A Functional Model for the Cysteine-Ligated Non-Heme Iron Enzyme Superoxide Reductase (SOR)

Terutaka Kitagawa, † Abhishek Dey, † Priscilla Lugo-Mas, † Jason B. Benedict, † Werner Kaminsky, † Edward Solomon, †,‡ and Julie A. Kovacs †,†

Department of Chemistry, University of Washington, Seattle, Washington 98195, and Department of Chemistry, Stanford University, Palo Alto, California 94304

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Super oxide reductases (SORs) are cysteine-ligated non-heme iron enzymes that reduce super oxide (O2•−) to H2O2 in anaerobic microbes. The cysteine of SOR is trans to the O2•− binding site and is proposed to play an important role in promoting the catalytic reaction. Herein, we report a rare example of a functional metallo enzyme active site model, that reduces O2•− via a trans thiolate-ligated Fe(III)-peroxo intermediate. The trans thiolate is shown to lower the redox potential, change the spin-state, and dramatically weaken the Fe—O bond, favoring O2•− reduction and H2O2 release.

Super oxide is a toxic byproduct of dioxygen chemistry that has been linked to a number of disease states. The proposed SOR mechanism involves the oxidative addition of O2•− to the open site of the square pyramidal FeII(N3H2S3) active site to afford a trans S3-ligated FeIII-peroxo intermediate. This intermediate displays an intense S-to-Fe(III) charge-transfer band at ~600 (~3500) nm, but has yet to be characterized by vibrational spectroscopy. Iron-peroxo species are extremely difficult to characterize since they are thermally unstable and photolabile. Vibrational data have been reported for mutant SOR (E47A) peroxides generated via the addition of H2O2. Whether these are identical to the catalytic SOR intermediate remains to be determined. Although a few well-characterized synthetic nitrogen-ligated iron-peroxos have been reported, there is a paucity of thiolate-ligated analogues. Since a thiolate is likely to influence the correlation between peroxide binding mode, vibrational parameters, and spin state, synthetic thiolate-ligated peroxos are needed to provide benchmark parameters.

Prior to the work reported herein, cis thiolate-ligated FeIII(S4H2N(tren)(OH))(OH)+ (1) was the only reported example of a synthetic thiolate-ligated FeIII-peroxo.

In situ depredation and deprotonation of the new macrocyclic ligand cyclam-PrS-Ac•-HCl, afforded [FeIII(cyclam-PrS)(BPh4)] (2) upon the addition of FeCl3 and NaBPh4. Single crystals were grown from pentane/THF at ~30 °C. As shown in the ORTEP (Figure 1), the Fe2+ ion of 2 is ligated by three secondary amines, one tertiary amine, and a tethered apical thiolate in a square pyramidal geometry (r = 0.13) resembling that of SOR. A related tertiary amine cyclam complex [FeIII(M2-cyclam-TeS)]+ (3) was recently reported that reacts with H2O2 to afford an FeIV=O. Like the SOR active site, 2 is high spin (S = 2; μeff = 5.03 μB (MeCN); 4.91 μB (solid)). The Fe—S bond length in 2 (2.286(1) Å) falls in the usual range for synthetic Fe(II)-thiolates, but is slightly shorter than that of SOR (Fe—S = 2.4 A), the cysteine sulfur of which is H-bonded to the protein backbone,2,5

Thiolate-ligated 2 reacts rapidly with O2•− (18-crown-6-K+ salt) in CH2Cl2 at ~78 °C to afford a metastable burgundy intermediate, as soon as a proton donor (MeOH; 82 equiv) is added. This intermediate is high-spin (g = 7.72, 5.40, 4.15), and displays an absorp-

![Figure 1](image-url)  
**Figure 1.** ORTEP of [FeIII(cyclam–PrS)]+ (2). Selected bond lengths (Å): Fe—S(1), 2.286(1); Fe—N(1), 2.181(4); Fe—N(2,3,4)avg 2.16(2).

