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Calculated Properties of the Active Site of Oxidized Rubredoxins

III. Zero Field Splittings, Magnetic Moments and Electron Spin Resonance Spectra

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For a molecular model of the Fe–S active site complex in oxidized rubredoxin, we have calculated the spin-orbit coupling between the ground sextet state and excited quartet and doublet states which gives rise to the observed zero field splitting of the sextet ground state into three spin-mixed Kramers doublets. Additionally, we have used the six spin-mixed sextet state components to calculate effective magnetic moments, magnetic field energies and nine g values corresponding to transitions between the three pairs of Kramers doublets in applied magnetic fields along three perpendicular axes. We have calculated these properties for eight conformational variations of the ligands around the Fe at the active site. The results of these calculations clearly show the origin of the observed $g = 4.3$ signal previously described only in terms of the phenomenological spin-Hamiltonian formalism. For the eight conformations considered, five have this characteristic signal. Zero field splitting comparable to the observed values could be obtained for all symmetries studied. In addition, the calculated values of magnetic moment in all symmetries correspond to that of high spin ferric ion and do not vary appreciably with temperature above 77° K, in agreement with experimental results. From comparison of all our calculated results with experiment, it appears that the active site in oxidized rubredoxins could have small conformational variations in different rubredoxins and under the various experimental conditions used.

Key words: Zero field splittings – Magnetic moments – Electron spin resonance spectra – Rubredoxins (oxidized)

1. Introduction and Background

The magnetic susceptibility of oxidized and reduced *C. Pasteurianum* rubredoxin has been measured by an NMR method in the temperature range of 150–250° K [1]. In this temperature range, the effective magnetic moment was found to be temperature independent and to have a value of 5.85 ± 0.20 Bohr magnetons for the oxidized state corresponding to the presence of a high-spin ferric ion and of 5.05 ± 0.2 in the reduced state corresponding to a high-spin ferrous ion. The electron spin resonance (ESR) spectra of rubredoxin from another anaerobic fermentative bacteria, *P. olevorans*, has recently been studied in the temperature range of 1.4–40° K using an X-band spectrophotometer [2], while a less extensive ESR investigation has also been made of rubredoxin from the green photosynthetic bacterium *Ch. ethylica* [3]. In common with other nonheme Fe proteins, such as transferrin and ferrichrome *A*, both oxidized rubredoxins exhibit a broad electron spin resonance spectra with g values ranging from 0.9–9.4 and a main absorption at $g = 4.3$. However, in the *P. olevorans* rubredoxin the absorption at $g = 4.3$ was more anisotropic.

The appearance of a $g = 4.3$ signal was first observed for iron impurities in glass [4] and ever since that time has been ascribed to high spin ferric ion in a "totally rhombic" environment. The original analysis of the origin of the $g = 4.3$ signal and all subsequent ones up to now have been made using the phenomenological spin-Hamiltonian:

$$H = D\,[S_z^2 - 1/3\,(S\,(S+1))] + E\,(S_x^2 - S_y^2) + \beta H \cdot S \qquad (1)$$

where D and E are the so called tetragonal and rhombic crystal field parameters related to the magnitude of the zero field splitting of the sextet ground state components into three Kramers doublets. In their original analysis, Caster, Newell, Holton, and Slichter [4] assuming maximum rhombicity, were able to attribute the $g = 4.3$ signal to a fairly isotropic magnetic field transition between the partners of the middle-energy Kramers doublet, and to determine that the upper and lower pairs have strongly anisotropic g values ranging from 9.6–0.6. A subsequent study of the similar ESR spectra of iron in ferrichrome A, by Wickman, Klein, and Shirley [5], using a spin-Hamiltonian analysis of the results, led to a verification of this original interpretation of the spectra, showing that $D = 0$ was equivalent to a maximum rhombicity condition of $E/D = 1/3$. In the recent temperature-dependent ESR study of the *P. oleovorans* rubredoxin [2], the $g = 4.3$ signal was attributed to the first excited Kramers doublet and its energy interval (ΔE_1) determined to be 5.6 cm^{-1}, and equal to the second interval.

