

Density functional theory calculations on Fe_2S_2 clusters: Effect of ligand environment on geometric/electronic structure and reduction potential

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The Fe_2S_2 clusters in nature are generally coordinated to the peptide backbone using thiolate ligands (from cysteines). A known variation of these are the imidazole (from histidine) coordinated active sites of Riesky proteins. Recently, a few newer modifications have been observed, e.g., the arginine coordinated cluster in biotin synthases. Very recently, a cysteine persulfide coordinated cluster has been observed in the hydrogenase maturase enzyme, HydE. Herein, density functional theory calculations are used to investigate the effect of the unusual arginine and cysteine persulfide coordinations on the geometric and electronic properties of these clusters. Further, the effect of these ligands in tuning the thermodynamic reduction potentials of these clusters has also been investigated.

Keywords: Bioinorganic chemistry, Theoretical chemistry, Density functional calculations, Iron, Sulfur clusters, Electronic structures, Reduction potentials

Iron-sulfur clusters are ubiquitous in nature. They catalyze a variety of biological transformations ranging from small molecule activation (CODH, nitrogenase, hydrogenase, etc.), electron transfer, DNA recognition, reductive cleavage to isomerization.¹⁻³ These clusters exist in several structural varieties, viz., mononuclear (e.g. rubredoxin, peptidyl deformylase), binuclear (plant ferredoxin, biotin synthase), trinuclear (ferredoxins), tetranuclear (bacterial ferredoxins, HiPIPs) and octanuclear (nitrogenase) clusters.⁴ Some of these active sites are inhomogeneous. While some clusters show inclusion of heterometals (e.g. NiFe hydrogenase, CODH, Mo and V dependent hydrogenases, etc.), others show a uniquely coordinated iron (aconitase, S-adenosinemethionine (SAM) activating tetranuclear clusters and Biotin synthase).⁵ The role of this heterogeneity has attracted considerable interest. In cases like that of the Fe_4S_4 clusters in aconitase and the SAM dependent Fe_4S_4 clusters, the biological significance of this unique coordination is clearer as it is the binding site of the substrate.⁶ In the case of the Riesky type proteins, two terminal cysteines of one of the two Fe's of the Fe_2S_2 cluster is replaced by two imidazoles. This change in ligation shifts the reduction potential of the Fe_2S_2 cluster by $\sim +700$ mV

relative to that of normal ferredoxins (Fd, Fig. 1 (A)). The histidine ligation also localizes the mixed valence state such that the histidine coordinated Fe acts as the redox active center.⁷

Crystal structures of members of the Biotin synthase family have revealed the presence of a unique Fe_2S_2 cluster where, one of the four cysteine ligands is replaced by a neutral arginine ligand (Fig. 1 (B)).⁸ Arginine, not a very common ligand, is known to bind transition metals like Ni^{II} , Zn^{II} , Co^{III} , etc., at neutral to alkaline pHs.¹⁰ Further, the arginine ligand coordinated to the iron in BioB is neutral which implies that the pK_a of this arginine is significantly lowered at this active site (pK_a of arginine > 10). Recently, Fontecave *et al.*⁹ reported the crystal structure of the hydrogenase maturase enzyme (HydE), which contains a Fe_2S_2 and an Fe_4S_4 cluster. In this enzyme, two of the cysteines of the Fe_2S_2 cluster are replaced with cysteinepersulfide (CysSS^-) ligands and one cysteine is replaced by a neutral arginine ligand (Fig. 1 (C)). The biological role of such a dramatic change in ligation around iron is not well understood as the enzyme is active without this cluster.¹¹ Recent DFT calculations on the HydE cluster indicate significant charge delocalization in this cluster.¹²

In this study we have used DFT calculations to investigate the electronic structure of these unnaturally ligated clusters of BioB and HydE, in both oxidized and reduced forms. Different modes of coordination of the arginine ligand are considered and its effect on the geometric and electronic structure of the cluster is probed. These calculations are extended to evaluate the contribution of these unusual ligands in tuning the redox properties of these Fe_2S_2 clusters.

