

CNDO and INDO MO calculations of a few properties of rubredoxins

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Abstract. The CNDO and INDO MO calculations have been performed on the oxidised and reduced species of rubredoxins using Clack, Hush and Yandle's parameterisation scheme for the transition metal complexes in conjunction with Pople and Nesbet's open-shell UHF method in an attempt to elicit information on the geometry of the conformation of the iron environment. The properties like isotropic and anisotropic hyperfine coupling constants and quadrupole splitting have been calculated and compared with the corresponding experimental values. The results suggest that both the oxidised and reduced species have the iron atoms attached to four sulphur atoms situated as pairs in two perpendicular planes as a result of a highly distorted tetrahedral arrangement.

Keywords. Iron sulphur proteins; rubredoxins; CNDO; INDO.

1. Introduction

Molecular evolution of metalloproteins has had to incorporate and preserve structural features that allow a fit of the metal atom at the binding site. Thus an understanding of the biological role, molecular properties and the co-ordination aspects of the metal binding site of the non-heme iron sulphur proteins has been one of the most fascinating but difficult topic of the time as is clearly evident from many books and reviews by several elegant research teams on this subject (Orme-Johnson 1973; Lovenberg 1973; Jensen 1974). Rubredoxins belong to the simplest class of iron sulphur proteins and take part in reversible oxidation-reduction processes in all forms of life and most of these function as one-electron carriers during photosynthesis, nitrogen fixation, mitochondrial respiration, etc. In contrast to the heme-proteins which are built around a preformed porphyrin moiety, the non-heme iron-sulphur proteins acquire the complete ligand arrangement by a suitable folding of the peptide chain and thus result in a low symmetry at the metal binding active site. This is caused by the presence of heavy conformational strain.

The first example of this class, the *Clostridium Pasteurianum* 1-Fe-S rubredoxin proteins are single polypeptide chain proteins of about 55 amino acid residues. This unit contains four cysteine sulphurs which act as liganding atoms around one iron atom in every protein molecule without any labile sulphurs. Rubredoxin has a redox potential of -57 mv, both the oxidised and reduced forms being stable.

X-ray structure determination of rubredoxin at RT by Watenpaugh *et al* (1972, 1973) reveals that the iron atom is surrounded by four cysteine sulphurs in a distorted tetrahedral array. After a series of refinements they finally reported the Fe-S bond lengths as 2.24, 2.32, 2.34 and 2.05 Å respectively which had an average of 2.24 Å with an rms deviation of 0.13 Å from the mean value. An extended x-ray absorption fine structure (EXAFS) study of rubredoxin by Schulman *et al* (1975) also decides on an average of 2.24 Å with a deviation of 0.1 Å for the same bond lengths. A very recent report by Sayers *et al* (1976) gives 2.30 ± 0.04 Å with a rms deviation of 0.06 ± 0.04 Å for this average Fe-S bond length again from an EXAFS study at -80 K. This average value of 2.30 Å is slightly larger than the values of earlier works and the reported deviations here are also smaller by a factor of two. This casts doubt on the existence of the anomalous short bond and indicates that all the Fe-S distances in rubredoxins proteins are approximately equal. This view is also supported by the x-ray crystal structures on synthetic analogues of rubredoxins (Lane *et al* 1975, 1977), unless the amino acid chain in rubredoxin protein happens to play a special role in shortening any one of the Fe-S distance. But Jensen (1974) feels that such an average bond length reported with very slight rms deviation for the Fe-S bonds does not necessarily imply a perfect tetrahedral geometry with all tetrahedral bond angles.

The other experimental properties available both for the oxidised and reduced states are the optical spectra (Lovenberg *et al* 1965; Eaton *et al* 1970, 1971), the ESR spin hamiltonian parameters (Peisach *et al* 1971), hyperfine coupling constant and quadrupole splitting from Mössbauer resonance (Phillips *et al* 1970; Rao *et al* 1972) and magnetic moments (Phillips *et al* 1970). Naturally these properties will markedly depend on the electron distribution, energy and stereochemistry of the active site of the complex and can be thought of as probes to deduce the structure of the active site. However until now the experimental results simply conform to the notion of high spin complexes both for oxidised and reduced state with a local rhombic symmetry around iron. What is needed is a one-to-one correlation between the experimental and theoretically calculated values of these properties to infer the most plausible conformation for the active site. Thus a knowledge of the magnitudes of these properties for various assumed conformations can be used for deducing the actual conformation and electronic distribution of the active site. A theoretical attempt in this direction had already been made by Loew and coworkers (1974) using an iterative extended Huckel theory. They had studied ten possible geometries which spanned a symmetry range from a completely tetrahedral to the no-symmetry crystal structure determined by Watenpaugh *et al* (1972, 1973). The empirical nature of extended Huckel calculations is well-known, and in this present study the more sophisticated CNDO and INDO.MO scheme from Clack *et al* (1972), Clack (1974) for the transition metal clusters which explicitly include electron-electron correlation have been used to investigate the above properties. We have confined ourselves to properties like isotropic and anisotropic

hyperfine coupling constants and secondly electric field-gradient and quadrupole coupling constant at the Fe^{57} nucleus. Calculations using some $X\text{-}\alpha$ scattered wave (SW) (Norman and Jackels 1975; Vaughan *et al* 1973) and generalised valence bond (GVB) (Bair and Gooddard 1977, 1978) have also been performed on such models of active site. GVB calculations are confined mainly to the interpretation of optical spectra and to the evaluation of field gradient tensor also to some extent while $X\text{-}\alpha$ SW calculations present the molecular orbitals and eigenvalues. Here we report a detailed investigation of the properties of the active site employing the sophisticated CNDO and INDO MO schemes.

2. Description of the set of conformers studied

Out of the 13 different geometric variations of the active site complex considered by us, ten of them $A, B, C, D, E, F, G, H, O_1$ and O_2 are the ones employed by Loew and co-workers in their IEHT calculations, while the remaining three A', B' and D' are included now by us*. Same geometries are assumed both for the oxidised and reduced forms. Any possibility of a geometry variation with change in oxidation state can naturally be taken care of by the abundance of the geometries included for computation. The molecular entity considered to represent the active site model contains one Fe atom, four cysteine sulphur atoms and four hydrogen atoms to replace the β carbon atoms bonded to sulphur in a manner identical to that assumed by Loew *et al* (1974a), i.e., the active site model is equivalent to considering $[\text{Fe}^{3+}, 4\text{SH}^-]$ for the oxidised species and $[\text{Fe}^{2+}, 4\text{SH}^-]$ for the reduced species.

