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

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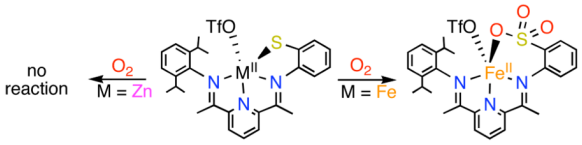
Abstract



The synthesis of structural and functional models of the active site of the non-heme 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 3 neutral nitrogen 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 non-heme 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 3 His ligands, in contrast to the 2-His-1-carboxylate "facial triad" that is the canonical motif for non-heme 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 species1f 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.

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 3 neutral N donors, 2) stabilize Fe^{II}, 3) allow for the facile incorporation of a thiolate donor

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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 htpp://pubs.acs.org.

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). 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 pseudo-equatorial 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

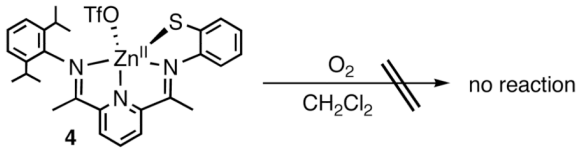
Addition of excess O_2 to **1** in CH_2Cl_2 leads to an immediate color change from black to brown. Analysis of the reaction mixture by laser-desorption ionization mass spectrometry (LDIMS) shows the complete loss of starting material after 24 h and the appearance of a prominent ion at *m/z* 532.1, consistent 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, corresponding to a doubly-oxygenated product which disappears as the reaction proceeds. The peak at 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 reverse-phase HPLC (H_2O/CH_3CN 95/5, 0.1% TFA) shows 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 $Fe^{II}Cl_2$, unsymmetrical ketone 2-(O=CMe)-6-(2,6-($^{i}Pr_2$-C_6H_3N=CMe)-C_5H_3N, 2-H_2N-$C_6H_4SO_3H$ and Et_3N followed by re-crystallization 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 (Figure 3 and Figure 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.



(1)

The role of the Fe^{II} ion in the *S*-oxygenation of **1** is not yet known, and mechanisms that involve both redox and non-redox pathways can be envisioned.1e However, synthesis of the redox-inert Zn^{II} analog [Zn(LN$_3$S)(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**.

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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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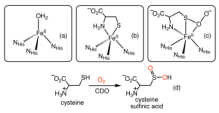


Figure 1.
Depiction of the active sites of CDO derived from X-ray crystallography for (a) the iron(II) resting state (b) the Cys-bound form and (c) a trapped persulfenate complex; (d) CDO reaction scheme.

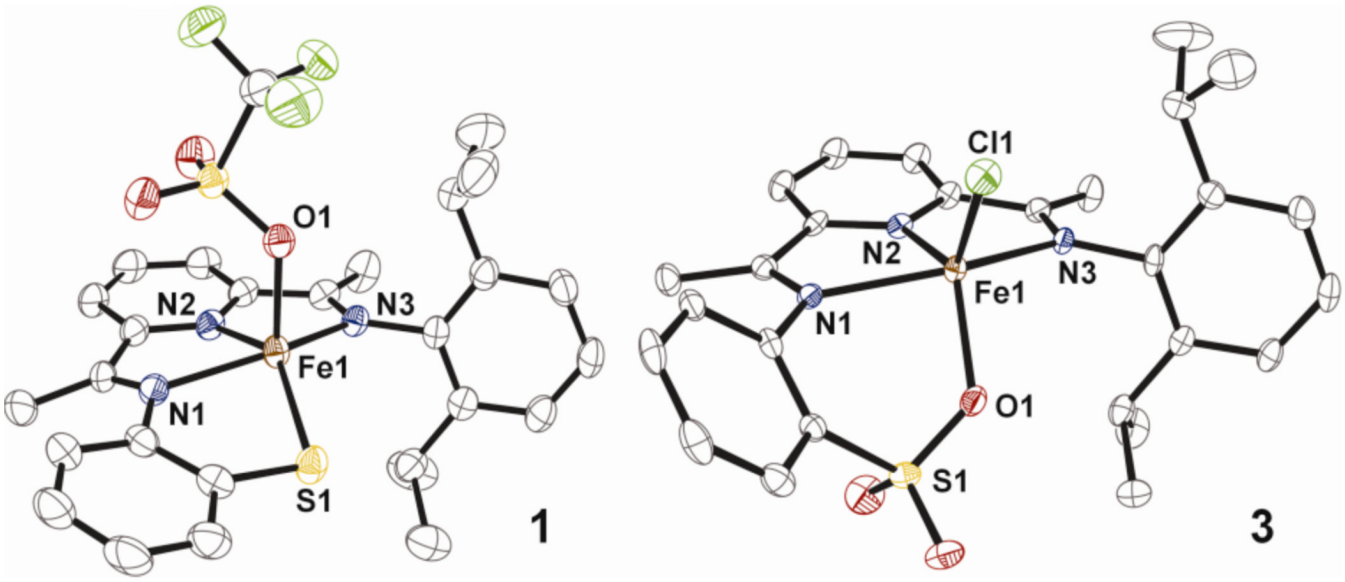


Figure 2.
Displacement ellipsoid plots (50% probability level) of **1** and **3**. The H atoms are omitted for clarity.

