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Oxoiron(IV) Complexes as Synthons for the Assembly of Heterobimetallic Centers such as the Fe/Mn Active Site of Class Ic Ribonucleotide Reductases

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Abstract

Nonheme oxoiron(IV) complexes can serve as synthons for generating heterobimetallic oxo-bridged dimetal complexes by reaction with divalent metal complexes. The formation of $\text{Fe}^{\text{III}}\text{--O--Cr}^{\text{III}}$ and $\text{Fe}^{\text{III}}\text{--O--Mn}^{\text{III}}$ complexes is described herein. The latter complexes may serve as models for the $\text{Fe}^{\text{III}}\text{--X--Mn}^{\text{III}}$ active sites of an emerging class of Fe/Mn enzymes represented by the Class Ic ribonucleotide reductase from *Chlamydia trachomatis* and the R2-like ligand-binding oxidase (R2lox) found in *Mycobacterium tuberculosis*. These synthetic complexes have been characterized by UV-Vis, resonance Raman, and X-ray absorption spectroscopy, as well as electrospray mass spectrometry. The $\text{Fe}^{\text{III}}\text{--O--Cr}^{\text{III}}$ complexes exhibit a three-band UV-vis pattern that differs from the simpler features associated with $\text{Fe}^{\text{III}}\text{--O--Fe}^{\text{III}}$ complexes. The positions of these features are modulated by the nature of the supporting polydentate ligand on the iron center, and their bands intensify dramatically in two examples upon the binding of an axial cyanate or thiocyanate ligand trans to the oxo bridge. In contrast, the $\text{Fe}^{\text{III}}\text{--O--Mn}^{\text{III}}$ complexes resemble $\text{Fe}^{\text{III}}\text{--O--Fe}^{\text{III}}$ complexes more closely. Resonance Raman characterization of the $\text{Fe}^{\text{III}}\text{--O--M}^{\text{III}}$ complexes reveals an ^{18}O -sensitive vibration in the range of 760 – 890 cm^{-1} . This feature has been assigned to the asymmetric $\text{Fe}^{\text{III}}\text{--O--M}^{\text{III}}$ stretching mode and correlates reasonably with the Fe–O bond distance determined by EXAFS analysis. The likely binding of an acetate as a bridging ligand to the $\text{Fe}^{\text{III}}\text{--O--Mn}^{\text{III}}$ complex **12** lays the foundation for further efforts to model the heterobimetallic active sites of Fe/Mn enzymes.

Keywords

heterobimetallic Fe–O–M complexes; oxoiron(IV) complexes; EXAFS analysis; resonance Raman spectroscopy; ribonucleotide reductase

Introduction

Enzymes that contain heterobimetallic active sites have recently been shown to be involved in O_2 activation.^{1,2,3,4} An interesting member of this growing class is the Class Ic ribonucleotide reductase (RNR) from *Chlamydia trachomatis*. This enzyme has substantial sequence homology with the Class 1a diiron RNRs found in *E. coli* and mammals and catalyzes the formation of deoxyribonucleotides from ribonucleotides but instead employs a

heterodinuclear Fe/Mn center.⁵ The reduced Fe/Mn center binds O₂ to generate transient high-valent Fe/Mn species involved in generating the functionally critical thiyl radical on the R1 subunit that initiates ribonucleotide reduction.^{6,7,8,9} A second example is the R2-like ligand-binding oxidase (R2lox or Rv0233) found in *Mycobacterium tuberculosis*. Its crystal structure¹⁰ (Figure 1) shows a 2-His-4-Glu bimetallic active site similar to that found for Class 1a RNRs and related diiron enzymes.⁴ One difference is the presence of a myristic acid moiety that acts as an additional bridge to the dimetal center, the function of which is not currently known. Based on anomalous diffraction maps, the metal binding is found to be specific with Mn bound to the site with only one terminal carboxylate. There is also a novel covalent cross-link found between a tyrosine and a valine residue in the protein scaffold, which presumably derives from a two-electron oxidation in the course of O₂ activation. EPR studies suggest a resting state with an iron(III)manganese(III) center,¹¹ and recent rapid kinetic and spectroscopic evidence has been reported for the trapping of peroxo and high-valent intermediates,¹² which are presumably involved in forming the Tyr-Val bond.

Due to fundamental interest in the nature of metal-metal interactions and their spectroscopic signatures, synthetic efforts to obtain analogs of these heterobimetallic active sites began even before the existence of such sites in biology was established. In early work, Que and coworkers utilized a symmetric dinucleating ligand to make Fe^{II}M^{II} and Fe^{III}M^{II} complexes, with phenolate and carboxylate bridges to hold the dinuclear site together.^{13,14} More recently, Latour and Nordlander in separate efforts synthesized and characterized Fe^{III}Mn^{II} complexes of related but unsymmetric dinucleating ligands that provide different binding sites for the two halves of the ligand.^{15,16} Using a self-assembly approach, Wieghardt and coworkers obtained heterobimetallic (μ -oxo)bis(μ -carboxylato)dimetal complexes (TACN)Fe^{III}-O-M^{III}(Me₃TACN) (M = Cr^{III} and Mn^{III}) by the hydrolysis of FeCl₃(TACN) and MCl₃(Me₃TACN) precursors.¹⁷

