

A Low-Spin Alkylperoxy–Iron(III) Complex with Weak Fe–O and O–O Bonds: Implications for the Mechanism of Superoxide Reductase

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Until recently, the removal of superoxide anion (O_2^-) from the biological milieu was believed to take place almost exclusively through the action of the superoxide dismutases. The discovery of superoxide reductase¹ (SOR), a mononuclear iron enzyme that converts O_2^- to H_2O_2 via a one-electron reduction pathway, has altered this view. The active site of SOR is unique, being comprised of a $[(N_{His})_4(S_{Cys})Fe^{II}]$ center (reduced active form, SOR_{red}). The four neutral N donors are positioned in the equatorial plane of a square pyramid, while the thiolate donor occupies an axial position trans to the putative O_2^- binding site. Thus, the iron coordination sphere in SOR resembles that of the heme iron in cytochrome P450. Spectroscopic evidence suggests that an $Fe^{III}-OO(H)$ intermediate forms during SOR turnover,¹ but unlike in P450, where this species undergoes O–O bond cleavage to form a high-valent $Fe=O$ species, the hydroperoxy group is released as H_2O_2 after protonation. It has been suggested that the spin state of the $Fe^{III}-OO(H)$ intermediate ($S = 1/2$ vs $5/2$) may play an important role in determining the outcome of this reaction,^{1b,2} but the mechanism of action of SOR and, in particular, the factors that account for the dramatically different outcomes of the chemistry of SOR versus P450 remain to be determined.

A few model complexes of SOR have recently emerged, including an example of an $Fe^{III}-OOH$ species ($S = 1/2$) that contains thiolate ligation³ and a high-spin $[N_4S_{thiolate}]Fe^{III}-OOtBu$ complex.^{2d} Here, we report the synthesis of a novel analogue of SOR_{red} , $[(15]aneN_4)Fe^{II}(SPh)]BF_4$ (**1**), and its reactivity with alkylperoxides. Specifically, we have characterized a low-spin $[N_4S_{thiolate}]Fe^{III}-OOR$ intermediate which exhibits a bonding pattern that is dramatically different from those of all previously characterized low-spin $Fe^{III}-OOR$ species.

The iron(II) complex **1** is obtained by reaction of $[15]aneN_4$ with $Fe^{II}(BF_4)_2$ followed by addition of NaSPh (Scheme 1). The structure of **1** (Figure 1) reveals that a single phenylthiolate ligand is coordinated to the iron(II) center, resulting in a five-coordinate geometry between that of square pyramidal (sp) and trigonal bipyramidal (tbp) ($\tau = 0.49$).⁴ The four neutral nitrogen donors and one thiolate ligand match the donor set found in SOR_{red} , although the geometry is closer to sp in the enzyme. A similar self-assembly reaction was used to prepare the SOR_{red} model $[(Me_4-cyclam)Fe^{II}(SPh-p-Ome)]^+$, which also exhibits a distorted geometry between sp and tbp ($\tau = 0.50$).^{5a} The Fe–N and Fe–S bond lengths for **1** are in line with other $Fe^{II}-N$ and $Fe^{II}-S$ bond distances for high-spin iron(II).⁶ The N–H groups are arranged so that H(2) points toward the axial thiolate ligand, while H(1), H(3), and H(4) lie on the opposite side of the macrocyclic plane. The NH(2)–S(1') and N(2)–S(1') distances of 2.60(5) and 3.489(4) Å,

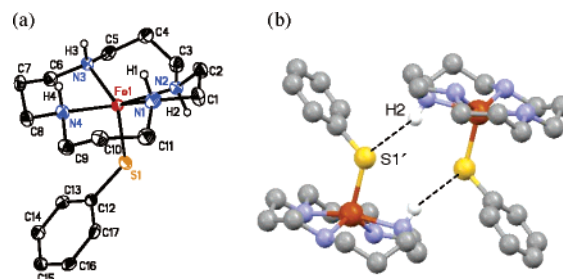
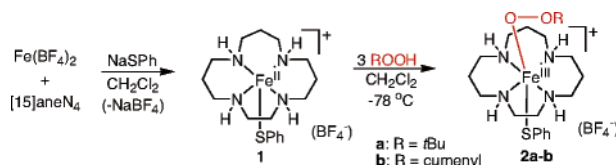


Figure 1. (a) ORTEP diagram of the cation of **1** showing 50% probability ellipsoids with some of the hydrogen atoms omitted for clarity. Selected bond lengths (Å): Fe(1)–N(1) 2.164(3), Fe(1)–N(2) 2.273(3), Fe(1)–N(3) 2.138(3), Fe(1)–N(4) 2.206(3), Fe(1)–S(1) 2.3316(11). (b) Packing diagram highlighting the intermolecular NH–S hydrogen bonds.

