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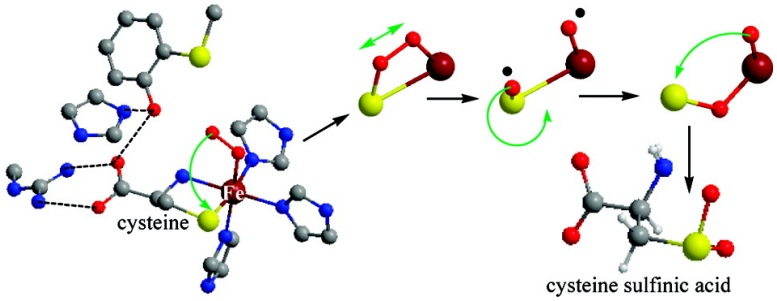

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The Mechanism of Cysteine Oxygenation by Cysteine Dioxygenase Enzymes

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Cysteine dioxygenase (CDO) is an essential enzyme in the human body involved in the metabolism and bioconversion of toxic cysteine.[1] This is a vital process in human health that regulates the cysteine concentration in the body. In some cases, the enzyme loses its efficiency, and this has been correlated to diseases such as Alzheimer's and Parkinson's diseases.[2] Furthermore, a decline in CDO activity has been linked with a neurological disorder associated with iron accumulation.[3] Therefore, understanding the catalytic mechanism by which CDO metabolizes cysteine to cysteine sulfinic acid is important. However, as the enzyme reacts very fast, only limited information regarding its mechanism is known. Surprisingly, no theoretical studies have been reported so far on this enzyme, and only a few experimental studies on intermediates of the catalytic cycle have been reported.[4] In particular, the mechanism after dioxygen binding is unknown and mostly based on speculation.

CDO is a nonheme enzyme that contains an active center with an iron that is connected to the protein backbone via three histidine bonds.[1,4] The remaining three ligand sites of iron are occupied by molecular oxygen and substrate (cysteine) that binds as a bidentate ligand via the sulfide and amide groups. Studies with isotopically labeled O_2 showed that both oxygen atoms are incorporated in cysteine sulfinic acid products.[5] The second sphere amino acids Tyr_{157}, Cys_{93}, and His_{155} have been implicated in stabilizing the reaction process through a tight hydrogen bonding network.[4] The catalysis of sulfoxidation reactions by this enzyme is not unique; it has also been reported for heme enzymes, such as the cytochromes P450 (P450).[6] The major difference, however, is that the P450s are monoxygenase enzymes that transfer only one oxygen atom to the substrate whereas CDO transfers both oxygen atoms.

To gain insight into the catalytic mechanism whereby CDO utilizes molecular oxygen and transfers both oxygen atoms to the substrate, we set up a model of the active site of CDO based on the 2IC1 pdb, which is the substrate-bound complex of human CDO.[7] We selected a large active site model that contains the first and second coordination spheres of iron, including the three metal-bound histidine groups (His_{86}, His_{88}, and His_{140}), cysteinate, methylguanidinium for Arg_{60}, phenol for Tyr_{157}, methyl mercaptane for Cys_{93}, and imidazolate for the second sphere His_{155}. We subsequently added hydrogen atoms and dioxygen to create a model with stoichiometry $FeC_{24}N_{12}H_{37}S_2O_5$ and overall charge +1.

All calculations were performed using previously described procedures which we will briefly summarize here.[8] The initial geometry optimizations were performed with the Jaguar 5.5 program package and utilized the unrestricted B3LYP hybrid density functional method in combination with a double-ζ quality LACVP basis set on iron and 6-31G on the rest of the atoms (basis set B1).[9] The optimized geometries were transferred to Gaussian 03 for a frequency analysis.[10] Subsequent, single point calculations with a triple-ζ quality LACV3P+ basis set on iron (that contains a core potential) and 6-311+G* on the rest of the atoms were performed (basis set B2). All energies reported in this work are obtained with basis set B2 corrected with ZPE calculated with basis set B1. Detailed test calculations into the effects of perturbations due to the surrounding protein or from a dielectric constant showed little effect on the rate-determining step in the reaction mechanism and the spin state ordering of the intermediates (see Supporting Information).

Figure 1 displays the calculated reaction mechanism of our CDO model starting from the dioxygen-bound complex (**A**), which reveals several new species not considered before. In the dioxygen-bound complex, the terminal oxygen atom forms a hydrogen bond with the amine group of cysteine, and as a result, the dihedral angle d_{OOFeS} is almost perpendicular. In the first step of the mechanism, the terminal oxygen atom of the O_2 moiety attacks the sulfur atom of cysteine to form a ring structure (**B**) containing an FeOOS ring via an S−O bond formation barrier ($\mathbf{TS_A}$). The formation of the ring structure elongates the O−O and Fe−S bonds and facilitates the oxygen transfer. The dioxygen bond breaks via a transition state $\mathbf{TS_B}$ to form the *cis*-sulfoxide structure **C**. This complex, however, has radical character on the sulfoxide group ($\rho_{CysO} = -0.79$). The sulfoxide group then undergoes an internal rotation around the S−C bond (dihedral angle OSCC) whereby in effect the Fe−S bond is broken and the oxygen atom of the sulfoxide binds to iron instead to form the *trans*-sulfoxide structure **D** via barrier $\mathbf{TS_C}$. Since the oxygen atom has a momentum in the direction away from the other oxygen atom (and iron), the rotation will be anticlockwise around the C−S bond. During this rotation, an electron transfer takes place from the sulfoxide to the oxoiron system. In structure **D**, the sulfur atom is accessible to the second oxygen atom to form the final product cysteine sulfinic acid (**E**) via a barrier $\mathbf{TS_D}$. We calculated this complete mechanism on the lowest lying singlet, triplet, and quintet spin states. Despite the fact that $^1\mathbf{A}$ is the lowest lying dioxygen-bound complex, its barrier leading to the ring structure **B** is significant and much higher than the barrier from $^5\mathbf{A}$ to $^5\mathbf{B}$ (i.e., $^5\mathbf{TS_A}$). In fact, the quintet spin state barriers are the lowest calculated barriers in most cases. Therefore, it is anticipated that starting from a singlet spin dioxygen-bound complex a fast spin state crossing to the quintet spin state will occur, which is the dominant state for the rest of the reaction mechanism. The rate-determining step in the reaction mechanism, therefore, is the initial S−O bond activation barrier via $^5\mathbf{TS_A}$. As a result of that, it is expected that products will be formed rapidly soon after this barrier has been crossed. It may be anticipated, therefore, that it will be difficult to experimentally trap and characterize complexes beyond $\mathbf{TS_A}$, due to the large exothermicity of the reaction so that the lifetimes of **B**, **C**, and **D** will be very short.

