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Density Functional Theory Calculations on the Fe_2S_2(Arg)(SCys)(SSCys)$_2$ Cluster in HydE: Unique Electronic Structure and Redox Properties

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A Fe_2S_2 cluster with unprecedented $CysSS^-$ (cysteinepersulfide) coordination has been observed crystallographically in the AdoMet-dependent hydrogenase maturase enzyme HydE. Geometry-optimized density functional theory calculations are used to develop an electronic structure description of this unusual cluster. The results indicate that the $CysSS^-$ ligand is unique because it can act as a donor as well as an acceptor ligand. This is due to the presence of S−S π^* (occupied) and S−S σ^* (unoccupied) orbitals in this ligand. Extensive back-bonding is observed between the cluster and the S−S σ^* orbital. The back-bonding is significantly higher in the reduced state, which is calculated to shift the reduction potential of this Fe_2S_2 cluster by +400 mV in the gas phase relative to a $CysS^-$-coordinated Fe_2S_2 cluster model of BioB.

Iron sulfur clusters are ubiquitous in nature. They are involved in electron transport [e.g., rubredoxin, ferredoxin (Fd), etc.], small-molecule activation (e.g., nitrogenase, carbon monoxide dehydrogenase, acetyl coenzyme A synthase, etc.), and reductive cleavage of adenosine methionine [e.g., biotin synthase (BioB), lysine aminomutase, etc.].[1−3] Their functional diversity is paralleled by their structural diversity. These clusters range from symmetric mononuclear, binuclear, trinuclear, tetranuclear, and octanuclear structures to asymmetric site-differentiated structures of different types.[4] Most clusters share two common structural features. They have bridging sulfide (S^{2-}) ligands and terminal cysteine ($CysS^-$) ligands. Although in some polynuclear clusters the terminal cysteine ligand is substituted with another amino acid derived ligand (e.g., histidine in Riesky-type proteins, arginine in BioB, etc.), there are no known cases of post-translational modification of the cysteine ligands akin to those observed in the active sites of nitrile hydratase and thiocyanate hydrolase.[5]

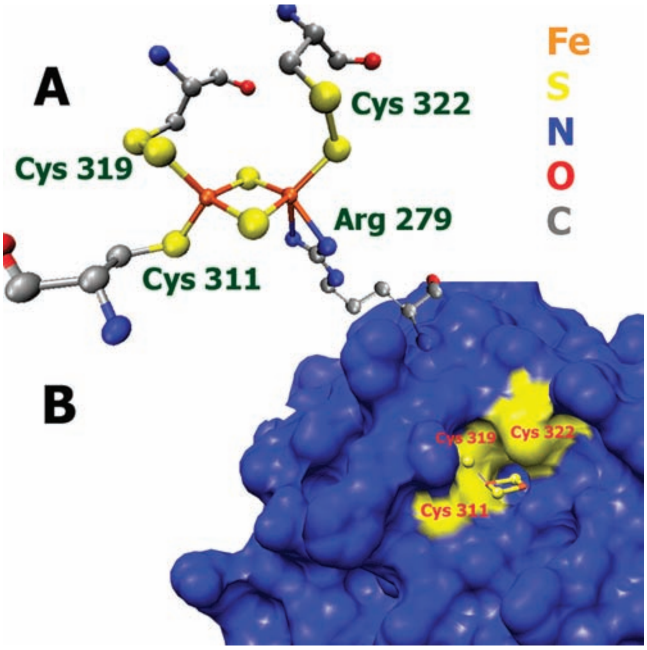

Figure 1. (A) Fe_2S_2 cluster of the HydE protein (pdb id: 3IIZ) containing a Fe_2S_2 core, a $CysS^-$ ligand, an arginine ligand, and two $CysSS^-$ ligands. (B) Solvent-exposed surface shown in blue. The yellow patches on the blue surface indicate the coordinating cysteinyl ligands.

In a recent report of the crystal structure of hydrogenase maturase enzyme HydE, an unprecedented Fe_2S_2 cluster ligation was observed.[6] Each of the two Fe atoms is coordinated to a cysteineperthiolate ($CysSS^-$) ligand (Figure 1A). This cluster, along with its coordinating ligands, is exposed to solvent (Figure 1B). The coordination by $CysSS^-$ is not only unique for Fe_2S_2 clusters but is also a relatively new entity in metalloenzymology.[7] It is also important to note that involvement of the CysSSH residue has been invoked as the sulfur source in the biosynthesis of biotin.[8] In this study, density

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functional theory (DFT) calculations have been used to explore the geometric and electronic structures of the Fe_2S_2 cluster of HydE. The results obtained for a $CysSS^-$ ligated cluster have been compared to those of a normal $CysS^-$ ligated cluster. The possible role of this modified ligand in tuning its reactivity has been explored.