The relatively recent availability of oxoiron(IV) complexes presented to us the possibility of assembling heterobimetallic complexes by reaction of these complexes with divalent metal precursors. Using this strategy, we recently confirmed the feasibility of this approach by characterizing a pair of (TMC)Fe^{III}-O-Cr^{III} isomers by the inner-sphere electron transfer reaction between [(TMC)Fe^{IV}(O_{anti/syn})](OTf)₂ and Cr^{II}(OTf)₂.^{18,19} In this paper, we extend this strategy to augment the series of Fe^{III}-O-Cr^{III} complexes starting with oxoiron(IV) complexes supported by pentadentate ligands TMC-py, N4Py and BnTPEN. Furthermore, we have synthesized novel Fe^{III}-O-Mn^{III} complexes relevant to the active sites of Class 1c RNRs and R2lox by reacting [(N4Py/BnTPEN)Fe^{IV}(O)]²⁺ with [Mn(dpaq)(OTf)] to construct [(N4Py/BnTPEN)Fe^{III}-O-Mn^{III}(dpaq)]³⁺ complexes. By replacing the pentadentate ligands used in the two previous examples with the tetradentate tripodal ligand TPA, we obtain an Fe^{III}-O-Mn^{III} complex with an available coordination site on each metal center that will allow us to investigate chemistry relevant to the Fe/Mn active sites of Class 1c RNRs and R2lox.

Results

Generation and characterization of Fe^{III}–O–Cr^{III} compounds

Building on our initial efforts to obtain heterodinuclear metal complexes by using oxoiron(IV) tetramethylcyclam (TMC) complexes as synthons,^{17,18} we have investigated the effect of the axial ligand on the properties of the Fe^{III}–O–Cr^{III} units of structural isomers **4** and **5**. Recently we reported the use of [Fe^{IV}(O)(TMC-py)]²⁺ as synthon in place of [Fe^{IV}(O)(TMC)]²⁺ to introduce a pyridine moiety as the axial ligand and found that the main effect of the pyridine was to blue-shift the ~400-nm feature of the three-band UV-vis pattern by 20 nm and downshift the asymmetric Fe–O–Cr vibration by 20 cm^{−1} (Table 1 and Chart 1).²⁰ We have also introduced cyanate and thiocyanate as anionic axial ligands to **5** by ligand exchange and found in both cases that the addition of one equivalent of anion was sufficient to elicit systematic changes in their UV-vis spectra.¹⁷ As illustrated in Figure 2, cyanate and thiocyanate binding to **5** resulted in an increase in the absorbances of the three UV-vis absorption bands that are associated with the Fe^{III}–O–Cr^{III} chromophore.

Further experiments show that this chemistry can be extended beyond the Fe^{IV}(O)TMC family, allowing the facile formation of Cr adducts to oxoiron(IV) complexes of pentadentate ligands N4Py or BnTPEN.²³ The addition of stoichiometric Cr(OTf)₂ to CH₃CN solutions of [(N4Py)Fe^{IV}(O)]²⁺ and [(BnTPEN)Fe^{IV}(O)]²⁺ immediately caused a decrease in the intensity of the near-IR features associated with these two Fe^{IV}(O) species concomitant with the appearance of a set of three bands in the visible region characteristic of the Fe^{III}–O–Cr^{III} unit and corresponding to the formation of **7** and **8**, respectively (Figure 3, Table 1). These are more intense than those of **5** but less so than those of **5-NCO** and **5-NCS** (Figure 2). Complexes **1**–**8** are all EPR-silent, due to antiferromagnetic coupling facilitated by the oxo bridge between both Fe(III) and Cr(III) centers. The EPR results for **5-NCO**, **5-NCS**, **7**, and **8** corroborate the notion that Cr(OTf)₂ has transferred an electron to the oxoiron(IV) complexes, as previously demonstrated for **4**, **5**, and **6**.^{18,19}

Resonance Raman spectra of **5-NCO**, **5-NCS** and **6** in frozen MeCN solution were obtained with either 515-nm or 561-nm excitation (Figure 4). These compounds exhibit a feature near 800 cm^{−1} that is sensitive to ¹⁸O-substitution and has been assigned to the asymmetric Fe–O–Cr stretch (Table 1). Only the asymmetric mode was enhanced significantly, as a consequence of the heterobimetallic nature of the Fe–O–Cr unit, as first noted by Sanders-Loehr *et al.* in their studies of a series of Fe–O–Fe complexes.²⁴ On the other hand, **7** and **8**, which also have 6-coordinate Fe sites, exhibit a resonantly enhanced Raman band near 900 cm^{−1} that is sensitive to ¹⁸O-substitution and has also been assigned to the asymmetric Fe–O–Cr stretch. The vibrational data for **7** and **8** are remarkably similar to the data for **1** and **4**. This shows that coordination number cannot be the determining factor in the frequency of the Fe–O–M asymmetric stretch.

Complexes **5-NCS** and **7** were further characterized by X-ray absorption spectroscopy (Table 2). The two complexes exhibit Fe K-edge energies of 7124.3 and 7124.0 eV, consistent with values found for Fe^{III}–O–Cr^{III} complexes **4**, **5** and **6**.^{18,19} Their pre-edge areas of 9 and 13 units, which reflect the extent to which an iron atom deviates from centrosymmetry,^{25,26} are both much smaller than that of **4** but comparable to those found for

5 (11 units) and **6** (15 units). As the iron centers of **4** and **5** are respectively 5- and 6-coordinate, the smaller pre-edge areas of **5-NCS** and **7** are indicative of 6-coordinate iron centers. Bond metrics for these compounds and other Fe^{III}–O–Cr^{III} compounds summarized in Table 2 also support the conclusion.

