear parameters of the complex.

Introduction

Dioxygen activation and substrate oxidation performed by metalloenzymes often involves high-valent reactive intermediates. The competent oxidants in several heme and non-heme iron enzymes are Fe^{IV}-oxo species. The reactivity of these Fe^{IV}-oxo centers is dependent on the reduction potential, spin state, and *pK_a* of the metal-oxo moiety; the combination of which allows for the transformation of relatively stable bonds of substrates, such as the cleavage of C-H and C-C bonds. In heme enzymes, the well characterized Compound I and II intermediates involve an Fe^{IV}-oxo center and there is growing evidence in literature for the role of the

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Supporting Information: Calculations for the *D*-value from the excited state energies and figure showing contour plots of Fe-based *d*-orbitals. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Fe^{IV}-oxo units in the activity of non-heme iron enzymes. In contrast to the intermediate-spin ($S = 1$) Fe^{IV}-oxo units of heme enzymes, the few non-heme enzymes for which an Fe^{IV} species has been observed are high-spin ($S = 2$). For instance, a short lived high-spin Fe^{IV}-oxo intermediate has been detected in an α -ketoglutarate dependent oxygenase, taurine dioxygenase (TauD).¹ This discovery led to the detection of other high-spin Fe^{IV}-oxo intermediates within α -ketoglutarate dependent enzymes, including halogenase SyrB2.²

Biomimetic complexes can be used to investigate the underlying chemistry of O₂ activation and substrate oxidation. These types of synthetic complexes can provide general insight into methods for detection, spectroscopic interpretation, and the electronic properties of Fe^{IV}-oxo centers. Various synthetic non-heme Fe^{IV}-oxo complexes with tetragonal symmetry have been reported,^{3,4} and typically have an $S = 1$ ground state with orbital configuration $d_{xy}^2 d_{xz}^1 d_{yz}^1$. The first example of a non-heme tetragonal Fe^{IV}-oxo complex with a high-spin ($S = 2$) ground state, arising from the weak ligand field of the penta-aquo coordination in Fe^{IV}(H₂O)₅(O), was reported by Bakac et al.⁵ A high-spin configuration can also arise in trigonal symmetry provided the two degenerate orbital pairs ($d_{xy/yz}$ and $d_{x^2-y^2/xy}$) are sufficiently close in energy. This suggestion is supported by two recent examples of high-spin Fe^{IV}-oxo complexes with ancillary tripodal ligands that support local C₃ symmetry.⁶⁻⁸

The effects of spin state on the reactivity of metal-oxo complexes have been addressed in a number of studies, and the possibility has been raised that high-spin Fe^{IV}-oxo complexes are more reactive than their low-spin counterparts.⁹⁻¹¹ However, in the case of hydrogen atom transfer other determinants of reactivity may prevail.^{12,13} Density functional theory (DFT) calculations and experimental measurements of $S = 1$ Fe^{IV}-oxo hemes suggest a significant degree of spin delocalization in the Fe-oxo bond. Considering that the electronic configurations for the Fe^{IV}-oxo units in the $S = 1$ state $d_{xy}^2 d_{xz}^1 d_{yz}^1$ and the $S = 2$ state $d_{xy}^1 d_{x^2-y^2}^1 d_{xz}^1 d_{yz}^1$ differ in the occupations of the in-plane d_{xy} and $d_{x^2-y^2}$ orbitals but retain the occupations of the orbitals involved in the Fe-oxo bond, it is expected that the spin delocalizations may be similar for heme and non-heme complexes. This paper presents the first experimental evidence to corroborate this expectation.

This report gives detailed electronic descriptions of Fe complexes of the ligand tris[(*N*'-tert-butylureaylato)-*N*-ethylene]aminato ([H₃buea]³⁻). The equatorial anionic aminato N-donors of the ligand enforce a trigonal symmetry at the metal center. A rare attribute of this ligand set is its ability of the butylureaylato arms to form intramolecular H-bonds with the axial oxygen. This ligand is capable of stabilizing a monomeric, high-spin Fe^{IV}-oxo complex (Fe^{IV}H₃buea(O)]⁻(**1**), as well as its Fe^{III}-oxo (Fe^{III}H₃buea(O)]²⁻(**2**), and Fe^{III}-hydroxo (Fe^{III}H₃buea(OH)]⁻(**2-OH**) analogs (Figure 1).^{6,14,15} The Fe^{III}-oxo complex is the only known example of a Fe^{III} unit with a terminal oxygen ligand, owing its stability to the protection offered by a network of intramolecular H-bonds provided by the ligand.¹⁴ The structures of the three complexes have been characterized by X-ray diffraction methods. In the present study, the electronic properties of these three complexes are examined using X- and Q-band, parallel- and perpendicular-mode EPR spectroscopy, high-field Mössbauer spectroscopy, and DFT calculations. Isotopic ¹⁷O labeling studies allow a quantitative determination of the spin delocalization in the Fe-O bond, which is the first such experimental measurement for non-heme complexes. In addition, the present work demonstrates the utility of these methods for characterization of Fe-oxo centers in metalloenzymes and biomimetic complexes.

Methods

Preparation of ⁵⁷Fe enriched salts.^{16,17}

The ⁵⁷Fe source (metal foil 94.69% enriched) was purchased from Pennwood Chemicals Inc. For a typical synthesis, a small piece of metal foil (174 mg, 3.05 mmol) was immersed in dilute

HCl and immediately poured onto a glass frit and washed with water. The metal was added to 3 mL of a 1:1 mixture of oxygen free glacial acetic acid and acetic anhydride in a Schlenk tube and the solution was then vigorously stirred and heated to ca. 90°C under a N₂ atmosphere. After 24 h, the mixture contained a white precipitate and ⁵⁷Fe metal. Volatiles were removed under reduced pressure and the solid was isolated on a glass-fritted funnel under an argon atmosphere, washed thoroughly with Et₂O, and dried for several hours, after which the ⁵⁷Fe metal was manually removed. The ⁵⁷Fe(OAc)₂ obtained was used without further purification (35 mg, 6% yield). This procedure was repeated several times with recycled ⁵⁷Fe until enough solid was obtained to conduct the subsequent experiments. The ⁵⁷Fe(OAc)₂ was used to prepare K[⁵⁷Fe^{III}H₃buea(OH)], K₂[⁵⁷Fe^{III}H₃buea(O)], and K[⁵⁷Fe^{IV}H₃buea(O)] following literature procedures.⁶

Preparation of ¹⁷O labeled samples

Water labeled with ¹⁷O (60.6% enriched) was purchased from Icon-Isotopes and used as received in the preparation of isotopically labeled samples. The K[Fe^{III}H₃buea(¹⁷OH)] salt was prepared following the literature procedure,⁶ in which the source of the hydroxo ligand was H₂¹⁷O. The K[Fe^{IV}H₃buea(¹⁷O)] salt was isolated by treating K[Fe^{III}H₃buea(¹⁷OH)] with [Fe^{III}Cp₂]BF₄ following the literature procedure; K₂[Fe^{III}H₃buea(¹⁷O)]²⁻ was synthesized from the reaction of K[Fe^{III}H₃buea(¹⁷OH)] and KOBu^t.