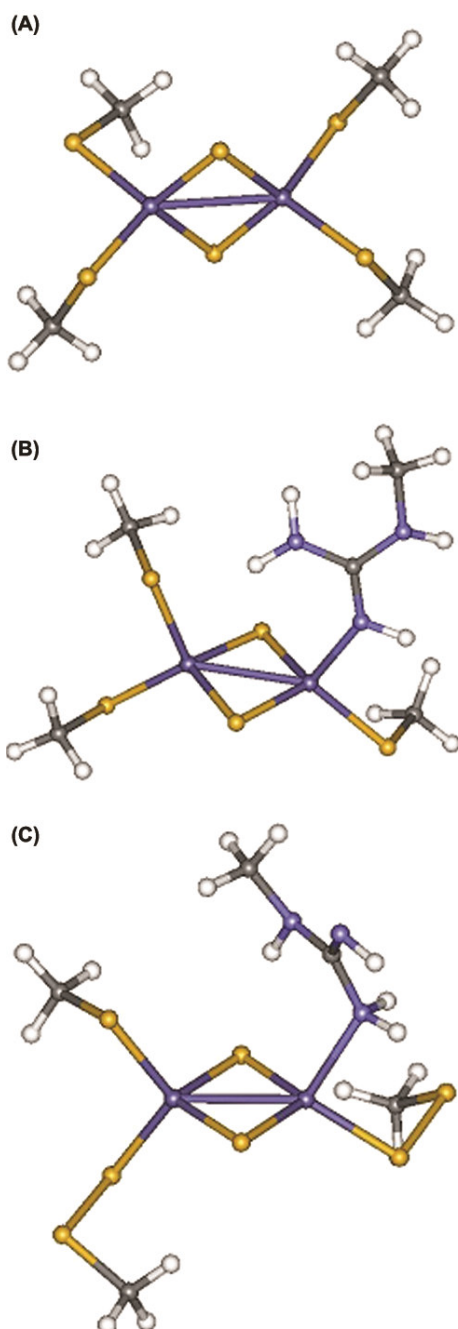


Fig. 1 – Crystal structures of the Fe_2S_2 clusters in Fd (A), biotin synthase⁸ (B) and hydrogenase maturase enzyme (HydE)⁹ (C).

Computational Method

All calculations were performed using Gaussian 03 program in the HPC cluster at IACS, Kolkata, using the BP86 functional.^{13,14} The starting structure of the $\text{Fe}_2\text{S}_2(\text{SCys})(\text{SSCys})_2(\text{Arg})$ cluster was obtained from the coordinates of HydE bound to SAM (3IIZ). The starting structure of $\text{Fe}_2\text{S}_2(\text{SCys})_3(\text{Arg})$ was obtained from the coordinates of BioB (1R30). The geometries were optimized using 6-311g* basis for Fe and S and 6-31g* basis for N, C and H atoms. Frequencies were computed to ensure a stable minimum. The energies and wavefunctions were calculated using the same method but using a 6-311+g* basis set on all atoms. The contour diagrams were obtained using Gaussview software. The reduction potentials were calculated by adding the entropic and zero point energy corrections (obtained during the frequency calculations) to the total electronic energies and using 4.31 eV as the energy of a free electron. Mulliken population analysis was used for spin density analysis.¹⁵

Results and Discussion

Geometric and electronic structure

(A) Oxidized

The optimized geometries of the clusters in Fd, BioB and HydE in their oxidized and reduced forms are presented in Table 1. Here, P refers to the proximal Fe, i.e., Fe bound to the arginine ligand, and D refers to the distal Fe, i.e., the Fe not ligated by arginine. The neutral arginine residue can coordinate either $\eta\text{-NH}$ or $\eta\text{-NH}_2$. The total energies reflect that the $\eta\text{-NH}_2$ coordination are almost iso-energetic for the oxidized state ($\eta\text{-NH}$ is more stable by 0.17 Kcal/mol).