Generation and Characterization of Fe^{III}–O–Mn^{III} complexes

Fe^{III}–O–Mn^{III} complexes could also be generated by the same strategy as the Fe^{III}–O–Cr^{III} compounds using suitable Mn^{II} complexes. Although no reaction occurred between [Mn^{II}(TPA)(OTf)₂] (Mn^{III/II} E_{p,a} = 1.82 V vs. SCE)³⁷ and oxoiron(IV) complexes supported by the pentadentate ligands N4Py and BnTPEN, reactions were observed with [Mn^{II}(dpaq)]⁺, a complex supported by a monoanionic tetradentate ligand (Mn^{III/II} E_{1/2} = –0.2 V vs. SCE),²⁷ which is more reducing than its neutral TPA counterpart. The near IR features associated with the Fe^{IV}(O) complexes decreased in intensity upon titration with [Mn^{II}(dpaq)]⁺ (Figure 7, Figure S7), and a new feature in the visible region appeared (Table 1). Figure 7 illustrates the titration of [Fe^{IV}(O)(N4Py)]²⁺ with stoichiometric Mn^{II}(dpaq)]⁺ in CH₃CN at –40 °C that results in the decrease of the 690-nm band associated with the oxoiron(IV) complex concomitant with the rise of a visible feature at 440 nm associated with [(N4Py)Fe^{III}–O–Mn^{III}(dpaq)]³⁺ (**10**). This molecular composition was confirmed by an ion at m/z 1174.0 and the associated isotope distribution (Figure 7 inset), which reproduced the simulated ion assuming a formulation of [(N4Py)FeOMn(dpaq)(OTf)₂]⁺ (Figure S4). The corresponding adduct of [(BnTPEN)Fe(O)]²⁺ (**11**) was also formed by a parallel reaction, which exhibited a visible band at 446 nm (Figure S7) and a molecular ion at m/z 1230.0 (Figure S8).

On the other hand, an immediate reaction was observed between [Mn^{II}(TPA)(OTf)₂] and [Fe^{IV}(O)(TPA)(NCMe)]²⁺ (Figure 8), suggesting that the latter is a more powerful oxidant than the corresponding N4Py and BnTPEN complexes. In this case, the 724-nm feature associated with [Fe^{IV}(O)(TPA)]²⁺ disappeared concomitant with the rise of features around 500 nm assigned to the (TPA)Fe^{III}–O–Mn^{III}(TPA) adduct **12**. An isosbestic point is found at 644 nm. The visible features of **12** at 500 and 560 nm red-shift to 540 and 577 nm upon addition of acetate (Figure S13), suggesting that the acetate acts to bridge the two metal centers, as documented for the corresponding [(TPA)₂Fe^{III}₂(μ-O)(μ-OAc)]³⁺ complex almost thirty years ago.²⁸ Indeed the two [(TPA)₂Fe^{III}M^{III}(μ-O)(μ-OAc)]³⁺ complexes share similar visible spectral features. The effect of adding acetate shows that **12** has coordination sites available for binding anions such as carboxylates that can bridge the two metal sites.

Complexes **10** – **12** have characteristic EPR spectra with a signal at *g* = 2 that exhibits a 6-line hyperfine splitting pattern (A_{iso} = 100 G) arising from ⁵⁵Mn (Figure 9). This observation is consistent with an *S* = 1/2 system where an *S* = 5/2 Fe^{III} center is antiferromagnetically coupled to an *S* = 2 Mn^{III} center. The hyperfine splitting indicates that the unpaired spin interacts strongly with the ⁵⁵Mn nuclear spin (*I* = 5/2). For comparison, the Fe^{III}Mn^{III} active site of the R2 protein of the Class 1c ribonucleotide reductase R2 exhibits a similar EPR signal at *g* = 2 with a comparable *A* value but with larger anisotropy.⁵

Resonance Raman spectra obtained for **10**, **11**, **12** and **12-OAc** at –40 °C reveal asymmetric Fe–O–Mn stretching modes that range from 780 cm^{–1} to 850 cm^{–1} and downshift

approximately 40 cm^{-1} upon ^{18}O -labeling (Table 1, Figure 10). In the case of **12**, a broad, low-intensity feature at 795 cm^{-1} is observed, which sharpens and downshifts to 785 cm^{-1} upon the introduction of acetate to form **12-OAc**. These spectral changes reflect the formation of an acetate bridge between the iron and manganese centers, thereby fixing the Fe–O–Mn angle. The latter value is quite close to the value of 770 cm^{-1} reported for the well characterized $[(\text{TPA})_2\text{Fe}^{\text{III}}_2(\mu\text{-O})(\mu\text{-OAc})]^{3+}$ complex.²⁹

XAS experiments were performed on **10**, **11**, and **12**. Their K-edge energies range from 7123.7–7124.6 eV and pre-edge areas fall between 11 and 15 units (Tables 1 and 3). These results point toward a series of complexes with 6-coordinate Fe^{III} centers and are supported by the bond metrics found from EXAFS analysis (Figure 11, Table 3). The Fe•••Mn distance changes from $\sim 3.6\text{ \AA}$ for the nearly linear **10** and **11** to $3.34\text{ \AA}$ for **12**, suggesting a decrease in the Fe–O–Mn angle of **12** that facilitates formation of the acetate bridge in **12-OAc**.

Discussion

In recent work, we have demonstrated that $\text{Fe}^{\text{IV}}(\text{O})$ species can serve as synthons for the formation of heterobimetallic $\text{Fe}^{\text{III}}\text{--O--M}$ ($\text{M} = \text{Cr}^{\text{III}}$, Sc^{III} and Ce^{IV}) complexes via an inner-sphere mechanism.^{17,18,19,20,21} In this paper, we increase the list of $\text{Fe}^{\text{III}}\text{--O--Cr}^{\text{III}}$ complexes to 7, a group that demonstrates how the coordination environment of the iron center affects the properties of the $\text{Fe}^{\text{III}}\text{--O--Cr}^{\text{III}}$ unit (Table 1). More importantly, we extend this chemistry to include four $\text{Fe}^{\text{III}}\text{--O--Mn}^{\text{III}}$ complexes (**10**, **11**, **12**, and **12-OAc**), which serve as the starting point for efforts to model the active sites of Fe/Mn enzymes like Class 1c RNRs² and R2lox^{4,11} and gain insight into how dioxygen is activated at this particular heterobimetallic active site.