EPR spectroscopy

X-band EPR spectra were recorded on a Bruker 300 spectrometer equipped with an Oxford ESR-910 liquid helium cryostat. Q-band (34.0 GHz) EPR spectra were recorded on a Bruker 200 spectrometer with a home-built microwave probe and cryostat.¹⁸ The quantification of all signals is relative to a CuEDTA spin standard. For both instruments, the microwave frequency was calibrated with a frequency counter and the magnetic field with a NMR gaussmeter. A modulation frequency of 100 kHz was used for all EPR spectra. The EPR simulation software (*SpinCount*) was written by one of the authors.¹⁹ The software diagonalizes the spin Hamiltonian where is the total spin of the complex unless explicitly stated:

$$H = \beta_e \mathbf{B} \cdot \mathbf{g} \cdot \mathbf{S} + \mathbf{S} \cdot \mathbf{D} \cdot \mathbf{S} + \mathbf{S} \cdot \mathbf{A} \cdot \mathbf{I} \quad (1)$$

where the parameters have the usual definitions, and performs least-squares fitting of the spectra. The quantitative simulations are least-squares fits of the experimental spectra generated with consideration of all intensity factors, which allows computation of simulated spectra for a specified sample concentration.

Mössbauer spectroscopy

Mössbauer spectra were recorded with two spectrometers, using a Janis Research Super-Vartemp dewar. Isomer shifts are reported relative to Fe metal at 298 K. The simulation of Mössbauer were calculated by least-square fitting performed *SpinCount* using the standard spin Hamiltonian:

$$H = \beta_e \mathbf{B} \cdot \mathbf{g} \cdot \mathbf{S} + \mathbf{S} \cdot \mathbf{D} \cdot \mathbf{S} + \mathbf{S} \cdot \mathbf{A} \cdot \mathbf{I} - g_n \beta_n \mathbf{B} \cdot \mathbf{I} + (eQV_{zz}/12)[3I_z^2 - I(I+1) + \eta(I_x^2 - I_y^2)] \quad (2)$$

DFT calculations

The DFT calculations were performed using the hybrid functional B3LYP and the basis set 6-311G provided by Gaussian 03 (Revision E.01) software package.²⁰ Geometry optimizations

were terminated upon reaching the default convergence criteria and were performed for **1** ($S = 1, 2$), **2** and **2-OH** ($S = 5/2$) without imposing any symmetry on the molecule. The optimizations of the structures for the $S = 1$ and 2 spin states of **1** were initiated using the crystal structure of **2**. Time-dependent (TD) DFT calculations were performed for the $S = 2$ and $S = 1$ states of **1** and the $S = 3/2$ spin state of **2** and **2-OH**. For all three complexes, the TD-DFT calculations gave positive excitation energies suggesting that the self consistent field (SCF) solutions represented the ground states. The vertical excitation energy calculations for the $S = 1$ state of **1** and $S = 1/2$ state of **2** and **2-OH** were performed by calculations on the equilibrium geometries of $S = 2$ spin state of **1** and $S = 5/2$ states of **2** and **2-OH**. The dipolar hyperfine tensor ($\mathbf{A}_{\text{dip}}$) and the electric field gradient tensor (EFG, V_{ii}) were calculated using the keyword PROP. The quadrupole splitting, ΔE_Q , and the symmetry parameter, η were calculated from the EFG tensor with the relations $\Delta E_Q = (eQV_{\text{zz}})/2 (1 + \eta^2/3)^{1/2}$ and $\eta = (V_{\text{xx}} - V_{\text{yy}})/V_{\text{zz}}$. The calculated isomer shifts (δ) were obtained from the electron density at the nucleus and calculated using the calibrations of Vrajmasu et al.²¹

Results

Spectroscopy of Fe^{IV}-oxo complex (**1**)

X- and Q- band measurements were performed on samples prepared by transferring aliquots of same reaction mixture of **1** to EPR tubes. The X-band spectrum, displayed in Figure 2A, shows a sharp signal at $g = 8.12$ and a much broader signal at 4.06. These signals originate from the $|2^\pm\rangle$ and $|1^\pm\rangle$ doublets, respectively, of an $S = 2$ spin manifold, where the $|m^\pm\rangle$ nomenclature refers to the symmetric and antisymmetric combinations of the magnetic spin states (see Discussion). The signals at $g = 4.30$ and 2.00 are from a small amount of ($< 1\%$) adventitious Fe^{III} and radical impurities. The presence of these weak signals in parallel mode was caused by a slight misalignment of the static and microwave magnetic fields. A plot of signal intensity times temperature (proportional to population) vs. temperature of the $g = 8.12$ and 4.06 signals is shown in Figure 3. The signal at $g = 8.12$ broadens for temperatures > 40 K. The solid lines give the percent populations of the spin doublets calculated with eq 1 for $D = +4.0$ (5) cm^{-1} . The simulations overlaid on the data are generated with the parameters given in Table 1 (Figure 2) accurately agrees with the signal shape and intensities of both transitions as a function of temperature. The Q-band spectrum showed a signal at $g = 8.12$ (Figure 2 Inset) from the $|2^\pm\rangle$ doublet. The simulation using the same parameter set accurately predicted the Q-band spectrum. The intensity is in quantitative agreement with the concentrations of the sample determined from the molar absorptivity of the optical spectrum and from the absorption of an equivalent Mössbauer sample.

The spectrum of **1** in acetonitrile shown in Figure 2B illustrates the effect of solvent on the EPR spectrum of **1**. The spectra in Figure 1 are scaled for equal concentration, showing that the intensity of the $g = 4.06$ resonance grows significantly with a change in solvent to acetonitrile. The signal-to-noise for the spectrum of **1** in acetonitrile is lower because of the lower concentration. The large increase in signal intensity of $g = 4.06$ is due to a relatively small increase in the spread of E/D , as indicated by the parameter $\sigma_{E/D}$ (see Discussion). The simulation of Figure 2B uses the same average zero-field parameters as Figure 2A, but with an increase in $\sigma_{E/D}$ from 0.012 to 0.016. This simulation is also in quantitative agreement with the expected amount of the Fe^{IV}-oxo complex in the sample.

