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# Calculated Properties of the Active Site Complex of Oxidized Rubredoxins

## II. Electric Field Gradient, Quadrupole Splitting and Hyperfine Interactions

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The active site of rubredoxins, the simplest class of iron-sulfur, electron-transfer proteins consists of a single Fe atom surrounded by a distorted tetrahedral array of four cysteine sulfur atoms. With the aid of a newly formulated computer program, we have calculated the electric field gradient at the $Fe^{57}$ nucleus, the resultant quadrupole splitting ($\Delta E_Q$) and the isotropic and anisotropic parts of the hyperfine interaction between the Fe nuclear spin and the net electron spin in the sextet ground state for ten conformational variations of the active site complex. For each conformer we used an electron distribution obtained from an Iterated Extended Huckel Theory (IEHT) molecular orbital calculation. Comparison of calculated and experimental results of ($\Delta E_Q$) obtained from Mossbauer resonance spectra indicated that a number of conformers have field gradient values in excellent agreement with experiment. Good agreement with experiment was also found for the calculated hyperfine coupling constant. In this interaction, the isotropic contribution is dominant while the anisotropic contribution is more symmetry dependent. Since a single average experimental value is observed, hyperfine interaction in this system is not a very sensitive probe of active site conformation.

*Key words:* Rubredoxins, oxidized – Iron-sulfur proteins – Mössbauer resonance – Hyperfine interactions

## 1. Introduction and Background

The Mossbauer resonance of at least two different $Fe^{57}$ enriched rubredoxin proteins has now been studied in detail. In the oxidized state, the quadrupole splitting ($\Delta E_Q$) obtained at 77 °K was 0.78 ± mm/sec [1]. From the low temperature Mossbauer resonance spectra, the hyperfine interaction of the $Fe^{57}$ nucleus with the unpaired electron density of the sextet ground state was also determined. A value of $375 \pm 55$ KGauss was obtained in one study [1] while a similar value of $370 \pm 3$ KGauss was obtained in the other [2]. Lack of resolution of the Mossbauer resonance spectra of the oxidized protein in a magnetic field prohibited the determination of the sign of the quadrupole splitting, but in the reduced state it was determined to be negative.

It is well known that the field gradient at the Fe nucleus is a sensitive function of the conformation of atoms surrounding the nucleus. Yet very little is known of the one-to-one correspondence of conformation and the magnitude of the field gradient. In order to study this dependence explicitly we have calculated the field gradient as a function of the conformation of the ligands about the Fe atom in the active site complex of oxidized rubredoxin. The basic model we have chosen for this active site is shown in Fig. 1 of the previous paper and described there. The active site complex is formally $Fe^{+3}$ plus 4 $SH^-$ ions, with 29 valence atomic orbitals and 37 valence electrons. Among the 10 conformations studied, is the one determined completely from the X-ray crystal structure [4] but with H atoms in the place of the $\beta$C atoms bound to the S of the cysteine residues ($0_1$). The 9 variations of the crystal structure considered are described in Paper I [3].

In the sextet configuration the electron occupancy of the molecular orbitals is as follows: $(1)^2 \ldots (16)^2 (17)(18)(19)(20)(21)$. The half-filled molecular orbitals 17–21 are the so-called ligand field, primarily $3d$, antibonding orbitals. In this calculation we have used the total set of 21 occupied orbitals obtained from an IEHT calculation [5, 6] for each symmetry, to calculate all 9 components of the electric field gradient tensor. We also obtain the principal components $V_{xx}$, $V_{yy}$ and $V_{zz}$ in a diagonal representation of this tensor and use these to calculate the quadrupole splitting in the Mossbauer resonance for each of the conformation studied.

The hyperfine coupling constants $A$ and $B$ depend only on the nature of the five half-filled molecular orbitals (17–21). These are used to calculate both the isotropic coupling constant ($A$) and the 9 components of the anisotropic coupling constant ($B$) for each of the 10 active site conformations studied. A more detailed account of the calculation of the field gradient and hyperfine interaction constants and the results obtained follows.