The geometry-optimized, broken-symmetry, DFT calculations (*Gaussian 03*, BP86, 6-311g*)[9,10] on the fully oxidized Fe_2S_2 cluster show a stable minimum. The optimized bond lengths are Fe−S 2.19 (2.19) Å, Fe−SCys 2.33 (2.05) Å, Fe−SSCys 2.31 (2.19) Å, Fe−N 2.05 (2.24) Å, and S−S 2.13 (2.04) Å. Although these vary significantly from those of the reported crystal structure (in parentheses), these values are well within the range of Fe−S distances reported for sulfide- and thiolate-ligated FeS clusters.[2] The Fe−S bonds on the proximal Fe (i.e., the Fe coordinated by the arginine) are shorter than those of the distal Fe (i.e., the Fe away from the arginine). The Fe−SSCys bonds are 2.3 Å, which are slightly shorter than the Fe−SCys bond (2.33 Å), and the S−S bond is 2.13 Å, which is 0.03 Å shorter than that calculated for free CH_3SSH.

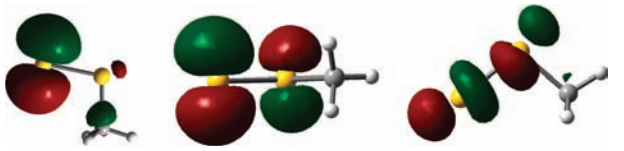

Figure 2. Valence orbital of a $CysSS^-$ ligand: the in-plane terminal S_{3p} (left), the out-of-plane S−S π^* (middle), and the in-plane S−S σ^* (right). The first two are occupied, while the last is unoccupied.

The $CysSS^-$ ligand has three valence orbitals (Figure 2). An occupied S_{3p} orbital on the terminal S atom (Figure 2, left) and an occupied S−S π^* orbital (Figure 2, middle) can have donor interaction with a vacant Fe orbital. In addition to the two donor orbitals mentioned above, there is a low-lying unoccupied S−S σ^* orbital (Figure 2 right) that can participate in back-bonding with an occupied Fe orbital.

The electronic structure of the Fe_2S_2 cluster is similar to the one observed for the $Fe_2S_2(SR)_4$ cluster in Fd.[11] Two high-spin ($S = {}^5/_2$) ferric centers having distorted tetrahedral geometry are antiferromagnetically coupled because of strong superexchange pathways provided by the covalently bridged sulfide ligands resulting in a diamagnetic ground state. The two Fe atoms are inequivalent, unlike in Fd, because of asymmetric coordination of the cluster. The arginine-coordinated Fe (β-spin manifold) has a stronger ligand field and thus higher orbital energies. The unoccupied α-spin manifold of this $CysSS^-$ ligated cluster (Figure 3; the β-spin manifold is not shown for clarity) shows that there are three orbitals on the $CysSS^-$ ligand that interact with the Fe center. Note that the occupied bonding orbitals are not shown in the molecular orbital diagram because they mix with other occupied orbitals of similar energy. Thus, the contours in Figure 3 are the antibonding counterparts of the corresponding bonding