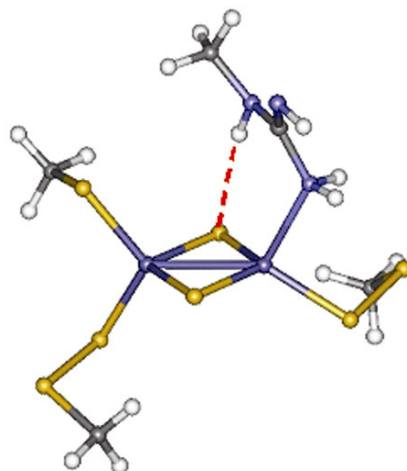
Replacing one of the terminal thiolate ligand with $\eta\text{-NH}_2$ guanidinium group produces significant changes in the geometry. Both the $\mu_2\text{S-Fe}$ and the RS-Fe bond lengths are shorter in the $\eta\text{-NH}_2$ model relative to the Fd model. This reflects the weaker donation of the neutral guanidine ligand relative to the thiolate ligand. This weak donation is compensated by stronger bonding from the $\mu_2\text{S}_{\text{sulfide}}$ and RS^- ligands which leads to the shorter Fe-S bond lengths. The Fe-N bond length for $\eta\text{-NH}_2$ is 2.23 Å which is much longer than the Fe-N bond length in the $\eta\text{-NH}$ model (2.06 Å) indicating a better donation from a NH group relative to a NH_2 group. Correspondingly, the Fe-S bond lengths are longer for the $\eta\text{-NH}$ model than the $\eta\text{-NH}_2$ model.

The $\text{Fe-}\mu_2\text{S}_{\text{sulfide}}$, Fe-SMe and the Fe-SSMe bond lengths of the optimized HydE model is in the range

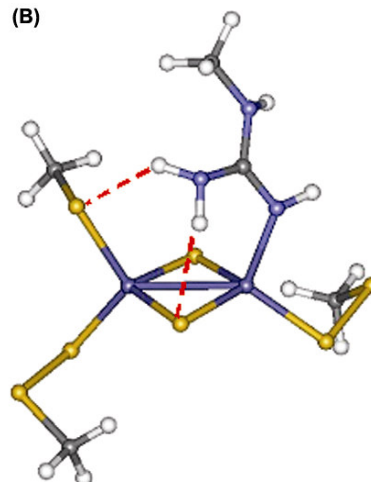
Table 1 – Bond lengths of optimized geometries of oxidized and reduced iron-sulfur clusters of Fd, BioB and HydE

Model	Fe-S _{sulfide}		Fe-SCys		Fe-SSCys		S-S		Fe-N	Fe-Fe	N-S _{thiolate} H-bond
	P	D	P	D	P	D	P	D			
Fd	2.20	2.20	2.33	2.34						2.72	
	2.20	2.28	2.44	2.44						2.80	
BioB	2.15	2.19	2.25	2.26,2.35					2.23	2.64	3.31
η -NH ₂	2.18	2.26	2.31	2.29,2.42					2.32	2.70	3.25
BioB	2.17	2.17	2.28	2.27,2.37					2.06	2.65	3.31
η -NH	2.20	2.28	2.33	2.31,2.40					2.14	2.77	3.28
HydE	2.15	2.19	2.28		2.28	2.31	2.14	2.12	2.19	2.66	3.45
η -NH ₂	2.18	2.20	2.24		2.16	2.12	2.46	2.26	2.26	2.65	3.40
HydE	2.19	2.19		2.33	2.29	2.30	2.13	2.12	2.05	2.71	3.36
η -NH	2.18	2.25		2.40	2.29	2.16	2.17	2.32	2.09	2.71	3.18

(A)



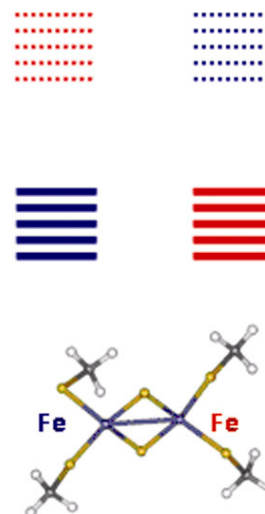
(B)

Fig. 2 – The different possible coordination of the arginine residue to Fe. [(A) η -NH₂ and (B) η -NH].

of accepted values. In particular the Fe-SMe and the Fe-SSMe bonds are of comparable lengths indicating similar ligand strengths. The S-S bonds are 2.14–2.12 Å. The changes of the Fe-S bond lengths relative to the Fd model and between the η -NH₂ and η -NH model parallels the observations in the BioB model. The total energies indicate that the η -NH₂ coordination is favored for the HydE cluster model by 5.5 kcal/mol.

There is a significant difference in the hydrogen bonding interactions present in the η -NH and η -NH₂ coordinated models. While for the η -NH₂ bound model the uncoordinated NH hydrogen bonds to the bridging μ_2 S_{sulfide} (Fig. 2A, red dashed line), the uncoordinated NH₂ in the η -NH model hydrogen bonds to both μ_2 S_{sulfide} and the thiolate ligand (Fig. 2B, red dashed line).