## 2. Isotropic Hyperfine Interaction

The isotropic hyperfine interaction between the nuclear spin of the Fe and the net electron spin at the nucleus is expressed by the so-called Fermi contact Hamiltonian [7]:

$$H_F = \frac{8\pi}{3} g_e \beta_e g_{Fe} \beta_n \sum_k I \cdot S \delta(r_k) \tag{1}$$

where $\beta_e$ and $\beta_n$ are the Bohr and nuclear magneton respectively, $g_e$ is the electron $g$ factor and $g_{Fe}$ is the $g$ value for $Fe^{57}$. The corresponding hyperfine coupling constant is given by $A = \langle H \rangle / IS$ where $I$ is the nuclear spin quantum number and $S$ the total spin quantum number of the electronic state of the molecule.

Using the molecular orbital description obtained from the IEHT program which ignores explicit electron correlation, we are forced to break the isotropic coupling constant into two parts: a direct term which is the result of direct interactions between the valence electrons and the nuclear spin, and the core-polarization term between the net spins in the inner-shell electrons due to polarization and the nuclear spin, neglecting the much smaller back-polarization of the valence electrons by the net spin of the inner shell electrons. Thus in our calculation $A_{\text{total}} = A_{\text{direct}} + A_{\text{polar}}$. The first term is obtained immediately by taking the expectation value of $H_F$ with respect to the sextet ground state. According to the molecular orbital description, the sextet ground state is the product of the five unpaired molecular orbitals and $A_{\text{direct}}$ is reduced to:

$$\text{(A)} \qquad A_{\text{direct}} = \frac{8\pi g_e \beta_e g_n \beta_n}{6S} \sum_{\substack{u \\ \text{unpaired}}}^{\text{mos}} |\Psi_u(\text{Fe})|^2 . \tag{2}$$

In our calculations we use $S=5/2$ and $g_{\mathrm{Fe}}=0.1806$. The sum is over the five half-filled molecular orbitals 17–21 and $\sum_u |\Psi_u(\mathrm{Fe})|^2$ is the electron density at the Fe nucleus. Following the formulation given by Han, Das and Rettig in their calculation of the hyperfine interaction at the $N$ nucleus in heme complexes [8], the quantity $\sum_u |\Psi_u(\mathrm{Fe})|^2$ can be divided into three contributions: a local term (2a), a cross term (2b) and a distant term (2c) as follows:

$$\sum_u |\Psi_u(\mathrm{Fe})|^2 = \sum (C^{\mathrm{Fe}}_{u,4s})^2\, |\Phi^{\mathrm{Fe}}_{4s}(\mathrm{Fe})|^2_{r=0} \tag{2a}$$

$$+\sum_u \sum_i C^{\mathrm{Fe}}_{u,4s}\, C^{\mathrm{S}}_{u,i}\, (\phi^{\mathrm{Fe}}_{ns}(\mathrm{Fe}))_{r=0}\, (\phi^{\mathrm{S}}_i(\mathrm{Fe}))_{r=r_{\mathrm{Fe-S}}} \tag{2b}$$

$$+\sum_u \sum_i (C^{\mathrm{S}}_{u,i})^2\, |\phi^{\mathrm{S}}_i(\mathrm{Fe})|^2_{r=r_{\mathrm{Fe-S}}} \tag{2c}$$

where $|\phi^{\mathrm{Fe}}_{4s}(\mathrm{Fe})|^2$ = electron density of the Fe(4$s$) atomic orbital at its nucleus.

$(\phi^{\mathrm{Fe}}_{4s}(\mathrm{Fe}))_{r=0}\,(\phi^{\mathrm{S}}_i(\mathrm{Fe}))_{r=r_{\mathrm{Fe-S}}}$ = pairwise product of the value of the Fe 4$s$ wave function and the value of the S ligand atomic orbitals at the Fe nucleus;

and

$|\phi^{\mathrm{S}}_i(\mathrm{Fe})|^2_{r=r_{\mathrm{Fe-S}}}$ = electron density of the $i^{\mathrm{th}}$ S ligand atomic orbital at the Fe nucleus.

$C_{u,4s}$ = coefficient of the Fe 4$s$ atomic orbital in the $u^{\mathrm{th}}$ molecular orbital;
$C_{u,i}$ = coefficient of the $i^{\mathrm{th}}$ S atomic orbital in the $u^{\mathrm{th}}$ molecular orbital.

Equation (2) and expressions 2a–c form the basis of the computer program we have written to calculate the value of $A_{\mathrm{direct}}$ from these three contributions. The atomic orbital coefficients needed are supplied to this program from the results of the IEHT molecular orbital calculations. These coefficients are used together with Clementi's [9] extended Slater Type Orbital (STO) expression for the 4$s$ Fe atomic orbital and for all the S atomic orbitals, to calculate the three contributions to the electron density at the Fe nucleus.