The original ten geometric variants considered and discussed in detail by Loew and coworkers can be summarised as follows. The conformation based entirely upon the final structure determination of Watenpaugh *et al* (1972) incorporating anomalous scattering is called O_1 . O_2 is a slight variation of O_1 where all the S-H bond lengths are shortened to 1.34 Å. In the conformers A to E the overall symmetry has been slowly increased (see figure 2 of Loew *et al* (1974a) on oxidised rubredoxins) so as to reach a totally regular tetrahedral arrangement of sulphur and hydrogen atoms in the conformer E . The four sulphur atoms in the conformers A to D are situated as pairs in two perpendicular planes yz and xz . In conformers A and B , Fe-S bond lengths as determined from an earlier refinement of crystal data by Watenpaugh *et al* (1972) are used. The S-H bond lengths are kept as 1.34 Å for all conformations A to H (i.e. for all except O_1). While in A and D hydrogen atoms lie in a pin wheel arrangement, they are arranged to have a C_{2v} symmetry about the z axis in B and C . Thus eventhough A and B have no overall symmetry, C exhibits a C_{2v} and D a D_2 symmetry. In F and G three S atoms undergo a three-fold rotation about the short bond axis considered as the z axis containing the fourth sulphur. The H atoms when attached yields a C_3 symmetry to F and a C_{3v} symmetry to G . The conformer H constitutes a planar arrangement.

* It is worth mentioning in this context that the recent derivation of a positive field gradient V_{zz} and quadrupole splitting for this oxidized form by Bair *et al* (1978) from their study on a most probable model for this oxidized rubredoxin is very questionable due to their high η and also due to the over simplified nature of their computation. They seem to have just evaluated the field gradient elements along their three space fixed axes instead of forming the full 3×3 efg tensor and then diagonalising it.

Among the three new conformers A' , B' and D' introduced, A' and B' are similar to A and B wherein we use the Fe-S bond lengths and S-Fe-S bond angles finally obtained by Watenpaugh *et al* (1973) on including anomalous scattering as used in O_1 and O_2 instead of the earlier lesser refined ones assumed by Loew *et al* (1974a) for A and B . Again in D' we are assuming Sayer's (1976) most recent average Fe-S bond length of 2.3 Å instead of the 2 Å adopted for D while in every other respect it is similar to D .

3. Computational details

The CNDO and INDO MO scheme developed on the basis of Clack *et al* (1972) and Clack (1974) parameterisation have been used to perform an unrestricted calculation on both the oxidised and reduced forms of rubredoxins for all 13 possible conformations of the active site. While the metal basis set consists of the valence $3d$, $4s$ and $4p$ orbitals, a minimum basis of $3s$ and $3p$ orbitals is used from sulphur atoms for the sake of computational ease. Zerner and Gouterman's (1966) exponents differentiating the $3d$ from the $4s$, $4p$ orbitals due to their different spatial distribution have been employed for metal orbitals. All such necessary parameters for Fe atom are those of Clack *et al* (1972) while for the ligand atoms the parameters employed by Santry and Segal (1967) have been used. The exchange parameters for S necessary at the INDO level are adopted from the works of Benson and Hudson (1970).

The level shift operator method of Saunders and Hilliers (1973) was used in some cases when there was some difficulty in achieving self-consistency. In some conformers of the reduced rubredoxin having a degenerate ground state (E , F , G) a symmetry restricted calculation was performed by averaging the occupancy of the highest occupied orbital (when degenerate) over the various components of the degenerate irreducible representation. This was done to maintain the molecular symmetry up to the final electronic distribution.

The co-ordinates of the 13 conformers were just fed as input into our CNDO and INDO MO programs developed in Fortran IV for IBM 370/155 computer here which automatically takes care of the above facts. Under the Pople and Nesbet's open-shell UHF formulation adopted here, the total electronic wavefunction ψ is represented as a normalised, anti-symmetrised product of all the filled α and β spin molecular orbitals $\psi_i^{\alpha/\beta}$ which are by themselves calculated as a linear combination (LCAO's) of the basis atomic orbital ϕ 's through our MO schemes.

The ground state of the high-spin oxidised species has 37 electrons occupying 21 α and 16 β MOs. However unlike in the IEHT MO calculations of Loew *et al* (1974) the five major metal $3d$ α MOs lie low among the set of the 21 filled α MOs while the corresponding five β MOs are high up among the set of the unfilled MOs, thus leading to a formal d^5 high spin $(3d^\alpha)^5 (3d^\beta)^0$ sextet configuration.

Similarly the corresponding reduced form with 38 electrons has 21 α orbitals and 17 β orbitals (except for E , F and G) occupied and once again the five major metal $3d$ α orbitals are situated low among the set of the filled 21 α orbitals. The 17th β orbital has (both the half filled 17th and 18th β MOs in the case of E , F and G have) mainly metal $3d$ component and the remaining $3d$ β orbitals are unoccupied giving a formal d^6 , i.e. $(3d^\alpha)^5 (3d^\beta)^1$ quintet configuration for the reduced species.

However as noted by Loew *et al* (1974a, d) in IEHT calculations, the ordering, energy and populations of these orbitals markedly vary from conformer to conformer. These variations have a remarkable effect in the various properties calculated using the resultant total electron density matrix and unpaired spin density matrix over the atomic orbital basis set as is to be seen below. For example, isotropic hyperfine coupling constant from Fe^{57} nucleus will probe the small but significant admixture of iron $4s$ orbital with Fe $3d$ orbitals and also the interaction of inner core s orbitals with the $3d$ orbitals. The anisotropic hyperfine coupling constant, electric field gradient and quadrupole splitting will gauge the anisotropic distribution of electrons among Fe $3d$ and $4p$ orbitals.

4. Hyperfine interaction

4.1. Magnetic hyperfine effect

Since the magnetic hyperfine interactions are dependent on the distribution of unpaired electrons in the molecular orbitals, UHF-MO calculation like this one can account for it in a more realistic way than the RHF open shell MO schemes as the variational equations corresponding to α and β MOs are treated separately in the earlier scheme. However, since the INDO calculations include the one-centre exchange integrals also they provide a more realistic account of these properties than the CNDO scheme which neglects these integrals. The magnetic hyperfine interaction between the magnetic moment of the nucleus (Fe^{57} here) and the electrons can be broken into two parts: isotropic and anisotropic.