The Mulliken population analysis (MPA) of the spin densities reveal that all of these clusters have high spin $S=5/2$ Fe^{III} centers which antiferromagnetically couple to the adjacent $S=5/2$ Fe^{III} centers through very covalent μ_2 S_{sulfide} bridges (Scheme 1).^{16,17} The spin density is



Generic MO diagram of the oxidized Fe₂S₂ cluster. Occupied orbitals are indicated in bold while unoccupied orbitals are indicated in dashed. Orbitals to the left are α spins and those to the right are the β spins. The orbitals are color coded according to the Fe atoms they originate from. Thus, the five bold blue orbitals to the left indicate the five occupied α spin orbitals on the Fe colored blue.

Scheme 1

delocalized onto the thiolate S due to covalent bonding to the Fe. The $\mu_2\text{S}_{\text{sulfide}}$ are bonded to both Fe with up and down spins resulting in negligible spin density on them. The spin densities on the ligated sulfur atoms in the BioB cluster are more than that of the Fd cluster. This indicates more covalent bonding between Fe and $\mu_2\text{S}_{\text{sulfide}}$ and CH_3S^- ligands compensating the weaker donation by the neutral guanidine ligand. The spin densities of the HydE cluster are similar to those of the BioB cluster. A significant difference is the presence of spin density on the non-coordinated sulfur atom of the MeSS^- ligand. This indicates charge/spin delocalization into the S-S unit of this ligand.

(B) Reduced

The reduced Fd cluster shows a valence localized antiferromagnetically coupled $\text{Fe}^{\text{III}}\text{Fe}^{\text{II}}$ state. This results from strong super-exchange interaction of the $\mu_2\text{S}_{\text{sulfide}}$ bridges which overcomes the double exchange interaction present in this mixed-valent system.^{18,19} The Fe- $\mu_2\text{S}_{\text{sulfide}}$ bond of the reduced Fe elongates by 0.08 Å. The spin density on the $\mu_2\text{S}_{\text{sulfide}}$ increases as the Fe^{III} ion with higher spin moment dominates the bonding. These results are consistent with previous reports.

Reduction of the BioB cluster also results in a localized mixed valence $\text{Fe}^{\text{III}}\text{Fe}^{\text{II}}$ state. The Fe- $\mu_2\text{S}_{\text{sulfide}}$ bonds of the distal Fe elongates by 0.07-0.09 Å depending on the nature of guanidine coordination. Alternatively, the proximal Fe- $\mu_2\text{S}_{\text{sulfide}}$ bond lengths elongate only by 0.03 Å. Additionally, the larger positive spin density on the proximal Fe and the positive spin polarization of the $\mu_2\text{S}_{\text{sulfide}}$ imply that the proximal Fe remains oxidized while the distal Fe coordinated to terminal thiolates gets reduced. Thus, coordination of the guanidine group (irrespective of the nature of coordination) lowers the reduction potential of the proximal Fe such that the distal Fe is preferentially reduced.

Reduction of the HydE cluster also produces a valence localized $\text{Fe}^{\text{III}}\text{Fe}^{\text{II}}$ state. However there is increased spin density on the non-coordinated MeSS^- sulfur (Table 2).¹² This indicates delocalization of the additional electron on to the S-S bond. This is better represented in the spin density contour of the oxidized state (Fig. 3 (A)) and the reduced state (Fig 3 (B)). There is a growth of spin density on the non-coordinated sulfur as indicated by the green lobe on it.

Table 2 – Spin densities of optimized geometries of oxidized and reduced iron-sulfur clusters of Fd, BioB and HydE

	Fe		$\mu_2\text{S}$	SCys		SSCys		SSCys		Arg
	P	D		P	D	P	D	P	D	
Fd	3.08	-3.09	0.02	-0.29	0.27					
	4.47	-3.20	0.28	0.20	-0.25					
BioB	3.07	-2.99	0.05	0.32	-0.31,-0.24					0.06
$\eta\text{-NH}$	3.16	-2.81	0.65	0.23	-0.18,-0.13					0.09
BioB	3.00	-2.98	0.19	0.34	-0.31,-0.27					0.07
$\eta\text{-NH}_2$	3.07	-2.72	0.72	0.24	-0.18,-0.14					0.05
HydE	3.04	-2.99	0.14		-0.27	0.23	-0.24	0.06	-0.06	0.09
$\eta\text{-NH}$	3.08	-2.63	0.53		-0.21	0.17	-0.19	0.03	0.08	0.10
HydE	2.97	2.94	0.34		-0.36	0.22	-0.25	0.04	-0.06	0.06
$\eta\text{-NH}_2$	2.73	-1.91	0.23		-0.11	0.13	-0.06	-0.17	0.06	0.08

