Theoret. Chim. Acta (Berl.) 32, 217–226 (1974)

Molecular Orbital Calculation of Hyperfine Interactions, Electric Field Gradient and Quadrupole Splitting in Reduced Rubredoxins

Gilda H. Loew and David Y. Lo

Department of Genetics, Stanford University School of Medicine, Stanford, California 94305

Received March 21, 1973/August 28, 1973

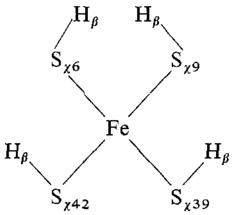
The active site of rubredoxins, the simplest class of iron-sulfur, electron-transfer proteins consists of a single Fe atom surrounded by a distorted tetrahedral array of four cysteine sulfur atoms. In a previous paper on the oxidized rubredoxins, we calculated the electric field gradient at the Fe^{57} nucleus, the resultant quadrupole splitting and the hyperfine field due to interaction between the Fe nucleus spins and the net electron spin of the molecular complex. In this paper, we are going to present our calculated results of the same set of properties for the reduced state, using the molecular orbitals similarly obtained from an Iterated Extended Hückel calculation. Significant contribution of the anisotropy to the hyperfine field as well as the sign of the electric field gradient, and hence the quadrupole splitting allow us to pin down conformations which give the best agreement with the experimental results. In fact, the conformation (conformer *A*) which represents a small movement of one of the S atoms from its crystal structure position in such a way that pairs of S atoms lie in ⊥ planes yield remarkably good results for all the physical properties calculated in both oxidation states. This consistency also suggests that there is no appreciable conformational change through the oxidation process.

Key words: Hyperfine interaction – Electric field gradient – Quadrupole splitting – Rubredoxin (reduced).

1. Introduction and Background

In an earlier paper [1], we have presented the results of our calculations on the hyperfine coupling constant and quadruple splitting for Fe^{57} in oxidized rubredoxins over ten conformational variations. It was seen that the quadrupole splitting provided a great deal of insight into the conformation of the molecular complex as a consequence of the tensorial character of the quadrupole moment. The hyperfine coupling constant, being highly sensitive to the geometry of the surrounding ions, would be an equally powerful piece of information for conformation study if the anisotropic contribution were not overshadowed by its isotropic counterpart. Whereas in the oxidized state, rubredoxin has negligible anisotropic contribution, the reduced state of the same complex has large anisotropy. Thus, in the absence of other pertinent information like the g-value, the hyperfine coupling constant becomes invaluable for conformation study.

In the present paper, results of our calculations on the hyperfine coupling constant and the electric field gradient for Fe^{57} in the reduced state of rubredoxins over nine conformations are presented and compared with the experimental results of Phillips [2] and of Rao *et al.* [3]. The model chosen for the active site



1Fe; 4 Cysteine S; 4H atoms in place of βC of cysteine; 38*e*: $[1\text{Fe}^{+2} \cdot 4\text{SH}^-]$ net(–)charge balanced by cation; 29 valence atomic orbitals: 9Fe (4*s*, 4*p*, 3*d*), 4S (3*s*, 3*p*), H (1*s*)

Fig. 1. Model for active site complex in reduced rubredoxin

of the compounds (see Fig. 1) remains the same as that chosen for the oxidized state [4], i.e., the Fe^{57} is surrounded by four S–H substituents, the spatial arrangement of which varies according to the hypothetical conformation under study. All the conformations we used for the oxidized state are retained except for the planar one which was discarded on ground of its lack of convergence in the Extended Huckel calculation and its unrealistic nature as has been shown in the oxidized state already. The conformation, based entirely upon Jensen's crystal structure data [5] is called O_1 and is a distorted tetrahedral array in which no Fe–S bond lengths and S–Fe–S bond angles are equal and one Fe–S bond is appreciably shorter than the others. The other eight conformations are variations of O_1 in gradation toward higher symmetries. O_2 is a slight variation on O_1 by merely shortening all the S–C bond length to 1.34, a value expected to allow more reasonable overlap between the sulfur and the hypothetical hydrogen substituent. *A* and *B* are similar variants of O_2 by forcing the four S–H substituents pairwise into the two perpendicular planes without altering the Fe–S bond lengths, and are different only in that the H atoms of *A* are arranged in a "pin-wheel" configuration whereas those of *B* are so arranged as to yield a C_{2v} symmetry. *C* is a variation on *B* in that Fe–S bond lengths are made pairwise equal resulting in a C_{2v} for the entire active site complex. *D* has the S's arranged in a tetrahedral array and the H's so arranged as to preserve the three two-fold axes of a D_2 symmetry. *E* has all the Fe–S–H bond angles made 180° to achieve a T_m symmetry for the entire complex. *F* and *C* are alike in having a 3-fold rotational symmetry axis, and are different in the arrangement of the H atoms such that *G* possesses a C_{3v} symmetry while *F* does not. Fuller description as well as figures of these configurations are given in Ref. [4].

