

### Theoretical Studies of the Oxidized and Reduced States of a Model for the Active Site of Rubredoxin

Sir:

Although non-heme iron-sulfur proteins are ubiquitous among living systems, their role in biological processes is understood in only a few systems and even in these cases the details of the electron transfer and chemical processes they promote have not been elucidated.<sup>1-5</sup> The physical properties are best understood for rubredoxin (Rd, hereafter), plant ferredoxin (Fd, hereafter), bacterial ferredoxin, and high potential iron-sulfur protein containing one, two, four, and eight irons, respectively, with each iron tetrahedrally coordinated to four sulfurs, and contained in clusters of one, two, or four irons.<sup>1-4</sup>

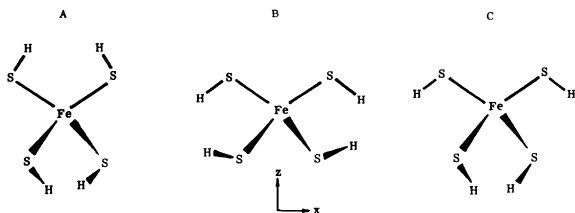


Figure 1. Geometries of  $\text{Fe}(\text{SH})_4$  model complexes.

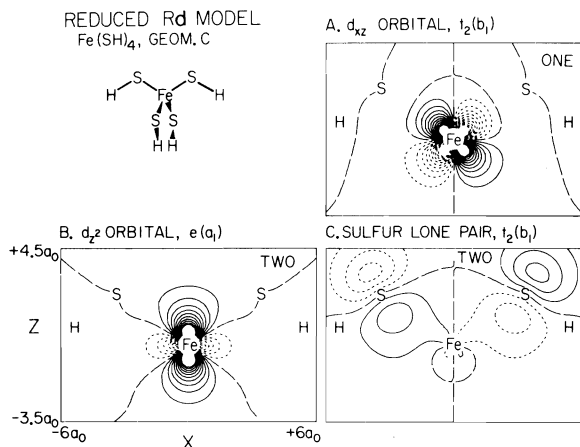


Figure 2. Amplitudes for the d orbitals for the quintet ground state of the reduced model,  $\text{Fe}(\text{SH})_4^{2-}$ . Long dashes indicate zero amplitude, solid and dotted lines indicate positive and negative amplitude (separation of adjacent contours is 0.05 au). In this state four d orbitals are singly occupied while the  $d_{z^2}$  orbital is doubly occupied.

In this initial study we use ab initio quality theoretical methods to examine the electronic properties of the active site of oxidized and reduced Rd for several geometries.

To model the active site of Rd, we have replaced the four cysteine ligands of the Fe with  $(\text{SH})^-$  groups. The Fe-S bond length was set to the average distance of 2.21 Å from the crystal structure studies<sup>6</sup> (more recent results<sup>7</sup> do not confirm the large inequalities of the Fe-S bond lengths of the early structural work<sup>6</sup>) and the sulfur atoms placed tetrahedrally. The S-H bond distance was fixed at 1.33 Å (as in  $\text{SH}_2$ ) and the H-S-Fe angle was chosen as the average value 107° of the  $\text{C}(\beta)\text{-S-Fe}$  angle from the crystal structure studies<sup>6</sup> (except for geometry A where the angle was taken as 92.2°). The spectrum of low excited states was examined for three different sets of S-Fe-S-H dihedral angles, as indicated in Figure 1. Geometry C most closely models the experimentally determined structure (oxidized Rd, we also find a lowest energy for this geometry).

We carried out Hartree-Fock (HF)<sup>8</sup> calculations on a number of excited states as discussed below. The calculations are ab initio except that the argon core of the Fe atom<sup>9</sup> and the Ne core of each S atom<sup>10</sup> were replaced with ab initio effective potentials. This allows ab initio quality calculations using an extensive "double zeta" basis set.<sup>11</sup>

The oxidized complex may be considered as  $\text{Fe}^{\text{III}}(\text{SH}^-)_4$  or  $[\text{Fe}(\text{SH})_4]^-$  and the reduced state as  $\text{Fe}^{\text{II}}(\text{SH}^-)_4$  or  $[\text{Fe}(\text{SH})_4]^{2-}$ . However, no restrictions were made on the shape or localization of the various orbitals; they were solved self-consistently for numerous states of both the oxidized and reduced forms.