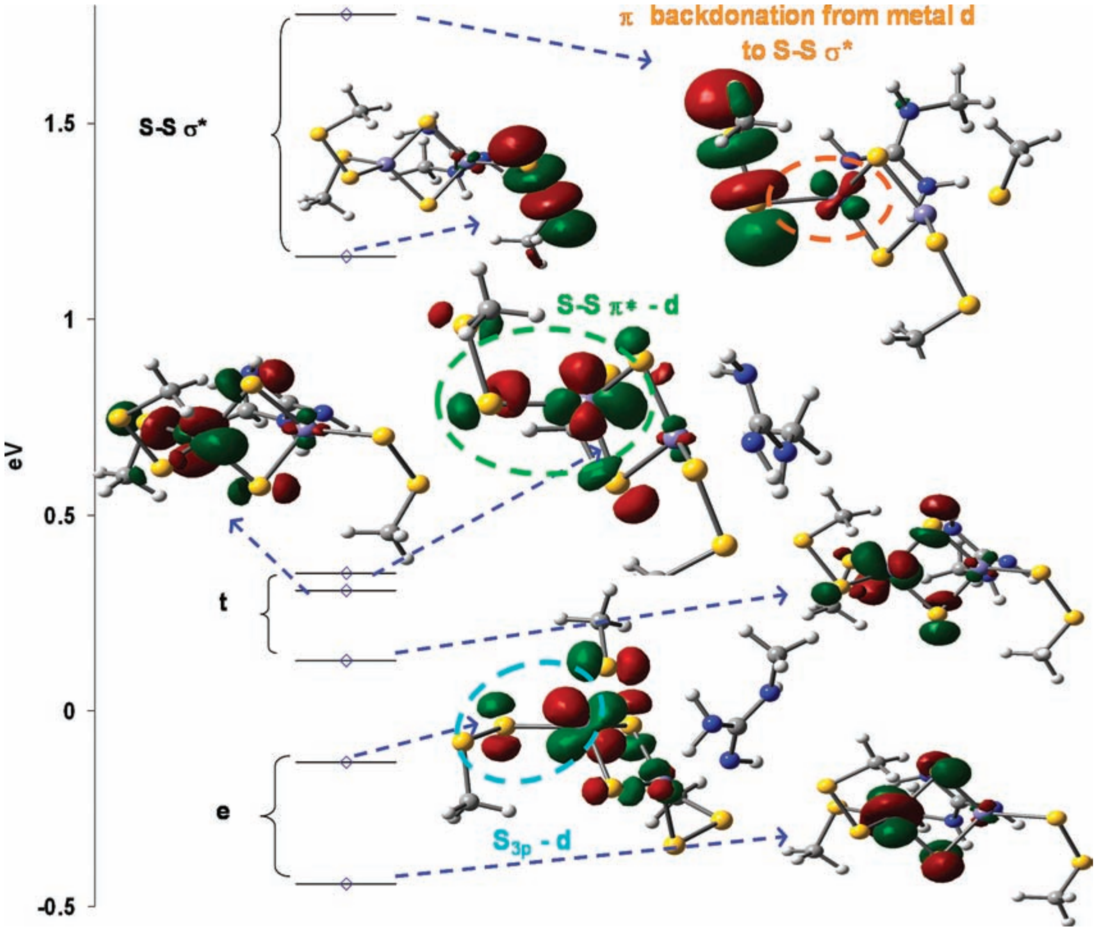

Figure 3. Molecular orbital diagram of the $CysSS^-$-coordinated Fe_2S_2 cluster of HydE. Only the antibonding counterpart of the interactions is shown (the occupied bonding orbitals are not shown). The blue diamonds represent the energy levels, and the corresponding contours are indicated with blue arrows. The interaction of the cluster with the $CysSS^-$ ligand is indicated by circles/ovals.

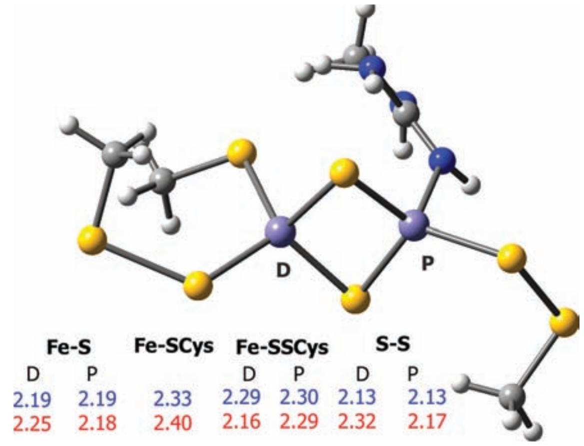

Figure 4. Geometric parameters of oxidized (blue) and reduced (red) clusters. Distal and proximal Fe atoms are indicated by D and P, respectively.

orbitals, which provide a cleaner representation of the interactions. The occupied S_{3p} orbital on the terminal S atom (Figure 2, left) and the S−S π^* orbital (Figure 2, middle) interact with the e (corresponding antibonding S_{3p}−Fe_{3d} π^* orbital shown in Figure 3, cyan circle) and t_2 orbitals (corresponding antibonding S−S π^*−Fe_{3d} π^* orbital shown in Figure 3, green circle) of the distal Fe, respectively. The low-lying unoccupied S−S σ^* orbital (Figure 2, right, and Figure 3, top) is absent in a normal $CysS^-$-coordinated cluster. The calculations show that these two S−S σ^* orbitals are in the same energy range as the unoccupied Fe_{3d} orbitals and have significant d−π back-bonding interaction with the occupied Fe_{3d} orbitals (corresponding antibonding orbital circled in orange in Figure 3). Mulliken population analysis (MPA) reveals that ∼15% Fe_{3d} character is mixed into the S−S σ^* orbitals. Thus, there is both donation (from terminal S_{3p} and S−S π^* orbitals) and back-donation (into the S−S σ^* orbitals) interaction between the Fe and the $CysSS^-$ ligand.

