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

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Superoxide reductases (SORs) are cysteinate-ligated non-heme iron enzymes[1] that reduce superoxide (O_2^-) to H_2O_2 in anaerobic microbes.[2] The cysteinate of SOR is trans to the O_2^- binding site and is proposed to play an important role in promoting the catalytic reaction. Herein, we report a rare example of a functional metalloenzyme active site model, that reduces O_2^- 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 O_2^- reduction and H_2O_2 release.

Superoxide is a toxic byproduct of dioxygen chemistry that has been linked to a number of disease states.[3] The proposed SOR mechanism involves the oxidative addition of O_2^- to the open site of the square pyramidal $Fe^{II}N_4^{His}S^{Cys}$ active site[2c] to afford a trans S^{cys}-ligated Fe^{III}-peroxo intermediate.[2d,e] 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) peroxos generated via the addition of H_2O_2.[2b,f,g] 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,[4a,c] there is a paucity of thiolate-ligated analogues.[4b] 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 $[Fe^{III}(S^{Me2}N_4(tren))(OOH)]^+$ (**1**)[4b] was the only reported example of a synthetic thiolate-ligated Fe^{III}-peroxo.

In situ deprotection and deprotonation of the new macrocyclic ligand cyclam-PrS-Ac·4HCl, afforded $[Fe^{II}(cyclam\text{-}PrS)](BPh_4)$ (**2**) upon the addition of $FeCl_2$ and $NaBPh_4$. Single crystals were grown from pentane/THF at −30 °C. As shown in the ORTEP (Figure 1), the Fe^{2+} ion of **2** is ligated by three secondary amines, one tertiary amine, and a tethered apical thiolate in a square pyramidal geometry ($\tau = 0.13$)[5] resembling that of SOR. A related tertiary amine cyclam complex $[Fe^{II}(Me_3\text{-}cyclam\text{-}EtS)]^+$ (**3**) was recently reported[6] that reacts with H_2O_2 to afford an Fe(IV)=O.[7] Like the SOR active site, **2** is high spin ($S = 2$; $\mu_{eff} = 5.03\ \mu_B$ (MeCN); 4.91 μ_B (solid)). The Fe−S bond length in **2** (2.286(1) Å) falls in the usual range for synthetic Fe(II)-thiolates,[6,8] but is slightly shorter than that of SOR (Fe−S = 2.4 Å), the cysteinate sulfur of which is H-bonded to the protein backbone.[2a,c]

Thiolate-ligated **2** *reacts rapidly with* $O_2^{-\bullet}$ (18-crown-6-K^+ salt) in CH_2Cl_2 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 absorption band at $\lambda_{max} = 530$ (1350) nm. Resonance Raman shows ν_{O-O}, ν_{Fe-O}, and ν_{Fe-S} stretches at 891 (Fermi doublet), 419, and 352 cm^{-1}, respectively (Figure 2). $K^{18}O_2$ (50% enriched; ICON) causes the ν_{O-O} and ν_{Fe-O} to shift to 856 and 400 cm^{-1}, respectively, and addition of D^+ (i.e., MeOD) causes the Fermi doublet to collapse. These data are consistent with the formation of an Fe−hydroperoxo species, $[Fe^{III}(cyclam\text{-}PrS)(OOH)]^+$ (**4**), via the proton-dependent oxidative addition of superoxide to **2**. No reaction occurs in the absence of a proton donor, and O_2^- does not convert to H_2O_2 in the absence of **2**, under the conditions examined (−78 °C, 82 equiv MeOH). Intermediate **4** represents the first example of a synthetic trans thiolate-ligated Fe^{III}-peroxo SOR intermediate analogue, the characterization of which provides important benchmark parameters.

The ν_{Fe-O} *stretch* of **4** is *significantly lower than all other reported synthetic iron peroxides* (range: 450−639 cm^{-1})[4a] but compares well with that of the only reported SOR peroxo (438 cm^{-1}).[2a,e] The ν_{O-O} stretch (891 cm^{-1}) is unusually high (reported range: 820−860 cm^{-1}).[4a] The DFT optimized structure of **4** minimizes with Fe−S and Fe−O distances of 2.36 and 1.95 Å,

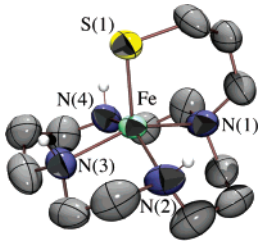

Figure 1. ORTEP of $[Fe^{II}(cyclam-PrS)]^+$ (**2**). Selected bond lengths (Å): Fe−S(1), 2.286(1); Fe−N(1), 2.181(4); Fe−$N(2,3,4)_{avg}$, 2.16(2).