Equivalent samples of **1** with ¹⁶O or enriched ¹⁷O were prepared in 1:1 DMF/THF from the oxidation of **2-¹⁶OH** or **2-¹⁷OH** (60% enrichment). The parallel-mode EPR spectra (Figure 4) of these samples showed a broadening from the ¹⁷O isotope. The red and green simulations shown in Figure 4 were calculated with the parameters given in Figure 2 in the absence and presence of an $I = 5/2$ nucleus (¹⁷O) at 60% enrichment. The simulations reproduced the broadening with a hyperfine value of $^{17}\text{O}A_z = 10$ MHz. The spectrum is only sensitive to the

z-component of the hyperfine tensor because the $|2^{\pm}\rangle$ doublet predominately magnetizes along this direction.

Low temperature (4.2 K) Mössbauer spectra of an ^{57}Fe enriched sample of **1** in acetonitrile for various magnetic fields are shown in Figure 5. In a magnetic field of 50 mT (Figure 5G), the complex showed a single quadrupole doublet with $\delta = 0.030(5)$ mm/s and $\Delta E_Q = 0.43(3)$ mm/s. At higher fields, the complex displayed a magnetic hyperfine pattern. The simulation parameters given in Table 1 predict the position and intensities of all the features at 3 and 8 T (Figure 5A and B). However, at 0.5 and 1 T, as shown in Figure 5D and F, the same parameters predict signals from the excited $m_s = -1$ state (marked with arrows) that are not apparent in the experimental spectra. The simulations overlaid on the data include a distribution in E/D ($\sigma_{E/D} = 0.016$). This distribution has no effect on the 3 and 8 T simulations, but broadens the $m_s = -1$ feature beyond detection in the 0.5 and 1 T spectra (Figure 5C and E, black trace on data). Consistently, the same distribution was required to simulate the EPR spectra for the same solvent to accurately predict the ratio of intensities of the $|1^{\pm}\rangle$ and $|2^{\pm}\rangle$ transitions. The zero-field parameters used for Mössbauer simulations are in agreement with those determined from EPR spectroscopy.

Spectroscopy of Fe^{III} -oxo complex (**2**)

Figure 6A shows the EPR spectrum of **2** in DMF at 10 K. The signals at $g = 5.8$ and 2.0 are indicative of an axial high-spin ferric complex.^{14,22} The signal at $g = 4.3$ is from a small amount ($< 1\%$) of an Fe^{III} impurity. Upon closer examination at higher field, an additional weak signal is evident at $g = 1.2$ (Figure 6, Inset). This signal is from a $|\pm 3/2\rangle$ transition, and its position and intensity are sensitive to the D-value. A fit to the temperature dependence of the $g = 5.8$ signal indicated that it originates from an excited doublet, and together with the position of the $g = 1.2$ resonance, give a value of $D = -0.7(2)$ cm^{-1} . A simulation (Figure 6B and Inset) with the parameters from Table 1 quantitatively agreed with the experimental spectrum. The simulations used a relatively large unresolved linewidth of 30 G, indicating the presence of intermolecular broadening. These intermolecular interactions are also believed to cause the asymmetric shape of the $g = 2$ feature that is not well predicted by the simulations. The ^{17}O enrichment of **2** showed no detectable broadening effects because the linewidths of the signals are dominated by intermolecular interactions.

Figure 7 shows Mössbauer spectra of ^{57}Fe enriched **2** in DMA at 4.2 K in an applied magnetic field. At 50 mT (Figure 7B), the sample showed a superposition of two magnetic patterns originating from a 70:30 mixture of **2** and **2-OH**. It is difficult to prepare a pure solution of **2** without small amounts of **2-OH** impurity because of the high basicity of the oxo group of **2** and residual acidic impurities present in the solvents. Figure 7A shows a spectrum of **2-OH** free of iron impurities. Figure 7C and D show the difference spectra at 0.05 T and 8 T after subtraction of the corresponding spectra of **2-OH** recorded under the same conditions. At 150 K (data not shown), the paramagnetic pattern does not collapse into a simple doublet indicating that the electronic spin is in the intermediate relaxation regime. Simulations using the parameters given in Table 1 are overlaid on the data. The zero-field parameters are in agreement with those from the EPR analysis.

Spectroscopy of **2-OH** (Fe^{III} -hydroxo complex)

An EPR spectrum of **2-OH** in DMF/THF is shown in Figure 6C. The complex showed sharp signals at $g = 10.1, 8.8, 5.3, 3.3$ and 2.6 which originate from the three $S = 5/2$ doublets, and are indicative of a non-axial ferric complex. The simulation (Figure 6C, black) for the parameters given in Table 1 quantitatively agrees with the experimental spectrum. Figure 6D, E and F show individual simulations for each doublet contributing to the spectrum (the quantum numbers in the limit $E/D = 0$ are $m_s = \pm 5/2, \pm 3/2$ and $\pm 1/2$, respectively). The relative intensities

of signals and their resonance position allowed an accurate determination of D and E/D of the complex. The $g = 4.3$ signal is from a minor amount of adventitious Fe^{III} impurity ($<0.1\%$).

Equivalent EPR samples of **2-OH** in DMF/THF were prepared with and without ^{17}O enrichment (60%) of the hydroxo ligand. A magnified view of EPR spectra in the region $10.5 < g < 8.0$ is shown in Figure 8. The ^{17}O labeled sample (Figure 8, red) showed broadening from the $^{17}\text{OH}^-$ ligand when compared to **2- ^{16}O H** (Figure 8, green). Based on simulations, the broadening from ^{17}O gave a hyperfine tensor for the ^{17}O nucleus of $\mathbf{A} = (\text{n.d.}, 8, 12)$ MHz (n.d. is not determined). The signal at $g = 3.3$ (Figure 6C and E) occurs for the molecular x-axis along the static magnetic field, but is too broad to allow a determination of A_x .

The Mössbauer spectra of an ^{57}Fe enriched sample of **2-OH** in DMA are shown in Figure 9. At 4.2 K and 50 mT (Figure 9A), the sample showed a six-line pattern indicative of a ferric species. As the applied magnetic field is increased from 3 to 8 T (Figure 9B and C, respectively), the six-line pattern moved inwards because the external magnetic field opposes the internal magnetic field of the Fe nucleus. The features in the 3 T spectrum (Figure 9B) marked with arrows are due to a small amount of adventitious Fe in the sample. At 120 K, the six-line pattern collapsed to a doublet with $\delta = 0.32$ mm/s and $\Delta E_Q = 0.82$ mm/s. The simulations (Figure 9, black) are generated for an $S = 5/2$ spin state using the parameters given in Table 1. The parameters from the simulation of the EPR and Mössbauer data are in agreement.

