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A Putative Fe^{2+}-bound Persulfenate Intermediate in Cysteine Dioxygenase

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Abstract

The common reactions of dioxygen, superoxide and hydroperoxides with thiolates are thought to proceed via persulfenate intermediates, yet these have never been visualized. Here we report a 1.4 Å resolution crystal structure of the Fe^{2+}-dependent enzyme cysteine dioxygenase (CDO) containing this putative intermediate trapped in its active site pocket. The complex raises the possibility that, distinct from known dioxygenases and proposed CDO mechanisms, the Fe-proximal oxygen atom may be involved in the primary oxidation event to yield a unique three-membered Fe-S-O cyclic intermediate. A non-polar environment of the distal oxygen would facilitate isomerization of the persulfenate to the sulfinate product.

The direct oxidation of thiols in both proteins and small molecules by reactive oxygen species (ROS) is widespread in living systems. Sometimes the oxidation causes toxicity but it can also serve to transduce oxidative signals regulating metabolism, cell growth, and development (1). In most organisms, major systems for defense against oxidative damage involve small molecule thiol-containing antioxidants, such as glutathione, that can directly detoxify ROS to form first sulfenic acid intermediates and then disulfides. In addition, the ubiquitous peroxiredoxin enzymes, involved both in protection from oxidative stress and in regulating oxidative signal transduction, undergo similar chemistry with the active site Cys reacting with peroxides to produce a stable cysteinesulfenic acid (2). All of these reactions are thought to proceed via reaction of a thiolate anion with O_2 or the ROS to yield a persulfenate intermediate (R-SOOH) that then typically undergoes O-O bond cleavage to produce a sulfenic acid (R-SOH). Studies with model thiol compounds have established the chemical reactivities of such intermediates (3).

A variation on this theme is the reaction catalyzed by cysteine dioxygenase (CDO). This Fe^{2+}-dependent reaction converts cysteine plus dioxygen to cysteinesulfinic acid in a reaction reported to proceed with the stoichiometric incorporation of both oxygen atoms into the product (4,5):

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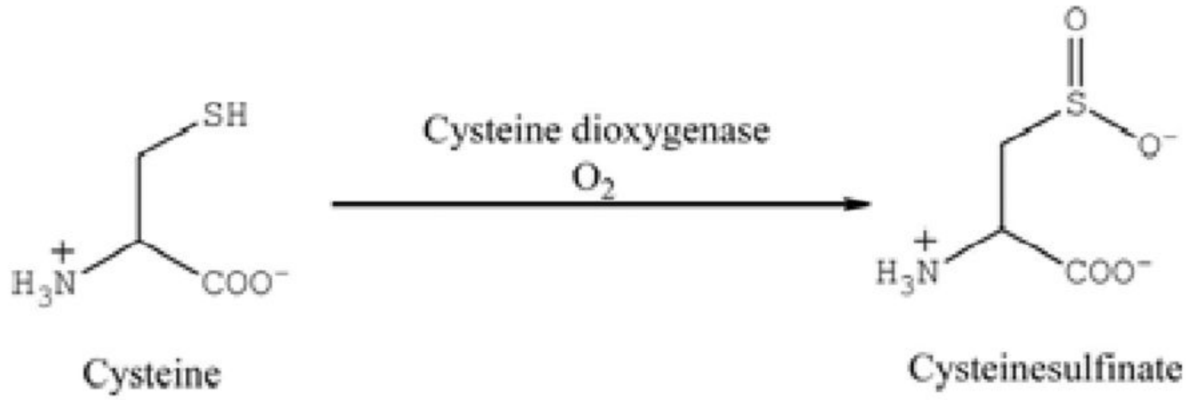

