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# Iron(II)-Thiolate *S*-Oxygenation by $O_2$: Synthetic Models of Cysteine Dioxygenase

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***Abstract:*** The synthesis of structural and functional models of the active site of the nonheme iron enzyme cysteine dioxygenase (CDO) is reported. A bis(imino)pyridine ligand scaffold was employed to synthesize a mononuclear ferrous complex, $Fe^{II}(LN_3S)(OTf)$ (**1**), which contains three neutral nitrogen donors and one anionic thiolato donor. Complex **1** is a good structural model of the Cys-bound active site of CDO. Reaction of **1** with $O_2$ results in oxygenation of the thiolato sulfur, affording the sulfonato complex $Fe^{II}(LN_3SO_3)(OTf)$ (**2**) under mild conditions. Isotope labeling studies show that $O_2$ is the sole source of O atoms in the product and that the reaction proceeds via a dioxygenase-type mechanism for two out of three O atoms added, analogous to the dioxygenase reaction of CDO. The zinc(II) analog, $Zn(LN_3S)(OTf)$ (**4**), was prepared and found to be completely unreactive toward $O_2$, suggesting a critical role for $Fe^{II}$ in the oxygenation chemistry observed for **1**. To our knowledge, *S*-oxygenation mediated by an $Fe^{II}-SR$ complex and $O_2$ is unprecedented.

The utilization of $O_2$ for the oxidation of organic substrates is a critical process carried out by metalloenzymes and a highly desirable one for synthetic chemists to replicate. Cysteine dioxygenase (CDO) is a mononuclear nonheme iron enzyme that catalyzes the *S*-oxygenation of cysteine to cysteine sulfinic acid with $O_2$ as oxidant (Figure 1).[1] Loss of CDO function has been correlated with Alzheimer's, Parkinson's, and other neurological disorders. CDO contains a mononuclear $Fe^{II}$ center bound by three His ligands, in contrast to the 2-His-1-carboxylate "facial triad" that is the canonical motif for nonheme Fe oxygenases. This unexpected structural variation suggests that the ligation of three neutral N donors may be important for CDO function.[1h] X-ray crystal structures of the native iron(II) CDO,[1b] a Cys-bound complex,[1c] and an intriguing Cys-persulfenate species[1f] have been determined (Figure 1). Little is known regarding the mechanism of CDO, although the persulfenate structure suggests an $Fe-O_2$ intermediate may be important.

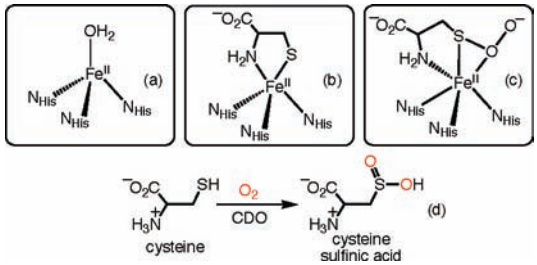
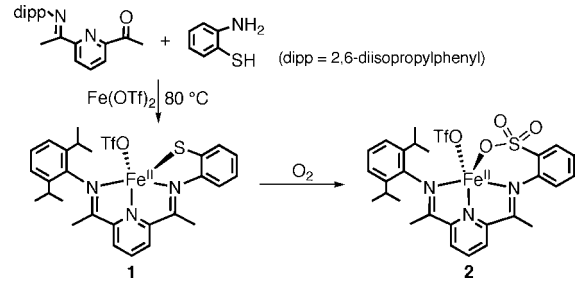



***Figure 1.*** Depiction of the active sites of CDO derived from X-ray crystallography for (a) the iron(II) resting state, (b) the Cys-bound form, and (c) a trapped persulfenate complex; (d) CDO reaction scheme.

Herein we describe the first structural and functional synthetic models of CDO. To obtain biologically relevant models, we targeted polydentate ligand platforms that would (1) provide three neutral N donors, (2) stabilize $Fe^{II}$, (3) allow for the facile incorporation of a thiolate donor, and (4) include steric protection against the formation of O- or S-bridged Fe complexes. These criteria were met with the metal-templated synthesis of $LN_3S$, a bis(imino)pyridine ligand in which a pendant thiolate donor has been incorporated.[2] Herein it is shown that an $Fe^{II}(LN_3S)$ complex reacts with $O_2$ via sulfur oxygenation. To our knowledge, *S*-oxygenation of a well-defined $Fe^{II}-SR$ species with $O_2$ is unprecedented.