In the reduced state complex there are altogether 38 valence electrons involved. The atomic orbitals chosen for chemical bonding are the Fe 3*d*'s, 4*s* and 4*p*'s, the 3*s*'s and 3*p*'s, and the H 1*s*'s. Since it has been known from experiments that the reduced state of rubredoxins corresponds to a total spin of 2, we assign an electronic configuration to the system in which the lowest 17 molecular orbitals are completely filled and the next 4 higher m.o.'s are singly occupied. These data together with the coordinates of the atoms are fed into the computer program which carries out the Extended Huckel calculations in an iterative manner [6, 7]. The resultant LCAO coefficients and the electron distribution, obtained from a Mulliken Population Analysis [8] are used to calculate the aforementioned properties.

2. Hyperfine Interaction

The Hamiltonian which describes the hypèrfine interaction between the nuclear spin and the electronic spins S_k's is given by

$$\hat{H}_{hf} = \hat{H}_1 + \hat{H}_2 \tag{1}$$

$$\hat{H}_1 = (8\pi/3)\,\gamma_e \gamma_{\mathrm{Fe}^{57}} \hbar^2 \sum_k \vec{I} \cdot \vec{s}_k \delta(\vec{r}_k) \tag{1a}$$

$$\hat{H}_2 = \gamma_e \gamma_{\mathrm{Fe}^{57}} \hbar^2 \sum_k \{3(\vec{s}_k \cdot \vec{r}_k)(\vec{r}_k \cdot I) - r_k^2 (\vec{s}_k \cdot \vec{I})\} r_k^{-5} \tag{1b}$$

where γ_i is the gyromagnetic ratio of the *ith* species and $\vec{r}_k$ is the position operator for the *kth* electron with the position of the nucleus as the origin of the coordinate system. $\hat{H}_1$ accounts for the isotropic hyperfine interaction because of the Dirac-delta function at the nucleus. $\hat{H}_2$ accounts for the anisotropy of the hyperfine field, and corresponds to the magnetic dipolar interaction term in the expansion of the classical electric potential energy of the system. In experiment, hyperfine interaction is observed as the expectation value of $\hat{H}_{hf}$ with respect to the true eigenfunction of the total Hamiltonian of the system. In our approximation, i.e., from the MO viewpoint, apart from the spins, the eigenfunctions are the products of the molecular orbitals, which are just the eigenfunctions for the Hamiltonian in the absence of the hyperfine term H_{hf}. In the cases where the lowest ligand orbitals are degenerate, the expectation value of the Hamiltonian is taken with respect to a wave-function which is a quantum mechanical averaging of the degenerate orbitals. For instance, in the case of C_3 where the two lowest ligand orbitals, MO(17) and MO(18), are degenerate, the wave-function used is

$$\psi = \frac{1}{\sqrt{2}} [(17)^2(18)(19)(20)(21) + (17)(18)^2(19)(20)(21)]$$

It should be noted that this quantum mechanical averaging is important in calculating the magnetic dipolar interaction and the field gradient to allow for proper manifestation of the symmetry of the molecular complex.