This reaction is the first step in the cysteine catabolic pathway and has crucial roles in generating inorganic sulfate and in the production of taurine, processes important not only for cysteine catabolism but also for the final steps of methionine sulfur oxidation (6). Declines in CDO activity have been associated with neurological disorders (7) and rheumatoid arthritis (8). The recent production of recombinant CDO has stimulated a series of detailed structural studies and mechanistic proposals (9-15). The resting enzyme has a non-heme iron tetrahedrally coordinated by three His residues and a solvent molecule (14), and a 2.7 Å resolution complex with cysteine (15) shows that cysteine displaces the solvent with both the α-amino group and the Sγ atom of cysteine coordinating to the iron. The Fe-center then is pentacoordinate with an open coordination site proposed to bind dioxygen, consistent with data showing that oxygen binds after cysteine (13). An unusual feature of eukaryotic CDO is a covalent linkage at the active site between Cys93-Sγ-Tyr157-Cε; this crosslink forms as an occasional side reaction during enzyme turnover and enhances the catalytic efficiency of the enzyme by 10-fold (16).

The CDO catalytic mechanism remains enigmatic (11), with all of the proposed mechanisms being guided by the fundamental assumption that after dioxygen activation by end on binding to the iron center, the initial thiolate oxidation involves the distal (terminal) oxygen atom. Here we report high-resolution analyses of crystals of rat CDO soaked in 100 mM cysteine (Table S1) that reveal a trapped cysteine persulfenate. If the trapped structure is a true catalytic intermediate, it suggests the possibility that the initial oxidation occurs by sulfur attacking the Fe-proximal oxygen with product formation involving an intramolecular isomerization.

The 1.42 Å resolution analysis (R=0.133, R_{free}=0.177) shows electron density for a Cys molecule ligating to the iron through both its N and S atoms with additional electron density that can be accounted for by two oxygen atoms (atoms O1 and O2 of a peroxy adduct) extending from the S-atom (Figure 1). In support of this interpretation, we note that the Cys-Sγ position has strong anomalous difference density consistent with it being a sulfur atom (Table S2) and that the Cys position is very similar to that seen previously (Figure S2). Also, the refined B-factors of all of the Cys atoms and atom O1 are similar to each other and to those of the surrounding protein atoms indicating the ligand is at least 70% occupied. The lower electron density for oxygen atom O2 is consistent with the assignment because atom O2 is in a loosely packed environment with no strong hydrogen bonding partners and would be expected to have significantly higher mobility than atom O1 which is wellfixed by interactions with both the iron and the sulfur. Given the presence in the crystals of just CDO, cysteine, dissolved air and buffer components, there is no other constellation of atoms that provides a plausible interpretation of the density.

In this complex, the metallocenter has distorted octahedral coordination with ligation distances ranging from 2.05 Å (for O1) to 2.47 Å (for Cys Sγ) (Table S2). The most distorted aspect of the coordination involves the S and O1 atoms, involved in a three-membered ring. Precedent for such an Fe-S-O cycle is found in two SO_2 iron-containing cluster compounds (17,18).

A broader look at the Cys-persulfenate binding site explains the remarkably high substrate specificity of CDO. The active site undergoes little change in conformation upon ligand formation (Figures S2 and S3), yet the bound Cys is fully buried with no room to spare for additional atoms and with the full hydrogen-bonding potential of both the α-amino and α-carboxylate satisfied (Figure 1). The phenolic hydroxyl of Tyr157 (11, 14) is clearly very important as it is located within 3.2 Å of four potential hydrogen-bond donors and acceptors (Figure 1b). This complex net of interactions makes the assignment of the Tyr157 hydrogen-bonding interactions challenging, but a reasonable proposal can be made that supports its assignment as a hydrogen-bond donor (and potential proton donor) to the proximal O1 oxygen (Figure 1b).

Is this a true catalytic intermediate or a dead-end complex and by what pathway does it form? These questions will take extensive study to answer definitively. However, the consideration of this complex as an intermediate is consistent with mechanistic studies to date, and its observation stimulates new thinking about the CDO mechanism and sulfur oxidation chemistry. Also, should it be a dead-end complex, it nevertheless gives important insight into the chemistry of CDO active site.