4.1a. *Isotropic contact hyperfine term*: The Fermi contact Hamiltonian depicting the isotropic hyperfine interaction between the nuclear spin of Fe^{57} and electron spin at the nucleus is given by

$$\mathcal{H}_F = \frac{8\pi g_e \beta_e g_N \beta_N}{3} \sum_k \mathbf{I} \cdot \mathbf{S} \delta(r_k). \quad (1)$$

The expectation values of \mathcal{H}_F with respect to the ground state total electronic wave function ψ yields the isotropic hyperfine constant A_{iso} as

$$A_{\text{iso}} = \frac{\langle \psi | \mathcal{H}_F | \psi \rangle}{\mathbf{I} \cdot \mathbf{S}} = \frac{8\pi g_e \beta_e g_N \beta_N}{6S} \times \begin{matrix} \text{(total unpaired spin density at} \\ \text{the Fe nucleus).} \end{matrix} \quad (2)$$

Here S is the total spin value for the electronic state of the molecule which is $5/2$ for the oxidised and $4/2$ for the reduced state. From the present valence-only CNDO and INDO MO calculations, contributions from valence electrons to A_{iso} called A_{val} is alone obtained from the net valence electron spin density $|\psi(\text{Fe})|_{\text{val}}^2$ at the Fe nucleus. Following the formulations of Han *et al* (1970), this $|\psi(\text{Fe})|_{\text{val}}^2$ can be written as

$$\begin{aligned} |\psi(\text{Fe})|_{\text{val}}^2 &= \sum_i^{\alpha} |\psi_i^{\alpha}(\text{Fe})|^2 - \sum_i^{\beta} |\psi_i^{\beta}(\text{Fe})|^2 \\ &= \left[\sum_i^{\alpha} (C_{4s,i}^{\alpha})^2 - \sum_i^{\beta} (C_{4s,i}^{\beta})^2 \right] |\phi_{4s}(\text{Fe})|^2 \end{aligned} \quad (3.1)$$

$$+ [\sum_i^a \sum_a C_{4si}^a C_{ai}^a - \sum_i^\beta \sum_a C_{4si}^\beta C_{ai}^\beta] \phi_{4s}(\text{Fe}) \phi_a(\text{Fe}) \quad (3.2)$$

$$+ [\sum_i^a \sum_a (C_{ai}^a)^2 - \sum_i^\beta \sum_a (C_{ai}^\beta)^2] |\phi_a(\text{Fe})|^2. \quad (3.3)$$

This valence density arises from (i) local Fe valence atomic orbitals out of which only the 4s contributes which is the direct local valence term (3.1), (ii) from cross terms between Fe 4s and ligand orbitals term (3.2) and (iii) from distant terms due to unpaired density at the Fe nucleus from the ligand orbitals, term (3.3). Here $|\phi_{4s}(\text{Fe})|^2$ is the electron density of the Fe 4s atomic orbital at the Fe nucleus; $\phi_{4s}(\text{Fe}) \phi_a(\text{Fe})$ is the pairwise product of the value of the Fe 4s wavefunction and the a th ligand atomic orbital at Fe; and finally $|\phi_a(\text{Fe})|^2$ is the electron density of the a th ligand atomic orbital at Fe. The summations \sum_i^a and \sum_i^β are over all the filled a and β molecular orbitals respectively while \sum_a denotes summation over the ligand basic atomic orbitals. $C_{4si}^{a/\beta}$ and $C_{ai}^{a/\beta}$ are the coefficients of Fe 4s atomic orbital and a th ligand orbital in the i th a or β spin molecular orbital respectively. Equation (3.1) is more reliable from the INDO scheme while it is underestimated in CNDO calculations due to the neglect of one-center exchange integrals; Equation (3.2) is equal to zero at the CNDO and INDO level of approximation as we neglect two-centre differential overlaps in both the cases. Also due to the exponential decay of electron density of ligand atomic orbitals from ligand origin, contributions from (3.3) can be considered negligible.

Besides this A_{val} , we have the additional A_{polar} contribution also resulting from the polarisation of the core electrons by the valence electrons. The much smaller back polarisation of the valence electrons by the net spin of the inner shell electrons is neglected. Thus in our calculation for the total $A_{\text{iso}} = A_{\text{valence}} + A_{\text{polar}}$, the first term is directly worked out from a knowledge of the spin density in the 4s orbital using equations (2) and (3.1). The second A_{polar} contribution has been evaluated following the pseudo-atom formulation of Rettig *et al* (1968) using the correlation formula

$$|\psi(\text{Fe})|_{\text{polar}}^2 = \frac{\sigma_{3d}}{4} \left[\sum_{n=1s} (|\phi_n^a(\text{Fe})|^2 - |\phi_n^\beta(\text{Fe})|^2) + \frac{\sigma_{4s}}{2} (|\phi_{4s}^a(\text{Fe})|^2 - |\phi_{4s}^\beta(\text{Fe})|^2) \right] \quad (4)$$

where $|\phi_n^a(\text{Fe})|^2 - |\phi_n^\beta(\text{Fe})|^2$ represents the difference in the density of a and β electrons at the Fe nucleus from the n th Fe atomic orbital as evaluated from an UHF calculation on the free iron atom in its 5D ground state by Bagus and Liu (1966). σ_{3d} and σ_{4s} are the total number of unpaired 3d and paired 4s electrons respectively of the complex, both of which are directly available from our MO calculations on the complex. The only difference between the 5D free iron and the pseudo-atom in a complex is assumed to be in the actual number of unpaired 3d and paired 4s electrons, in each case. Keeping in mind that these are four and two respectively for a free atom; $\frac{1}{4}(\sigma_{3d})$ and $\frac{1}{2}(\sigma_{4s})$ represent the scaling factors which account for the changed populations as the free atom becomes dressed in

the molecular complex to become a pseudo-atom. The core polarisation by the small amount of unpaired $4p$ electrons is ignored. Thus, calculated isotropic hyperfine constants A_{valence} , A_{polar} and the total A_{iso} for our 13 conformers in its both oxidised and reduced form, from the INDO scheme alone are given in the upper halves of the tables 1 and 2 since these alone are considered for the discussion of this unpaired spin-dependent property.

The experimental hyperfine field at the Fe^{57} nucleus as reported for the oxidised rubredoxin (Phillips *et al* 1970; Rao *et al* 1972) in its sextet ground state from a low temperature Mössbauer resonance measurement is 370–375 KG with no specific information regarding as to which particular component of an anisotropic field, if any, this single value closely approximates. For the reduced species the available experimental work of Rao *et al* (1972) reports a value of -246 KG for the spin part of the hyperfine field which is suggested by them to be the transverse component to the assumed symmetry axis of the active site. In addition a 300 KG value is also estimated by them for the isotropic contribution of the reduced entity.