Reaction of the unsymmetrical ketone 2-(O=CMe)-6-(2,6-($^i$Pr$_2$—$C_6H_3N$=CMe)—$C_5H_3N$ with 2-aminothiophenol in the presence of $Fe^{II}(OTf)_2$ and $Et_3N$ at 80 °C in ethanol affords the desired dark brown $Fe^{II}$ complex $[Fe^{II}(LN_3S)(OTf)]$ (**1**) in good yield (86%) (Scheme 1).

***Scheme 1***




The molecular structure of **1** is shown in Figure 2. The $Fe^{II}$ ion is bound by the three neutral N donors and the thiolate S donor of the $LN_3S$ ligand in a distorted square pyramidal geometry ($\tau = 0.12$), with the $OTf^-$ anion occupying the axial position. The diisopropyl substituents are projected orthogonal to the pseudoequatorial $N_3S$ plane, providing significant steric protection of the metal center. The Fe—N/S/O distances are consistent with a high-spin $Fe^{II}$ complex.[3]

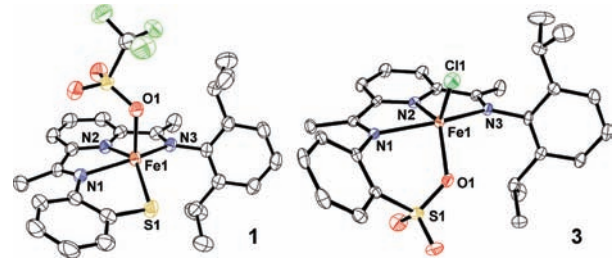



***Figure 2.*** Displacement ellipsoid plots (50% probability level) of **1** and **3**. The H atoms are omitted for clarity.

Addition of excess $O_2$ to **1** in $CH_2Cl_2$ leads to an immediate color change from black to brown. Analysis of the reaction mixture by laser-desorption ionization mass spectrometry (LDIMS) shows the complete loss of starting material after 24 h and the appearance of a prominent ion at *m*/*z* 532.1, consistent with the triply oxygenated cation $[Fe^{II}(LN_3SO_3)]^+$ of **2** (Scheme 1). The reaction is solvent independent, giving the same product in $CH_3CN$ or THF. Reaction mixtures at earlier times (e.g., 5—180 min) contain starting material **1** ($[Fe^{II}(LN_3S)]^+$, *m*/*z* 484.2) and **2**, together with a smaller peak at *m*/*z* 516.1,



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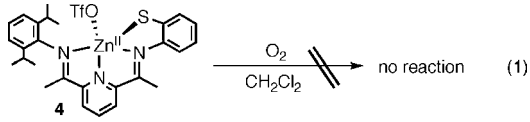
corresponding to a doubly oxygenated product which disappears as the reaction proceeds. The peak at *m/z* 516.1 is consistent with either a sulfinato ($RSO_2^-$) complex or a persulfenate species analogous to that seen for CDO. A sulfenato ($RSO^-$) complex is not observed.

Attempts to crystallographically characterize **2** after $O_2$ addition were unsuccessful. However, demetalation and acid hydrolysis (1 M HCl), followed by quantitative reversed-phase HPLC ($H_2O/CH_3CN$ 95/5, 0.1% TFA), show that the expected oxygenated organic fragment 2-$H_2N-C_6H_4SO_3H$ is formed in good yield (60%). These data confirm that *S*-oxygenation occurs upon reaction of $O_2$ with **1**. EPR spectra at 15 K of mixtures of **1** + $O_2$ reveal a signal for high-spin $Fe^{III}$ ($g$ 4.3), but double integration shows this signal accounts for less than 5 ± 2% of the total iron content. The lack of a significant EPR signal indicates a +2 oxidation state for **2**. Quantitation with 1,10-phenanthroline yields a total $Fe^{II}$ content of 91% after $O_2$ addition (see Supporting Information).

