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 that reduce superoxide (O\textsubscript{2}\textsuperscript{-}) to H\textsubscript{2}O\textsubscript{2} in anaerobic microbes. The cysteinate of SOR is trans to the O\textsubscript{2}\textsuperscript{-} 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\textsubscript{2}\textsuperscript{-} via a trans thiolate-ligated Fe(III)-peroxo intermediate.

Figure 1. ORTEP of [Fe\textsuperscript{III}(cyclam-PrS)]\textsuperscript{2+} (2). Selected bond lengths (Å): Fe-S(1), 2.286(1); Fe-N(1), 2.181(4); Fe-N(2,3,4)\textsubscript{avg}, 2.10(2).

Figure 2. rRaman spectra of 4 generated from \textsuperscript{18}O\textsubscript{2}\textsuperscript{-} (blue), \textsuperscript{18}O\textsubscript{2}\textsuperscript{-} (red), and “decayed” product (dashed black) (571 nm excitation @ 183 K in THF/MeOH (upper panel); at 77 K in CH\textsubscript{2}Cl\textsubscript{2}/THF/MeOH (lower panel)).
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(II)–O–O (peroxo) structures (1.76–1.86 Å)4,5a reflecting the trans influence of the thiolate sulfur. The calculated νFe=O (345 cm⁻¹), νFe–O (400 cm⁻¹), and νO–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,4,5 then the calculated νFe=O (495 and 420 cm⁻¹), respectively is considerably higher. These vibrational data, along with the calculated force constant (kFe=O = 1.20 mdynes/cm² for 4 vs reported range = 2.2–2.1 mdynes/cm²),4,5a indicate that the Fe–O (peroxo) 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 H₂O₂ (as detected using an amplex red assay), and cleanly affords a new aqua blue species λmax = 604 (1350) nm (Figure 3). As 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 νO–O and νFe–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 [Fe⁴⁺(cyclam-PrS)(OAc)]⁺ (5), a model for Glu-bound SOR.

Addition of a sacrificial reductant (Cp₂Co) to 5 at low temperatures (−78 °C) regenerates 2, which then reacts with a second equivalent of O₂⁻ to re-afford peroxo 4. Addition of a second equivalent of HOAc releases H₂O₂ (Figure 4), thereby mimicking the proposed SOR catalytic cycle involving glutamic acid 2d,e and demonstrating that reduction of O₂⁻ 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 [Fe⁴⁺(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 to S = 5/2; the majority of nitrogen-ligated Fe(III)-OOH’s are S = 1/2,4,5a as is cis-thiolate- ligated 1.4b Third, the thiolate dramatically shifts the νFe=O stretch and decreases the kFe=O force constant well-below all other reported iron peroxides.4,5a Peroxo 4 partially converts to methoxide-bound [Fe⁴⁺(cyclam-PrS)(OMe)]⁺ (6; g = 2.34, 2.26, 1.95; νFe=O = 357 cm⁻¹) within minutes at −78 °C, whereas cis-ligated peroxo 1 takes hours (t½ = 63.9 h) to convert to [Fe⁴⁺(SMe₂N(trn))(OMe)]⁺ under the same conditions. Methoxide-bound 6 was identified via its independent synthesis involving Cp₁Fe⁺ oxidation of 2 in MeOH, in the presence of Pr₃EtN⁺.

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 πKₐ 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, 1H NMR, ESI mass spectrometry (ligand, S) EPR, 1Jg vs F 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