The theoretical estimations, however, indicate the dominance of the core polarisation contribution over the valence contribution. Besides, as the variation of σ_{3d} with change in symmetry is less marked this dominant core-polarisation is less symmetry dependent, diminishing the sensitivity of the total A_{iso} to conformational change, a conclusion in total accord with Loew and Lo (1974b, d). However, our values are more negative than those of Loew *et al* (1974) which were closer to experimental values if we consider the $-(370-375)$ KG and -300 KG as the experimental isotropic field for the oxidised and reduced forms respectively. The pseudo-atom expression assumed here is only a rough approximation for A_{polar} where we have totally neglected the direct polarisation of the Fe core orbitals by ligand valence electrons. Hence the very large calculated values of the total A_{iso} may not be due to the poor quality of CNDO and INDO wave functions. Eventhough the results of Loew and Lo (1974b, d) are surprisingly close to the experimental values, certain examples can be cited where the employment of Das formulation in conjunction with IEHT yield very negative values like ours. Even Freeman and Watson (1964) in a theoretical approach to evaluate A_{iso} from a core-polarisation approach for the $3d$ metal ions like Mn^{2+} , Fe^{2+} , Fe^{3+} , etc arrived at a large negative value. From the same computational attempt using this INDO scheme and the pseudo-atom correlation formula of Han *et al* on some simple well-established Fe complexes we could work out a scaling factor of 0.7 from an one-to-one matching of their experimental values with the ones theoretically arrived at by us. This scaling factor of 0.7 when applied to our theoretical A_{iso} could bring them down nearer to experimental values. But any attempt to derive the possible conformation from isotropic hyperfine coupling alone is meaningless due to (i) the total A_{iso} calculated is quite insensitive to conformational change and also we could bring them down nearer to experimental values only after a scaling and (ii) the experimental value for hyperfine field at the Fe nucleus (370–375) KG reported for the oxidised species need not necessarily be the isotropic one as assumed by Loew and Lo (1974b, d) since no mention was made about its anisotropic nature.

4.1b. *The anisotropic (dipolar) hyperfine term*: This anisotropic contribution to the hyperfine interaction is represented by the Hamiltonian

Table 1. Isotropic and anisotropic hyperfine interaction elements for the oxidised rubredoxin as evaluated from the INDO.

	A	A'	B	B'	C	D	D'	E	F	G	H	O ₁	O ₂
A_{valence} (MHz)	0.92	0.90	0.92	0.90	0.96	0.47	1.04	0.58	0.87	0.89	1.55	2.15	0.91
A_{polar} (MHz)	-28.09	-28.12	-28.07	-28.10	-28.15	-27.93	-28.24	-28.73	-28.20	-28.16	-28.20	-27.99	-28.14
Total A_{iso} (MHz)	-27.17	-27.22	-27.15	-27.20	-27.18	-27.46	-27.20	-28.14	-27.32	-27.27	-26.65	-25.84	-27.23
Total A_{iso} (KG)	-497.2	-498.2	-496.8	-497.8	-497.5	-502.6	-497.8	-515.0	-500.0	-499.1	-487.7	-472.9	-498.3
Total A_{iso} (KG) scaled down by 0.7	-348.0	-348.7	-347.8	-348.5	-348.2	-351.8	-348.4	-360.5	-350.3	-349.4	-341.4	-331.0	-348.8
B'_{xx} (KG)	7.13	7.19	9.38	9.48	7.02	1.75	3.60	0.0	8.48	0.18	-38.13	3.84	3.48
B'_{yy} (KG)	16.53	11.52	15.80	12.41	11.12	1.76	3.66	0.0	8.48	0.18	-39.99	7.53	6.04
B'_{zz} (KG)	-23.66	-18.71	-25.18	-21.89	-18.14	-3.50	-7.26	0.0	-16.96	-0.37	78.12	-11.37	-9.52

Table 2. Isotropic and anisotropic hyperfine interaction elements for the reduced rubredoxin as evaluated from the INDO.

	A	A'	B	B'	C	D	D'	E	F	G	H	O ₁	O ₂
A_{valence} (MHz)	1.58	1.46	1.53	1.46	1.45	0.93	1.58	0.82	1.30	1.38	1.54	2.91	1.55
A_{polar} (MHz)	-30.95	-30.91	-30.95	-30.96	-30.96	-30.21	-31.00	-30.33	-30.91	-30.84	-30.71	-30.26	-30.96
Total A_{iso} (MHz)	-29.38	-29.44	-29.42	-29.50	-29.51	-29.28	-29.42	-29.51	-29.61	-29.46	-29.17	-27.34	-29.40
Total A_{iso} (KG)	-430.2	-431.1	-430.8	-431.9	-432.1	-428.6	-430.8	-432.1	-433.5	-431.3	-427.0	-400.3	-430.5
Total A_{iso} (KG) scaled down by 0.7	-301.1	-301.8	-301.6	-302.3	-302.4	-300.0	-301.5	-302.4	-303.4	-301.9	-298.9	-280.2	-301.3
B'_{xx} (KG)	80.81	86.58	81.16	86.50	85.44	85.14	89.13	0.0	-54.03	-37.12	8.60	-24.41	-22.05
B'_{yy} (KG)	94.08	92.53	93.91	94.14	93.79	86.68	90.06	0.0	-54.03	-37.12	64.95	-144.6	-142.9
B'_{zz} (KG)	-174.9	-179.1	-175.1	-180.6	-179.2	-171.8	-179.2	0.0	108.1	74.23	-73.55	169.1	165.0

$$\mathcal{H} = g_e \beta_e g_{Fe} \beta_N \sum_k \frac{3 (\mathbf{S}_k \cdot \mathbf{r}_k) (\mathbf{I} \cdot \mathbf{r}_k) - r_k^2 (\mathbf{S}_k \cdot \mathbf{I})}{r_k^5} \quad (5)$$

This contribution can be represented in the spin Hamiltonian by a 3×3 tensor B , whose components in the present UHF-MO formalism may be evaluated by the relation

$$B_{pq} = \frac{g_e \beta_e g_{Fe} \beta_N}{2S} \sum_{\mu\nu} (P_{\mu\nu}^\alpha - P_{\mu\nu}^\beta) \left\langle \phi_\mu \left| \frac{3r_p r_q - r^2 \delta_{pq}}{r^5} \right| \phi_\nu \right\rangle \quad (6)$$

where p and q are direction co-ordinates and $P_{\mu\nu}^\alpha$ and $P_{\mu\nu}^\beta$ are the α and β density matrix elements over our atomic orbital basis set. The matrix elements

$$\left\langle \phi_\mu \left| \frac{3r_p r_q - r^2 \delta_{pq}}{r^5} \right| \phi_\nu \right\rangle$$

can be further split into a radial part depending on the radial integral

$$\left\langle \phi_\mu \left| \frac{1}{r^3} \right| \phi_\nu \right\rangle$$

and an angular part involving the remaining operators which in turn can be expressed in terms of spherical harmonics. On evaluating the B tensor elements from the above expression all multi-centre integrals involving ligand orbitals are neglected due to the CNDO and INDO approximations adopted by us and also due to the fast decay of the $\langle 1/r^3 \rangle$ term as we move away from the central Fe atom. Thus the above sum over the basis valence atomic orbitals is restricted to only Fe orbitals, of which only the d and $4p$ have to be considered for this anisotropic interaction. For the radial part we have used a single value of

$$\left\langle \phi_\mu \left| \frac{1}{r^3} \right| \phi_\nu \right\rangle$$

2.0369 a.u. for all combinations of $4p$ orbitals and a 5.0 a.u. for all combinations of $3d$ orbitals (Rettig *et al* 1968). The $3 \times 3B$ tensor hence built out for all our conformers from the INDO results alone once again, for the oxidised and reduced form are diagonalised to yield the principal components B'_{xx} , B'_{yy} and B'_{zz} which are expressed in KG in the lower halves of tables 1 and 2.