Taking the expectation value of H_1 with respect to the spatial coordinates only, we arrive at a definition for the isotropic term of the hyperfine coupling constant A_0 as

$$\langle \hat{H}_1 \rangle_{\vec{r}\,\mathrm{only}} = A_0 \vec{I} \cdot \vec{S} \tag{2}$$

where S is the spin for the electronic state of the molecular complex. A_0 is now explicitly given by

$$A_0 = \frac{8\pi\gamma_e\gamma_{\mathrm{Fe}}\hbar^2}{6S} \left\{ \sum_{k=1}^{4} |C_{k,4s}|^2 |\Phi_{4s}|^2 + \sum_{k,i} (C_{k,4s} C_{ki} \Phi_{4s} \Phi_i + C_{ki}^2 |\Phi_i|^2) \right\} \tag{2a}$$

where C_{ki}'s are the LCAO coefficients obtained from the Extended Huckel calculations and Φ_i's are the atomic orbitals evaluated at the nucleus. The AO's we use are taken from the Clementi Table of Atomic Functions, and are discussed in detail in our previous paper [1]. However, our approximations using first-order perturbation as well as ignoring electron-correlation in the molecular orbital method lead us to grossly underestimating the value of A. In effect, we have ignored the existence of an additional amount of net electronic spin density at the nucleus due to the inner-core electrons as a result of polarization by the "d" valence electrons. This additional amount of spin density, compared to that due to the $4s$ electrons and the distant overlap of the sulfur orbitals, turns out to be great for Fe^{++} and Fe^{+++} for two reasons. Firstly, the polarizing strength made of 5 or 6 d-electrons is vastly significant. Secondly, the large nuclear charge as well as the size of Fe results in greater probability density at the nucleus for the inner s-electrons than for the outer electrons. The correlation we use, following Das *et al.* [9], is given by

$$A^p = \frac{\sigma_d}{4}\left\{\sum_{n=1s}^{3s}\{|\Phi_n^{\alpha}|^2 - |\Phi_n^{\beta}|^2\} + \frac{\sigma_{4s}}{2}\{|\Phi_{4s}^{\alpha}|^2 - |\Phi_{4s}^{\beta}|^2\}\right\} \tag{3}$$

where Φ_n^{χ} is the probability density for an electron in the *nth* orbital of a free Fe atom with spin $\chi = \left\{\begin{smallmatrix}\alpha\\ \beta\end{smallmatrix}\right\}$ or evaluated at the nucleus; σ_d is the total number of unpaired d-electrons in the molecular complex, and is obtained by a Mulliken Population Analysis of the electron distribution of the system. Bearing in mind that there are 4 unpaired electrons in a free Fe atom, we see that $\sigma_d/4$ represents a scaling factor as the free atom becomes "dressed" in the molecular complex. σ_{4s} is the Fe-$4s$ population in the complex, obtained similarly.

It should be noted that (3) is a rough approximation since it is arrived at only from a plausible physical reasoning rather than from a rigorous derivation using many-particle theory. Additionally, use of the Mulliken Population Analysis to obtain values for σ_d and σ_{4s} introduced another degree of approximation into Eq. (3). Now the corrected hyperfine coupling constant is given by

$$A = A_0 + A_p \tag{4}$$

Taking the expectation value of H_2 with respect to the spatial coordinates only, we arrive at a similar definition for the hyperfine coupling tensor B as

$$\langle \hat{H}_2 \rangle_{\vec{r}\,\text{only}} = \vec{I}\cdot\overleftrightarrow{B}\cdot\vec{S} = \sum_{i,j=1}^{3} I_i B_{ij} S_j \tag{5}$$

where B_{ij} is explicitly given by

$$B_{ij} = \frac{\gamma_e \gamma_{\text{Fe}^{57}} \hbar^2}{5} \sum_{k=1}^{4} \sum_{\alpha,\beta} C_{k\alpha} C_{k\beta} \left\langle \Phi_\alpha \left| \frac{3x_i x_j - r^2\delta_{ij}}{r^5} \right| \Phi_\beta \right\rangle \tag{5a}$$

$C_{k\alpha}$'s are LCAO coefficients from the Extended Hückel calculations, and $\left\langle \frac{3x_i x_j - r^2\delta_{ij}}{r^5} \right\rangle$ have been evaluated for the d and p orbitals of a given atom [1]. As a first approximation, the matrix elements between orbitals of different atoms will be ignored for our calculations because of their complexity and of the rapid falling-off of the expectation value of $1/r^3$; although such cross-terms have recently been shown to have some significance by the work of Das *et al.* [11]. We shall also ignore the so-called distant terms, i.e. those involving sulfur atomic orbitals since in addition to the effect of $1/r^3$ the LCAO's from the sulfurs are small. A rough comparison with the corresponding terms for the A value should substantiate this point.