The optimized geometries are typical for nonheme and heme iron complexes, and the bond distances are in good agreement with related structures found for other enzyme models.[8,11]

In summary, DFT studies on the dioxygenation mechanism of cysteine by the dioxygen iron cluster in CDO reveal the following

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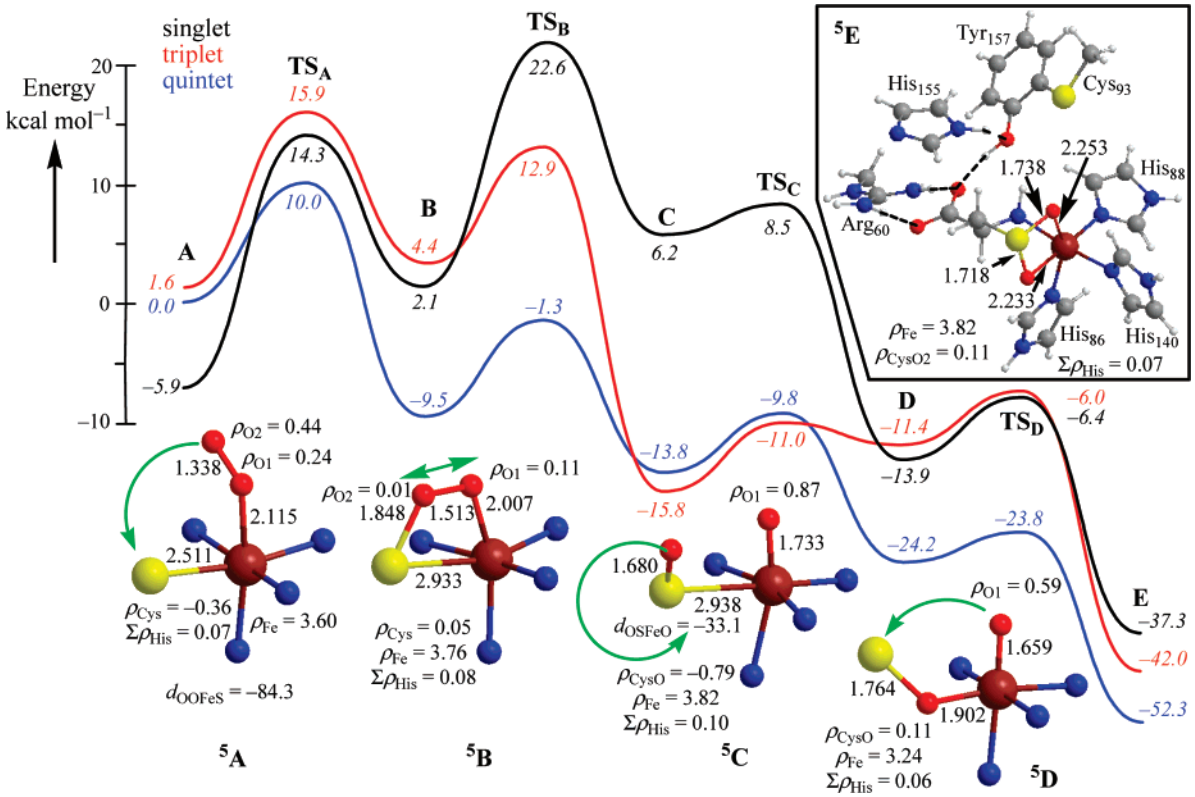

Figure 1. Potential energy profile of the dioxygenation reaction of cysteine starting from the dioxygen-bound complex of a CDO enzyme model. All energies are in kcal mol^{-1} relative to ^{5}**A**. Also shown are extracts of the optimized geometries in the quintet spin state with group spin densities (ρ), bond lengths (in Å), and angles (in degrees) identified; $\Sigma\rho_{His}$ is the sum of the spin densities on the three histidine ligands of iron.

key features: (1) The rate-determining step is the attack of the terminal oxygen atom of O_2 on the sulfur atom of cysteinate, which weakens the O−O and Fe−S bonds to form a sulfoxyl radical. (2) An internal rotation in concert with electron transfer then makes the sulfur center accessible to the second oxygen atom, and at the same time, the existing S−O bond is weakened due to the pull effect of the iron on the oxygen atom. (3) The final oxygen abstraction leads to cysteine sulfinic acid in a highly exothermic process. (4) The reaction mechanism is mainly on a high-spin (quintet) state surface with a low rate-determining barrier and large exothermicity.

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Supporting Information Available: Cartesian coordinates of all structures described in this work, tables with group spin densities and charges, detailed geometry scans for the various reaction processes, and complete ref 10. This material is available free of charge via the Internet at http://pubs.acs.org.

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