Since the 370-375 KG field measured in the oxidised form need not necessarily be the isotropic one, it is more appropriate first to compare this with all the three principal components H' of the internal magnetic field at Fe, namely, $H'_{zz} = A_{1so} + B'_{zz}$; $H'_{yy} = A_{1so} + B'_{yy}$ and $H'_{xx} = A_{1so} + B'_{xx}$. Secondly in the thus evaluated H' the A_{1so} term needed a scaling as mentioned already though the B' calculated by us is quite reasonable. For these two reasons it is difficult to conclude anything positive from such a comparison. Since the theoretically evaluated components of H' are of negative sign for all the 13 possible conformers we can just say that the experimental 370-375 KG reported for the oxidised form should have a negative sign in agreement with the suggestion of Rao *et al* (1972).

Similarly for the reduced form the 300 KG estimated for the isotropic field from experiment can again be assigned a negative sign on the basis of calculated A_{1so} for all conformers, once again in agreement with Rao *et al* (1972). Now the experi-

mental transverse spin part of the hyperfine field component of 246 KG which should be either $A_{1so} + B'_{xx}$ or $A_{1so} + B'_{yy}$ can also have the same negative sign similar to the isotropic field (once again in conformity with Rao *et al* (1972) finding) since the magnitude of the components of B' is very low compared to that of A_{1so} values. This gives us an experimental value of + 54 KG for the transverse spin anisotropic part B' of the reduced state. This has to be compared with the calculated B'_{xx} and B'_{yy} for all 13 forms. Conformer F , G , O_1 and O_2 giving negative transverse B' components B'_{xx} and B'_{yy} can be rejected as impossible conformers. A , A' , B , B' , C , D and D' giving + 80 to + 90 KG transverse B' values together with the planar H which also gives +ve B'_{xx} and B'_{yy} (of course with high rhombicity) can only be the possible conformers for the reduced form from the magnetic hyperfine interaction point of view.

4.2. Electric field gradient at the Fe nucleus and quadrupole splitting in Mössbauer resonance

The Hamiltonian representing this quadrupole interaction in the principal axis system of the electric field gradient (efg) tensor is given by

$$\mathcal{H} = \frac{eQ}{4I(2I-1)} [V'_{zz}(3I_z^2 - I(I+1)) + (V'_{xx} - V'_{yy})(I_x^2 - I_y^2)]. \quad (7)$$

Here Q is the quadrupole moment of the nuclear state with spin I and V'_{xx} , V'_{yy} and V'_{zz} are the principal values of the efg tensor. $|V'_{zz}| > |V'_{yy}| \geq |V'_{xx}|$ such that z' is the axis of highest symmetry and $\eta = V'_{xx} - V'_{yy}/V'_{zz}$ is the rhombicity parameter depicting the extent of rhombic distortion. The Mössbauer quadrupole splitting for our Fe^{57} nucleus in its excited state where $I = 3/2$ is then given by

$$\Delta E_Q = \frac{eQV'_{zz}}{2} \left[1 + \frac{\eta^2}{3} \right]^{1/2} \quad (8)$$

The sign convention is such that for positive V'_{zz} , the state $|3/2, \pm 1/2\rangle$ lies lower. However, when $V'_{yy} \approx -V'_{zz}$ and $V'_{xx} \approx 0$, i.e. when $\eta \approx -1$, the quantisation axis is itself not defined uniquely and therefore the sign of ΔE_Q remains indeterminate.

Since the magnitude of this splitting is regarded as a very sensitive probe of the electron distribution and hence of the conformation about the Fe nucleus, we have investigated the quantitative variations of the calculated efg and quadrupole splitting with specific conformational variations of the active site in both the oxidised and reduced states. The expectation values of all the 9 components of the efg tensor V with respect to our UHF total electronic ground wave function ψ were first evaluated using the relation

$$V_{\nu\alpha} = \sum_{\mu\nu} P_{\mu\nu} \left\langle \phi_\mu \left| \frac{3r_\nu r_\alpha - r^2 \delta_{\nu\alpha}}{r^5} \right| \phi_\nu \right\rangle. \quad (9)$$

Here $P_{\mu\nu} = P_{\mu\nu}^a + P_{\mu\nu}^b$ represent the total electron density matrix elements. All the remaining terms have the same meaning as in (6). The summation over μ and ν is restricted to the iron valence atomic orbitals alone; in particular to the $3d$ and $4p$ as was done earlier with the B tensor evaluation. The (3×3) V tensor hence constructed for all the 13 conformers for their oxidised and reduced states

from both the CNDO and INDO were subsequently diagonalised to yield the principal values V''_{xx} , V''_{yy} and V''_{zz} and are given in tables 3 to 6 along with the corresponding rhombicity parameter η . Since we deal with just a charge-dependent property, both the CNDO and INDO results are analysed with equal care to investigate the relative merits. It can be seen from these tables that the value of each principal component of V' is indeed highly symmetry-dependent. Non-vanishing elements for the field gradient tensor arise as a result of the deviation from a spherical distribution of the valence electrons of Fe^{57} . In other words, the differential population in the various d orbitals due to a lower ligand field symmetry would contribute to the efg tensor. The direct lattice contribution to field gradient is ignored as being small. Due to the addition of one more electron, the magnitude of efg tensor elements in the reduced species is much higher than the corresponding values for the oxidised form, as evident from the V' component presented in tables 3 to 6. Also the magnitude of V''_{zz} and hence of ΔE_Q for the reduced species is not very sensitive to symmetry, in spite of the higher magnitude of the V' components quite in contrast to the oxidised species. We can definitely make use of the calculated sign variation for V''_{zz} and ΔE_Q of the reduced species to guess the most possible conformer. The conformer E has a null-field gradient tensor at the Fe nucleus both for the oxidised and reduced species from our symmetry restricted calculation as should be expected for this tetrahedral conformer with a total cubic environment around Fe. In the case of F and G with axial symmetry, η has come out as zero. The x-ray determined structures O_1 and O_2 on the other hand have a substantial rhombic contribution to the field gradient. The sensitivity of the components of the field-gradient to small changes in the environment around the central metal is evident from the largely different values obtained for the efg tensor elements even when just the positions of H atoms are changed. (cf. O_1 and O_2 , A and B , A' and B' , D and E , F and G). This is in agreement with the findings of Bair and Gooddard (1978) who explain this strong effect of second nearest H atoms on Fe orbitals as due to the orientation of the S-H bond restricting the orientation of the two lone pairs on each S which in turn can interact strongly with the Fe.