The anisotropy of the hyperfine coupling is more conveniently expressed in terms of the principal axes, a coordinate system in which $B_{ij} = 0$ for $i \neq j$. In terms of these principal components of the hyperfine tensor the expectation value of $\hat{H}_2$ with respect to all the coordinates is given by

$$\langle \hat{H}_2 \rangle = I'_x B'_{xx} S'_x + I'_y B'_{yy} S'_y + I'_z B'_{zz} S'_z \tag{6}$$

when I'_i, S'_i are the expectation values of the corresponding spin operators $\hat{I}_i$, $\hat{S}_i$, with respect to the spin states of the system. Note that each quantity in the principal axis system is indicated by a prime. It is a common practice to define the so-called internal field H_n at the nuclear site for the system as

$$\vec{H}_n = \frac{A\vec{S} + \vec{B} \cdot \vec{S}}{S} \tag{7}$$

because H_{eff} defined as

$$\vec{H}_{\text{eff}} = \vec{H}_0 + \vec{H}_n \tag{8}$$

where $\vec{H}_0$ is the external magnetic field, is directly measurable from the splittings of the corresponding NMR spectra. In the principal axis system, H_n becomes

$$\vec{H}_n = \begin{pmatrix} H'_{nx} \\ H'_{ny} \\ H'_{nz} \end{pmatrix} = \frac{1}{S} \begin{pmatrix} (A + B'_{xx})S'_x \\ (A + B'_{yy})S'_y \\ (A + B'_{zz})S'_z \end{pmatrix} \tag{9}$$

S'_i is usually evaluated with the aid of the density matric ϱ as

$$S'_i = \text{Trace}(\varrho \hat{S}_i) \tag{10}$$

To simplify our presentation, we will assume that the condition

$$\varrho_{m'_s m_s} = \begin{cases} 1 & m'_s = m_s = s \\ 0 & \text{otherwise} \end{cases} \tag{11}$$

is fulfilled such that $S'_i = S$. This condition is equivalent to experimentally polarizing the electronic spin state completely in the direction of the *ith* axis of the principal-axis system, which leads to

$$H_{ni} = A + B'_{ii}\,, \qquad i = 1{,}2 \quad \text{or} \quad 3 \text{ (as } x, y \text{ or } z). \tag{12}$$

Our calculated values of A and H_{ni} for each conformation are given in Table 1. We see from this table that A stays fairly constant around the value of 360 *KG*, whereas H_{ni} varies considerably with conformational change, ranging from 252 *KG* (O_1) to 470 *KG*(*G*) for the *x*-component and 314 *KG*(*B*) to 214 *KG*(*G*) for the *z*-component. The degree of anisotropy, i.e. the difference in magnitude between the *z*-component and the *x*-component falls in the range of ~200 *KG* for all conformations. In the work of Rao *et al.* (see Ref. [3]), only one value of H_n, estimated to be ~246 *KG*, is observed which they interpret as a transverse component to the assumed axial symmetry axis. In our study, conformer O_1, O_2, A and B do not possess any axis of symmetry (the *z*-axis for these systems are so chosen as to become coincident with the axis of highest symmetry of the conformers evolved from them); but the *x*-components of the hyperfine field of these conformers yield good agreement with the measured value of 246 *KG*. Conformer C has a 2-fold rotation axis of symmetry, and the *x*-component of its hyperfine field, although slightly high, yields acceptable agreement with experiment. On

Table 1

Conformation	$A(KG)$	$H_{nx}(KG)$	$H_{ny}(KG)$	$H_{ny}(KG)$
Expt.	300	246	—	—
O_1	370.6	252.0	392.0	474.0
O_2	368.0	256.0	368.0	481.0
A	360.0	264.0	309.5	505.5
B	359.5	261.8	305.5	514.5
$C(C_{2v})$	360.1	276.0	295.6	508.0
$D(D_2)$	313.1	451.8	194.1	293.1
$E(T_D)$	387.6	461.6	466.6	233.0
$F(C_3)$	445.1	491.9	479.3	363.7
$G(C_{3v})$	433.3	483.1	482.8	334.1