While descriptions of the ESR spectra based on the spin-Hamiltonian formalism have been very valuable in giving the initial insight and explanation of the origin of the $g = 4.3$ signal and other characteristics of the spectra of high spin ferric ion in a "rhombic environment", it remains a phenomenological and incomplete description. With such a description, the actual symmetry of the ligand atoms surrounding the Fe need not be specified. Furthermore, the spin-orbit coupling between the ground sextet state and the excited quartet and doublet states, the fundamental interaction leading to the zero field splitting of the sextet state and g values different from 2, is not actually calculated. Nor are the true spin-mixed functions used as eigenfunctions to calculate the magnetic field behavior.

In order to more fully understand the origin of the $g = 4.3$ signal and in general to obtain a more precise correspondence between conformation and g values of ferric ion in its so-called rhombic environment in proteins, we have undertaken a more rigorous calculation of these properties than can be provided by the use of the spin-Hamiltonian formalism. In our calculation, we consider 8 conformational variations of the active site complex of the oxidized rubredoxin. For each of these we use the actual Hamiltonian to describe both zero field and magnetic field interactions and solve the corresponding problem to some degree of approximation. Thus, we obtain values for observable properties as a function of conformation. X-ray crystal structure analysis of one rubredoxin has revealed that the single Fe atom is surrounded by a distorted tetrahedral array of four cysteine S atoms [6, 7]. Using this information, we have chosen the molecular model, schematically represented in Fig. 1 of paper I, which includes the Fe atom, the cysteine S atoms and H atoms placed in the position of the βC atoms bound to the S. We have explicitly considered 8 conformational variation of this active site. These are described in detail in paper I of this series. They include 1) the X-ray structure itself [6, 7] with H atoms replacing the βC atoms in exactly the positions determined from the X-ray structure analysis (conformer O_1) and a variation in which the S – H bonds are shortened but all else is kept the same (O_2).

The 6 additional conformational variations of the experimentally determined structure we considered can be found in paper I. (Conformers *A*, *B*, *C*, *D*, *F*, *H*.) Seven conformers have different kinds of "rhombic" symmetry, while $C_3(F)$ has axial symmetry. One of the main objects of studying these conformations was to determine the sensitivity of the g values to conformation and specifically to further understand the symmetry conditions required to obtain the characteristic $g = 4.3$ signal. In addition to g values, we have also calculated the effective magnetic moments and zero-field splittings as a function of conformation.

2. Spin-Orbit Coupling Interactions of the Active Site Complex

The sextet ground state in high spin ferric ion complexes is orbitally non-degenerate and to first order has no spin-orbit coupling. Thus, in this approximation, the zero field splittings among components of this state are zero and the observed g values in ESR spectra would be 2 and isotropic. However, the sextet state can undergo spin-orbit coupling to excited quartet and doublet states. In molecular environments of lower than cubic symmetry this interaction leads to spin-mixing and a splitting of all states, and specifically of the ground state sextet into three spin-mixed Kramers doublets. A calculation of this spin-orbit interaction then takes the place of the phenomenological zero-field spin-Hamiltonian where the parameters D and E are directly related to the resulting zero field splitting in less than cubic fields.

In this study then, we have formulated a computer program to calculate the spin-orbit interaction among the sextet ground state and those quartet and doublet states lying close enough to it in energy to appreciably interact with it. To do this, we must evaluate matrix elements of the form:

$$\mathscr{H}_{ij} = \langle \psi_i(T, S, M_s) \,|\, \lambda L \cdot S \,|\, \psi_j(T', S', M_s') \rangle \tag{2}$$

where ψ_j is a state function arising from electron configuration T with spin S and a particular value of M_s. In the oxidized state, the active site complex has 29 valence molecular orbitals and 37 electrons. We have characterized this system by use of a semi-empirical molecular orbital program based on the so-called Iterative Extended Hückel Theory (IEHT). Spin-orbit interactions involving the sextet, four quartet and three doublet states were considered, the choice of which has been determined energetically by our calculation of approximate term energies presented in paper I (10). Thus, we were using a 28×28 spin-orbit matrix.