Unlike $\text{Fe}^{\text{III}}\text{--O--Fe}^{\text{III}}$ complexes which show an intense band near $350(\pm 30)\text{ nm}$ with ϵ values ranging from $4000 - 13000\text{ M}^{-1}\text{ cm}^{-1}$,²⁹ each of the $\text{Fe}^{\text{III}}\text{--O--Cr}^{\text{III}}$ complexes exhibits a rich and characteristic UV-Vis spectrum consisting of three clusters of bands of decreasing intensity (Table 1). The most intense cluster of bands is found at 350 nm with ϵ values (Table 1) of $4000 - 10000\text{ M}^{-1}\text{ cm}^{-1}$, followed by a second band at $400\text{--}500\text{ nm}$ with ϵ values of $2000 - 9000\text{ M}^{-1}\text{ cm}^{-1}$ and a third band between $520 - 560\text{ nm}$ ($\epsilon = 400 - 2000\text{ M}^{-1}\text{ cm}^{-1}$). These bands are sensitive to the nature of the polydentate supporting ligand as well as the nature of the axial ligand on the Fe center (Figs 2 and 3). In particular, the introduction of cyanate and thiocyanate as axial ligands to **5** significantly increases the intensities of the UV-vis bands (Figure 2). Interestingly, the electronic spectra of **4 – 8** differ significantly from those of the $\text{Fe}^{\text{III}}\text{--O--Cr}^{\text{III}}$ complexes reported by Wieghardt and coworkers in the early '90s.³⁰ This difference probably arises from a change in the $\text{Fe}^{\text{III}}\text{--O--Cr}^{\text{III}}$ angle. Complexes **4 – 8** have linear $\text{Fe}^{\text{III}}\text{--O--Cr}^{\text{III}}$ units, while those for the Wieghardt complexes must have bent $\text{Fe}^{\text{III}}\text{--O--Cr}^{\text{III}}$ units due to the presence of the two carboxylate bridges that make the $\text{Fe}^{\text{III}}\text{--O--Cr}^{\text{III}}$ angle significantly less than 180 degrees.³¹ Less dramatic differences are observed upon formation of $\text{Fe}^{\text{III}}\text{--O--Mn}^{\text{III}}$ complexes (Figs 8 and 10).

Resonance Raman and XAS have been used to probe the nature of the $\text{Fe}^{\text{III}}\text{--O--M}^{\text{III}}$ (Tables 1 and 2). A dramatic difference is observed between the XAS pre-edge areas of complexes

with 5- and 6-coordinate iron centers. Indeed the pre-edge areas of **1** and **4** at ~30 units stand in sharp contrast to values of 9 – 15 units found for the other complexes in Table 1, which correlate with the coordination number of the iron center. On the other hand, the vibrational data we have collected for this set of Fe^{III}–O–M complexes (Table 1) provide a more nuanced assessment of the differences among these complexes. We have identified asymmetric Fe–O–M vibrations for these complexes with frequencies that span a range of over 100 cm⁻¹. The initially small subset of data available for Fe^{III}–O–M^{III} complexes suggested that the 104-cm⁻¹ difference between **4** and **5** could be simply rationalized by the difference in coordination number of the iron center, with **4** having a 5-coordinate iron center and a much higher stretching frequency than **5**, which has a 6-coordinate iron center. However the additional results presented in this paper show that coordination number is not the correct rationale, as some complexes with 6-coordinate iron centers, e. g. **7** and **8**, exhibit asymmetric Fe–O–M vibrations at the high end of the observed range. Instead, Figure 12 shows that the vibrational data for Fe–O–Cr and other Fe–O–M compounds can be reasonably correlated with the Fe–O bond length, which is perhaps not an unexpected outcome.

In order to synthesize Fe–O–Mn compounds relevant to the active sites of Class 1c RNRs and R2lox, various [(L)Fe^{IV}(O)](OTf)₂ complexes were reacted with Mn^{II} compounds. Our initial experiments using (TPA)Mn^{II}(OTf)₂ to react with [(N4Py)Fe^{IV}(O)]²⁺ and [(BnTPEN)Fe^{IV}(O)]²⁺ were not successful, but the use of a more reducing Mn^{II} complex supported by the mono-anionic dpaq ligand resulted in the reduction of the Fe^{IV}(O) precursors and formation of Fe^{III}–O–M^{III} adducts **10** and **11** with a new absorption with λ_{max} ~ 450 nm. ESI-MS, EPR and XAS confirmed the formulation of these new species as Fe–O–Mn complexes (Table 1). On the other hand, (TPA)Mn^{II}(OTf)₂ reacted with [(TPA)Fe^{IV}(O)(NCMe)]²⁺ to form the Fe^{III}–O–Mn^{III} species **12**. These results show that [Fe^{IV}(O)TPA(NCMe)₂]²⁺ is more oxidizing than the corresponding complexes supported by the pentadentate ligands N4Py and BnTPEN.

Our results have expanded on our previous work in using Fe^{IV}=O species as synthetic precursors to heterobimetallic complexes. Our results show that the asymmetric Fe–O–M vibration of these complexes is correlated with the Fe–O bond length. We have also demonstrated the first directed synthesis of an Fe–O–Mn complex, **12**, that is capable of binding exogenous ligands. This work thus provides a foundation for future efforts to synthesize high-valent mimics of the RNR 1c active site that shed light on the mechanism for O₂ activation and on the factors that modulate the redox properties of the Fe–O–Mn center.