Scheme 1



together with an N(2)–H(2)–S(1') angle of 158(3)°, provide good evidence for the presence of an intermolecular N–H–S hydrogen bond. It is not known at this time if these intermolecular H-bonds persist in solution, but it is worth noting that, in SOR, the coordinating S_{Cys} appears to be hydrogen bonded to two N–H_{peptide} groups.^{1a}

Reaction of **1** with the alkyl hydroperoxides *t*BuOOH or cumeneOOH in CH_2Cl_2 at low temperature (-78 °C) leads to the formation of dark red intermediates **2a** (*t*BuOOH) and **2b** (cumeneOOH) (Scheme 1). These dark red species, not observed at room temperature, exhibit relatively short lifetimes at -80 °C (**2a**: $k_{obs} = 5.4 \times 10^{-3} s^{-1}$), persisting for ~ 10 min at -80 °C. This brief window of stability was enough to characterize the red intermediate by low-temperature UV–vis spectroscopy. The red intermediate for the *t*BuOOH reaction gives rise to a 526 nm absorbance ($\epsilon = 2150 M^{-1} cm^{-1}$ assuming total conversion of **1** to **2a**) (Figure 2). In comparison, the cumenyl derivative exhibits a λ_{max} at 527 nm ($\epsilon = 1650 M^{-1} cm^{-1}$). Both the position and intensity of these bands are indicative of alkylperoxy-to-iron(III) LMCT transitions, suggesting that the red intermediate is an $Fe^{III}-OOR$ complex.^{2d,7}

The EPR spectra of **2a,b** reveal characteristic patterns of low-spin iron(III) complexes, with $g = 2.20$ and 1.97 (Figure S1).⁸ Definitive identification of the chromophoric intermediate as an $Fe^{III}-OOR$ species is provided by resonance Raman (RR) spectra obtained with a 514 nm excitation in the transient absorption. The RR spectrum of **2a** exhibits bands at 439, 483, 612, and 803 cm^{-1}

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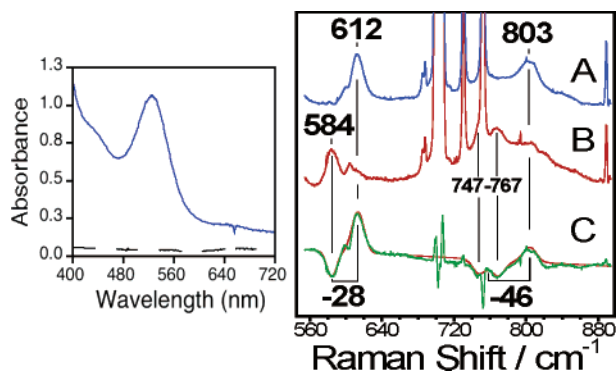


Figure 2. UV-vis spectra of **1** (black, dashed line) and **2a** (blue, solid line) in CH_2Cl_2 at -80°C , and RR spectra of ^{16}O -**2a** (A) and ^{18}O -**2a** (B). The ^{16}O - ^{18}O difference spectrum (C, green) is overlapped with a simulated trace composed of Gaussian peaks (C, red).

Table 1. Spin States and Vibrational Data for Fe^{III} -OOR Species

complex	spin state ^a	$\nu_{\text{Fe-O}}$ (cm^{-1})	$\nu_{\text{O-O}}$ (cm^{-1})	ref
$[\text{Fe}^{\text{III}}(\text{[15]janeN}_4)(\text{SPh})(\text{OOrBu})]^+$	LS	612	803	<i>b</i>
$[\text{Fe}^{\text{III}}(\text{[15]janeN}_4)(\text{SPh})(\text{OOCm})]^+$	LS	615	795	<i>b</i>
$[\text{Fe}^{\text{III}}(\text{TPA})(\text{OH})_2(\text{OOrBu})]^{2+}$	LS	696	796	7f
$[\text{Fe}^{\text{III}}(\text{L}^{\text{b}}\text{py}_2)(\text{SAr})(\text{OOrBu})]^+$	HS	623	830, 874	2d

^a LS = low-spin ($S = 1/2$); HS = high-spin ($S = 5/2$). ^b This work.

