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H. Eicher, F. Parak, D. Bade, J. Tejada. ELECTRONIC STRUCTURE OF THE IRON IN DEOXIGENATED MYOGLOBIN FROM MÖSSBAUER SPECTROSCOPY. *Journal de Physique Colloques*, 1974, 35 (C6), pp.C6-363-C6-366. 10.1051/jphyscol:1974665 . jpa-00215822

**HAL Id: jpa-00215822**

**<https://hal.science/jpa-00215822>**

Submitted on 4 Feb 2008

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## ELECTRONIC STRUCTURE OF THE IRON IN DEOXYGENATED MYOGLOBIN FROM MÖSSBAUER SPECTROSCOPY

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**Résumé.** — On présente de nouvelles mesures de la variation en fonction de la température de l'interaction quadropolaire du fer dans la myoglobine désoxygénée du cachalot. La séparation en énergie entre les termes électroniques d'énergie basse du fer est obtenue par un lissage direct (méthode des moindres carrés) à la variation du gradient de champ électrique en fonction de la température. En prenant le terme  ${}^5B_2$  comme niveau de référence ( $E = 0$ ), on déduit la séquence énergétique suivante :  ${}^1A_1(60 \text{ cm}^{-1})$ ;  ${}^5E(106 \text{ cm}^{-1})$ ;  ${}^3E(809 \text{ cm}^{-1})$ ;  $D(-298 \text{ cm}^{-1})$  ce qui donne  $\eta(77 \text{ K}) = -13,3$ .

Ces résultats sont compatibles avec les spectres Mössbauer du fer dans des monocristaux de myoglobine présentés récemment par Gonser *et al.*

**Abstract.** — New measurements of the temperature dependence of the quadrupole splitting of iron in deoxygenated sperm whale myoglobin are reported. The energy separation of the low lying electronic terms of the iron is obtained by a direct least squares fit to the temperature variation of the electric field gradient. Taking the  ${}^5B_2$  term as the reference level ( $E = 0$ ) the following energy scheme was deduced :  ${}^1A_1(60 \text{ cm}^{-1})$ ;  ${}^5E(106 \text{ cm}^{-1})$ ;  ${}^3E(809 \text{ cm}^{-1})$ ;  $D(-298 \text{ cm}^{-1})$  yielding  $\eta(77 \text{ K}) = -13.3$ . These results are consistent with Mössbauer spectra of the iron in single crystals of deoxygenated myoglobin recently reported by Gonser *et al.*

**1. Introduction.** — The temperature dependent quadrupole splitting of  $\text{Fe}^{(2+)}$  porphyrin compounds as measured by the Mössbauer effect, provides highly useful information on the electronic structure of the ferrous ion in these compounds. In order to achieve this goal a theory had been developed [1, 2] which allowed the evaluation of the eigenvalues and eigenvectors of the proper Hamiltonian within the  $3d^6$  configuration of the ferrous ion. This Hamiltonian contains the Coulomb repulsion of the 3d-electrons, the nearly tetragonal point symmetry of the iron cation, the effects of a small rhombic perturbation of the  $C_{4v}$  point symmetry, and the spin-orbit coupling of the 3d-electrons. Thus, the temperature dependent electric field gradient (efg)-tensor at the iron nucleus as produced by the low-lying electronic levels, can directly be compared with the experimental data. This then provides the needed information about the energy term scheme of  $\text{Fe}^{(2+)}$  in the various porphyrin compounds.

We have recently measured with high accuracy the temperature dependence of the quadrupole splitting of deoxygenated samples of sperm whale myoglobin (Mb) of human haemoglobin (HbA) of isolated  $\alpha$  and  $\beta$  chains of HbA, of haemoglobin of *Chironomus thummi thummi*, and finally of a haemoglobin-haptoglobin complex. Mössbauer experiments on single crystals of Mb which have also been performed have been reported

elsewhere [3]. In this communication we wish to discuss on the basis of these experiments the electronic structure of the iron cation in Mb according to the theoretical approach outlined in [1, 2]. A similar treatment for the other compounds measured together with a comparison of the Mössbauer results with respect to physiological properties is still in progress.

**2. Theory.** — The efg-tensor at the iron nucleus is produced by the combined action of the electrons in the perturbed  $3d^6$ -configuration and the second order components of the crystalline electric field [1, 4]. Because of the nearly tetragonal symmetry of the iron in Mb, the direct contribution from the crystalline field which is rather small can be taken into account by a temperature independent and axially symmetric efg  $V_{zz}$  (lattice). The major contribution to the efg-tensor arises from the 3d-electrons of the iron cation. Starting with the tetragonal point symmetry  $C_{4v}$  of the iron site in Mb, the corresponding Hamiltonian depends on three adjustable parameters  $\epsilon_1$ ,  $\epsilon_2$ ,  $\epsilon_3$  which determine the crystalline field splitting of the one-electron states of the  $3d^6$ -configuration [1]. Taking into account the Coulomb repulsion of the 3d-electrons it is found that the many electron levels  ${}^1A_1$ ,  ${}^3E$ ,  ${}^5B_2$ , and  ${}^5E$  lay lowest in energy as shown in figure 1. Figure 2 explains how these levels are related to the one-electron terms of the  $3d^6$ -configuration. Note that