Now the quadrupole splitting ΔE_Q evaluated using the relation (8) are tabulated in the lower-halves of tables 3 to 6. There is some ambiguity in the value of Q for the excited $I = 3/2$ state of Fe^{57} nucleus which ranges from a $Q = 0.187$ to $Q = 0.47$ barns. Hence the values of ΔE_Q (max) and ΔE_Q (min) using the upper $Q = 0.48$ and lower $Q = 0.18$ limits of Q are also evaluated besides the ΔE_Q using the conventional $Q = 0.21$ barns (Stevens and Stevens 1975). Besides, the Sternheimer shielding correction (Sternheimer 1950, 1951; Sternheimer and Foley (1956) is incorporated by multiplying the above calculated ΔE_Q by the shielding factor $(1 - R)$. Using Clementi's Hartree-Fock atomic functions for Fe orbitals, a value of 0.68 has been reported (Rettig *et al* 1968) for the ferric ion which is used for both the oxidised and reduced forms.

The ΔE_Q for the oxidised species has been reported as 0.78 ± 0.02 mm/sec with no determination of the sign from a 77° K measurement (Phillips *et al* 1970). So the tetrahedral conformer E predicting a null field gradient tensor and hence zero quadrupole splitting for the oxidised species is ruled out. With the O_1 and O_2 , INDO scheme gives a negative ΔE_Q while the sign of the ΔE_Q is left indeterminate from the CNDO approach due to high η parameters. Now among the remaining

Table 3. Electric field gradient tensor components (with $|V'_{xx}| > |V'_{yy}| \geq |V'_{zz}|$) at Fe nucleus and quadrupole splitting ΔE_Q (mm/sec) of Mössbauer resonance for the oxidised rubredoxin as calculated from the CNDO analysis.

	A	A'	B	B'	C	D	D'	E	F	G	H	O ₁	O ₂
V'_{xx}	0.118	0.165	0.157	0.200	0.201	0.041	0.112	0	0.120	-0.084	-0.800	0.009	0.034
V'_{yy}	0.379	0.266	0.386	0.272	0.227	0.049	0.112	0	0.120	-0.084	-0.854	0.209	0.162
V'_{zz}	-0.498	-0.430	-0.543	-0.472	-0.428	-0.090	-0.224	0	-0.240	0.168	1.653	-0.218	-0.196
η	0.524	0.235	0.421	0.152	0.061	0.080	0.002	0	0	0	0.033	0.916	0.657
ΔE_Q (min)*	-0.947	-0.791	-1.017	-0.862	-0.780	-0.164	-0.408	0	-0.438	0.305	3.011	\mp 0.450	\mp 0.381
ΔE_Q (max)**	-2.526	-2.109	-2.713	-2.299	-2.081	-0.438	-1.088	0	-1.168	0.814	8.029	\mp 1.201	\mp 1.017
ΔE_Q †	-1.105	-0.922	-1.187	-1.006	-0.910	-0.192	-0.476	0	-0.511	0.356	3.513	\mp 0.525	\mp 0.445
ΔE_Q (min) (1-R) ††	-0.644	-0.538	-0.692	-0.586	-0.531	-0.112	-0.277	0	-0.298	0.207	2.047	\mp 0.306	\mp 0.259
ΔE_Q (max) (1-R)	-1.718	-1.434	-1.845	-1.564	-1.415	-0.298	-0.740	0	-0.794	0.553	5.460	\mp 0.816	\mp 0.692
ΔE_Q † (1-R)	-0.751	-0.627	-0.807	-0.684	-0.619	-0.130	-0.324	0	-0.348	0.242	2.389	\mp 0.357	\mp 0.302

* with $Q = 0.18$ barns; ** with $Q = 0.48$ barns; † with $Q = 0.21$ barns; †† Sternheimer correction (1-R) = 0.68.

Table 4. Electric field gradient tensor components (with $|V'_{xx}| > |V'_{yy}| \geq |V'_{zz}|$) at Fe nucleus and quadrupole splitting ΔE_Q (mm/sec) of Mössbauer resonance for the oxidised rubredoxin as calculated from the INDO analysis.

	A	A'	B	B'	C	D	D'	E	F	G	H	O ₁	O ₂
V'_{xx}	0.071	0.103	0.111	0.125	0.107	0.013	0.054	0	0.098	-0.064	-0.588	0.045	0.043
V'_{yy}	0.261	0.173	0.248	0.180	0.147	0.023	0.055	0	0.098	-0.064	-0.644	0.097	0.080
V'_{zz}	-0.332	-0.276	-0.359	-0.305	-0.254	-0.036	-0.109	0	-0.196	0.127	1.232	-0.143	-0.123
η	0.574	0.252	0.380	0.181	0.157	0.285	0.002	0	0	0	0.045	0.366	0.304
ΔE_Q (min)*	-0.636	-0.508	-0.670	-0.559	-0.465	-0.066	-0.199	0	-0.358	0.232	2.244	-0.265	-0.228
ΔE_Q (max)**	-1.696	-1.355	-1.786	-1.491	-1.241	-0.176	-0.531	0	-0.954	0.618	5.984	-0.708	-0.608
ΔE_Q †	-0.742	-0.593	-0.781	-0.652	-0.543	-0.077	-0.232	0	-0.417	0.270	2.618	-0.310	-0.266
ΔE_Q (min) (1-R) ††	-0.432	-0.346	-0.455	-0.380	-0.316	-0.045	-0.135	0	-0.243	0.157	1.526	-0.180	-0.155
ΔE_Q (max) (1-R)	-1.153	-0.921	-1.215	-1.014	-0.844	-0.120	-0.361	0	-0.648	0.320	4.069	-0.481	-0.414
ΔE_Q † (1-R)	-0.504	-0.403	-0.531	-0.444	-0.369	-0.052	-0.158	0	-0.284	0.184	1.780	-0.210	-0.181

* with $Q = 0.18$ barns; ** with $Q = 0.48$ barns; † with $Q = 0.21$ barns; †† Sternheimer correction (1-R) = 0.68.