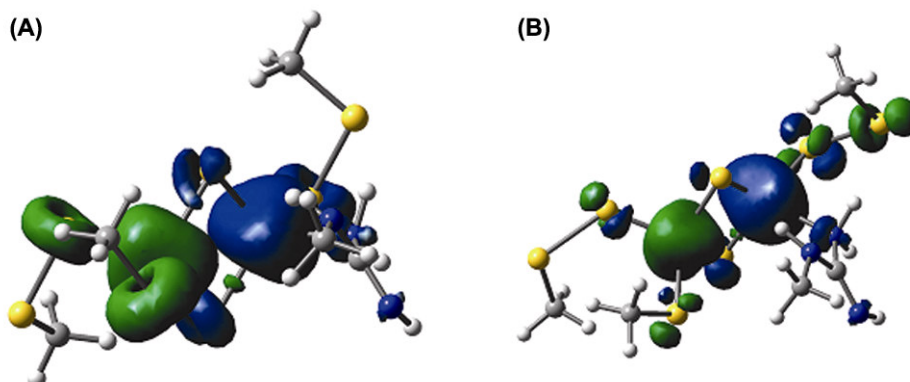


Fig. 3 – Spin density contour of the oxidized (A) and reduced (B) HydE cluster.

Table 3 – The calculated E_o values for BioB and HydE clusters

Model	E_o (V)	ΔE_o (V) (NH-NH ₂)	ΔE_o (V) (S-S)
BioB	2.89		
η -NH BioB	3.15	0.26	
η -NH ₂ HydE	3.40		0.51
η -NH HydE	3.76	0.36	0.61
η -NH ₂			

Reduction potential

The Fe_2S_2 clusters generally perform one electron oxidation and reductions, and an important parameter is their reduction potentials. The calculated values in the gas phase for the clusters in BioB and HydE are listed in Table 3. In both the cases, switching the mode of coordination from η -NH to η -NH₂ of the guanidine group leads to 0.26-0.36 V shift in the calculated reduction potentials. This observed shift has two contributions: (a) the weaker donor strength of the -NH₂ group relative to the =NH group, and, (b) hydrogen bonding difference between the η -NH₂ and η -NH models. As discussed in the earlier, the hydrogen bonding is stronger in the η -NH coordinated model as the free NH₂ hydrogen bonds to both $\mu_2\text{S}_{\text{sulfide}}$ and thiolate ligands. Thus, this will shift the reduction potential of the η -NH₂ coordinated model more negative and not positive as calculated.^{20,21} Note that the calculated net positive shift reflects the compensation of this hydrogen bonding effect by the weaker donation of the -NH₂ group.

The presence of the S-SMe ligand produces a remarkable positive shift in the reduction potential relative to a normal SMe coordinated model. The potential is shifted by 0.51 V and 0.61 V in the =NH and -NH₂ coordinated models, respectively. This is due to the efficient delocalization of the excess electron density of the reduced state into the S-S σ^* orbitals.¹² This is possible due to strong backbonding interactions present between the HOMO of the reduced state and the unoccupied S-S σ^* orbital of the MeSS^- ligand bound to the Fe.¹² This delocalization preferentially stabilizes the reduced state, causing the calculated positive shift in reduction potential of these Fe_2S_2 clusters.

Conclusions

DFT calculations have been used to compare the geometric and electronic structures of the unusual Fe_2S_2 clusters present in BioB and HydE. The

calculations indicate that both the η -NH and η -NH₂ coordination modes result in stable structures. While the η -NH coordination is slightly favored for the BioB site, the η -NH₂ coordination is stable by more than 5 kcal/mol for the HydE cluster using the current level of theory. The coordination of the nitrogen ligand lowers the potential of the proximal iron and results in the reduction of the distal iron atom. The reduction potential of the η -NH coordinated cluster is lower due to its stronger donation. The presence of the MeS-S^- ligand raises the potential by 0.5-0.6 eV due to stabilization of the reduced cluster via backbonding into the S-S σ^* orbital.

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