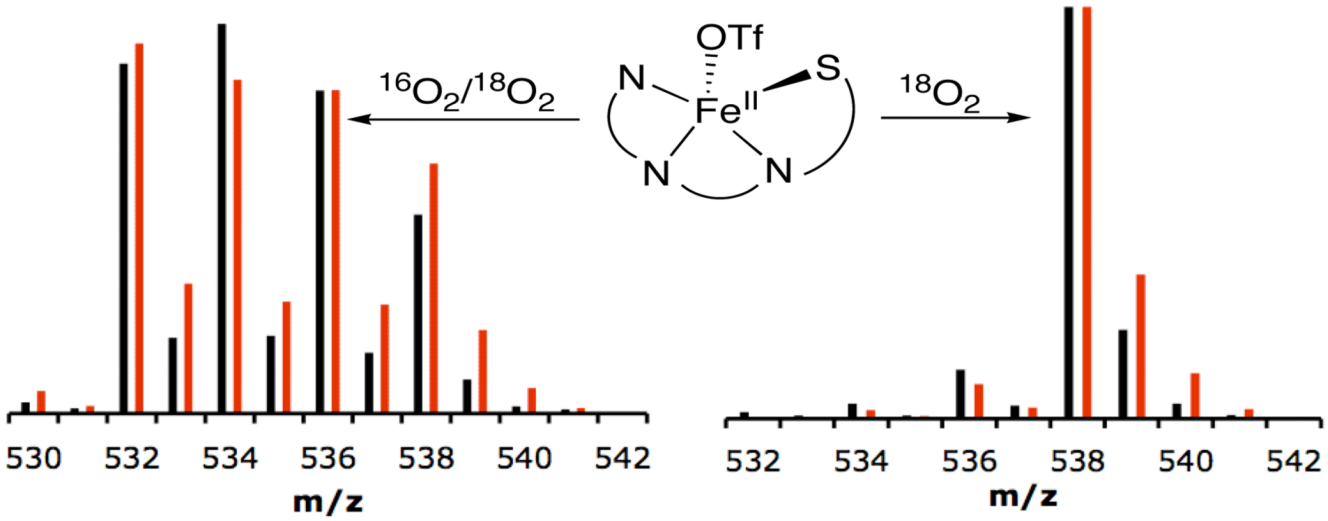
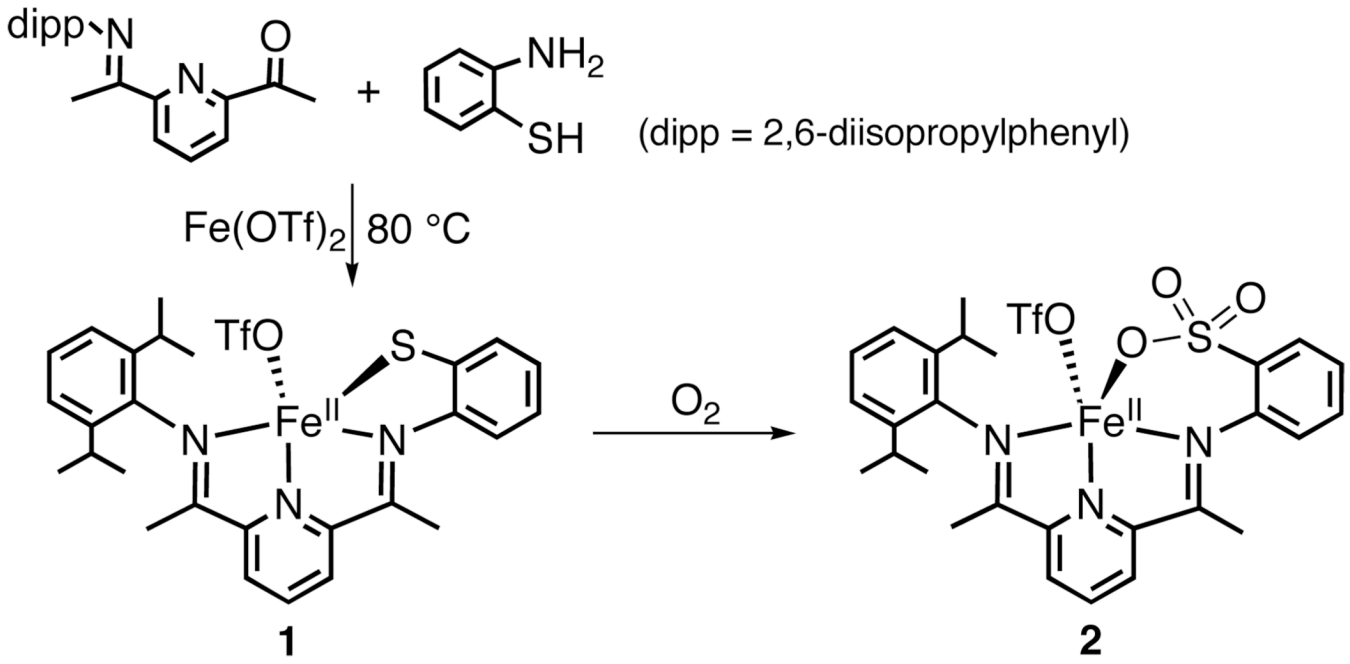


Figure 3.
Oxygen isotope studies using LDIMS. $^{18}O_2/^{16}O_2$ (~ 49/51) mixture (left) and $^{18}O_2$ (98%) (right). Exptl (black), simulation (red).



1 2

Scheme 1.