Role of Protons in Superoxide Reduction by a Superoxide Reductase Analogue

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Superoxide reduction by thiolate-ligated [FeII(SMe2N4(tren))]+ (1) involves two proton-dependent steps and a single peroxide intermediate, [FeIII(SMe2N4(tren))(OOH)]2+ (2). An external proton donor is required, ruling out mechanisms involving H3O+ or H-atom abstraction from the ligand N-H. The initial protonation step affording 2 occurs with fairly basic proton donors (EtOH, MeOH, NH4+) in THF. More acidic proton donors are required to cleave the Fe–O(peroxide) bond in MeOH, and this occurs via a dissociative mechanism. Reaction rates are dependent on the pKa of the proton donor, and a common [FeIII(SMe2N4(tren))(MeOH)]2+ (3) intermediate is involved. Acetic acid releases H2O2 from 2 under pseudo-first-order conditions ([HOAc] = 138 mM, [2] = 0.49 mM) with a rate constant of 8.2 × 10−4 s−1 at −78 °C in MeOH. Reduction of 3 with Cp2Co regenerates the active catalyst 1.

Superoxide reductases (SORs) are non-heme iron enzymes that reduce superoxide (O2−) to H2O2 in anaerobic microbes.1a–j The catalytically active form of this enzyme contains reduced Fe2+ ligated by four N bind and a S trans to an open coordination site (Figure 1).1i The oxidized SOR resting state contains a highly conserved Glu-CO2− (14Glu or 47Glu) coordinated to this site.1e,h Upon reduction, this Glu-CO2− dissociates to regenerate the open binding site necessary for catalysis (reaction 3, Figure 1). The mechanism by which

Figure 1. Proposed mechanism of superoxide reduction by the non-heme iron enzyme superoxide reductase (SOR).

SOR is proposed to reduce superoxide has been controversial, particularly with regard to the number of intermediates involved.1a,b,e,g It is generally agreed that the mechanism involves initial coordination of O2− to the open coordination site and is thus inner-sphere.1f After this step, two intermediates are observed in the reaction between D. baarsii SOR and O2−.1b,2 whereas only one intermediate is observed in the reaction between D. vulgaris SOR and O2−.1a,e,g DFT calculations indicate that an end-on FeIII−OOH intermediate is most likely involved.1g It has been suggested that the nearby 47Glu-CO2− displaces peroxide from the final intermediate.1b,c However, it is not clear whether the Glu-bound state is involved in the catalytic cycle or whether a solvent-bound form1a would be catalytically more competent. Protons clearly play an important role in the SOR mechanism. The source of protons (in both the first and second protonation steps) has also been a point of discussion. Under acidic conditions, 14Glu-COOH has been proposed to serve as a proton source.1a,e Solvent was shown to provide protons in the D. baarsii SOR mechanism.1b A highly conserved Lys-NH3+ residue (15Lys or 48Lys), essential for catalytic activity,2 has been shown to affect rates of H2O2 formation, thus suggesting its involvement in the mechanism.1a,e A condensed version of the SOR mechanism is presented in Figure 1.

The active site of SOR closely resembles that of P450,3 and both enzymatic mechanisms are proposed to involve an

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Fe\textsuperscript{III}−OOH intermediate and initial protonation of the distal oxygen. The site to which the second proton is delivered, as well as the spin state,\(^{4b}\) determines whether the Fe−O or O−O bond is cleaved.\(^{4a−c}\) Double protonation of the distal oxygen and an S = 1/2 spin state\(^{4b}\) would result in heterolytic O−O bond cleavage to form H\(_2\)O and a high-valent Fe\textsuperscript{V}O species, whereas protonation of the proximal oxygen and an S = 3/2 spin state would result in Fe−O bond cleavage to afford H\(_2\)O\(_2\). The only observed peroxide in SOR is an S = 3/2 (generated via H\(_2\)O\(_2\) addition to a Glu → Ala mutant),\(^{1c}\) thus it is likely that H\(_2\)O\(_2\) release by SOR involves protonation at the Fe\textsuperscript{III}−OOH proximal oxygen.