![Figure 2](image-url)  
**Figure 2.** Raman spectra of 4 generated from 18O2– (blue), 18O2– (red), and “decayed” product (dashed black) (571 nm excitation @ 183 K in THF/MEOH (upper panel); at 77 K in CH2Cl2/THF/MEOH (lower panel).
respectively, and a protonated peroxo O–O distance of 1.44 Å. This Fe–O–O (peroxo) distance is significantly longer than the few reported Fe–O(π-OH) structures (1.76–1.86 Å)\(^{4,5}\) reflecting the trans influence of the thiolate sulfur. The calculated \(v_\text{Fe-O} = 345\) cm\(^{-1}\), \(v_\text{Fe-O} = 400\) cm\(^{-1}\), and \(v_\text{O-O} = 933\) cm\(^{-1}\) stretches are in reasonable agreement with the experimental data. When the thiolate is replaced with an amine or alkoxy, trans to the peroxo,\(^{7}\) then the calculated \(v_\text{Fe-O} = 495\) and 420 cm\(^{-1}\), respectively is considerably higher. These vibrational data, along with the calculated force constant \((k_{\text{Fe-O}} = 1.20\) mdynes/cm\(^{2}\) for 4 vs reported range = 2.2–2.1 mdynes/cm\(^{2}\)),\(^{4,6}\) indicate that the Fe–O (peroxide) bond is significantly weakened upon the introduction of a trans thiolate into the coordination sphere.

Addition of HOAc to metastable 4 at \(-78^\circ\)C releases H\(_2\)O\(_2\) (as detected using an amplex red assay), and cleanly affords a new aqua blue species \(\lambda_{\text{max}} = 604\) (1350) nm (Figure 3). If this reaction is monitored by EPR, the high-spin signal associated with 4 is replaced with a new low-spin signal at \(g = 2.37, 2.30, 1.89\). The \(v_\text{O-O} = 400\) and \(v_\text{O-O} = 933\) stretches disappear in the rRaman spectrum, and new stretches are observed at 339, 409, and 421 cm\(^{-1}\). Although this aqua blue species proved too unstable to isolate, it was unambiguously identified by ESI-mass spectrometry as acetate-bound [Fe\(^{III}\)(cyclam-PrS)(OAc)]\(^+\) (5), a model for Glu-bound SOR.

Addition of a sacrificial reductant (Cp-Co) to 5 at low temperatures \((-78^\circ\)C) regenerates 2, which then reacts with a second equivalent of O\(_2\) to re-afford peroxo 4. Addition of a second equivalent of HOAc releases H\(_2\)O\(_2\) (Figure 4), thereby mimicking the proposed SOR catalytic cycle involving glutamic acid \(^{2,3,4}\) and demonstrating that reduction of O\(_2\) by 2 is catalytic. Thus far, five turnovers have been achieved.

The thiolate ligand and its trans positioning relative to the substrate appear to contribute significantly to the function of our biomimetic catalyst. First, the pendant thiolate arm of 2 causes the redox potential to shift anodically by +480 mV relative to [Fe\(^{III}\)(cyclam)(MeCN)]\(^+\) (from +700 to +220 mV vs SCE), making it better suited to promote superoxide reduction. Second, the trans thiolate changes the spin state from \(S = \frac{1}{2}\) to \(S = \frac{5}{2}\). The majority of nitrogen-ligated Fe(III)-OOH’s are \(S = \frac{1}{2}\),\(^{17}\) as is cis-thiolate-ligated 1.\(^{4,6}\) Third, the thiolate dramatically shifts the \(v_\text{Fe-O} = 357\) cm\(^{-1}\) stretch and decreases the \(k_{\text{Fe-O}} = 0.1\) force constant well-below all other reported iron peroxides.\(^{4,6}\) Peroxo 4 partially converts to methoxide-bound [Fe\(^{III}\)(cyclam-PrS)(OMe)]\(^+\) (6; \(g = 2.34, 2.26, 1.95\); \(v_\text{Fe-O} = 357\) cm\(^{-1}\) within minutes at \(-78^\circ\)C, whereas cis-ligated peroxo 1 takes hours \((t_1/2 = 63.9\) h) to convert to [Fe\(^{III}\)(S\(^2\)Me\(^2\)N\(^2\)N\(^2\)tren)](OMe)\(^+\) under the same conditions. Methoxide-bound 6 was identified via its independent synthesis involving Cp\(_2\)Fe\(^2\) oxidation of 2 in MeOH, in the presence of Pr\(_3\)EtN.

In conclusion, the data described herein indicate that like the enzyme, SOR intermediate-analogue 4 is better suited to promote Fe–O, as opposed to O–O, bond cleavage. This is in contrast to P450 and its analogue 3. Kinetics studies and studies aimed at determining the pK\(_a\) of the proximal and distal peroxo oxygens of 4 are currently underway.

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Supporting Information Available: Detailed ligand syntheses and description of UV/vis monitored catalytic turnover, \(^{1}H\) NMR, ESI mass spectrometry (ligand, 5), EPR, \(1/2\ G\) vs \(T^2\) plot and CV of 2; UV/vis of 4. Amplex red assay, and X-ray tables. This material is available free of charge via the Internet at http://pubs.acs.org.

References


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