Table 5. Electric field gradient tensor components (with $|V'_{xx}| > |V'_{yy}| \geq |V'_{zz}|$) at Fe nucleus and quadrupole splitting ΔE_Q (mm/sec) of Mossbauer resonance for the reduced rubredoxin as calculated from the CNDO analysis.

	A	A'	B	B'	C	D	D'	E	F	G	H	O ₁	O ₂
V'_{xx}	1.232	1.382	1.221	1.354	1.324	1.379	1.416	0	-1.008	-0.762	0.071	-0.446	-0.344
V'_{yy}	1.517	1.482	1.502	1.485	1.484	1.395	1.431	0	-1.008	-0.763	0.995	-2.222	-2.271
V'_{zz}	-2.750	-2.864	-2.723	-2.839	-2.808	-2.774	-2.847	0	2.016	1.525	-1.066	2.668	2.615
η	0.104	0.035	0.103	0.046	0.057	0.006	0.005	0	0	0	0.867	0.666	0.737
ΔE_Q (min)*	-5.016	-5.215	-4.966	-5.171	-5.116	-5.051	-5.184	0	3.671	2.777	\mp 2.171	\pm 5.204	\pm 5.174
ΔE_Q (max)**	-13.376	-13.907	-13.244	-13.789	-13.644	-13.470	-13.824	0	9.790	7.405	\mp 5.789	\pm 13.878	\pm 13.798
ΔE_Q †	-5.852	-6.084	-5.794	-6.033	-5.969	-5.893	-6.048	0	4.283	3.240	\mp 2.533	\pm 6.672	\pm 6.037
ΔE_Q (min) (1 - R) ††	-3.411	-3.546	-3.377	-3.516	-3.479	-3.435	-3.525	0	2.496	1.888	\mp 1.476	\pm 3.539	\pm 3.518
ΔE_Q (max) (1 - R)	-9.096	-9.457	-9.006	-9.376	-9.278	-9.160	-9.400	0	6.657	5.035	\mp 3.936	\pm 9.437	\pm 9.383
ΔE_Q † (1 - R)	-3.979	-4.137	-3.940	-4.102	-4.059	-4.007	-4.113	0	2.912	2.203	\mp 1.722	\pm 4.129	\pm 4.105
ΔE_Q (oxidised)													
ΔE_Q (reduced)	0.189	0.152	0.205	0.167	0.152	0.032	0.079	..	-0.120	-0.110	\pm 1.387	\pm 0.086	\pm 0.074

* with $Q = 0.18$ barns; ** with $Q = 0.48$ barns; † with $Q = 0.21$ barns; †† Sternheimer correction $(1 - R) = 0.68$.

Table 6. Electric field gradient tensor components with $|V'_{xx}| > |V'_{yy}| \geq |V'_{zz}|$ at Fe nuclei; and quadrupole splitting ΔE_Q (mm/sec) of Mössbauer resonance for the reduced rubredoxin as calculated from the INDO analysis.

	A	A'	B	B'	C	D	D'	E	F	G	H	O ₁	O ₂
V'_{xx}	1.243	1.356	1.234	1.340	1.318	1.319	1.401	0	-0.891	-0.662	0.102	-0.458	-0.043
V'_{yy}	1.496	1.449	1.481	1.462	1.462	1.338	1.415	0	-0.891	-0.662	1.059	-2.239	-2.197
V'_{zz}	-2.739	-2.805	-2.715	-2.802	-2.779	-2.656	-2.816	0	1.783	1.324	-1.160	2.697	2.628
η	0.093	0.033	0.091	0.043	0.052	0.007	0.005	0	0	0	0.825	0.660	0.672
ΔE_Q (min)*	-4.994	-5.108	-4.950	-5.104	-5.063	-4.836	-5.127	0	3.246	2.410	± 2.340	± 5.256	± 5.132
ΔE_Q (max)**	-13.319	-13.621	-13.200	-13.610	-13.501	-12.897	-13.673	0	8.656	6.427	± 6.240	± 14.016	± 13.685
ΔE_Q †	-5.827	-5.959	-5.775	-5.955	-5.907	-5.642	-5.982	0	3.787	2.812	± 2.730	± 6.132	± 5.987
ΔE_Q (min) (1-R)††	-3.396	-3.473	-3.366	-3.471	-3.443	-3.289	-3.486	0	2.207	1.639	± 1.591	± 3.574	± 3.490
ΔE_Q (max) (1-R)	-9.057	-9.262	-8.976	-9.255	-9.181	-8.770	-9.298	0	5.886	4.370	± 4.243	± 9.531	± 9.306
ΔE_Q † (1-R)	-3.962	-4.052	-3.927	-4.049	-4.017	-3.837	-4.068	0	2.575	1.912	± 1.856	± 4.170	± 4.071
ΔE_Q (oxidised)													
ΔE_Q (reduced)	0.127	0.099	0.135	0.110	0.092	0.014	0.039	..	-0.110	-0.096	± 0.959	± 0.050	± 0.044

* with $Q = 0.18$ barns; ** with $Q = 0.48$ barns; † with $Q = 0.21$ barns; †† Sternheimer correction (1-R) = 0.68.

conformers, except the totally unimaginable planar conformer *H* and the conformer *G*, both the CNDO and INDO methods predict a negative ΔE_Q value for all the remaining geometries. Hence the sign of the experimental splitting of 0.78 mm/sec can be safely taken as negative. Considering ΔE_Q with the conventional $Q = 0.21$ barns and matching the predictions from the CNDO and INDO schemes with the experimental value, conformers *A* and *B* seem to be the best possibilities.

The experimental value of ΔE_Q for the reduced rubredoxin at 77° K has been determined by two investigations to be -3.16 (Rao *et al* 1972) and -3.38 mm/sec (Phillips *et al* 1970) respectively. The average value of -3.27 mm/sec with the established negative sign, rules out conformer *E* (predicting zero splitting) and conformers *F* and *G* (predicting positive splitting). The conformers *H*, O_1 and O_2 have high η values and hence ΔE_Q calculated with indeterminate sign out of which even the magnitude of splitting from the planar *H* is unreasonably far off from the 3.27 mm/sec. For the remaining seven conformers *A* to *D'*, negative ΔE_Q 's have been evaluated. Of these, *A* and *B* giving the nearest calculated splitting to experimental one once again turn out to be the best fit.