In our approximation any one state $\psi_i(T, S, M_s)$ is a product of a space function and a spin function. The space function is in turn a product of the molecular orbital functions of the 5 electrons specified by each configuration.

The spin-orbit coupling operator is a one-electron operator. Thus, each matrix element H_{ij} in expression (2) can be reduced to a sum of matric elements over molecular orbitals:

$$\mathscr{H}_{ij} = \langle \psi_i(T, S, M_s) | \lambda L \cdot S | \psi_j(T', S', M_s') \rangle = \sum_{k,l} C_{ik} C_{jl} \langle \phi_m^k | \lambda L \cdot S | \phi_n^l \rangle \tag{3}$$

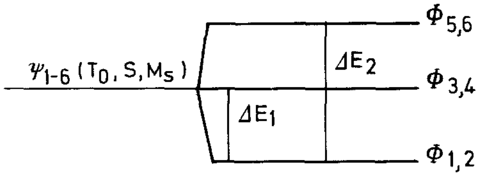
where ϕ_m^k is the m^{th} ligand molecular orbital associated with the k^{th} spin-configuration.

In order to actually obtain numerical values for these matric elements, the set of ligand-field molecular orbitals 17–21 are expressed as linear combination of atomic orbitals, i.e. $\phi_i = \sum_j^{a.o.} a_{ij} X_j$. The coefficients of the atomic orbitals for the set of five molecular orbitals needed are supplied to the spin-orbit coupling calculation from the results of the IEHT program for the 8 conformers studied. In addition to supplying the sets of a_{ij} coefficients, we have evaluated the spin-orbit coupling matric elements over the atomic basis set. There are no cross terms in the *p* and *d* contributions, *s* contributions are zero, and the contributions are independent of the principal quantum number of the *p* and *d* electrons.

The magnitude of the spin-orbit coupling is determined by the value of the matrix elements we have just described and of the energy separation between the sextet, quartet and doublet states which are interacting. There are no experimental values for such relative term energies for oxidized rubredoxin or any model compound analogues of the active site complex, since all $d-d$ transitions are spin-forbidden and none have been observed [8, 9]. Thus, we have used the results of a semi-empirical method described in paper I to obtain relative term energy values needed in the spin-orbit coupling calculation. In this approach the energy difference between the sextet and lowest quartet state is used as a parameter (Δ_1) in our calculations. The magnitude of Δ_1 is a measure of how far from the sextet ground state the lowest quartet is. Then for a given value of Δ_1, calculated values of the relative term energies ($\Delta_2 - \Delta_7$) are used for the remaining six excited quartet and doublet terms considered.

In our calculations, we have fixed the value of the spin-orbit coupling constant, another parameter needed in the calculation, at 200 cm^{-1}. The value for atomic Fe is 400 cm^{-1} and the interaction is known to be diminished for Fe in molecules. For this value of λ, then we have diagonalized the spin-orbit coupling matrix obtained for each geometric conformer studied for a set of different values of Δ_1, and corresponding values of $\Delta_2 - \Delta_7$.

The results of the diagonalization of the 28×28 spin orbital coupling matrix is a set of 28 doubly degenerate, spin-mixed eigenfunctions and their corresponding energies. The first six such doubly degenerate states Φ_{1-6} are the spin-mixed, primarily sextet states. In these, the components of the sextet term with different M_s values, ψ_{1-6}, mix appreciably while in addition there are small amounts of quartet and doublet components ($\psi_7 \ldots \psi_{28}$). The energy intervals between the three sets of double degenerate states Φ_{1-6} are the so-called zero-field splittings: i.e.



The results of our calculations indicate that the values of zero field splittings obtained, scale to the value of the quantity (λ^2/Δ_1) when the values of all other Δ_i are fixed by their calculated ratio to Δ_1. For each conformer, then, it was possible to find a value of Δ_1, for $\lambda = 200\,\mathrm{cm}^{-1}$, which gave good agreement with the experimentally determined value of ΔE_1. In the first row of Table 1 is given the value of Δ_1 which produced the best value of the zero field splittings (ΔE_1), listed for each conformer in the second row. Exact agreement with experiment could have been obtained in each case, as it was for O_1, the totally experimental conformer, if we had varied Δ_1 in smaller intervals. Thus, since experimental values of Δ_1 are not available, agreement with the experimental value of the first zero field interval per se does not distinguish among the various conformers of the active site complex. However, direct measurements of ΔE_2 would, since the value of the ratio of $\Delta E_2/\Delta E_1$ is symmetry dependent.