Materials and Methods

Materials

All reagents and solvents were purchased from commercial sources and used as received unless specified. Iodosylbenzene,³¹ and ¹⁸O-labeled iodosylbenzene³² were synthesized following published procedures, as were the complexes [(TMC)Fe^{II}(OTf)](OTf),³³ [(N4Py)Fe^{II}(NCCH₃)](OTf)₂,²² [(BnTPEN)Fe^{II}(OTf)](OTf),²² [Mn^{II}(dpaq)(OTf)],^{34,26} and [Mn^{II}(TPA)(OTf)₂].³⁵ Solutions of [(TMC)Fe^{IV}(O)(NCCH₃)]²⁺, [(N4Py)Fe^{IV}(O)]²⁺ and

$[(\text{BnTPEN})\text{Fe}^{\text{IV}}(\text{O})]^{2+}$ were prepared by adding solid PhIO to a solution of the iron(II) complex in CH_3CN at room temperature for 30 min as described previously.^{22,30} The turbid liquid was kept at -40°C for 20 min and then filtered by a PTFE syringe filter for use. All moisture- and oxygen-sensitive compounds were prepared using standard Schlenk-line techniques.

Methods

Elemental analysis was carried out by Atlantic Microlab (Norcross, GA). UV-vis absorption spectra were recorded on an HP 8453A diode array spectrometer. Low-temperature visible spectra were obtained using a cryostat from UNISOKU Scientific Instruments, Japan. Electrospray mass spectrometry was performed on a Finnigan LCQ ion trap mass spectrometer. Raman spectra were collected with an Acton AM-506 monochromator equipped with a Princeton LN/CCD data collection system, with 514.5/568.2-nm excitation from a Spectra-Physics model 2060 argon-ion by Cobolt Lasers, Inc. Spectra in acetonitrile were obtained at 77 K using a 135° backscattering geometry. The detector was cooled to -120°C prior to the experiments. Spectral calibration was performed using the Raman spectrum of acetonitrile/toluene 50:50 (v:v). The collected data was processed using Spekwin32 and a multipoint baseline correction was performed for all spectra. X-band EPR spectra were collected on a Bruker Eleksys E-500 spectrometer equipped with an Oxford ESR-910 cryostat. EPR spectral integrations were carried out using a Windows software package (SpinCount v3.1.2).

Iron K-edge X-ray absorption spectra were collected on samples at SSRL beam line 7-3 using a 30 element solid state Ge detector (Canberra) with a SPEAR storage ring current of ~ 500 mA at a power of 3.0 GeV. The incoming X-rays were unfocused using a Si(220) double crystal monochromator, which was detuned by 40% of the maximal flux to attenuate harmonic X-rays. Seven scans were collected from 6882 eV to 8000 eV at a temperature (10 K) that was controlled by an Oxford Instruments CF1208 continuous flow liquid helium cryostat. An iron foil was placed in the beam pathway prior to I_0 and scanned concomitantly for an energy calibration, with the first inflection point of the edge assigned to 7112.0 eV. A 3 μm Mn filter and a Soller slit were used to increase the signal to noise ratio of the spectra. Photoreduction was monitored by scanning the same spot on the sample twice and comparing the first derivative peaks associated with the edge energy during collection.

The detector channels from the scans were examined, calibrated, averaged, and processed for EXAFS analysis using EXAFSPAK to extract $\chi(k)$. Theoretical phase and amplitude parameters for a given absorber-scatterer pair were calculated using FEFF 8.40³⁶ and utilized by the "opt" program of the EXAFSPAK package during curve fitting.³⁷ Parameters for each species were calculated using a model derived from the crystal structures of $[\text{Fe}^{\text{III}}(\text{OCH}_3)(\text{N4Py})]^{2+}$ ³⁸ and $[\text{Mn}^{\text{III}}(\text{OH})(\text{dpaq})]^{+}$.³¹ In all analyses, the coordination number of a given shell was a fixed parameter and was varied iteratively in integer steps, while the bond lengths (R) and mean-square deviation (σ^2) were allowed to freely float. Pre-edge analysis was performed on data normalized in the "process" program of the EXAFSPAK package, and pre-edge features were fit as described elsewhere²³ between 7108

eV to 7118 eV using the Fityk program with pseudo-Voigt functions composed of 50:50 Gaussian/Lorentzian functions.³⁹

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

BnTPEN	<i>N</i> -benzyl- <i>N</i> , <i>N</i> ', <i>N</i> '-tris(2-pyridylmethyl)-1,2-diaminoethane
Hdpaq	2-[bis(pyridin-2-ylmethyl)]amino- <i>N</i> -quinolin-8-yl-acetamide
N4Py	<i>N</i> , <i>N</i> -bis(2-pyridylmethyl)- <i>N</i> -bis(2-pyridyl)methylamine
TMC	1,4,8,11-tetramethylcyclam
TMC-py	1-(pyridyl-2'-methyl)-4,8,11-trimethyl-1,4,8,11-tetrazacyclotetradecane
TPA	tris(2-pyridylmethyl)amine
H₂ICIMP	2-(<i>N</i> -carboxylmethyl)-[<i>N</i> -(<i>N</i> -methylimidazolyl-2-methyl)aminomethyl]-[6-(<i>N</i> -isopropylmethyl)-[<i>N</i> -(<i>N</i> -methylimidazolyl-2-methyl)aminomethyl-4-methylphenol]]
HBPMP	2,6-bis[(bis(2-pyridylmethyl)-amino)methyl]-4-methylphenol
TACN	1,4,7-triazacyclononane
Me₃TACN	1,4,7-trimethyl-1,4,7-triazacyclononane