The ambiguities present in the above theoretical evaluations of ΔE_Q for the oxidised and reduced forms due to the range of values for Q and inclusion or non-inclusion of Sternheimer correction, etc. can be overcome if we try to correctly predict their ratio as was done by Loew and Lo (1974b, d). This, however, makes an inherent assumption that the active site geometry for oxidised and reduced species are identical. While the experimental value of this ratio [ΔE_Q (oxidised)/ ΔE_Q (reduced)] works out to be $(-0.78/-3.27) = +0.24$; the theoretically computed ratios for the various conformers through the CNDO and INDO analysis are found at the bottom of tables 5 and 6. Next to conformer *E* with zero quadrupole splitting, conformers *F* and *G* predicting a negative value for this ratio can also be neglected. Now among the conformers *H*, O_1 and O_2 possessing calculated values for these ratios with indeterminate sign (due to the uncertainty in the sign of ΔE_Q (oxidised) and ΔE_Q (reduced) computed for them), conformer *H* giving a quite an unreasonable value is definitely next to impossible. Even the magnitudes of the corresponding ratios worked out for O_1 and O_2 are far too low from the 0.24 value. Of the remaining conformers *A* to *D'* even though the calculated values for this ratio is generally lower than the experimental 0.24, conformer *A* and *B* giving the nearest values are once again the best possibilities considering the approximations involved in the calculations.

5. Conclusions

Based on the correlations of magnetic hyperfine interaction constants for the reduced species conformer *A* to *D'* are suggested as the best possibilities. If we also consider the quadrupole splittings only the conformers *A* and *B* turn out to be the best possibilities considering the individual ΔE_Q splitting of oxidised and reduced forms and their ratio. Thus we suggest that this rubredoxin has conformers *A* and *B* as the best possibilities both with the iron atoms attached to four sulphur atoms situated as pairs in two perpendicular planes as a result of highly distorted tetrahedral arrangement. The attached hydrogen atoms (and hence the carbon atom in the actual protein) are in a pin wheel arrangement in *A* and in a

mirror image form in *B* as mentioned already. This inference of ours is in agreement with that of Loew and coworkers (1974) who have suggested earlier conformer *A* alone as the best possibility from their IEHT calculations, though the individual values of these properties calculated from the IEHT and our CNDO, INDO approach for the various conformers differ considerably. We feel that more confirmatory experimental measurements regarding the sign and anisotropic nature of hyperfine constants and quadrupole splitting ΔE_Q will be assets for a better conclusion. From the theoretical side, the effect of inclusion of *3d* orbitals on sulphur for the calculation of these properties has to be tested. Meanwhile a number of other properties deduced from ESR measurements like *g* values, zero field splitting, etc., are to be correlated which we hope will further help us to elucidate the conformation of active site complex for oxidised and reduced species.

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References

- Bagus P S and Liu B 1966 *Phys. Rev.* **148** 79
 Bair R A and Gooddard W A (III) 1977 *J. Am. Chem. Soc.* **99** 3505
 Bair R A and Gooddard W A (III) 1978 *J. Am. Chem. Soc.* **100** 5669
 Benson H G and Hudson A 1970 *Theor. Chim. Acta* **23** 259
 Clack D W, Hush N S and Yandle J R 1972 *J. Chem. Phys.* **57** 3503
 Clack D W 1974 *Mol. Phys.* **27** 1513
 Eaton W A and Lovenberg W 1970 *J. Am. Chem. Soc.* **92** 7195
 Eaton W A, Palmer G, Fee J A, Kimura T and Lovenberg W 1971 *Proc. Natl. Acad. Sci. USA* **68** 3015
 Freeman A J and Watson R E 1964 *Hyperfine interactions in magnetic materials in magnetism* eds G T Rado and H Suhl (New York: Academic Press) Vol. 2
 Han P S, Das T P and Rettig M F 1970 *Theor. Chim. Acta* **16** 1
 Jensen L H 1974 *Ann. Rev. Biochem.* **43** 461
 Lane R W, Ibers J A, Frankel R B and Holm R H 1975 *Proc. Natl. Acad. Sci. USA* **72** 2868
 Lane R W, Ibers J A, Frankel R B and Holm R H 1977 *J. Am. Chem. Soc.* **99** 84
 Loew G H, Chadwick M and Steinberg D A 1974a *Theor. Chim. Acta* **33** 125
 Loew G H and Lo D 1974b *Theor. Chim. Acta* **32** 217
 Loew G H, Chadwick M and Lo D 1974c *Theor. Chim. Acta* **33** 147
 Loew G H and Lo D 1974d *Theor. Chim. Acta* **33** 137
 Lovenberg W and Sobel B E 1965 *Proc. Natl. Acad. Sci. USA* **54** 193
 Lovenberg W 1973 *Iron-sulphur proteins* (New York: Academic Press) 1-2
 Norman J G Jr. and Jackels S G 1975 *J. Am. Chem. Soc.* **97** 3833
 Orme-Johnson W H 1973a Ferredoxins and other iron sulphur proteins; in *Inorganic bio-chemistry* 2 ed. G L Eichhorn (New York: Elsevier)
 Orme-Johnson W H 1973b *Ann. Rev. Biochem.* **42** 159
 Peisach J, Blumberg W E, Lode E T and Coon M J 1971 *J. Biol. Chem.* **246** 5877
 Phillips W D, Poe M, Weiher J F, McDonald C C and Lovenberg W 1970 *Nature (London)* **227** 574
 Pople J A and Beveridge D L 1970 *Approximate molecular orbital theory* (New York: McGraw-Hill) and references therein

- Pople J A, Santry ——— and Segal ——— 1970
Rao K K, Evans M C W, Cammack R, Hall D O, Thomson C L, Jackson P J and Johnson C E
1972 *Biochem. J.* **129** 1063
Rettig M F, Han P S and Das T P 1968 *Theor. Chim. Acta* **12** 178
Santry D and Segal G A 1967 *J. Chem. Phys.* **47** 158
Saunders V R and Hillier I H 1973 *Int. J. Quantum Chem.* **7** 699
Sayers D E, Stern E A and Herriott J R 1976 *J. Chem. Phys.* **64** 427
Schulman R G, Eisenberger P, Blumberg W E and Stombaugh N A 1975 *Proc. Natl. Acad. Sci.
USA* **72** 4003
Sternheimer R 1950 *Phys. Rev.* **80** 102
Sternheimer R 1951 *Phys. Rev.* **84** 244
Sternheimer R and Foley H M 1956 *Phys. Rev.* **102** 731
Stevens J G and Stevens V E 1975 *Mössbauer effect data index* (New York: Plenum Press) p 54
Vaughan D J, Tossell J A and Johnson K H 1973 *Geochim. Cosmochim. Acta* **38** 993
Watenpaugh K D, Sieker L C, Herriott J R and Jensen L H 1972 *Cold Spring Harbor Symp.
Quant. Biol.* **36** 359
Watenpaugh K D, Sieker L C, Herriott J R and Jensen L H 1973 *Acta Crystallogr.* **B29** 943
Zerner M and Gouterman M 1966 *Theor. Chim. Acta* **4** 44