Table 1. Properties of active site complex of oxidized rubredoxin as a function of conformation ($\lambda = 200\,\mathrm{cm}^{-1}$)

Conformer	O_1(exp)	O_2(exp$S$)[a]	A(exp$S$)[a]	B(exp$S$)[a]	$C(C_{2v})$	$D(D_2)$	$F(C_3)$	$H(PL.C_2)$	exp
$\Delta_1(\mathrm{cm}^{-1})$	500	500	700	800	800	1200	250	750	
$\Delta E_1(\mathrm{cm}^{-1})$	5.60	5.75	5.11	5.21	4.75	5.35	5.88	5.00	5.60
$\Delta E_2(\mathrm{cm}^{-1})$	13.16	13.68	11.4	10.96	7.17	16.27	12.65	12.8	(11.20)
$\Delta E_2/\Delta E_1$	2.35	2.38	2.23	2.10	1.51	3.04	2.15	2.56	(2.00)
$\mu^2_{\mathrm{eff}}(4.2°)$	33.5	34.6	35.2	35.0	35.4	36.4	38.1	33.8	
$\mu^2_{\mathrm{eff}}(\geqq 77°)$	36.4	37.8	37.7	37.4	37.1	42	41.0	35.2	(34.2 ± 2.5)
g_{12}	1.31(x)	1.42(x)	0.95(x)	0.77(x)	0.045(x)	4.7 (x)	1.02(x)	2.1 (x)	0.9
g_{12}	1.46(y)	1.75(y)	1.44(y)	1.12(y)	0.128(y)	4.7 (y)	1.07(y)	4.9 (y)	1.2
g_{12}	9.65(z)	9.71(z)	9.95(z)	10.1 (z)	10.3 (z)	6.7 (z)	10.6 (z)	7.1 (z)	9.4
g_{34}	3.7 (z)	3.62(z)	4.0 (z)	4.3 (z)	0.77 (y)	4.7 (x)	4.52(z)	0.78(z)	4.0
g_{34}	4.3 (y)	4.26(y)	4.3 (y)	4.3 (y)	0.96 (x)	4.7 (y)	4.75(x)	1.02(y)	4.3
g_{34}	4.7 (x)	4.93(x)	4.9 (x)	4.7 (x)	6.25 (z)	0.07(z)	4.77(y)	5.4 (x)	4.7
g_{56}	0.38(z)	0.27(z)	0.40(z)	0.52(z)	2.0 (z)	7.7 (x)	0.63(z)	0.13(z)	
g_{56}	6.18(y)	8.45(y)	0.68(y)	1.12(y)	5.2 (x)	7.7 (y)	0.92(y)	1.14(y)	
g_{56}	8.02(x)	8.82(x)	10.24(x)	10.12(x)	6.9 (y)	0.0 (z)	10.5 (x)	8.2 (x)	
$H^{\mathrm{eff}}_A(KG)$	372	364	360	362	354	312	365	209	370 – 375
$H^{\mathrm{eff}}_B(KG)$	90	97.5	52	34	56	70	17	181	—
$\Delta E_{1_{\mathrm{min}}}$ ($Q = 0.187$) mm/sec	1.79	1.62	0.89	0.57	0.86	1.26	0.24	3.00	0.78 ± 0.02
$\Delta E_{\mathrm{max}}(Q = 0.45)$	4.58	4.14	2.29	1.45	2.20	3.21	0.62	7.61	

[a] Conformer O_2, A, B have experimental position of S atoms with H atom portions varied.