References

1. Högbom M, Stenmark P, Voevodskaya N, McClarty G, Gräslund A, Nordlund P. Science. 2004; 305:245–248. [PubMed: 15247479]
2. Bollinger JM, Jiang W, Green MT, Krebs C. Curr Opin Struct Biol. 2008; 18:650–657. [PubMed: 19046875]
3. Carboni M, Latour J-M. Coord Chem Rev. 2011; 255:186–202.
4. Griesse JJ, Srinivas V, Högbom M. J Biol Inorg Chem. 2014; 19:759–774. [PubMed: 24771036]
5. Jiang W, Yun D, Saleh L, Barr EW, Xing G, Hoffart LM, Maslak M-A, Krebs C, Bollinger JM. Science. 2007; 316:1188–1191. [PubMed: 17525338]
6. Jiang W, Hoffart LM, Krebs C, Bollinger JM. Biochemistry. 2007; 46:8709–8716. [PubMed: 17616152]
7. Jiang W, Yun D, Saleh L, Bollinger JM Jr, Krebs C. Biochemistry. 2008; 47:13736–13744. [PubMed: 19061340]
8. Younker JM, Krest CM, Jiang W, Krebs C, Bollinger JM, Green MT. J Am Chem Soc. 2008; 130:15022–15027. [PubMed: 18937466]

9. Martinie RJ, Blaesi EJ, Krebs C, Bollinger JM, Silakov A, Pollock CJ. *J Am Chem Soc.* 2017; 139:1950–1957. [PubMed: 28075562]
10. Andersson CS, Högbom M. *Proc Natl Acad Sci USA.* 2009; 106:5633–5638. [PubMed: 19321420]
11. Shafaat HS, Griese JJ, Pantazis DA, Roos K, Andersson CS, Popovi -Bijeli A, Gräslund A, Siegbahn PE, Neese F, Lubitz W. *J Am Chem Soc.* 2014; 136:13399–13409. [PubMed: 25153930]
12. Miller EK, Trivelas NE, Maugeri PT, Blaesi EJ, Shafaat HS. *Biochemistry.* 2017; 56:3369–3379. [PubMed: 28574263]
13. Holman TR, Andersen KA, Anderson OP, Hendrich MP, Juarez-Garcia C, Münck E, Que L Jr. *Angew Chem Intl Ed Engl.* 1990; 29:921–923.
14. Holman TR, Wang Z, Hendrich MP, Que L Jr. *Inorg Chem.* 1995; 34:134–139.
15. Carboni M, Clemancey M, Molton F, Pecaut J, Lebrun C, Dubois L, Blondin G, Latour J-M. *Inorg Chem.* 2012; 51:10447–104460. [PubMed: 22989001]
16. Das B, Daver H, Singh A, Singh R, Haukka M, Demeshko S, Meyer F, Lisensky G, Jarenmark M, Himo F, Nordlander E. *Eur J Inorg Chem.* 2014:2204–2212.
17. Hotzelmann R, Wiegardt K, Floerke U, Haupt HJ, Weatherburn DC, Bonvoisin J, Blondin G, Girerd JJ. *J Am Chem Soc.* 1992; 114:1681–1696.
18. Zhou A, Kleespies ST, Van Heuvelen KM, Que L Jr. *Chem Commun.* 2015; 51:14326–14329.
19. Zhou A, Prakash J, Rohde GT, Klein JEMN, Kleespies ST, Draksharapu A, Fan R, Guo Y, Cramer CJ, Que L Jr. *Inorg Chem.* 2016; 55:518–527. [PubMed: 28001379]
20. Ching W, Zhou A, Klein JEMN, Fan R, Knizia G, Cramer CJ, Guo Y, Que L Jr. *Inorg Chem.* 2017; 56:11129–11140. [PubMed: 28858496]
21. Prakash J, Rohde GT, Meier KK, Jasiewski AJ, Van Heuvelen KM, Münck E, Que L Jr. *J Am Chem Soc.* 2015; 137:3478–3481. [PubMed: 25743366]
22. Draksharapu A, Rasheed W, Klein JEMN, Que L Jr. *Angew Chem Int Ed.* 2017; 56:9091–9095.
23. Rohde J-U, Torelli S, Shan X, Lim MH, Klinker EJ, Kaizer J, Chen K, Nam W, Que L Jr. *J Am Chem Soc.* 2004; 126:16750–16761. [PubMed: 15612713]
24. Sanders-Loehr J, Wheeler WD, Shiemke AK, Averill BA, Loehr TM. *J Am Chem Soc.* 1989; 111:8084–8093.
25. Roe AL, Schneider DJ, Mayer RJ, Pyrz JW, Widom J, Que L Jr. *J Am Chem Soc.* 1984; 106:1676–1681.
26. Westre TE, Kennepohl P, DeWitt JG, Hedman B, Hodgson KO, Solomon EI. *J Am Chem Soc.* 1997; 119:6297–6314.
27. Wijeratne GB, Corzine B, Day VW, Jackson TA. *Inorg Chem.* 2014; 53:7622–7634. [PubMed: 25010596]
28. Norman RE, Yan S, Que L Jr, Sanders-Loehr J, Backes G, Ling J, Zhang JH, O'Connor CJ. *J Am Chem Soc.* 1990; 112:1554–1562.
29. Kurtz DM Jr. *Chem Rev.* 1990; 90:585–606.
30. Hotzelmann R, Wiegardt K, Flörke U, Haupt H-J, Weatherburn D, Bonvoisin J, Blondin G, Girerd J-J. *J Am Chem Soc.* 1992; 114:1681–1696.
31. Havare N, Plattner DA. *Org Lett.* 2012; 14:5078–5081. [PubMed: 22992207]
32. Schardt BC, Hill CL. *Inorg Chem.* 1983; 22:1563–1565.
33. Rohde J-U, In J-H, Lim MH, Brennessel WW, Bukowski MR, Stubna A, Münck E, Nam W, Que L Jr. *Science.* 2003; 299:1037–1039. [PubMed: 12586936]
34. Wang Z-W, Chen Q-Y, Liu Q-S. *Transition Met Chem.* 2014; 39:917–924.
35. Ward AL, Elbaz L, Kerr JB, Arnold J. *Inorg Chem.* 2012; 51:4694–4706. [PubMed: 22458367]
36. Ankudinov A, Ravel B, Rehr J, Conradson S. *Phys Rev B.* 1998; 58:7565–7576.
37. George, GN. EXAFSPAK Stanford Synchrotron Radiation Laboratory Stanford, CA. 1990.
38. Roelfes G, Lubben M, Chen K, Ho RY, Meetsma A, Genseberger S, Hermant RM, Hage R, Mandal SK, Young VG. *Inorg Chem.* 1999; 38:1929–1936. [PubMed: 11670967]
39. Wojdyr M. *J Appl Cryst.* 2010; 43:1126–1128.