One plausible proposal for the CDO-catalyzed reaction mechanism, based on assuming that the structure described here is a true catalytic intermediate, is shown in Scheme 1. Cysteine binding to the free enzyme alters the coordination of the iron and creates the oxygen binding site (13). Starting from this enzyme cysteine complex **1**, the binding of molecular oxygen to the active site ferrous iron gives **3**. This is converted to **4** by a nucleophilic addition mechanism. Addition of the negatively charged distal oxygen to the sulfur then gives thiadioxirane **5**. This undergoes heterolytic cleavage of the O-O bond, facilitated by a hydrogen bond (or proton transfer) between Tyr 157 and the proximal oxygen, to give **6**. This reaction is possible because of the low energy empty d orbitals on the sulfur. Product release completes the reaction. We favor this mechanism over an alternative involving heterolytic cleavage of the O-O bond in **4** followed by hydroxide addition to the resulting electron-deficient sulfur because the relatively nonpolar active site, with the distal oxygen surrounded by Leu95, Cys93-Sγ, Ile133 is poorly suited to stabilize negative charge on the distal oxygen of **4**. Assuming this mechanism, we note that the trapping of intermediate **4** suggests that the rate-limiting step of the reaction, at least as the enzyme is cooled to cryotemperatures, is the intramolecular cyclization to give the thiadioxirane **5**. Other mechanistic possibilities for the formation of intermediate **4** cannot yet be excluded. For example, **4** could be formed directly from **2** by the coupling of a thiyl radical with a radical on the proximal oxygen. Alternatively **4** could be formed by addition of a thiyl radical to the distal oxygen of **2** followed by rearrangement or by addition of the thiolate to side-bound oxygen. Differentiating between these possibilities will require additional experimentation.

The conversion of **4** to product involves an intramolecular rearrangement, which implies a perfect incorporation of both atoms of molecular oxygen into product. A previous labeling study using rat liver cytoplasm suggested that this is the case (4), but was not conclusive. We have repeated the oxygen labeling experiment using pure recombinant rat CDO, shorter reaction times, and a much more sensitive and rapid MSbased analysis. These experiments demonstrated unequivocally that both oxygen atoms of the sulfinic acid are derived from a single molecule of molecular oxygen and that no exchange with buffer is occurring (Figure S4).

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One model system that may be closely analogous to the CDO reaction is the Ni(II)-catalyzed oxidation of thiols to sulfinate. Those reactions also show the incorporation of both atoms of molecular oxygen into the sulfinate suggesting that intramolecular rearrangement to sulfinate is an intrinsic property of metal bound persulfenates (19,20) and need not be a consequence of restricted exchange at the enzyme active site. Our mechanism also maintains a central role for Tyr157 as an active site acid facilitating OO bond cleavage in agreement with other current models (11). As has been discussed before, the crosslink between Tyr157 and Cys93 may enhance activity by modulating the precise positioning and possibly the pKa of Tyr157.

While the chemistry of thiol and thioether oxidation has been extensively investigated, persulfenate intermediates have not previously been directly observed (3,21). The stabilization of the cysteine persulfenate **4** in the CDO active site at cryo-temperatures is fortuitous. This was an intermediate in all four previously proposed mechanisms for CDO (11), but none of the proposals predicted the observed coordination of this intermediate to the active site iron, nor did they suggest that the cysteine thiol would bind to the proximal (iron-bound) oxygen rather than to the distal oxygen. Thus, if this is a true intermediate, the CDO chemistry is different from what has been anticipated based on current paradigms for thiol oxidation. These potentially unique aspects of the CDO mechanism, compared with dioxygenases that oxidize C=C bonds (22-24), reflect the electron-rich character of the thiol functional group and the availability of low energy empty d orbitals on the sulfur. The visualization of this putative intermediate and its unexpected coordination chemistry is likely to stimulate further studies on thiol oxidation chemistry.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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SUPPORTING INFORMATION AVAILABLE Materials and Methods, Figures. S1, S2, S3 and S4 and Tables S1, S2 and S3. This information is available free of charge via the internet at http://pubs.acs.org