Table 2 lists the remaining values of the term energies ($\Delta_2-\Delta_7$) that were used to obtain these zero field splittings. Since the results scale to the quantity (λ^2/Δ_1), the transition energies listed in this table could be as much as a factor of 4 greater, corresponding to a maximum value of $\lambda=400\,\text{cm}^{-1}$, the free Fe atom value. If these transitions were not spin-forbidden, at least one of them should have been observed in the region 3500–10000 cm^{-1} which was examined without success for the oxidized rubredoxins. Were such $d\rightarrow d$ transition energies available together with our calculated results, they could further help distinguish the conformation of the active site complex.

Table 2. Optimum[a] values of term energies $(\Delta_i)^{b,c}$ $(\lambda=200\,\text{cm}^{-1})^c$

Conformer	$O_1(O_2)$ (cm^{-1})	A (cm^{-1})	B (cm^{-1})	C (cm^{-1})	D (cm^{-1})	F (cm^{-1})	H (cm^{-1})
$^6E_1$	0	0	0	0	0	0	0
$^4E_1$	500	700	800	800	1200	250	750
$^4E_2$	5000	3500	3200	2400	1200	2500	900
$^4E_3$	1000	1400	1332	2400	1800	500	900
$^4E_4$	6000	4200	3600	3660	4800	3000	1200
$^2E_1$	3000	4200	4000	2000	2400	1250	374
$^2E_2$	3100	4340	4400	2000	2160	1375	824
$^2E_3$	3250	4550	4800	4000	2160	1500	1050

[a] Optimum for best agreement with exp zero-field splitting values.

[b] $\Delta_i={}^sE_i-{}^6E_1(\text{cm}^{-1})$.

[c] Zero-field splitting values scale to λ^2/Δ_j. (e.g. for $\lambda=400\,\text{cm}^{-1}$ multiply all energy values in table by 4).

3. Effective Magnetic Moments as a Function of Temperature

The three spin-mixed, doubly degenerate sextet state functions Φ_{1-6} and their zero field energies determine the magnetic properties of the active site complex in its oxidized state. The magnetic moment operator for such a complex which obeys Russell-Sounders coupling is:

$\mu_q=(L_q+2S_q)$ in units of Bohr Magnetons where $q=x,\ y\ z$

L_q is the corresponding orbital angular moment operator

S_q is the corresponding spin angular moment operator

and the total value of $\mu^2_{\text{eff}}=\mu^2_x+\mu^2_y+\mu^2_z$.

The field-independent value of magnetic moment to second order, including the contribution to all components of this operator from the six spin-mixed sextet state functions is given by:

$$\mu^2_{\text{eff}}(T)=\sum_{N,q}\left[\langle N|L_q+2S_q|N\rangle^2+2kT\sum_{M\neq N}\frac{\langle N|L_q+2S_q|M\rangle^2}{E_M-E_N}\right]\cdot e^{-\Delta E_N/kT}\Big/\sum_N e^{-\Delta E_N/kT} \qquad (4)$$

where $q=x,\ y,\ z$.

N, M stand for six spin-mixed sextet states which are also eigenfunctions of each of the components of the angular momentum operator. Such eigenfunctions must be used in both the first and second order expressions of Eq. (9) and are obtained as linear combinations of pairs of degenerate partners: $\Phi_{1,2}$;

$\Phi_{3,4}$; $\Phi_{5,6}$. ΔE_N is the zero field energy values for each sextet states relative to the lowest energy state, i.e. $\Delta E_{1,2}=0$; $\Delta E_{3,4}=\Delta E_1$ $\Delta E_{5,6}=\Delta E_2$.

To obtain numerical values for the effective magnetic moment, each matrix element is expressed as a sum over matrix elements for molecular orbitals and finally over atomic orbitals in a manner similar to that described for the $(\lambda L\cdot S)$ operator. The coefficients of these atomic orbital contributions are again supplied by the results of the IEHT calculation and are the same as were used for the spin-orbit coupling calculation. A master table of (L_q+2S_q) matrix elements over the *s*, *p* and *d* atomic orbitals was generated and is available upon request.