(Figures 2A and S2-A). Intermediate **2b** displays similar frequencies at 430, 490, 615, and 795 cm^{-1} (Figure S2-B). On the basis of earlier studies,⁷ signals below 500 cm^{-1} can be assigned to (C–C–C) and (C–C–O) deformation modes of the alkylperoxy ligand. The bands at 803 and 795 cm^{-1} are within the expected range of O–O stretching vibrations in metal–alkylperoxy complexes, and they compare well with other low-spin Fe^{III} -OOR species (Table 1). In contrast, O–O stretching modes from high-spin Fe^{III} -OOR species are higher in energy, as seen in Table 1. RR bands at 612 cm^{-1} for **2a** and 615 cm^{-1} for **2b** are consistent with Fe–O stretching vibrations, but they are *dramatically lower in energy* than those observed for low-spin Fe^{III} -OOR adducts.^{2d,7}

To confirm these assignments, intermediate **2a** was prepared with $t\text{Bu}^{18}\text{O}^{18}\text{OH}$. The RR spectrum of ^{18}O -labeled **2a** is shown in Figure 2B (middle), along with the ^{18}O - ^{16}O difference spectrum. The $\nu(\text{Fe}-^{18}\text{O})$ is observed at 584 cm^{-1} which represents a 28 cm^{-1} ^{18}O -downshift. The $\nu(^{18}\text{O}-^{18}\text{O})$ is downshifted 46 cm^{-1} and splits as a Fermi doublet centered at 757 cm^{-1} (Figure 2B). These ^{18}O -shifts are in complete agreement with those expected for isolated Fe–O and O–O diatomic oscillators. In contrast, vibrations observed below 500 cm^{-1} show only marginal ^{18}O -shifts, consistent with their assignment to alkyl C–C–C and C–C–O deformation modes (Figure S3). The perfect match between observed ^{18}O -shifts and predicted values for isolated diatomics supports an analysis of the observed $\nu(\text{Fe}-\text{O})$ and $\nu(\text{O}-\text{O})$ frequencies as group vibrations and permits correlations between vibrational frequencies and bond strengths.

Many mononuclear non-heme iron enzymes have been proposed to proceed via Fe^{III} -OOH reaction intermediates. Experimental and theoretical work on enzymes and models have led to a widely invoked hypothesis regarding spin state and Fe–O and O–O bond strengths in Fe^{III} -OOR complexes.^{2d,7} Low-spin intermediates have been shown to exhibit high $\nu(\text{Fe}-\text{O})$ and low $\nu(\text{O}-\text{O})$, suggesting *strong* Fe–O and *weak* O–O bonds, respectively, while high-spin species exhibit the opposite pattern (Table 1). This trend is in

agreement with the presumed mechanism of cytochrome P450 in which a low-spin Fe^{III} -OO(H) leads to O–O cleavage and formation of a high-valent $\text{Fe}=\text{O}$ intermediate. In SOR, a high-spin Fe^{3+} -OO(H) intermediate with a weak Fe–O bond could favor H_2O_2 release rather than O–O bond cleavage.²

The vibrational signatures of intermediates **2a** and **2b** show that these Fe^{III} -OOR species exhibit *weak Fe–O bonds*, in sharp contrast with the data from other low-spin Fe^{III} -OOR complexes. The weaker Fe–O bonds in **2** may be due to a trans effect from the sulfur ligand, provided it is coordinated trans and not cis to the peroxide.⁹ The sulfur ligand is believed to remain coordinated to the iron in **2** since preliminary experiments with substituted arylthiolate ligands show shifted transient absorption maxima (data not shown). Interestingly, recent DFT calculations have determined that a low-spin Fe^{III} -OOH intermediate should be energetically favored for SOR.^{2a} Our results support these predictions and the possibility of a low-spin intermediate with a weak Fe–O bond in the course of O_2^- reduction by SOR.

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Supporting Information Available: Experimental details, EPR spectra, RR data, and X-ray structure files for **1** (CIF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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- (8) Quantitation of the low-spin EPR signal reveals that it accounts for 10–30% of the starting iron(II) complex. This relatively low value is likely due to decay of the Fe^{III} -OOR species during the manual mixing time in the EPR tube. In contrast, the UV-vis spectra for **2** are obtained within a few seconds after addition of ROOH. Moreover, the ~5-fold increase in concentration of reactants in the EPR experiments compared to those used in the UV-vis will accelerate intermolecular decay pathways. Thus, it is reasonable to estimate ϵ values for **2** based on 100% conversion despite the EPR quantitation measurements.
- (9) Deuteration of **1** at the N–H positions does not change the $\nu(\text{Fe}-\text{O})$, ruling out hydrogen bonding as the cause of the low frequency.

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