To evaluate the core polarization contribution to $A$ we use the "pseudo-atom" formulation of Das and Han in their calculation for Fe in heme complexes [10]. In their approximation the

$$A_{\mathrm{polar}} = (d_{\mathrm{den}}/4)\Bigg\{ \sum_{l=1s}^{3s} [|X_{l\alpha}(0)|^2 - |X_{l\beta}(0)|^2] \tag{3a}$$

$$+\frac{(4s\ \mathrm{dens})}{2}[|X_{4s\alpha}(0)|^2 - |X_{4s\beta}(0)|^2]\Bigg\} \tag{3b}$$

where $d_{\mathrm{den}}$ = total number of 3$d$ electrons with unpaired spin
4$s$ dens = total number of 4$s$ electrons with paired spin

and the quantity $[|X_{l\alpha}(0)|^2 - |X_{l\beta}(0)|^2]$ is the difference in the density of the $\alpha$ and $\beta$ spin components of each $s$ function at the Fe nucleus obtained from an unrestricted Hartree-Fock calculation for $^5D$ atomic Fe [12]. The only important

Table 1. Values of $A$ and $B$ for 10 conformers of oxidized rubredoxin

| Conformer | $0_1$ [a] | $0_2$ [b] | $A$ [c] | $B$ [c] | $C(C_{2v}$ | $D(D_2)$ | $E(T_D)$ [d] | $F(C_3)$ | $G(C_{3v})$ | $H^{(\mathrm{planar})}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| $A_{\mathrm{direct}}^{(\mathrm{local})}$ (MHz) | 0.59 | 0.91 | 0.26 | 0.16 | 0.20 | 1.56 | 0 | 0.12 | 0.46 | 5.81 |
| $A_{\mathrm{direct}}^{(\mathrm{cross\ term})}$ | − 0.25 | − 0.29 | 0.26 | 0.16 | 0.22 | − 0.56 | 0 | 0.17 | − 0.01 | 0.59 |
| $A_{\mathrm{direct}}^{(\mathrm{distant})}$ | 0.28 | + 0.31 | 0.23 | 0.19 | 0.19 | 0.82 | 0.76 | 0.39 | 0.15 | 1.27 |
| $A_{\mathrm{diret}}^{(\mathrm{total})}$ | 0.62 | + 0.93 | 0.76 | 0.51 | 0.61 | 1.82 | 0.76 | 0.68 | 0.60 | 7.67 |
| $d_{\mathrm{dens}}$ | 3.08 | 3.03 | 2.90 | 2.90 | 2.86 | 2.54 | 3.10 | 2.91 | 2.90 | 2.61 |
| $A_{(\mathrm{polar})}$ | −21.86 | −20.87 | −20.31 | −20.20 | −20.01 | −18.92 | −21.71 | −19.95 | −20.13 | −19.57 |
| $A_{\mathrm{total}}$ (MHz) | −20.24 | −19.94 | −19.56 | −19.69 | −19.40 | −17.08 | −20.95 | −19.14 | −19.48 | −11.90 |
| $B_{xx}$ (MHz) | − 2.19 | − 2.55 | − 1.12 | − 0.69 | − 1.27 | − 1.38 | 0 | − 0.32 | − 1.67 | − 3.76 |
| $B_{yy}$ (MHz) | − 0.54 | − 0.23 | − 0.60 | − 0.45 | − 0.60 | − 1.07 | 0 | − 0.32 | − 1.67 | − 2.38 |
| $B_{zz}$ (MHz) | + 2.73 | 2.78 | + 1.72 | + 1.15 | 1.87 | + 2.45 | 0 | 0.64 | 3.33 | 6.14 |
| $H_A^{\mathrm{eff}}$ (kG) [f] | 372 | 364 | 360 | 362 | 354 | 312 | 383 | 365 | 365 | 209 |
| $H_B^{\mathrm{eff}}$ (kG) [e] | 90 | 97.5 | 52 | 34 | 56 | 70 | 0 | 175 | 91.5 | 181 |

[a] Totally experimental conformer.

[b] Totally experimental conformer with shortened S–H bond = 1.34 Å.

[c] S atoms in experimentally determined position; H atom positions varied.

[d] Calculated values of $B$ are zero in $T_D$ symmetry; a good test of formulation.