DFT studies

The DFT optimized structure for the $S = 2$ state of **1** is in good agreement with the crystal structure as indicated by the comparison of the selected bonds and angles in Table 2. The table also presents the data for the optimized structure of the $S = 1$ state. As expected from the orbital occupancies in the two spin states, the three Fe- N_{eq} distances are virtually equal in the $S = 2$ structure, but are not equal in the $S = 1$ structure. On the average, the Fe- N_{eq} distances in the $S = 2$ structure ($d_{xz/yz}^2 d_{xy/x2-y2}^2$) are 0.09 Å longer than in the $S = 1$ structure ($d_{xz/yz}^3 d_{xy/x2-y2}^1$). The resonance conditions for these doublets is 1 , whereas the Fe-O and Fe- N_{ax} distances in the $S = 2$ structure are shorter by 0.03 Å and 0.10 Å, respectively. Like for **1**, DFT optimizations gave for **2** and **2-OH** structures that are in good agreement with the X-ray diffraction. The DFT values for the spectroscopic parameters obtained for the optimized structures of **1**, **2**, **2-OH** are presented in Table 1.

Discussion

Spectroscopy of Fe^{IV} -oxo complex (**1**)

The catalytic cycles of the non-heme α -ketoglutarate dependent oxygenase and halogenase enzymes involve high-spin Fe^{IV} -oxo intermediates.^{1,2} The present work demonstrates that high-spin Fe^{IV} -oxo species can be characterized with EPR spectroscopy. The software we have developed allows spin concentration determinations directly from EPR spectra. The ability to detect and quantify EPR signals represents a valuable tool for kinetic and mechanistic studies aimed at quantifying the intermediates formed during the reaction cycles of biomimetic and enzymatic systems.

The Fe^{IV} -oxo complex, **1**, showed signals from transitions within the $|1^\pm\rangle$ and $|2^\pm\rangle$ doublets of the $S = 2$ spin manifold. The detection of signals from both doublets of an $S = 2$ state is rare. For integer-spin systems with near axial symmetry, the non-Kramers states in zero magnetic field are approximately the symmetric and antisymmetric combinations of the m_s states: $|m^\pm\rangle = (|+m\rangle \pm |-m\rangle)/\sqrt{2}$ where $m = 1$ or 2 .²³ The resonance conditions for these doublets is $(h\nu)^2 = A_m^2 + (gz\beta B \cos\theta)^2$ where θ is the angle between the molecular z-axis and the external field (B), and $\Delta_1 = 6E$ and $\Delta_2 = 3D(E/D)^2$ are the zero-field splittings of the two doublets. The

signal intensity from each doublet is proportional to Δ_m^2 . For **1**, the ratio $\Delta_2/\Delta_1 = 67$ implies that the signal from the $|1^\pm\rangle$ transition is a factor of 4500 more intense than the signal from the $|2^\pm\rangle$ transition. However, the distribution in Δ_m values are significantly greater for the $|1^\pm\rangle$ transition. The distribution in Δ_1 is shown in Figure 10. The center of the distribution is at 0.83 cm^{-1} , meaning that a large fraction of molecules have $\Delta_1 > h\nu = 0.3 \text{ cm}^{-1}$ (X-band) and cannot satisfy the resonance condition. As the width of the distribution in E/D increases, a greater fraction of molecules can satisfy the resonance condition at X-band resulting in increased signal intensity. This is evident from the comparison of the EPR data of **1** in DMF and acetonitrile. The solvent interactions in acetonitrile induce a wider distribution in E/D ($\sigma_{E/D} = 0.016$) as compared to DMF ($\sigma_{E/D} = 0.012$) resulting in a greater fraction (11% vs 6%) of molecules with $\Delta_1 < h\nu$, and thus a higher intensity for the resonance in acetonitrile. The average value of Δ_1 is less than the microwave quantum at Q-band (1.2 cm^{-1}), thus a majority of molecules can be brought into resonance (Figure 10). Despite the larger fraction, the simulations indicated that the distribution severely broadens the signal, rendering it undetectable against the baseline.

For the $|2^\pm\rangle$ doublet of **1**, the distribution in Δ_2 is sharp and is entirely less than the microwave energy, resulting in a detection limit for **1** of 0.5 mM. Enzymatic active sites are expected to have lower symmetry and higher E/D values, and since the intensity is proportional to $(E/D)^4$, it is anticipated that the detection limit will drop significantly for metalloenzymes (i.e. $\ll 0.5 \text{ mM}$). The resonance condition for an integer-spin signal contains two molecular parameters: Δ_m and g_z . Dual frequency EPR spectra allowed accurate measurement of these parameters for quantitative analysis. The combination of the ratio of intensities of the transitions in $|1^\pm\rangle$ and $|2^\pm\rangle$, the data at two microwave frequencies, and the simulations, all allow accurate determination of E/D, D , the distribution in E/D ($\sigma_{E/D}$), and the concentration of species. The concentration of **1** determined by the EPR simulations is in agreement with the amount determined from an equivalent Mössbauer sample.

The ^{57}Fe isomer shift of **1** (0.02 mm/s) is significantly lower than the values for other $S = 2$ Fe^{IV} -oxo species, including TauD-J (the Fe^{IV} -oxo intermediate of TauD), and the synthetic complexes $\text{Fe}^{\text{IV}}(\text{H}_2\text{O})_5(\text{O})$ and $[\text{Fe}^{\text{IV}}(\text{TMG}_3\text{tren})(\text{O})]^{2+}$ ($\delta = 0.3, 0.38$, and 0.09 mm/s , respectively).^{5,7} The lower magnitude of the isomer shift in **1** indicates higher covalency of the Fe- N_{eq} bonds, resulting in a higher s-electron density at the iron nucleus. This is consistent with the overall shorter Fe-N bond lengths of **1** relative to that of $[\text{Fe}^{\text{IV}}(\text{TMG}_3\text{tren})(\text{O})]^{2+}$ complex (Table 2). The Fe-oxo bond for **1** is longer, which as discussed below, is presumably because of the hydrogen bonding present in **1**. The ^{57}Fe A-tensor for **1** is similar to those of other high-spin Fe^{IV} complexes (Table 3). The isotropic value (A_{iso}) for **1** is -25.9 MHz while those for $\text{Fe}^{\text{IV}}(\text{H}_2\text{O})_5(\text{O})$, TauD-J, and $[\text{Fe}^{\text{IV}}(\text{TMG}_3\text{tren})(\text{O})]^{2+}$ are -34 , -30.6 , and -26.6 MHz , respectively.^{1,5,7,24} The $|A_{\text{iso}}|$ for the series $\text{Fe}^{\text{IV}}(\text{H}_2\text{O})_5(\text{O}) > \text{TauD-J} > [\text{Fe}^{\text{IV}}(\text{TMG}_3\text{tren})(\text{O})]^{2+} > \textbf{1}$ also reflects an increase in covalency and reduction of the spin polarization of the s-electrons by the d-electrons, consistent with the trend deduced from the isomer shift. The anisotropic dipolar hyperfine tensor for **1** is $\mathbf{A}_{\text{dip}} = (+5.6, +5.3, -10.9) \text{ MHz}$, which is within 5% of the values from $[\text{Fe}^{\text{IV}}(\text{TMG}_3\text{tren})(\text{O})]^{2+}$ and TauD-J (Table 3). The three complexes have axial $\mathbf{A}_{\text{dip}}$ tensors with similar principal values, as they all have a strongly coordinating oxo ligand. The dipolar A-tensor of **1** predicted by DFT calculations has the eigenvalues $(+4.7, +4.5, -9.2) \text{ MHz}$, which are in reasonable agreement with the experimental values.