Further support for the identity of **2** comes from the synthesis of a close analog. A template reaction with $Fe^{II}Cl_2$, the unsymmetrical ketone 2-$H_2N-C_6H_4SO_3H$, and $Et_3N$ followed by recrystallization from $CH_3CN/iPr_2O$ affords [$Fe^{II}(LN_3SO_3)$(Cl)] (**3**) (Figure 2). The sulfonato group coordinates as expected to the $Fe^{II}$ center, completing a distorted square pyramidal geometry ($\tau$ = 0.33) with the N and Cl donors. Thus complex **3** is a reasonable structural analog of the sulfonato product **2** proposed in Scheme 1.

Isotopic labeling studies provide important mechanistic information regarding the oxygenation reaction. Addition of $^{18}O_2$ (98%) to **1** results in fully labeled [$Fe^{II}(LN_3S^{18}O_3)$]$^+$ (Figure 3). In contrast, no $^{18}O$ incorporation is observed when the reaction is run in the presence of excess $H_2{}^{18}O$. Thus $O_2$ is the source of *S*-oxygenation in **2**, which parallels the results obtained from $^{18}O$-labeling studies with CDO.[1f] Two mechanistic possibilities for the formation of complex **2** are (1) incorporation of an intact molecule of $O_2$ before or after the addition of a single O atom (2 + 1 case) or (2) single O atom addition for all three sulfonato oxygens (1 + 1 + 1 case). Reaction of **1** with a mixture of $^{18}O_2/^{16}O_2$ (~49:51), followed by LDIMS and statistical simulation of the isotopic distribution pattern in **2**, provides a means for distinguishing these two possibilities.[4] Simulations of the isotopic envelope show that the 2 + 1 mechanism is the dominant pathway (Figures 3 and S1). This pathway indicates that a dioxygenase-type reaction is occurring, as seen for CDO. The failure to detect a singly oxygenated sulfenato complex at earlier reaction times suggests that the third O atom is incorporated after dioxygenation, not before.




The role of the $Fe^{II}$ ion in the *S*-oxygenation of **1** is not yet known, and mechanisms that involve both redox and nonredox pathways can be envisioned.[1e] However, synthesis of the redox-inert $Zn^{II}$ analog [$Zn(LN_3S)(OTf)$] (**4**) provides some initial insights.[5] Exposure of **4** to $O_2$ for up to 7 d at 25 °C (eq 1) gives no reaction as determined by $^1$H NMR and LDIMS. Thus the requirement for iron(II), the native metal in CDO, appears to be critical for the *S*-oxygenation of **1**.

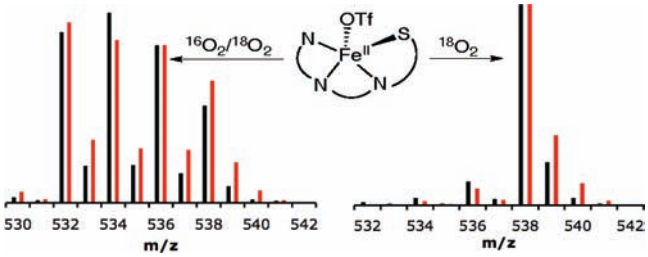



***Figure 3.*** Oxygen isotope studies using LDIMS. $^{18}O_2/^{16}O_2$ (~49/51) mixture (left) and $^{18}O_2$ (98%) (right). Exptl (black), simulation (red).

There are only a few reports of $O_2$-mediated *S*-oxygenation of $Fe^{III}-SR$ complexes.[6,7] However, prior to the present study, the reaction of $O_2$ with $Fe^{II}-SR$ complexes has led only to the formation of $Fe^{III}-O-Fe^{III}$ complexes, in lieu of *S*-oxygenates.[8] Interestingly, Darensbourg observed that the site of O-capture (Fe vs S) in the reaction of $Fe^{II}-SR$ + $O_2$ resulted in the exclusive selection of Fe over S.[8a] Our findings establish that an $Fe^{II}-SR$ complex, in the appropriate ligand environment, can selectively react with $O_2$ to yield *S*-oxygenates. Further examination of **1** and related complexes should provide new, general insights regarding Fe/S/$O_2$ reactivity.

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**Supporting Information Available:** Experimental details, spectra, and crystallographic data for complexes **1**, **3**, and **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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