Our group has demonstrated that thiolate-ligated [Fe\textsuperscript{II}, (S\textsuperscript{Me2}N\(_4\)(tren))]\(^2\+\) (1) (Figure 2) will react with O\(_2\)\(^−\) in MeOH at low temperatures to generate the first synthetic example of an Fe\textsuperscript{V}−O−O species containing sulfur in the coordination sphere, [Fe\textsuperscript{III}(S\textsuperscript{Me2}N\(_4\)(tren))(OOH)]\(^2\+\) (2).\(^{3a,b}\) Intermediate 2 is low-spin (S = 1/2) and displays a \�\(N\textsubscript{o-N}\) value of 784 cm\(^−1\) (that shifts to 753 cm\(^−1\) upon isotopic labeling with \^18O\(_2\)). A charge-transfer transition at 455 (2900) nm, and a coordinated diatomic oxygen ligand with one short, and one long Fe−O distance at 1.86(3) and 2.78(3) Å, respectively, as determined by EXAFS.\(^{5a}\) The charge-transfer transition associated with 2 is unusually high in energy for a \textsuperscript{S} → Fe(III) CT transition.\(^{6,7}\)

In this work, we explore the addition of proton and electron sources to our functional model\(^{5b}\) in order to model reaction steps 1–3 of the proposed SOR mechanism (Figure 1). Pre-

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**Figure 2.** Biomimetic SOR analogue reduces superoxide in two proton-dependent steps via an Fe\textsuperscript{III}−OOH intermediate 2. The reduced catalyst 1 is regenerated via HOAc-promoted H\(_2\)O\(_2\) release, followed by reduction with cobaltocene.
2 reacts with HOAc with a rate constant of $8.2 \times 10^{-4}$ s$^{-1}$ at $-78 \, ^\circ$C in MeOH. Reaction rates are dependent on the pK$_a$ of the proton donor; reactions are complete in seconds with stronger acids (HBF$_4$, HClO$_4$) vs hours with HOAc. The fact that a common MeOH-bound intermediate is observed in these reactions suggests that H$_2$O$_2$ release occurs via a proton-induced dissociative mechanism. An associative mechanism involving nucleophilic displacement is ruled out by the fact that NH$_4^+$OAc$^-$ does not release H$_2$O$_2$ from 2 in MeOH.\textsuperscript{13} That a similar mechanism is most likely involved in H$_2$O$_2$ release from the SOR enzyme.\textsuperscript{1a} The proton dependence of the first step of our mechanism agrees with Sawyer’s reported thermodynamic and kinetic data for superoxide-induced oxidation reactions\textsuperscript{10} but differs from that of SOR.\textsuperscript{1a,b} The cis, instead of trans, arrangement between the thiolate and the peroxide of our model, versus the enzyme, might be responsible for the mechanistic differences between our model and the enzyme. The fact that we observe only one (hydro)peroxide-bound intermediate, as opposed to two, suggests that the mechanism differs from that of the \textit{D. baarsii} SOR enzyme.\textsuperscript{1b,2} Possible mechanisms consistent with the proton dependence of the formation of 2 would involve either initial protonation of superoxide to afford HO$_2^-$, a more potent oxidant than O$_2^-$, or initial protonation of the thiolate sulfur of 1 to afford a dicationic species that has a higher affinity for O$_2^-$. More detailed kinetic studies of both the proton-induced formation of Fe$^{III}$-OOH (2) and the proton-induced release of H$_2$O$_2$ are currently underway in our laboratory.

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**Supporting Information Available:** Experimental section describing compound preparation; kinetic plot for 2 + HOAc (Figure S-1); electronic absorption spectra showing HClO$_4$-induced conversion of 2 to 3 (Figure S-2), HBF$_4$-induced conversion of 6 to 3 (Figure S-3), and HBF$_4$-induced conversion of 2 to 3 (Figure S-4). This information is available free of charge via the Internet at http://pubs.acs.org.

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