^{17}O hyperfine interaction and Fe-oxo bond covalency

The EPR resonance at $g = 8.12$ is sufficiently sharp to allow detection of broadening from ^{17}O isotopic enrichment. A large broadening from ^{17}O of a $g = 8$ signal was recently observed from an Fe^{III} -superoxo species in a catechol dioxygenase. The broadening allowed measurement of a component of the ^{17}O hyperfine tensor, which was found to be large and indicative of localization of an unpaired electron on the superoxide.²⁵ The ^{17}O enriched

samples of **1** in the present study showed much less broadening. Nevertheless, as described next, this result is indicative of significant spin polarization of oxo, and covalency, by binding to the paramagnetic Fe^{IV} ion.

The ¹⁷O magnetic hyperfine interaction is described with the Hamiltonian $^{170}\mathbf{I}^{170}\mathbf{A}\cdot\mathbf{S}$, where $\mathbf{S}$ is the spin of the iron center. Except where explicitly stated, the $\mathbf{A}$ -values are given for a Hamiltonian of total spin $S = 2$ for **1** or $S = 5/2$ for **2** and **2-OH**. The isotropic hyperfine constant ($^{170}A_{\text{iso}}$) of ¹⁷O has been expressed as:²⁶

$$^{170}A_{\text{iso}} = a_p \rho_p = ^{170}A_z - ^{170}A_z^{\text{dip}} \quad (3)$$

It has been assumed that $^{170}A_{\text{iso}}$ is proportional to the spin population ρ_p of the p-orbitals of the oxo ligand ($\rho_p = \rho_{p_x} + \rho_{p_y} + \rho_{p_z}$). The constant a_p is the isotropic hyperfine coupling for an unpaired electron in a p-orbital of oxygen ($\rho_p = 1$). The magnitude of ρ_p is indicative of the delocalization of the oxygen p-electrons into the Fe d-orbitals, which gives rise to spin polarization on the oxo ligand. For example, $\rho_p = 0$ would correspond to an ionic Fe^{IV}-O²⁻ bond (no delocalization), whereas a value of $\rho_p = 1$ would correspond the transfer of a net amount of electron spin density representing one unpaired electron. In eq 3, the z-component of the dipolar hyperfine tensor is $^{170}A_z^{\text{dip}}$. The simulation of the $g = 8.12$ resonance gave an experimental value of $^{170}A_z = \pm 10$ MHz (see Figure 4 and Results). This resonance is only sensitive to the component of the $\mathbf{A}$ -tensor along the Fe-oxo bond (molecular z-axis), and the sign of hyperfine values cannot be determined from EPR spectroscopy. DFT calculations of Fe^{IV}-oxo species with the functional and basis set adopted here have the propensity to underestimate the s-orbital polarization of the electrons at the iron nucleus, giving a lower magnitude of $^{57}\text{Fe}A_{\text{iso}}$ than the experimental value. The analysis presented below suggests that a similar problem arises for $^{170}A_{\text{iso}}$. In contrast to the isotropic value, the dipolar $\mathbf{A}$ -tensor ($\mathbf{A}_{\text{dip}}$) can be calculated with reasonable accuracy. DFT calculations of the optimized structure gave $^{170}\mathbf{A}_{\text{dip}} = (-13.4, -13.4, +26.8)$ MHz and $^{170}A_{\text{iso}} = -11$ MHz for ¹⁷O-isotopomer. Using the DFT value for $^{170}A_z^{\text{dip}} = +26.8$ MHz and the experimental value of $A_z = \pm 10$ MHz, the isotropic value was evaluated from eq 3 to give two possible values for $^{170}A_{\text{iso}} = -16.8$ or -36.8 MHz. A value of $a_p^{S=1/2} = -120$ MHz has been previously reported for a single unpaired electron in an oxygen p-orbital.^{26,27} This value can be converted to that appropriate for an $S = 2$ Hamiltonian as $a_p = a_p^{S=1/2}/4 = -30$ MHz.^{26,28} The value of the total unpaired spin density, ρ_p , in the oxo 2p orbitals can then be solved from eq 3 using the two values of $^{170}A_{\text{iso}}$ to give $\rho_p = 0.56$ ($^{170}A_{\text{iso}} = -16.8$ MHz) or 1.23 ($^{170}A_{\text{iso}} = -36.8$ MHz). The value of δ and the ⁵⁷Fe $\mathbf{A}$ -tensor obtained from Mössbauer spectroscopy were in the range expected for a +4 oxidation state of Fe, favoring the value of $\rho_p = 0.56$. The value of $\rho_p = 1.23$ would be expected to give a +3 oxidation state of Fe. Furthermore, the Mulliken spin populations for the d_{xz} and d_{yz} orbitals calculated by DFT are equal to 1.25 (Table S1). The total spin density transferred from the oxo $p_{x/y}$ orbitals into the $d_{xz/yz}$ orbitals is $2 \times (1 - 1.25) = -0.5$, resulting in spin populations of +0.25 in each of the p_x and p_y orbitals of oxygen. Thus, the Mössbauer data and the Mulliken population analysis are most consistent with the value of $\rho_p = 0.56$. This large value of spin polarization of the oxo indicates significant covalency of the Fe-oxo bond. ¹⁷O ENDOR studies of HRP Compound I have estimated a delocalization of 25% for the $S = 1$ Fe^{IV}-O heme center,²⁹ yielding a net spin population of $\rho_p = 2 \times 0.25 = 0.50$ shared between p_x and p_y , which is comparable to the net spin population of **1**.