the other hand, conformers D, E, F and G, the last three of which also show axial symmetry (see Table 1), fail to have a $\perp$ component which agrees reasonably well with the experimental value of 246 KG, instead, the $\|$ components are all in the range of 200–300 KG. Naturally, one is tempted to conclude that conformers D, E, F and G are less likely candidates for the true conformation of the molecular complex in the reduced state since their calculated hyperfine property appears to have failed to agree with experiment. Nevertheless, we feel that a definitive statement on this subject cannot be reached until we have a thorough knowledge about the behaviour of the g-values of the system. The reason is that whether the $\|$ or the $\perp$ component of the effective field should be observed with a powder sample which was being used in the experiment depends strongly upon whether $g_{\|}$ or $g_{\perp}$ predominates. The latter condition in turn depends upon the electronic states of the system. (For instance, in the oxidized state $g_{\|}(1,2) \gg g_{\perp}(1,2)$ while $g_{\|}(3,4) \sim g_{\perp}(3,4) \sim 4.6$ (see Ref. [10]). If further information suggests that the $\|$ component should be observed, then the actual conformation of the molecular complex should resemble more those of D, E, F and G because their $\|$ components agree well with 246 KG. Otherwise, we may infer that the 3-fold rotation axis unique to these 4 conformations causes disconcerted electronic destribution in the complex and hence fails to provide good agreement with experiment for this particular physical property.

3. Electric Field Gradient and Quadrupole Splitting

The quadrupole Hamiltonian is given by

$$\hat{H}_Q = \frac{eQ}{6I(2I-1)} \sum_{i,j=1}^{3} \hat{V}_{ij}\left[\tfrac{3}{2}(\hat{I}_i\hat{I}_j + \hat{I}_j\hat{I}_i) - \delta_{ij}\hat{I}^2\right] \tag{13}$$

where Q is the quadrupole moment of the nucleus; $\hat{V}_{ij}$ is the i–j^{th} component of the electric field gradient tensor due to an electron at $\vec{r}$ from the origin treated as an operator, and is given by

$$\hat{V}_{ij} = \frac{1}{r^5}\left[3\hat{x}_i\hat{x}_j - \delta_{ij}\hat{r}^2\right] \quad \left(r^2 = \sum_{i=1}^{3} x_i^2\right). \tag{13a}$$

In the spin-Hamiltonian $\langle \hat{H}_Q \rangle_{\vec{r}\,\text{only}}$, $\langle V_{ij} \rangle_{\vec{r}}$ has an explicit form of

$$\langle V_{ij} \rangle_{\vec{r}} = -\sum_{k=1}^{\alpha 1} n(k) \left\{ \sum_{\alpha,\beta} C_{k\dot{\alpha}} C_{k\beta} \left\langle \Phi_\alpha \left| \frac{3x_i x_j - r^2 \delta_{ij}}{r^5} \right| \Phi_\beta \right\rangle \right\}$$

where $C_{k\alpha}$'s are LCAO coefficients from the Extended Hückel calculations and the atomic matrix elements, $\left\langle \Phi_\alpha \left| \frac{3x_i x_j - r^2 \delta_{ij}}{r^5} \right| \Phi_\beta \right\rangle$ have been evaluated for the d and p orbitals for a given atom (see Ref. [1]); $N(k) = 1$ for the top four MO's and $= 2$ for the rest which are all doubly occupied. For the same reason as stated for the calculation of the magnetic dipolar interaction, we will ignore matrix elements between orbitals of Fe and S atoms and of S atoms themselves. In the principal-axis system (in general different from the one appropriate for the hyperfine tensor) where $\langle V_{ij} \rangle_{\vec{r}} = 0$ for $i \neq j$, the quadrupole Hamiltonian can be rewritten, using the condition $\sum_i V_{ii} = 0$, to yield

$$\langle \hat{H}_Q \rangle_{\vec{r}} = \frac{eQ \langle V'_{zz} \rangle}{4I(2I-1)} [3\hat{I}'^2_z - \hat{I}^2 + \eta(\hat{I}'^2_x - \hat{I}'^2_y)] \tag{14}$$

where $\eta \equiv \frac{\langle V'_{xx} \rangle - \langle V'_{yy} \rangle}{\langle V'_{zz} \rangle}$ is usually called the asymmetry parameter. If we choose our axes such that the condition

$$|\langle V'_{zz} \rangle| > |\langle V'_{yy} \rangle| \geqq |\langle V'_{xx} \rangle| \tag{15}$$