In oxidized Rd, the Fe can be thought of as ferric  $d^5$  and hence can lead to either high spin ( $S = 5/2$  or sextet), intermediate spin ( $S = 3/2$  or quartet), or low spin ( $S = 1/2$  or doublet). Magnetic susceptibility results<sup>12</sup> clearly indicate an  $S = 5/2$  ground state which is also consistent with ESR<sup>13</sup> and Mössbauer<sup>14</sup> experimental results. We considered the lower states of each spin and found (see Table I) that the ground state is clearly high spin with the lowest quartet state higher by over 1.5 eV and the lowest doublet state higher by over 3 eV. This result confirms experimental observation and indicates our methods are accurate enough to predict ground state spins.

A  $d^5$  configuration leads to only one sextet state and consequently the lower excited states are found to result from spin-forbidden (sextet to quartet) d-d transitions, as indicated in Table I. In oxidized Rd, the charge transfer bands are found to start at  $\sim 2.2$  eV;<sup>15</sup> however, there is a weak band observed at 1.66 eV<sup>16</sup> which, on the basis of these calculations, could be

**Table I.** Excited States of Fe(SH)<sub>4</sub> (energies in eV). A, B, and C Indicate the Geometries (See Figure 1 for coordinate system)

	Configuration <sup>a</sup>					Calculated					Exptl <sup>b</sup>	
	<i>z</i> <sup>2</sup>	<i>xy</i>	<i>x</i> <sup>2</sup> - <i>y</i> <sup>2</sup>	<i>xz</i>	<i>yz</i>	A	B	C				
Oxidized	1	1	1	1	1	<sup>6</sup> A <sub>1</sub>	0 <sup>c</sup>	<sup>6</sup> A <sub>1</sub>	0 <sup>c</sup>	<sup>6</sup> A <sub>1</sub>	0 <sup>c</sup>	0
	2	1	1	1	0	<sup>4</sup> E	1.76	<sup>4</sup> E	1.87 <sup>e</sup>	<sup>4</sup> B <sub>2</sub>	1.72	1.66
	2	1	0	1	1			<sup>4</sup> B <sub>2</sub>	1.61			
	1	2	1	1	0	<sup>4</sup> E	2.06	<sup>4</sup> E	2.21			
	1	1	2	1	0	<sup>4</sup> E	2.25	<sup>4</sup> E	2.48			
	2	2	1	0	0	<sup>2</sup> B <sub>2</sub>	3.06					
Reduced	2	1	1	1	1	<sup>5</sup> A <sub>1</sub>	0 <sup>d</sup>	<sup>5</sup> A <sub>1</sub>	0 <sup>d</sup>	<sup>5</sup> A <sub>1</sub>	0 <sup>d</sup>	0
	1	2	1	1	1	<sup>5</sup> B <sub>1</sub>	0.39	<sup>5</sup> B <sub>1</sub>	0.42	<sup>5</sup> A <sub>2</sub>	0.37	
	1	1	2	1	1	<sup>5</sup> B <sub>2</sub>	0.59	<sup>5</sup> B <sub>2</sub>	0.70	<sup>5</sup> A <sub>1</sub>	0.52	≤0.50
	1	1	1	2	1	<sup>5</sup> E	0.72	<sup>5</sup> E	0.75	<sup>5</sup> B <sub>1</sub>	0.72	0.68-0.87
	1	1	1	1	2	<sup>5</sup> E	0.72	<sup>5</sup> E	0.75	<sup>5</sup> B <sub>2</sub>	0.80	
	2	2	1	1	0	<sup>3</sup> E	2.56					
	2	2	2	0	0	<sup>1</sup> A <sub>1</sub>	4.70					

<sup>a</sup> With our coordinate system, *z*<sup>2</sup> and *xy* are e<sub>g</sub>-type d orbitals while *x*<sup>2</sup> - *y*<sup>2</sup>, *xz*, and *yz* are t<sub>2g</sub> type. <sup>b</sup> Eaton et al., ref 16. <sup>c</sup> Total energies for the <sup>6</sup>A<sub>1</sub> state are -65.2598, -65.2637, and -65.2706 h for geometries A, B, and C, respectively. <sup>d</sup> Total energies for the <sup>5</sup>A<sub>1</sub> state are -65.2461, -65.2579, and -65.2554 h for geometries A, B, and C, respectively. <sup>e</sup> ORD and CD spectra suggest the presence of a band at 1.91 eV: D. J. Newman and J. R. Postgate, *Eur. J. Biochem.*, **7**, 45 (1968).