As described in above calculations from the hyperfine ¹⁷O value, the unpaired spin density on the oxo is 0.56. In comparison to the Fe^{III}-superoxo species of catechol dioxygenase in which nearly a full unpaired electron is localized on the superoxo, the rather small ¹⁷O broadening effect observed for **1** was unexpected, and much less than that based on the ratio of unpaired spin densities for the two complexes. The small hyperfine value observed for **1** is a consequence

of two factors: the lower overall spin polarization, and the O p-orbital occupations. For superoxide, the localization of an unpaired electron in a single π^* orbital gives (for $S = 1/2$) $^{170}\text{A}_{\text{dip}}^{S=1/2} = (-160, +80, +80)$ MHz.³⁰ In contrast, for **1**, the two singly occupied $d_{xz/yz}$ iron orbitals form π bonds with the p_x and p_y oxo orbitals, inducing polarization of electron spin in both oxo orbitals. The two-orbital polarization changes the sign of the largest component of $^{170}\text{A}_{\text{dip}}$ to give (for $S = 1/2$) $^{170}\text{A}_{\text{dip}}^{S=1/2} = (-54, -54, +108)$ MHz. For both the Fe^{III} -superoxo species of catechol dioxygenase and **1**, the EPR resonance was sensitive to the largest component of $^{170}\text{A}_{\text{dip}}$. For the Fe^{III} -superoxo species, the largest component (-160 MHz) has the same sign as A_{iso} , whereas for **1** the sign of the largest component (+108 MHz) is opposite that of A_{iso} , and consequently the two values effectively cancel to give the small A-value observed experimentally.

DFT calculations of **1**

The DFT optimized structure of **1** gives the d-orbital scheme shown in Figure S1 with an electronic configuration of $d_{xz}^1 d_{yz}^1 d_{xy}^1 d_{x^2-y^2}^1$. The trigonal symmetry results in degenerate d_{xz} and d_{yz} orbitals, which are lower in energy than the degenerate set of d_{xy} and $d_{x^2-y^2}$ orbitals. The Fe ion is displaced above the plane formed by the equatorial nitrogens (N_{eq}) as evident from the average O-Fe- N_{eq} angle of 97.5° . DFT calculations indicate that total energy for the optimized structure of **1** in the $S = 1$ spin state is 0.66 eV (5300 cm^{-1}) higher than the energy for the optimized structure of **1** in the $S = 2$, in agreement with the experimentally observed $S = 2$ ground state configuration for **1**. As observed with the other $S = 1$ Fe^{IV} complexes, the Fe-oxo bond lengths for the two DFT optimized structures are close (1.680 vs 1.706 Å).^{3,6,31} However, the Fe- N_{eq} bond lengths differ significantly indicating that the equatorial ligands play a role in spin-state determination as suggested by previous studies.³²

The Mulliken spin on the oxo ligand from DFT ($\rho_{\text{p}}^{\text{DFT}}$) is 0.38. This value is significantly lower than the value of 0.56 determined from the experimental ^{170}A hyperfine constant. The difference between these numbers may reflect that DFT underestimates the spin polarization or that the magnitude of the constant, $a_{\text{p}}^{S=1/2}$ used above is too small.

Considering the trend in ρ_{p} from DFT calculations, the value $\rho_{\text{p}}^{\text{DFT}} = 0.38$ of **1** is significantly lower than the reported values of 0.63 for $[\text{Fe}^{\text{IV}}(\text{TMG}_3\text{tren})(\text{O})]^{2+}$ and 0.6 for **TauD-J**.^{7,33} While spin populations calculated from DFT may depend on the functional and basis set used, this dependence is not the cause for the difference as the calculation for $[\text{Fe}^{\text{IV}}(\text{TMG}_3\text{tren})(\text{O})]^{2+}$ and **1** used the same functional and basis set. The $[\text{Fe}^{\text{IV}}(\text{TMG}_3\text{tren})(\text{O})]^{2+}$ complex with similar C_3 symmetry and nitrogen based equatorial ligands is expected to have a similar spin polarization of the oxo ligand as **1**. However, $[\text{Fe}^{\text{IV}}(\text{TMG}_3\text{tren})(\text{O})]^{2+}$ lacks the intramolecular H-bonding network provided by the butylureaylato arms of the $[\text{H}_3\text{buea}]^{3-}$ ligand. These H-bonds would have the propensity to lower the spin density on the oxo ligand which may be reflected by the smaller magnitude of $\rho_{\text{p}}^{\text{DFT}}$ in **1** as compared to $[\text{Fe}^{\text{IV}}(\text{TMG}_3\text{tren})(\text{O})]^{2+}$. The effect of H-bonding is also consistent with the observed lengthening of the Fe-O bond of **1** (1.68 Å) relative to $[\text{Fe}^{\text{IV}}(\text{TMG}_3\text{tren})(\text{O})]^{2+}$ (1.65 Å). A similar EPR study of $[\text{Fe}^{\text{IV}}(\text{TMG}_3\text{tren})(\text{O})]^{2+}$ may provide proof of the DFT prediction that $[\text{Fe}^{\text{IV}}(\text{TMG}_3\text{tren})(\text{O})]^{2+}$ has larger $^{170}\text{A}_{\text{iso}}$.

TD-DFT calculations were performed to obtain a theoretical value for the zero-field splitting of **1**. The first four excited electronic configurations having $S = 2$ electronic spin are two degenerate pairs at 1.5 and 3.0 eV above the ground $S = 2$ state as shown in Figure 11. These excited configurations are due to the promotion of an electron from the $d_{x^2-y^2}/d_{xy}$ (1.5 eV) and d_{xz}/d_{yz} (3.0 eV) orbitals to the unoccupied d_{z^2} orbital. The matrix elements of the angular momentum operator (L) between d_z^2 and $d_{x^2-y^2}/d_{xy}$ vanish, consequently, the ^5E configuration at 1.5 eV does not contribute to the D-tensor. For the ^5E configuration at 3.0 eV, however, the

matrix elements of $\mathbf{L}$ between d_z^2 and d_{xz}/d_{yz} are non-zero, and this configuration contributes to the D-tensor.

The triplet spin configurations may also contribute to the D-tensor. The lowest triplet configuration, calculated by computing the SCF energy for a vertical excitation from the ground state, is 1.1 eV higher in energy than the quintet ground state. TD-DFT calculations reveal four triplet states near this energy due to the transitions given in Figure 11. In addition, there are excitations to two other pairs of triplet states at 1.7 and 1.9 eV. Using the excitation energies calculated from TD-DFT, a theoretical D-value has been computed as described in SI. The computed D-value contributions from the $S = 2$ and $S = 1$ spin states are $D_2 = +0.73 \text{ cm}^{-1}$ and $D_1 = +3.2 \text{ cm}^{-1}$, respectively, giving a total value of $D = D_1 + D_2 = +3.9 \text{ cm}^{-1}$. The calculated zero-field splitting is in reasonable agreement with the experimental value of $+4.7 \text{ cm}^{-1}$. The D-tensor for the low-spin ($S = 1$) Fe^{IV} complexes been shown to have contributions from the $S = 2$ electronic excited states.³⁴ Notably, the calculations here also demonstrate that the $S = 1$ excited electronic states have major contributions to the D-value of **1**. A similar effect of excited state contributions to the D-value due to spin state mixing was also observed for $\text{Fe}^{\text{IV}}(\text{H}_2\text{O})_5(\text{O})$.⁵