is fulfilled, then in the case of axial symmetry, i.e. $\langle V'_{xx} \rangle = \langle V'_{yy} \rangle$, $\eta = 0$ and $\hat{H}_Q$ is reduced to

$$\langle \hat{H}_Q \rangle_{\vec{r}} = \frac{eQ \langle V'_{zz} \rangle}{4I(2I-1)} (3\hat{I}'^2_z - \hat{I}^2) \tag{16}$$

which has eigenvalues E' given by

$$E' = \frac{e^2 Q \langle V'_{zz} \rangle}{4I(2I-1)} [3m_I^2 - I(I+1)] \qquad |m_I| = I, I-1, \ldots \geqq 0 .$$

In general, (14) has eigenvalues E given by

$$\text{a)}\ E = \frac{e^2 Q}{4I(2I-1)} [3m_I^2 - I(I+1)] \left[\pm \sqrt{-\frac{4}{3} \sum_{j>i=1}^{3} \langle V'_{ii} \rangle \langle V'_{jj} \rangle} \right]$$

or

$$\text{b)}\ E = \frac{e^2 Q \langle V'_{zz} \rangle}{4I(2I-1)} [3m_I^2 - I(I+1)] \sqrt{1 + \eta^2/3} . \tag{18}$$

Expression (18a) shows complete symmetry in the permutation of x, y and z and is indeterminate with regard to the sign of the square root. However, the fact that (17) is the asymptotic solution of (18) suggests that E depends upon the sign of the largest component, i.e. the component with the largest magnitude. This criterion fails when $V_{xx} \cong -V_{zz}$ and $V_{yy} = 0$, i.e. when which corresponds to a maximum mixing between the states of $|m_I| = \frac{3}{2}$ and $\frac{1}{2}$. Under this circumstance, we can no longer make the kind of statement that $|m_I| = \frac{3}{2}$ lie lowest and vice

Table 2

Conformation	V_{zz}	η	$\Delta E_Q^{\mathrm{red}}$(mm/sec)	ΔE_Q^{ox}(mm/sec)$\gamma =$	$\dfrac{\Delta E_Q^{\mathrm{ox}}}{\Delta E_Q^{\mathrm{Re}}}$
O_1	−1.37	0.88	(±)2.56	1.79	(±)0.70
O_2	−1.72	0.99	(±)3.00	1.62	(±)0.54
A	−2.29	0.23	−3.45	0.98	−0.26
B	−2.48	0.22	−3.75	0.57	−0.15
$C(C_{2v})$	−2.41	0.08	−3.61	0.86	−0.24
D	(±)1.98	1	(±)3.44	1.26	(±)0.37
$E(T_D)$	+2.43	0	+3.64	0	0
F	+0.99	0.16	+1.49	0.24	0.16
$G(C_{3v})$	+1.25	0	+1.87	1.65	0.88
Exp (C. Past)			−3.16/−3.34	0.78	−0.25/0.23

versa. For Fe^{57}, $I=\frac{3}{2}$, and splitting between the doubly degenerate pairs $M_1=\pm\frac{3}{2}$ and $M_1=\pm\frac{1}{2}$ in the absence of an external magnetic field yields

$$\Delta E=\frac{eQ\langle V'_{zz}\rangle}{2}\left(1+\frac{1}{3}\eta^2\right)^{\frac{1}{2}}; \tag{19}$$

and the sign of $\langle V'_{zz}\rangle$ determines whether $m=\pm\frac{3}{2}$ or $m=\pm\frac{1}{2}$ lie lowest when η is small compared with unity, Q for Fe^{57} being in general accepted as positive.

In our calculations, *EFG* caused directly by the surrounding atoms is ignored. A non-vanishing field gradient tensor arises as a result of the deviation from a spherical electronic distribution around the Fe. In the oxidized state where the outer shell of Fe^{57} has five *d*-character electrons, the apparent deviation from a spherical electronic distribution is actually a result of charge distortion caused by interaction with the surrounding atoms. Therefore, it was no surprise to see that for a total tetrahedral symmetry (conformer *E*), $V_{ii}=0$ for all *i* in the oxidized state. This striking effect is absent in the reduced state due to the fact that the contribution of the 6th *d*-electron is much larger than that of a d^5-configuration. In Table 2 are presented the values of $\langle V'_{zz}\rangle$, η, $\Delta E_Q^{\mathrm{red}}(Q=1{,}81)$ calculated for all the conformations studied. For the sake of comparison, also given is the value of $E_Q(Q=0.187)$ for the oxidized state, E_Q^{ox} which we have previously calculated (see Ref. [1]), and the ratio $\gamma=\Delta E_Q^{\mathrm{ox}}/\Delta E_Q^{\mathrm{red}}$.