The optimized geometry of the reduced cluster shows that the Fe−S and Fe−SCys bonds of the distal Fe (D in Figure 4) are elongated, indicating a localized reduced state (with the distal Fe reduced) similar to those observed in the Fe_2S_2 cluster in Fd's (Figure 4). This also indicates that arginine coordination lowers the reduction potential of the proximal Fe (P in Figure 4) such that the distal Fe is specifically reduced. Interestingly, the HydE cluster model also shows dramatic elongation of the CysS−S bond (0.19 Å) and shortening of the CysSS−Fe bond (0.13 A) upon reduction.

The contour of the highest occupied molecular orbital (HOMO) of the reduced state (Figure 5, left) shows significant back-bonding interaction (22% S−S σ^* mixed in the Fe_{3d} HOMO) with the S−S σ^* orbital of the $CysSS^-$ ligand coordinated to the distal Fe. Simultaneously, there is 20% Fe_{3d} mixing into the corresponding S−S σ^* unoccupied orbital. This back-bonding interaction is also observed in the oxidized state but is weaker owing to the larger energy difference between the occupied Fe_{3d} orbitals and the unoccupied S−S σ^* orbital relative to the reduced state. Upon reduction, the Fe_{3d} manifold of the reduced distal Fe is raised in energy and the back-bonding interaction is significantly increased. MPA indicates that 0.44 e charge (22% of $\alpha + \beta$) is transferred into the S−S σ^* orbital upon reduction of this cluster. This charge delocalization is clearly visible in the contour of the HOMO of the reduced state, which shows significant mixing of the S−S σ^* orbital. This is also reflected in the total spin density plot for the reduced state, where significant spin is observed in the noncoordinated S of the $CysSS^-$ ligand (Figure 5, right). Thus, MPA and total spin density analysis indicate charge delocalization from Fe to the S−S σ^* orbital because of the presence of back-bonding interaction between the two.

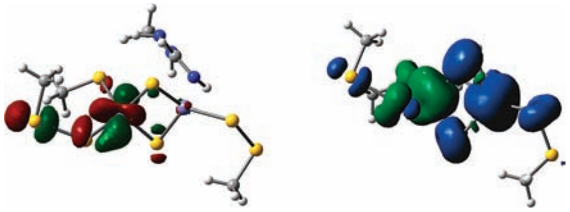

Figure 5. HOMO (left) and the total spin density (α is blue and β is green) of $CySS^-$ bound to the distal Fe of the reduced Fe_2S_2 site of HydE.

The calculated ionization energy for the $CysSS^-$-coordinated cluster in the gas phase is 3.31 eV, while that calculated for the $CysS^-$ cluster is 2.91 eV. Thus, the presence of the $CysSS^-$ ligand shifts the reduction potential ($E°$, assuming entropic corrections to be similar) by 400 mV relative to a $CysS^-$ ligand in the gas phase. This is because the additional electron density of the reduced cluster is effectively delocalized into the S−S σ^* orbital of the $CysSS^-$ ligand, unlike in a $CysS^-$ ligand. This stabilizes the reduced state relative to the oxidized state, resulting in the calculated positive shift in $E°$.

In summary, DFT calculations indicate that this unique $CysSS^-$ ligand acts as a donor as well as an acceptor ligand. An occupied 3p orbital on the coordinating S and an occupied S−S π^* orbital act as donor orbitals, while an unoccupied S−S σ^* orbital acts as an acceptor orbital. Back-bonding interaction between the Fe and S−S σ^* orbitals significantly stabilizes the reduced state because of electron delocalization. This is predicted to shift the $E°$ of this cluster by 400 mV relative to a $CysS^-$-bound cluster. While the physiological role of such an unusual $CysSS^-$ ligation is uncertain,[12] the calculations presented in this study show significant back-bonding interaction between the Fe and S−S σ^* orbitals and predict a positive shift in $E°$ of the Fe_2S_2 cluster because of $CysSS^-$ ligation relative to $CysS^-$ ligation.

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Supporting Information Available: Computational details and optimized geometries. This material is available free of charge via the Internet at http://pubs.acs.org.

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