[e] $H_B^{\mathrm{eff}}$ (kG) = 18.3 $(Bzz - Bxx)$ MHz.

[f] $H_A^{\mathrm{eff}}$ (kG) = $A$ (MHz) · $S/\beta_n g_{\mathrm{Fe}}$ = 18.3 $A$ (MHz).

difference between the $^5D$ Fe and the Fe pseudo-atom in its active site complex is assumed to be in the actual number of unpaired $d$ electrons and paired 4$s$ electrons, present in each case. The core polarization is assumed to be proportional to these differences, i.e. $d$ dens/4 + 4$s$ dens/2. Both $d$ dens and 4$s$ dens are obtained from a Mulliken population analysis [11] of the half-filled molecular orbitals in the IEHT calculation [3]. The core polarization by the small amount of unpaired 4$p$ electron density is ignored in these expressions. Typically, the ratio of unpaired 3$d$ to unpaired 4$p$ electron density in the active site complex was 100/1.

Using the working program just described, we have calculated the isotropic hyperfine interaction constant for each of the 10 geometric conformations of the active site of oxidized rubredoxin. In the results given in Table 1, the three contributions to $A_{\mathrm{direct}}$ are included (in units of MHz where 1 MHz = $3.34 \times 10^{-5}$ cm$^{-1}$). We see that the magnitude of the three individual contributions are quite symmetry dependent. The value of $A_{\mathrm{direct}}^{\mathrm{local}}$ is a direct reflection of the %Fe (4$s$) character in the half-filled molecular orbitals. The value of $A_{\mathrm{direct}}^{\mathrm{distant}}$ reflects the amount of unpaired ligand electron density at the Fe nucleus and varies by a factor of ten.

Looking at the core polarization contribution to $A$, also given in Table 1, ($A$ polar) we see that it totally dominates the direct contribution for each conformer by a factor ranging from 3 in planar symmetry to 30–40 in most other conformers and its magnitude is much less symmetry dependent because the variation in $d_{\mathrm{dens}}$ with symmetry is not marked. Since total values of $A$ are dominated by the contribution from the core polarization, its sensitivity to conformation is diminished compared to the values of $A_{\mathrm{direct}}$.

## 3. Dipolar Contribution to Electron-Nuclear Spin Coupling

The electron-nuclear spin dipolar Hamiltonian is given by:

$$H_{\mathrm{dip}} = \beta e\, ge\, \beta n\, gn \sum_i \frac{3(S_i \cdot r_i)(I \cdot r_i) - r_i^2 (S_i \cdot I)}{r^5}$$

where the constants are as defined in Eq. (1), $I$ is the nuclear spin, $r_i$ is the vector from the origin at the nucleus to the $i^{\mathrm{th}}$ electron and $S_i$ is the spin of the $i^{\mathrm{th}}$ electron.

The contributions of $H_{\mathrm{dip}}$ to the spin-Hamiltonian is given by: $H_{\mathrm{dip}}^{\mathrm{spin}} = \boldsymbol{I} \cdot B \cdot \boldsymbol{S}$. The components of the tensor $B$ are obtained by equating the matric elements of $H_{\mathrm{dip}}$ over the total wave function to the matrix elements of $H_{\mathrm{dip}}^{\mathrm{spin}}$ over the spin-functions only:

$$\langle \Psi | H_{\mathrm{dip}} | \Psi \rangle = \langle H_{\mathrm{dip}}^{\mathrm{spin}} | S \rangle \tag{5}$$

where

$$\Psi = \Phi S \tag{5a}$$

and

$$\Phi = |\phi_1(1)\, \phi_2(2)\, \phi_3(3)\, \phi_4(4)\, \phi_5(5)|\,, \tag{5b}$$

$$S = \alpha(1)\, \alpha(2)\, \alpha(3)\, \alpha(4)\, \alpha(5)\,. \tag{5c}$$

The $\phi_i(i)$ are the spatial parts of the one electron MO's obtained from the IEHT calculation. Only the half-filled MO's need be considered. Using this procedure the components of the $B$ tensor are given by:

$$B_{ij} = \frac{g_e \beta_e g_{\mathrm{Fe}} \beta_n}{5} \sum_{\substack{u \\ \text{unpaired} \\ \text{MO}}} \sum_i \sum_j C_{ui} C_{uj} \langle \chi_i | \frac{1}{r^3} \left\{ \frac{3x_i y_j - \delta_{ij} r^2}{r^2} \right\} | \chi_j \rangle\,. \tag{6}$$

In Eq. (6), the matric elements of each component of $B$: $\langle X_i | B | X_j \rangle$ are over an atomic orbital basis set and $c_{ui}$, $c_{uj}$ are the coefficients of the $X_i$ and $X_j$ atomic orbitals in the $u^{\mathrm{th}}$ half-filled molecular orbital. The $(1/r^3)$ factor is the radial part of the operator equivalent of $B$ while the expression in brackets is the angular part of the $B$ tensor operator. Each angular component of this operator can be expressed as a sum of spherical harmonic functions.