Values of $\mu^2_{\text{eff}}(T)$ obtained from Eq. (9) are summarized in Table 1 for the spin-mixed functions which gave the best values of zero field splitting ΔE_1. For each conformer, there is no temperature dependence of the effective magnetic moment for $T \geqq 77°$ K in agreement with experimental results obtained in the range of 150–250° K [1] and the total temperature dependence is not marked. Calculated high temperature values of $\mu^2_{\text{eff}}(\geqq 77°)$ are somewhat dependent on symmetry. While generally higher than the experimental value, calculated values of $\mu^2_{\text{eff}}(\geqq 77°)$ for most conformers agree quite well with it ($\leqq 20\%$) and certainly bear out the picture of an active site with a "high-spin" ferric ion ground state.

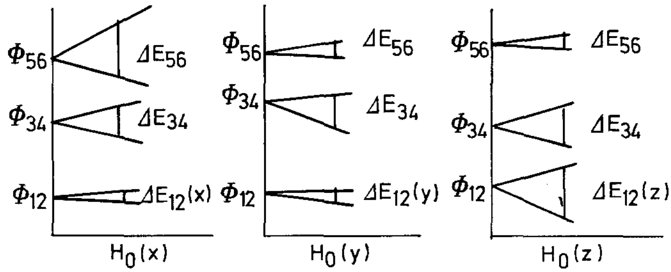
4. Magnetic Field Energies and "g" Values in Electron Spin Resonance Spectra

The Hamiltonian operator for the magnetic field energy is:

$$H(q) = -\boldsymbol{\mu}\cdot\boldsymbol{H}_0 = -(L_q+2S_q)\cdot\beta H_0(q) \qquad q=x,y\;z \tag{5}$$

where $\boldsymbol{\mu}$ = the magnetic moment defined previously and $H_0(q)$ = applied magnetic field in three perpendicular directions. This Hamiltonian takes the place of the phenomenological magnetic field spin-Hamiltonian $g\beta HS$ given in Eq. (1). To describe the behavior of the active site complex in a magnetic field we use as a zero field description of the system the six spin-mixed, doubly degenerate sextet state functions and their zero field energies obtained from the results of the spin-orbit coupling calculation. For each spin-orbit coupling calculation, our program allows these six states to mix in a magnetic field, and the resultant (6×6) energy matric is solved for all three magnetic field directions for a set of values of H_0 ranging from 1000–20000 Gauss.

For a given zero-field description, the results of the diagonalization of the sets of 3 (6×6) magnetic field energy matrices for a series of values of H_0, were sets of six distinct magnetic field energies for $H_0(x)$, $H_0(y)$ and $H_0(z)$ as a function of the magnitude of H_0, i.e.:



Nine g values corresponding to transitions between Kramers doublets Φ_{12}, Φ_{34}, and Φ_{56} with magnetic fields in the three perpendicular directions were defined as follows:

$$g_{ij}(q) = \Delta E_{ij}/H_0(q) \qquad i, j = 1, 2;\ 3, 4;\ \text{and}\ 5, 6;\ q = x, y, z\,.$$

Since the magnetic field energies were almost linear with field up to 10000 Gauss, the corresponding g values did not vary appreciably in this region. The nine g values calculated by the procedure outlined above, for the spin-orbit couplings yielding experimental values of zero field splittings, are given in Table 1 for each conformer studied.