Figure 1. Bimetallic active site structure for R2lox (or Rv0233, PDB 3EE4) showing the 2-His-4-Glu binding motif and the additional fatty acid carboxylate moiety that bridges the two metals. The Fe center is shown in brown, and the Mn center is shown in violet. The Tyr-Val crosslink is shown in the bottom left quadrant.

Figure 2.

UV-Vis absorption spectra for **4** (0.2 mM, pink trace), **5** (0.21 mM, black trace), **5-NCO** (0.21 mM, red trace), and **5-NCS** (0.21 mM, blue trace).

Figure 3.

UV-Vis absorption spectral changes upon titration of $Cr^{II}(OTf)_2$ to a 0.20 mM solution of $[(N4Py)Fe^{IV}(O)]^{2+}$ in MeCN at $-40^{\circ}C$ (blue line) to form **9** (brown line). The characteristic near-IR feature of $[(N4Py)Fe^{IV}(O)]^{2+}$ at 690 nm decreases in intensity concomitant with the growth of bands at 362, 382, 434 and 520 nm due to **9** upon addition of 0.25, 0.5, 0.75, and 1.1 eq of $Cr(OTf)_2$. Red lines correspond to spectral changes at intermediate stages of the titration.

Figure 4.

Resonance Raman spectra of **4** (maroon), **5** (blue), **5-NCS** and **5-NCO**. Black, ^{16}O ; red, ^{18}O . 5 mM in CH_3CN ; $\lambda_{\text{ex}} = 514.5$ (**4**) and 561 (**5**, **5-NCS**, **5-NCO**) nm). Spectra were collected at 77 K. Asterisk denotes CH_3CN solvent peak, and ampersands denote trifluoroethanol solvent peak using ^{18}O for the ^{18}O -labeled samples.

Figure 5. Resonance Raman spectra of **7** (top) and **8** (bottom) (5 mM in CH₃CN; $\lambda_{\text{ex}} = 514.5$ nm, 77 K). Black, ¹⁶O; red, ¹⁸O. Asterisk denotes solvent peak. The ¹⁸O-**7** sample has ~80% label incorporation from PhI¹⁸O, while the ¹⁸O-**8** sample has ~60% label incorporation from PhI¹⁸O.

Figure 6.

Top row: XANES spectra (black dots) of **5-NCS** (left) and **7** (right); baseline (green), pre-edge peak components (blue), fit (red). Bottom row: Fourier-transformed EXAFS spectra (black squares) of **5-NCS** (left) and **7** (right) overlaid with the best fits (red lines) corresponding to fit number 7 in Table S1 and fit number 8 in Table S2. Insets: corresponding unfiltered k -space data (black dots) and best fits (red lines).

Figure 7.

UV-vis absorption spectral titration of 0.3 mM $[Fe^{IV}(O)(N4Py)]^{2+}$ in CH_3CN at $-40\text{ }^{\circ}C$ with $[Mn^{II}(dpaq)](OTf)$ to generate $Fe^{III}-O-Mn^{III}$ adduct **10**. Black, 0 eq.; red, 0.25 eq.; blue: 0.5 eq.; magenta, 0.75 eq.; green, 1 eq. Inset: Isotope distribution pattern for the molecular ion of **10** (m/z 1174.0) obtained by electrospray mass spectrometry.

Figure 8.

UV-Vis absorption spectral changes upon titration of $[(\text{TPA})\text{Fe}^{\text{IV}}(\text{O})(\text{NCMe})]^{2+}$ (1 mM) (black) in MeCN at -40°C with $[\text{Mn}^{\text{II}}(\text{TPA})(\text{OTf})_2]$ to form **12** (green). Amounts of $[\text{Mn}^{\text{II}}(\text{TPA})(\text{OTf})_2]$ added: 0.25 eq (red), 0.50 eq (blue), 0.75 eq (pink), 1 eq (green).

Figure 9.

EPR spectrum of 0.5 mM **12** in frozen CH_3CN solution. Conditions: $T = 2$ K; microwave power attenuation: 30 dB. Asterisks denote features due to hyperfine splitting. Nearly identical EPR spectra of **10** and **11** are shown in Fig S10 and Fig S14, respectively.

Figure 10.

Resonance Raman spectra of Fe–O–Mn complexes in CH₃CN solution. Spectra for 0.5 mM **10** were collected using 515-nm excitation at 77 K, while spectra for 1.25 mM **11** and 2.5 mM **12-OAc** were collected with 457-nm excitation at –40 C. * denotes a CH₃CN solvent peak, # denotes a CD₃CN solvent peak, and & denotes a trifluoroethanol solvent peak. ¹⁸O-**10** has ~70% ¹⁸O incorporation from PhI¹⁸O, while ¹⁸O-**12-OAc** has ~60% ¹⁸O incorporation from H₂¹⁸O exchange into the oxo bridge.