Using Eq. (6), we have included the calculation of each component of $B$ in our hyperfine interaction program. In the evaluation of $B$ at the Fe nucleus we have considered only the one-centered local terms. Thus, the sum over atomic orbitals in (6) is limited to the $4p$ and $3d$ Fe orbitals. The two-centered non-local terms were neglected because of the sharp decay of the $1/r^3$ term as distance increases from the origin. We have used a single value of $\langle X_i | 1/r^3 | X_j \rangle$ of 2.0369 a.u. for all combinations of $4p$ atomic orbitals [10] and of 5.0 a.u. for all combinations of $3d$ atomic orbitals [10]. Additionally, the $3 \times 3$ $B$ tensor matrix obtained is diagonalized to yield values of $Bxx$, $Byy$, and $Bzz$ in a principal axis representation of $B$.

Using the program just described we have calculated the $B$ tensor components of the anisotropic hyperfine coupling for the 10 conformational variations of the active site complex of the oxidized rubredoxins. Table 1 gives the values of $Bxx$, $Byy$ and $Bzz$ obtained for each symmetry in a diagonal representation (in units of MHz). We see from this table that the $B$ values are zero in the totally tetrahedral field as expected from symmetry considerations alone. For all other symmetries the anisotropic contribution to the hyperfine splittings are highly symmetry-dependent and of the same order of magnitude or greater than the direct contribution to the isotropic interaction. In Table 1 we have indicated the total anisotropy as $H_B^{\text{eff}}$ (kG), by the value of $(B_{zz}-B_{xx})$ converted to an effective field at the Fe nucleus.

As we have mentioned, the magnitude of the hyperfine field at the $\text{Fe}^{57}$ nucleus in the oxidized rubredoxin obtained experimentally was 370–375 kG [1, 2]. For the oxidized state, this is an average value with no attempt to determine to which component of an anisotropic effective field, if any, this single observed value most closely approximates. Thus for the conformers studied, the experimental value of $H^{\text{eff}}$ (kG) is best compared with our calculated value of $H_A^{\text{eff}}$ (kG) which represents an isotropic value of the effective field, while bearing in mind that the value of $H_B^{\text{eff}}$ (kG) represents the possible anisotropic spread in this value which would be of interest to observe experimentally. On this basis we see that all symmetries except the planar and tetrahedral ones give good agreement with experiment and that as we have said the predominance of $A_{\text{polar}}$ greatly diminishes the sensitivity of the hyperfine interaction as a probe of molecular conformation at the active site in the oxidized high spin rubredoxins.

## 4. Electric Field Gradient at the Fe Nucleus and Quadrupole Splitting in Mossbauer Resonance

The nuclear transition in Mossbauer Resonance of $\text{Fe}^{57}$ is split into a doublet due to the interaction of the quadrupole moment of the excited nuclear state with $I=3/2$ and the electric field gradient at the Fe nucleus. The nuclear electric quadrupole Hamiltonian in the principal axis system of the field gradient tensor may be expressed by:

$$H=\frac{eQ}{4I(2I-1)}\,V_{zz}[3I_3^2-I(I+1)]+(V_{xx}-V_{yy})(I_x^2-I_y^2) \tag{7}$$

where $Q$ = Quadrupole moment of nuclear state with spin $I$ and $V_{zz}$, $V_{xx}$, $V_{yy}$ are principal values of the electric field gradient.

For $I=3/2$ the corresponding splitting between the $M_I=\pm 3/2$ and $M_I=\pm 1/2$ states is:

$$\Delta E_Q=1/2\,eqQ[1+n^2/3] \tag{8}$$

where $q=V_{zz}$; $n=(V_{xx}-V_{yy})/V_{zz}$ and the sign convention is such that for $V_{zz}$ positive the $M_I=\pm 1/2$ state lies lower.