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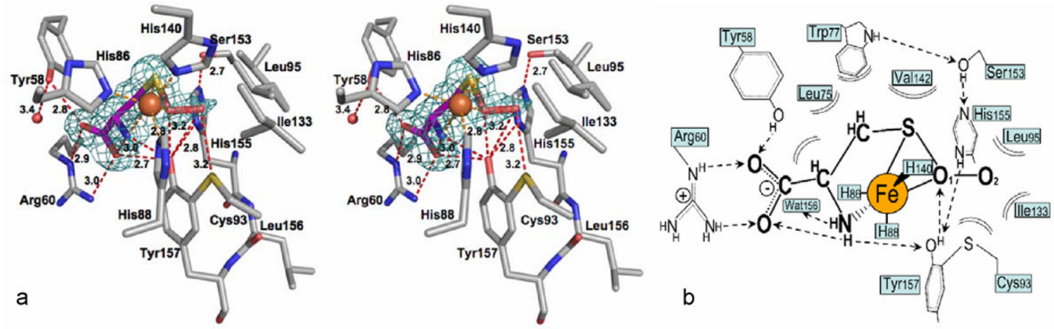
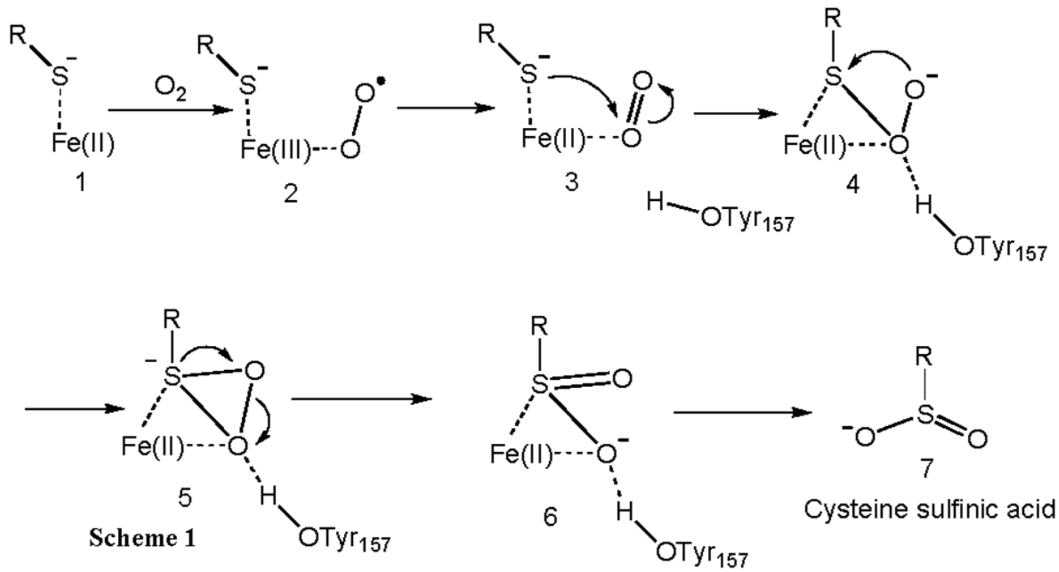


Figure 1.

The trapped persulfenate intermediate in the active site of CDO. (a) Stereoview of the active site of CDO with electron density for the ligand shown at 0.8*ρ_{rms}. Bonds to the iron (orange dashed lines) and potential hydrogen bonds (red dashed lines) are shown. See Figure S1 for a close-up of omit density for the ligand. (b) Schematic of the active site interactions and metallocenter geometry based on the same view shown in panel (a). Close non-polar approaches surrounding the ligand (double arcs) and potential hydrogen bonds have been inferred from the environment. The geometry is such that one of the two protons of the sp3 hybridized α-amino group points toward a bound water (Wat156) and the other points toward where it can make a bifurcated hydrogen bond to both Tyr157-OH and the α-carboxylate oxygen. This is the main interaction allowing us to propose that the Tyr157 hydroxyl donates a hydrogen bond to atom O_1.



Scheme 1.
Proposed CDO mechanism

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