The first quintet excited state appears at energy of 12000 cm^{-1} (1.5 eV) above the ground state (Figure 11, marked with an arrow). This energy gap corresponds to a wavelength of 800 nm for a spin allowed d-d electronic transition (^5A to ^5E). The electronic absorption spectrum of **1** shows a broad absorbance at 808 nm with $\epsilon = 280 \text{ M}^{-1}\text{cm}^{-1}$. The extinction coefficient is in the range expected for d-d transition.⁷ A similar absorbance was observed in the $[\text{Fe}^{\text{IV}}(\text{TMG}_3\text{tren})(\text{O})]^{2+}$ complex at 866 nm and this complex has a similar D-value ($+5 \text{ cm}^{-1}$). Based on this comparison, this absorbance is assigned to the d-d transition between the ground and the first excited quintet states.

Spectroscopic properties of **2** and **2-OH**

The isotropic ^{57}Fe A-value ($A_{\text{iso}} = -24.9 \text{ MHz}$) of **2** is lower than that of **2-OH** (-27.0 MHz) and other observed ferric complexes. The lower magnitude is due to increased covalency of the Fe-O bond in **2**, which reduces the spin polarization of the s-electrons. Such an effect has been reported for other ferric states with significant covalent interactions (e.g., rubredoxin).³⁵ The covalency of metal-ligand bonds also affects the magnitude of isomer shift (δ). For both complexes, the three deprotonated nitrogen atoms of the $[\text{H}_3\text{buea}]^{3-}$ ligand afford strong in-plane Fe-N bonds, which results in lower δ than that commonly observed for ferric complexes. The quadrupole splitting, ΔE_{Q} , for **2** (-1.54 mm/s) has a larger negative value than ΔE_{Q} for **2-OH** (-0.87 mm/s) indicating a larger electric field gradient for the stronger oxo ligand as compared to the hydroxo ligand. This is consistent with the longer Fe-O bond of **2-OH** (1.813 and 1.931 Å for **2** and **2-OH**, respectively). The theoretical values of δ and ΔE_{Q} for these complexes from DFT calculations are in reasonable agreement with the experimental results. The magnitude of η for the complex is close to 1 ($\eta^{\text{exp}} = 0.65$, $\eta^{\text{DFT}} = 0.72$), thus the sign of quadrupole splitting is indeterminate for **2-OH**.

Simulations of the ^{17}O isotopic broadening observed in the EPR spectrum of **2-OH** gave $^{170}\mathbf{A} = (\text{n.d.}, \pm 8, \pm 12) \text{ MHz}$ (Figure 8). This effect is similar to the broadening observed in other water ligated ferric heme complexes.³⁶ The A_x value and the sign of these values cannot be determined from the data. The dipolar contribution to the hyperfine tensor determined from DFT calculations was $^{170}\mathbf{A}_{\text{dip}} = (5.25, -0.35, -4.90) \text{ MHz}$. From the two possible $^{170}\mathbf{A}$ experimental values and the $^{170}\mathbf{A}_{\text{dip}}$ tensor from DFT calculations, two possible isotropic values were calculated from Eq. 3: $^{170}A_{\text{iso}} = +8.4$ or -7.6 MHz . The $^{170}A_{\text{iso}}$ value of $+8.4 \text{ MHz}$ can be discarded since the sign of the Fermi contact term for ^{17}O is negative.³⁷ From eq 3, the total spin density on the hydroxo was found to be $\rho_{\text{p}} = 0.32$. Iron(III) has three singly occupied

orbitals that interact with the oxygen p-orbitals, thus the percent delocalization of the spin density for the Fe-OH bond is $(\rho_p/3) \times 100\% = 11\%$. As expected, the spin polarization of the single Fe-hydroxo bond of **2-OH** is lower in comparison to the double Fe-oxo bond of **1**.

Isotope labeling with ^{17}O resulted in observable broadening for **2-OH** as described above. The Fe-O bond in **2** is stronger than the Fe-OH bond in **2-OH** and is expected to show a larger effect from ^{17}O labeling, however, this effect cannot be detected in the present samples of **2** for the following reason. The broadening observed in **2** is due to intermolecular interaction which can be established from simulation. For **2**, the simulations require an excessive phenomenological broadening that cannot be attributed to distributions in zero-field parameters. The crystal structure of **2** shows a close packed intermolecular Fe distance of 10 Å. A magnetic dipolar interaction between two $S = 5/2$ molecules (with zero-field parameters of **2**) that are 10 Å apart, will result in signal shifts of up to 14 mT. This shift is comparable to the phenomenological broadening used in the simulation of Figure 6B. This indicates short range aggregation for **2** in frozen solution. We have considered a variety of solvent systems to remove the intermolecular interactions: DMA, DMF, acetonitrile, THF. The best was observed for the glass forming solvent mixture, 1:1 DMA:THF. This mixture gave the extremely sharp lines for **2-OH**, and while it significantly sharpened the signals of **2**, the dipolar broadening from intermolecular interaction still overwhelms the expected broadening from isotopic labeling.

Conclusion

The present work demonstrates the detection and quantitative characterization of the high-spin $[\text{Fe}^{\text{IV}}\text{H}_3\text{buea}(\text{O})]^-$ complex and associated ferric complexes using EPR and Mössbauer spectroscopies. ^{17}O -EPR studies of $[\text{Fe}^{\text{IV}}\text{H}_3\text{buea}(\text{O})]^-$ gave $^{170}\text{A}_{\text{iso}} = -16.8$ MHz and a quantitative determination of the spin polarization ($\rho_p = 0.56$) of oxo p-orbitals. The magnitude of spin polarization is indicative of significant covalency in the Fe-oxo bond, but apparently mitigated by the H-bonds of the butylureaylato arms of the ligand. The experimental values of the zero-field parameter D for $[\text{Fe}^{\text{IV}}\text{H}_3\text{buea}(\text{O})]^-$ and the dipolar ^{57}Fe A-tensors are found to be in good agreement with DFT calculations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Structures of Fe^{III} and Fe^{IV} complexes characterized in this report.

Figure 2.

X-band EPR spectrum of **1** in DMF (A) and in acetonitrile (B). The black lines are simulations with the parameters given in Table 1. Inset: Q-band EPR spectrum of **1** in DMF. Experimental conditions: microwave frequency, 9.3 GHz (X), 33.9 GHz (Q); power 2 mW; temperature, 10 K; microwave mode, $B_1 \parallel B$.

Figure 3.