Equation (8) can be written in terms of the usual units used in Mossbauer Resonance:

$$\Delta E_Q\,(\text{mm/sec})=9.18\,Q\,(\text{barns})\,q\left(\frac{\text{esu}}{\text{cm}^3}\right)[1+n^2/3]^{1/2}\,. \tag{9}$$

The magnitude of this splitting is regarded as a very sensitive probe of the conformation and electron distribution about the Fe nucleus and has been used as such in a variety of heme proteins and in the two iron ferredoxins as well as for rubredoxin. However, one-to-one correlations between specific conformational

Table 2. Electric field gradient components at Fe nucleus and quadrupole splitting ($\Delta E_Q$) in Mossbauer resonance

| Conformer | $0_1$(Exp) | $0_2$(Exp) | $A$(Exp) | $B$(Exp) | $C(C_{2v})$ | $D(T_D-D_2)$ | $E(T_D)$ | $F(C_3)$ | $G(C_{3v})$ | $H(PL.C_2)$ |
|---|---|---|---|---|---|---|---|---|---|---|
| $Vzz\left(\frac{\text{esu}}{\text{cm}^3}\right)$ | 0.95 | 0.85 | 0.52 | 0.315 | 0.50 | 0.73 | 0 | 0.14 | 0.96 | 1.65 |
| $Vxx$ | $-0.09$ | $-0.09$ | $-0.34$ | $-0.27$ | $-0.19$ | $-0.43$ | 0 | $-0.07$ | $-0.48$ | $-1.33$ |
| $Vyy$ | $-0.86$ | $-0.76$ | $-0.19$ | $-0.04$ | $-0.31$ | $-0.30$ | 0 | $-0.07$ | $-0.48$ | $-0.32$ |
| $\eta\left(\frac{Vxx-Vyy}{Vzz}\right)$ | 0.81 | 0.79 | 0.29 | 0.73 | 0.24 | 0.18 | 0 | 0 | 0 | 0.61 |
| $\Delta E_{\min}(Q=0.18)$ | 1.79 | 1.62 | 0.89 | 0.57 | 0.86 | 1.26 | 0 | 0.24 | 1.65 | 3.00 |
| $\Delta E_{\max}(Q=0.48)$ | 4.58 | 4.14 | 2.29 | 1.45 | 2.20 | 3.21 | 0 | 0.62 | 4.22 | 7.61 |
| $(1-R)\Delta E_{\min}$ | 1.22 | 1.10 | 0.76 | 0.39 | 0.58 | 0.86 | 0 | 0.16 | 1.12 | 2.04 |
| $(1-R)\Delta E_{\max}$ | 3.11 | 2.82 | 1.56 | 0.99 | 1.50 | 2.18 | 0 | 0.42 | 2.87 | 5.18 |

variations and values of this splitting cannot readily be made experimentally. Rather values of splittings have been linked to such gross properties as the spin state and formal oxidization state of the Fe in the active site complex. In a recent calculation for the oxidized state of two-iron ferredoxins, we have shown that a wide variation in field gradient values can be obtained by systematic variations in the symmetry of the active site complex while maintaining its oxidation state and spin state constant [13]. We wished to further investigate the quantitative variations of field gradient (and hence quadrupole splitting) with specific conformational variations of the oxidized state of the active site complex of the one-iron proteins rubredoxins in their sextet ground state. To do this, we have written a program which calculates the expectation value of all nine components of the electric field gradient tensor for the sextet ground state using the same product form for the total state function as we used to calculate the $B$ tensor components. Since each molecular orbital $\emptyset_i$ is itself a linear combination of atomic orbitals, the expectation value of each component of the electric field gradient is:

$$\langle\psi_n|efg|\psi_n\rangle = \sum_{\substack{i\\ \text{filled mos}}} n_i\left[\sum_j \sum_k C_{ij}C_{ik}\langle X_j|efg|X_k\rangle\right] \tag{10}$$

where $n_i =$ occupancy of the $i^{\text{th}}$ mo and is either 2 or 1 and $c_{ij}$, $c_{ik}$ are the coefficients of the $X_j$ and $X_k$ atomic orbitals in the $i^{\text{th}}$ molecular orbital, obtained from the results of the IEHT calculation. In this expression the matrix elements of each component of the electric field gradient $\langle X_j|efg|X_k\rangle$ over the atomic orbital basis set are exactly the same, except for sign, as those for the components of the $B$ tensor. The same values of $\langle 1/r^3\rangle$ $4p$ and $\langle 1/r^3\rangle$ $3d$ are also used and only the local contribution of the Fe atomic orbitals are included. The $3\times 3$ field gradient tensor component matrix is diagonalized yielding values for $V_{zz}$, $V_{xx}$ and $V_{yy}$ in the principal axis representation.