Figure 11.

Fourier-transformed EXAFS spectra of **10** (top), **11** (middle) and **12** (bottom). Insets show corresponding unfiltered k -space EXAFS data. Black dots represent the data and red lines show the best fits. See Figures S6, S9, and S12 and Tables S3 and S4 for further details.

Figure 12.

ν_{as} values of various Fe-O-M complexes plotted versus Fe-O bond length. Black triangles represent compounds where Fe-O distances have been crystallographically characterized, while red squares represent compounds where the Fe-O distance was determined by EXAFS ($R^2 = 0.77$).

Chart 1.

Structures of the complexes listed in Table 1. Structures of complexes **1**, **2**, **3**, and **9** have been established by X-ray crystallography; those of the others are either proposed or supported by EXAFS analysis.

Table 1
UV-Vis absorption, resonance Raman, and selected XAS and XRD data for Fe^{III}-O-M^{III} complexes

Complexes	$\lambda_{\max}$ (e, M ⁻¹ cm ⁻¹)	$\nu_{\text{as}}(\text{Fe-O-M})$ (¹⁸ O) (cm ⁻¹)	Pre-edge area (units)	d(Fe-O) (Å) (XRD, XAS)	d(Fe•••M) (Å) (XRD, XAS)
(TMC)Fe ^{III} -O _{syn} -Sc ^{III} (OTf) ₄ (NCMe) ₁₈ , 2 ¹ (1)	310	878	32	1.748(5)	3.6358(17)
[(TMC)Fe ^{III} -O _{syn} -Fe ^{III} Cl ₃ (OTf) ₁₈ (2)	–	888 (–49)	–	1.742(3) ^a	3.4994(14)
(Cl)(TMC)Fe ^{III} -O _{syn} -Fe ^{III} Cl ₃ ¹⁸ (3)	–	800 (–32)	–	1.8510(12)	3.6213(5)
(TMC)Fe ^{III} -O _{syn} -C ^{III} (OTf) ₄ ¹⁸ (4)	395, 446, 515, 560	877 (–45)	30	1.77	3.58
(MeCN)(TMC)Fe ^{III} -O _{syn} -C ^{III} (OTf) ₄ ¹⁸ (5)	358, 398 (3700) 447 (3000) 558 (720)	773 (–43), 751	11	1.81	3.65
[(SCN)(TMC)Fe ^{III} -O _{syn} -C ^{III} (OTf) ₄] [–] (5-NCS)	395 (7500) 447 (6300) 558 (1400)	767 (–49) 751	9	1.85	3.67
[(OCN)(TMC)Fe ^{III} -O _{syn} -C ^{III} (OTf) ₄] [–] (5-NCO)	381 (6000) 447 (4200) 558 (850)	773 (–59) 751	–	–	–
(TMC-py)Fe ^{III} -O _{syn} -C ^{III} (OTf) ₄ ¹⁹ (6)	381 (3800) 451 (3200) 558 (920)	760 (–40)	15	1.84	3.65
(N4Py)Fe ^{III} -O-C ^{III} (OTf) ₄ (7)	368 (5200) 382 (5000) 434 (2600) 520 (640)	886 (–42) 873	13	1.78	3.56
(BnTPEN)Fe ^{III} -O-C ^{III} (OTf) ₄ (8)	376 (5200) 438 (2400) 538 (720)	877 (–47) 866	–	–	–
(N4Py)Fe ^{III} -O-Ce ^{III} (NO ₃) ₄ (OH ₂) ₂₂ (9)	490	707 (–31)	14	1.825	3.825
[(N4Py)Fe ^{III} -O-Mn ^{III} (dpaq)] ³⁺ (10)	440	846 (–41)	13	1.80	3.56
[(BnTPEN)Fe ^{III} -O-Mn ^{III} (dpaq)] ³⁺ (11)	446	815	11	1.80	3.58
[(TPA)Fe ^{III} -O-Mn ^{III} (TPA)] ⁴⁺ (12)	500, 550	795	15	1.81	3.34
[(TPA)Fe ^{III} (μ-O)(μ-OAc)-Mn ^{III} (TPA)] ³⁺ (12-OAc)	540, 577	785 (–38)	–	–	–

Table 2

Data obtained from XAS analysis of Fe–O–Cr complexes

Complex	Pre-edge area	K-Edge energy (eV)	Fe–N/O			Fe–Cr			Reference
			n	r (Å)	σ^2	n	r (Å)	σ^2	
4	30	7123.6	4	2.16	3.28	1	3.58	1.33	18
			1	1.77	4.12				
5	11	7124.0	5	2.17	4.12	1	3.65	1.00	18
			1	1.81	2.55				
5-NCS	9	7124.3	5	2.17	4.2	1	3.67	0.0	This work
			1	1.85	2.9				
6	15	7124.2	5	2.16	5.28	1	3.65	0.33	19
			1	1.84	0.43				
7	13	7124.0	5	2.15	5.27	1	3.56	–0.6	This work
			1	1.78	1.25				

Table 3

Data obtained by XAS analysis of Fe–O–Mn complexes.

Complex	Pre-edge Area (units)	K-edge energy (eV)	Fe–O/N			Fe...Mn		
			n	r	σ^2	n	r	σ^2
10	12.7	7123.7	5	2.16	4.56	1	3.56	1.90
			1	1.80	3.06			
11	11.1	7124.0	5	2.16	7.01	1	3.58	0.22
			1	1.80	3.04			
12	15.0	7124.6	5	2.13	4.64	1	3.34	1.12
			1	1.81	2.57			