We see from Table 2 that for all conformations the calculated value of E_Q is much larger in the reduced state. Unlike the oxidized state, the magnitude of E_Q^{red} is not very sensitive to symmetry, but the sign of E_Q does vary. The experimental value of E_Q^{red} for rubredoxins from *C.* Pasteurianeum has been determined by two investigators [2, 3] to by 3.16 [2] and −3.34 [3] (mm/sec). Reasonable agreement with experiment is obtained for all symmetry except conformers *E*, *F* and *G* which give the wrong sign of V_{zz} and therefore E_Q. The sign of V_{zz} for O_1, O_2 and *D* is ambigous due to $\eta \sim 1$. The disparate behaviour of *D*, *E*, *F* and *G* conformer can be traced back to the distribution in the electron density among the ligand orbitals as a result of having a C_3 symmetry imposed upon the molecular complex. From the energy diagrams of the ordered ligand field orbitals (17–21), given in Fig. 2, we see that in conformers *D*, *E*, *F* and *G* there is a large

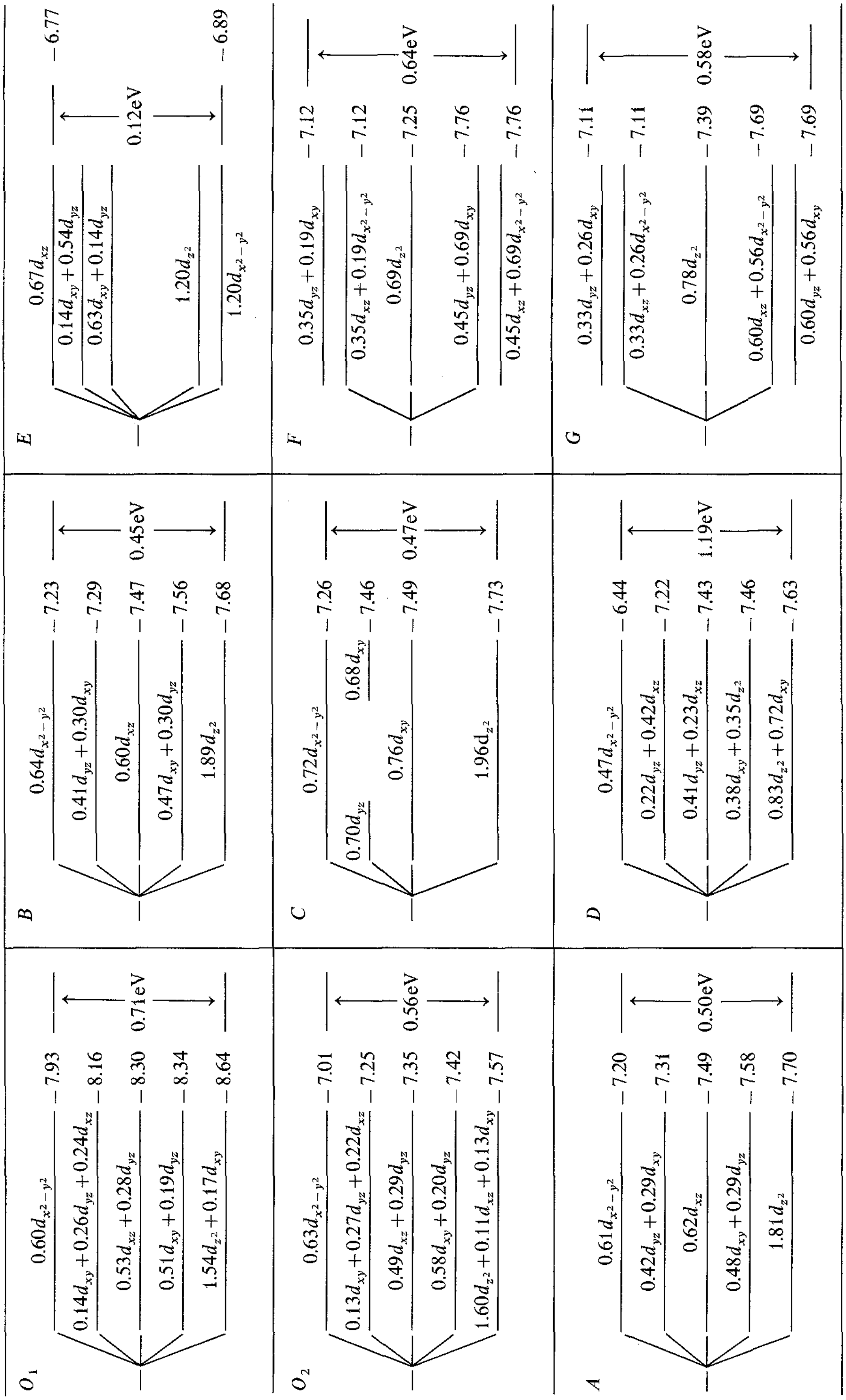


Fig. 2. Nature and energy of the 5 Fe (3*d*) "ligand orbitals"

contribution from d_{xy} or $d_{x^2-y^2}$ to the 17th and 18th m.o., the lowest-lying ligand field orbitals. Ignoring the H atoms in D, all of these 4 conformations possess a 3-fold rotation axis. Therefore, the presence of a C_3 symmetry seems to result in another failure to yield agreement with experiment. On the other hand, conformers A, B and C which give good agreement with experiment have a lowest-lying ligand-field orbital with predominantly pure d_{z^2} character. The fact that conformers O_1 and O_2 have appreciable contribution from other d orbitals than d_{z^2} probably explains the sign ambiguity of their field gradient.