The field gradient components thus obtained for the 10 conformers studied are given in Table 2. We see in this table that the value of each principal component of $V$ is indeed highly symmetry dependent. The non-zero values of $V_{zz}$ varies from 0.14 in $C_3$ symmetry to 1.65 (esu/cm$^3$) in planar symmetry, a factor of 12. All values of $V_{xx}$ and $V_{yy}$ are negative. The extent of "rhombic" distortion as measured by the so-called rhombicity parameters $n=(V_{xx}-V_{yy}/V_{zz})$ was also calculated. The $C_3$ and $C_{3v}$ conformers have axial symmetry and indeed we find $n=0$. The X-ray determined structures on the other hand have a substantial "rhombic contribution" to the total field gradient.

The sensitivity of the field gradient components to small changes in the environment around the Fe is apparent in the great effect the change in position of H atoms which are twice removed from the Fe has, i.e. compare results for $0_1$, $0_2$, $A$ and $B$; $D$ with $E$ and $F$ with $G$.

To calculate the value of the quadrupole splitting observed in Mossbauer resonance spectra of the $Fe^{57}$ we use Eq. (9) together with the calculated values of the principal components of the field gradient, i.e. ($V_{zz}=q$ and $V_{xx}-V_{yy}/V_{zz}=n$). However there is the problem that the value of the quadrupole moment $Q$ for the excited $I=3/2$ state of $Fe^{57}$ is not known (13). Ambiguity in the value of $Q$ leads to consideration of a range of acceptable values from 0.187 – 0.47 Barns [13, 14]. Values of $\Delta E_Q$ (max) (min) are given in Table 2 using the upper and lower limits respectively. We see that the value of the quadrupole splitting (for a given value of $Q$) is extremely symmetry dependent and varies by a factor of 10 from $C_3$ symmetry with the lowest non-zero value to planar symmetry with the highest. $T_D$ symmetry has no splitting. These results strongly indicate that one does not need to invoke large differences in the Fe atom condition such as differences in its formal oxidization state or its spin state to account for substantial variations in observed values of quadrupole splitting in Mossbauer resonance of $Fe^{57}$ in going from one Fe site to another, either in the same or different proteins.

Since we use only the sextet ground state to evaluate the field gradient, values of $\Delta E_Q$ which we calculate should be compared with low temperature experimental results. The experimental value of $\Delta E_Q$ for the oxidized state of rubredoxin has been determined to be $0.78 \pm 0.02$ mm/sec at 77 °K with no determination of the sign of the splitting available. Our calculations yield a positive sign for $\Delta E_Q$ for all symmetries studied, i.e. the $M_I = \pm 1/2$ state is the lower energy component. There are three conformers which give excellent agreement with the experimental value of $\Delta E_Q$ in the oxidized state: $A$, $B$, and $C$. $A$ and $B$ are variations of the crystal structure with the H atoms moved, while $C$ has total $C_{2v}$ symmetry obtained by making pairwise equal S atoms. Were we to invoke the $(1-R)$ factor, which is often used to account for the polarizability of the inner core electrons by the valence electrons of the Fe atom, the uniformly reduced results for each symmetry, given in Table 2 would be obtained with $(1-R)=0.68$ [10, 14]. However, invoking this factor adds no insight into the rubredoxin results for the following reasons:

1. The corrected values still lead us to the same conclusion: conformer $A$, $B$ and $C$ agree best with experiment and now conformer $D$ results do as well.
2. Uncertainties in the value of $Q$ already introduce a greater lee-way into the range of acceptable values of field gradient than that provided by consideration or non-consideration of $(1-R)$.
3. The best test of our model and description of the active site and our method of calculation of the field gradient will be if we can predict the correct ratio of values of $\Delta E_Q$ for the oxidized and reduced states of rubredoxin. In this ratio

uncertainties in the $Q$ values and whether or not $(1-R)$ is used should cancel to a large extent.

Meanwhile there are a number of other properties of the oxidized state of rubredoxin which we hope to explain by our model and which can also help to further monitor the conformation of the active site complex.

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