One of the most striking results of our calculation is that this more rigorous method of calculating spin-orbit coupling, and magnetic field energies, succeeds in accounting for the observed $g = 4.3$ signal and in general for all other salient features of the observed ESR spectra of the oxidized rubredoxins. This is the first time that an actual calculation, outside the phenomenological spin-Hamiltonian formalism, has led to such a result. The description of the ESR spectra which we obtained is qualitatively similar to that obtained from the spin-Hamiltonian formalism. In both cases the origin of the $g = 4.3$ signal is a fairly isotropic transition between the components of the middle energy Kramers doublet (Φ_3, Φ_4). Transitions between partners of the other two Kramers doublets lead to a diffuse spectra with widely varying g values from less than 1 to almost 10. However, our calculations also provide a one-to-one correspondence between specific active site conformations and g values. In striking contradiction to the previous spin-Hamiltonian analysis, it appears that "rhombicity" is neither a necessary or suffiecient condition for the appearance of a $g = 4.3$ signal. Conformers O_1, O_2, A and B all have rhombic i.e. no symmetry and indeed all have one set of g values with varying degrees of anisotropic deviations about a $g = 4.3$ value. However, the planar (C_2), the $T_D(D_2)$ and the C_{2v} symmetry which are also rhombic do not have the characteristic $g = 4.3$ pattern. On the other hand, the idealization of the X-ray structure in which the unique short bond was preserved and the other three Fe–S bonds were made equivalent to produce C_3 (or C_{3v}) axial symmetry (a three-fold z axis) also exhibit a nearly isotropic "$g = 4.3$" signal with the difference from "rhombic" symmetries that two of the g values are equal. Extremely careful analysis of the $g = 4.3$ signal, not always possible, is required to distinguish between 1, 2, and 3 nearly superimposed resonance absorptions. Further as our results show, the degree of anisotropy of $g = 4.3$ transition also varies within the class of conformation which have the "required" rhombic distortion to produce it. Thus, the appearance of a signal of this type per se cannot be equated immediately with "rhombic" distortion, nor can its absence be construed to mean lack of "rhombicity".

Variations in the magnitude of spin-orbit coupling for a given active site conformer cannot qualitatively transfer the g value pattern from one which has the "$g = 4.3$" signal to one which does not and vice versa, but can only account for small numerical differences in g values. Also, the choice of detailed geometric parameter such as Fe–S or S–H bond lengths, within a specific symmetry type, i.e. totally tetrahedral, (four equivalent Fe–S) bonds, or pairwise equivalent Fe–S bond etc. has a much more limited effect on g values compared to the symmetry effect, i.e. also cannot cause qualitative change in the observed ESR spectra.

In the ESR spectra of *P. oleovorans* rubredoxin six g values were tentatively identified and attributed to the first and second Kramers doublets as shown in the last column of Table 1. Within the accuracy of the experimental results, it can be seen from this table that all of the conformers which closely resemble the active site conformer determined by X-ray crystal analysis (O_1, O_2, A and B) give good agreement with experiment, with conformer A the best. As indicated above, conformer $F(C_3)$ with axial symmetry obtained by retaining the short Fe–S bond along the z axis and equalizing the other three Fe–S bonds, also give good agreement with experimental g values, even though two g values are equal. None of the other symmetries give results which agree with the experimental ESR results.

Another type of observation which could be explained from the results of Table 1, is the small quantitative difference in the ESR of oxidized rubredoxin proteins from different sources. For example, the increased isotropy of the $g = 4.3$ signal observed in the rubredoxin from *Ch. ethlica* over that for *P. oleovorans* could correspond to a small change in conformation of the cysteine residue which leaves the position of the sulfur atoms the same and alters the "βC" atom positions, i.e. from conformer A to B.

For the sake of completion, we have presented in Table 1, along with our calculated values of zero-field splittings, $\mu^2_{\mathrm{eff}}(T)$ and g values, the values of the isotropic and anisotropic hyperfine fields at the Fe nucleus and of the quadrupole splitting in the Mössbauer resonance of Fe^{57} which we have previously calculated for the same 8 conformers of the active site of oxidized rubredoxins [12]. Table 1 then represents all the properties of these proteins which we have calculated in a self-consistent systematic way.

From the results presented in Table 1, we see that these magnetic and electronic properties taken together make a more sensitive probe of conformation than any one of them separately. Best correspondence between calculated experimental behavior for all of these properties is obtained with conformer A and somewhat less so for B. Each represents a specific displacement of the H atoms from the positions of the $\beta - C$ atoms determined experimentally, while leaving the four S atoms in their experimentally determined positions. The two conformers closest to the experimentally determined one, O_1 and O_2, both give somewhat too high values of the field gradient, while agreeing with experiment in every other property.

Our results seem to indicate that there are small conformational differences in the cysteine residues, which do not affect the S atoms, between the crystal structure of one of the rubredoxin proteins and the powder and solution samples used in the experimental determination of its properties, and also between it and other rubredoxins.

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