a spin-forbidden d-d transition (calculated at 1.72 eV, for geometry C). This band has been previously assigned as a <sup>4</sup>T<sub>1</sub> ← <sup>6</sup>A<sub>1</sub> d-d transition by Rawlings et al.<sup>17</sup>

In reduced Rd, the Fe can be thought of as ferrous d<sup>6</sup>, and again magnetic susceptibility experiments clearly indicate a high spin (*S* = 2 or quintet) ground state, rather than intermediate spin (*S* = 1 or triplet) or low spin (*S* = 0 or singlet). We find that the ground state is high spin with the lowest triplet and singlet states over 2 and 4 eV higher, respectively. Again, this result is in agreement with experimental observation.

The self-consistent d orbitals of the quintet ground state (geometry C) are shown in Figure 2. Note here that for our coordinate system the tetrahedral e orbitals are d<sub>z<sup>2</sup></sub> and d<sub>xy</sub> (not d<sub>z<sup>2</sup></sub> and d<sub>x<sup>2</sup>-y<sup>2</sup></sub>) while the t<sub>2</sub> orbitals are d<sub>xz</sub>, d<sub>yz</sub>, and d<sub>x<sup>2</sup>-y<sup>2</sup></sub> (not d<sub>xz</sub>, d<sub>yz</sub>, and d<sub>xy</sub>). On each (SH)<sup>-</sup> group there is a lone pair perpendicular to the H-S-Fe plane. There is a second lone pair and an S-H bond pair in this plane. These pairs have different effects upon the Fe orbitals, leading to a symmetry lower than *T<sub>d</sub>*.

There are five low-lying quintet d<sup>6</sup> states. In *T<sub>d</sub>* symmetry this would lead to <sup>5</sup>E and <sup>5</sup>T<sub>2</sub> states. We find that even with four S in *T<sub>d</sub>* symmetry the orientations of the four S-H bonds cause significant splitting, leading to <sup>5</sup>E-like states at 0.0 and 0.37 eV and <sup>5</sup>T<sub>2</sub>-like states at 0.52, 0.72, and 0.80 eV (geometry C). Thus we find dipole-allowed d-d transitions at 0.52, 0.72, and 0.80 eV, in excellent agreement with the spectroscopic studies of Eaton and co-workers<sup>16</sup> who find one electronic transition with a peak at <0.50 eV and another broad transition at 0.68-0.87 eV, both with circular dichroism consistent with d-d transitions. We find no other spin-allowed d-d transitions and no spin-forbidden transitions below the energy (2.2 eV) expected for charge transfer transitions; this is consistent with the experimental results.<sup>14</sup>

Averaging appropriate subcomponents leads to a <sup>5</sup>E-<sup>5</sup>T<sub>2</sub> splitting of 10*Dq* = 0.50 eV = 4030 cm<sup>-1</sup>. This is a bit less than the value of 4400 cm<sup>-1</sup> observed for tetrahedral aquo ferrous iron complexes,<sup>18a</sup> as would be expected for sulfur ligands.<sup>18b</sup>

The sulfur lone pairs interact sufficiently with the iron d<sub>xz</sub> and d<sub>yz</sub> orbitals to cause the states with two electrons in either of these d orbitals to be highest. A comparison of the reduced states of all three geometries shows that the energy separation of the d-d transitions is sensitive to the dihedral angle of the S-H bond. The position of the S-H bond pair strongly influences the orientation of the S lone pair pointing toward the Fe, which in turn directly influences the energies of the Fe d<sub>xz</sub> and

d<sub>yz</sub> orbitals. This sensitivity may lead to a mechanism for protein conformation to provide control over redox potentials and related properties of the protein.<sup>19</sup>

Similar models of the active site of Rd have previously been calculated with the extended Hückel<sup>20</sup> and Xα methods.<sup>21</sup>

In summary, using ab initio calculations on a model of the active site of Rd, we find electronic and spectroscopic properties in excellent agreement with experiment. Thus we find unequivocally that the oxidized and reduced forms are high spin, and we obtain excellent agreement (~0.06 eV error) with experimental excitation energies.

These calculations, being ab initio, allow us to compare energies for various geometries and hence to solve for the local geometry of the oxidized and reduced active sites, even for excited states.

Similar studies should be particularly useful for the ferredoxins where one could determine the geometric distortions for various oxidized and reduced states and investigate models for the electron transfer mechanisms. Such studies are in progress.

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