Signal intensity times temperature versus temperature of the $g = 4.06$ (blue) and $g = 8.12$ (red) EPR signals of **1** recorded under non-saturating conditions. The black lines, generated from the parameters of Table 1, give the theoretical percent population of the magnetic sublevels of the $S=2$ manifold involved in the transitions as a function of temperature. Signal times temperature is proportional to population of a specific doublet.

Figure 4. EPR spectra (top) and simulations (bottom) in the $g = 8$ region for **1** in DMF/THF with 60% enriched ^{17}O (green) and ^{16}O (red). The experimental conditions are the same as in Figure 2.

Figure 5. Enriched ^{57}Fe Mössbauer spectra of **1** in acetonitrile in a magnetic field of: (A) 8 T, (B) 3T, (C) 1 T, (E) 0.5 T, and (G) 50 mT. The black traces are simulations for the parameters of Table 1. (D, F) Simulation of C and E, respectively, with $\sigma_{E/D} = 0$. A high-spin ferric impurity, accounting for 12% of the Fe in the sample, has been subtracted from all the data except spectrum E and G. The arrows indicate excited state signals from the $m_s = -1$ spin state. Experimental conditions: temperature, 4.2K; magnetic field parallel to γ -ray direction.

Figure 6.

EPR spectra (red) and simulations (black) of **2** (A, B) and **2-OH** (C) in DMF/THF. (D, E, F) Simulations for **2-OH** from transitions within the m_s doublets $|\pm 5/2\rangle$, $|\pm 3/2\rangle$, $|\pm 1/2\rangle$, (C, black) is the sum of D, E, and F. Inset: High field expanded view of **2** from the region indicated. Experimental conditions: microwave frequency, 9.64 GHz; power 0.2 mW; temperature, 10 K; microwave mode, $B_1 \perp B$. The simulation parameters are given in Table 1.

Figure 7.

^{57}Fe Mössbauer spectrum of **2** and **2-OH** in DMA in applied magnetic field. (A) **2-OH** at 50mT; (B) **2** at 50mT; (C) Difference spectrum: B - 0.3A; (D) Difference spectrum of **2** at 8T after subtracting **2-OH**. The black traces are simulations for the parameters of Table 1. Experimental conditions are the same as in Figure 6. The central region in the spectrum C is from a small amount (<10%) of a diamagnetic impurity.

Figure 8. EPR spectra of $2\text{-}^{16}\text{OH}$ (green) and $2\text{-}^{17}\text{OH}$ (red) in DMF/THF. Experimental conditions are the same as in Figure 6.

Figure 9.

Mössbauer spectra of ^{57}Fe enriched **2-OH** in DMA in a magnetic field of (A) 50 mT; (B) 3T; (C) 8T. The black traces are simulations for the parameters of Table 1. The arrows indicate signals from ferric impurity in the sample. Experimental conditions are the same as in Figure 5.

Figure 10.

The distribution in Δ_1 values of the $|1^\pm\rangle$ doublet for $\sigma_{E/D} = 0.012$ (red) and 0.016 (green) for **1** in DMF and acetonitrile, respectively. The vertical lines indicate the microwave energy quanta at X- and Q-band. The shaded region indicates region of the distribution that can be detected at X-band.

Figure 11.

Relative energies of quintet and triplet electronic excited states of **1** from TD-DFT and vertical SCF calculations. The orbitals connected by horizontal arrows indicate the d-d electronic transition with respect to the ground state configuration. The vertical arrow represents the electronic transition observed in optical spectra near 800 nm.

Table 1

Experimental and calculated EPR and Mössbauer parameters of **2**, **2-OH** and **1**.

	2		2-OH		1	
	Exp	DFT	Exp	DFT	Exp	DFT
D (cm ⁻¹)	-0.7		-2.4		+4.7	+3.9
E/D, $\sigma_{E/D}$	0.005, 0.03		0.157, 0.01		0.03, 0.012	
g	2.00, 2.00, 2.00		2.010, 2.009, 2.012		-, -, 2.02	
⁵⁷ Fe A/g _{eff}	-24.8, -25.5, -24.4		-26.3, -28.4, -26.3		-20.3, -20.6, -37.0	-21.3, -21.5, -35.2 ^a
δ (mm/s)	0.32	0.36	0.32	0.32	0.02	0.04
ΔE_Q (mm/s)	-1.54	-1.40	-0.82	+0.98	+0.43	+1.05
η	0	0	0.65	0.74	0	0

^a A-tensor (in MHz) calculated with addition of experimental A_{iso} plus theoretical A_{dip}.

Table 2

Comparison of calculated and experimental values of selected bond distances (Å) and angles of **1** and [Fe^{IV}(TMG₃tren)(O)]²⁺.

	X-ray	DFT (S = 2)	DFT (S = 1)	TMG ^a (X-ray)
Fe-O	1.680	1.677	1.706	1.661
Fe-N_{eq}1	1.981	1.999	1.874	1.994
Fe-N_{eq}2	1.988	2.001	1.932	2.006
Fe-N_{eq}3	1.997	2.001	1.933	2.020
Avg. Fe-N_{eq}	1.989	2.000	1.913	2.005
Fe-N_{ax}	2.064	2.105	2.211	2.121
Avg< O-Fe-N_{eq}	97.59	97.44	98.55	97.81

^a [Fe^{IV}(TMG₃tren)(O)]²⁺

Table 3

Comparison of EPR and Mössbauer parameters of selected $S = 2$ Fe^{IV} -oxo complexes.

Complex	D^a	E/D	g^b	ΔE_Q^b	$^{57}\text{Fe } A/g_H \beta_h \text{ (dip)}^c$	$^{57}\text{Fe } A/g_H \beta_h \text{ (iso)}^c$	Ref.
$[\text{Fe}^{\text{IV}}\text{H}_3\text{bueat}(\text{O})]$	+4.7	0.03	0.03	0.43	+5.6,+5.3,-10.9	-25.9	This work
$[\text{Fe}^{\text{IV}}(\text{TMG}_3\text{tren})(\text{O})]^{2+}$	+5.0	0.02	0.09	-0.29	+5.4,+6.4,-11.8	-26.6	7
TauD-J	+10.5	0.01	0.31	-0.88	+5.4,+6.5,-11.9	-30.6	1
$[\text{Fe}^{\text{IV}}(\text{H}_2\text{O})_5(\text{O})]^{2+}$	+9.7	0	0.38	-0.33	-34.0	-34.2 ^d	5
$[\text{Fe}^{\text{IV}}(\text{tpaPh})(\text{O})]^-$	+4.32	0.098	0.09	0.51	n.d.	n.d.	8

^a in cm^{-1} ;

^b in mm/s;

^c in MHz;

^d the value for this complex was calculated from the two experimental A-values and the dipolar A-tensor from DFT calculations