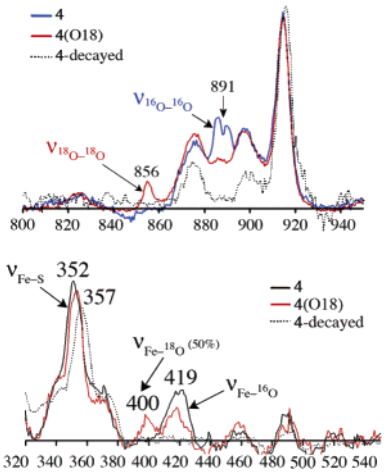

Figure 2. rRaman spectra of **4** generated from $^{16}O_2^-$ (blue), $^{18}O_2^-$ (red), and "decayed" product (dashed black) (571 nm excitation @ 183 K in THF/MeOH (upper panel); at 77 K in CH_2Cl_2/THF/MeOH (lower panel).

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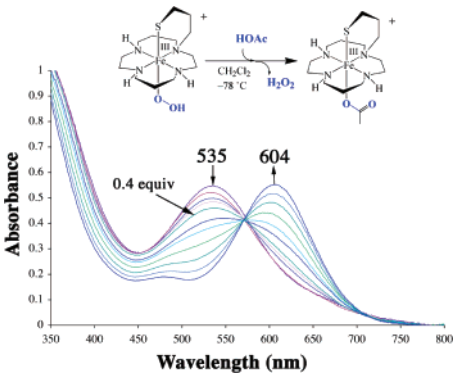

Figure 3. Conversion of peroxo-bound **4** (0.5 mM in CH_2Cl_2) to acetate-bound $[Fe^{III}(cyclam\text{-}PrS)(OAc)]^+$ (**5**) via the addition of HOAc (0.1 equiv aliquots every 2 min) at −78 °C.

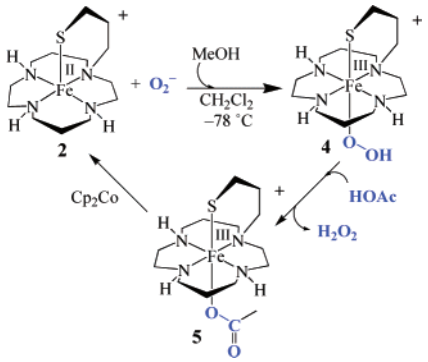

Figure 4. The catalytic cycle involving $[Fe^{II}(cyclam\text{-}PrS)]^+$ (**2**) induced superoxide (O_2^-) reduction

respectively, and a protonated peroxo O−O distance of 1.44 Å. This Fe−O (peroxo) distance is significantly longer than the few reported Fe−(η^1-OOH) structures (1.76−1.86 Å)[4a,b] reflecting the trans influence of the thiolate sulfur. The calculated ν_{Fe-S} (345 cm^{-1}), ν_{Fe-O} (400 cm^{-1}), and ν_{O-O} (933 cm^{-1}) stretches are in reasonable agreement with the experimental data. When the thiolate is replaced with an amine or alkoxide, trans to the peroxo,[9] then the calculated ν_{Fe-O} (495 and 420 cm^{-1}, respectively) is considerably higher. These vibrational data, along with the calculated force constant (k_{Fe-O} = 1.20 mdynes/cm^2 for **4** vs reported range = 2.2−2.1 mdynes/cm^2),[4a] 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 °C releases H_2O_2 (as detected using an amplex red assay), and cleanly affords a new aqua blue species λ_{max} = 604 (1350) nm (Figure 3). When 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 ν_{O-O} and ν_{Fe-O} 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 $[Fe^{III}(cyclam\text{-}PrS)(OAc)]^+$ (**5**), *a model for Glu-bound SOR*.

Addition of a sacrificial reductant (Cp_2Co) to **5** at low temperatures (−78 °C) regenerates **2**, which then reacts with a second equivalent of $O_2^{-\bullet}$ to re-afford peroxo **4**. Addition of a second equivalent of HOAc releases H_2O_2 (Figure 4), thereby mimicking the proposed SOR catalytic cycle involving glutamic acid,[2d,e,i] and demonstrating that reduction of $O_2^{-\bullet}$ 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 $[Fe^{II}(cyclam)(MeCN)_2]$ (from +700 to +220 mV vs SCE), making it better suited to promote superoxide reduction. Second, the trans thiolate changes the spin state from $S = {}^1/_2$[4b] to $S = {}^5/_2$: the majority of nitrogen-ligated Fe(III)-OOH's are $S = {}^1/_2$,[4a] as is cis thiolate-ligated **1**.[4b] Third, the thiolate dramatically shifts the ν_{Fe-O} stretch and decreases the k_{Fe-O} force constant well-below all other reported iron peroxides.[4a] Peroxo **4** partially converts to methoxide-bound $[Fe^{III}(cyclam\text{-}PrS)(OMe)]^+$ (**6**; g = 2.34, 2.26, 1.95; ν_{Fe-S} = 357 cm^{-1}) within minutes at −78 °C, whereas cis-ligated peroxo **1** takes hours ($t_{1/2}$ = 63.9 h) to convert to $[Fe^{III}(S^{Me2}N_4(tren))(OMe)]^+$ under the same conditions. Methoxide-bound **6** was identified via its independent synthesis involving Cp_2Fe^+ oxidation of **2** in MeOH, in the presence of $^iPr_2EtN$.

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/χ vs T 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.

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