In summary, it appears from the two physical properties we have calculated that the reduced rubredoxin does not possess any higher symmetry than C_{2v} as in conformer C. However, in the oxidized state, conformer C yields poor result for the g-values [10]. If there is not any significant change in conformation in the oxidation process, which seems highly probable in view of the properties we have calculated, we may safely conclude that rubredoxin does not possess any symmetry higher than a 2-fold rotation symmetry. While conformers O_1, O_2, A and B all give acceptable agreement with experimental values for all the properties we have calculated in both oxidation states, conformer A has by far the most striking agreement and consistency with experimental results. As discussed in our previous work (see Ref. [10]), conformer A represents a small movement of one of the S atoms from its crystal structure position in such a way that pairs of S atoms lie in planes. The results of our calculations for both oxidized and reduced states then appear to be most consistent with such a small deviation from crystal structure.

Acknowledgement. The author wishes to gratefully acknowledge financial support for this work from NSF Grant No. GB 17980.

References

1. Loew, G., Lo, D., submitted to: Theoret. Chim. Acta (Berl.) (1972)
2. Phillips, W., Poe, M., Weiher, J. F., McDonald, C. C., Lovenberg, W.: Nature **227**, 574 (1970)
3. Rao, K. K., Evans, M. C. W., Cammack, R., Hall, D. O., Thompson, C. L., Jackson, P. J., Johnson, C. E.: Biochem. J. **129**, (1972)
4. Loew, G., Chadwick, M., Steinberg, D., submitted to: Theoret. Chim. Acta (Berl.) (1972)
5. Jensen, L. H.: Latest X-ray results received 10/12/72 by private communication
6. Zerner, M., Gouterman, M.: Theoret. Chim. Acta (Berl.) **4**, 44 (1966)
7. Zerner, M., Gouterman, M., Kobayashi, H.: Theoret. Chim. Acta (Berl.) **6**, 363 (1966)
8. Mulliken, R. S.: J. Chem. Phys. **23**, 1833, 2338, 2343 (1955)
9. Rettig, M. F., Han, P. S., Das, T. P.: Theoret. Chim. Acta (Berl.) **12**, 178 (1968)
10. Loew, G., Chadwick, M., Lo, D., submitted to: Theoret. Chim. Acta (Berl.) (1972)
11. Han, P. S., Rettig, M. F., Ikenberry, D., Das, T. P.: Theoret. Chim. Acta (Berl.) **22**, 261 (1971)

Dr. G. H. Loew
Department of Genetics
Stanford University
School of Medicine
Stanford, California 94305
USA