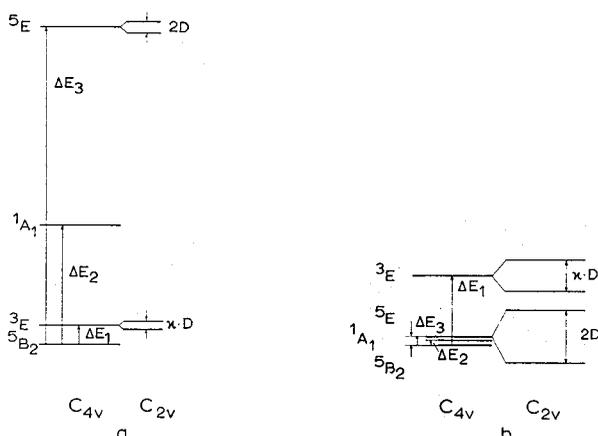


FIG. 1. — Lowest energy levels of the  $3d^6$  configuration. Both sequences use as the groundstate a high spin configuration. The rhombic distortion of the  ${}^3E$  state is  $D\kappa(\Delta E_1, \Delta E_2, \Delta E_3)$  (myoglobin, Fig. 1b:  $\kappa \approx 1.2$ ).

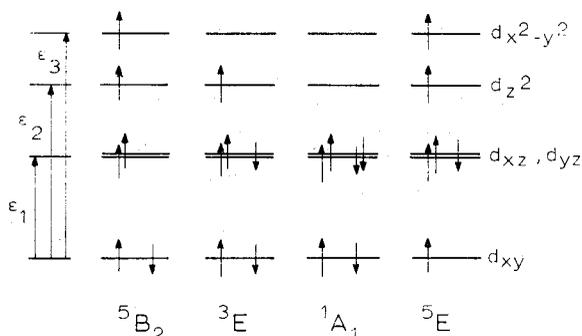


FIG. 2. — Correlation between one-electron levels of the  $3d^6$  configuration and the multielectron scheme. The degeneracy corresponds to a  $C_{4v}$  point symmetry. The energy parameters  $\varepsilon_1, \varepsilon_2$  and  $\varepsilon_3$  determine the crystalline field splitting.

in the exact treatment [1] some further distributions of the six electrons in the one-electron terms are admixed slightly to the low-lying  ${}^3E$  and  ${}^1A_1$  levels. As the next step, the eigenvalues and eigenvectors of these four levels, still dependent on the adjustable parameters  $\varepsilon_1, \varepsilon_2, \varepsilon_3$  are used as the base-vectors for the diagonalization of the relatively small spin-orbit interaction (the proper coupling constant in the molecule has shown to be  $\lambda = 70 \text{ cm}^{-1}$ ) and the rhombic perturbation, being defined by the adjustable parameter  $D$  [2]. The spin and orbital degeneracy of the base vectors is removed by these two interactions, giving rise to 22 singlets as the low-energy electron levels of the  $\text{Fe}^{(2+)}$  cation; the spin remains no longer an exact quantum number. The eigenvectors and eigenvalues of the singlets depend on the four adjustable parameters ( $\varepsilon_1, \varepsilon_2, \varepsilon_3, D$ ). Equivalently one may also choose  $\Delta E_1, \Delta E_2, \Delta E_3$  and  $D$  as parameters where the  $\Delta E_\mu$  are referred to the relative energies of the base vectors as shown in figure 1. The corresponding efg-tensors of the singlets contribute to the total efg at the iron nucleus according to the

Boltzmann population of each level. In this manner the quadrupole splitting is obtained as a function of temperature with four adjustable parameters to be fitted to the experimental data.

In general the quadrupole splitting of the 14.4 keV niveau of  ${}^{57}\text{Fe}$  is given by

$$\Delta E_Q(T) = \frac{1}{6} eQ \sqrt{9 V_{zz}^2(\text{eff}) + 12 V_2^2(\text{eff})} \quad (1)$$

where [4]

$$V_{zz}(\text{eff}) = (1 - R) \langle V_{zz}(\text{val}) \rangle_T + (1 - \gamma_\infty) V_{zz}(\text{lat})$$

$$V_2(\text{eff}) = (1 - R) \langle V_2(\text{val}) \rangle_T. \quad (2)$$

The symbols « val » and « lat » refer to the charge distribution of the aspherical 3d « valence » electrons of the ferrous cation and to the charge distribution of the neighbouring ions, respectively.  $(1 - R)$  and  $(1 - \gamma_\infty)$  are the appropriate Sternheimer factors.  $V_{zz}(\text{val})$  and  $V_2(\text{val})$  acts as an operator on the 22 singlet-eigenfunctions of our secular problem (eq. (9) of ref. [1]), and its matrix elements are averaged according to Boltzmann statistics.

The magnitude of the valence efg is governed by the factor  $e \langle r^{-3} \rangle$ . Thus we can redefine eq. (2) as follows

$$\begin{aligned} V_{zz}(\text{eff}) &= (1 - R) e \langle r^{-3} \rangle v_{zz}(\text{eff}) \\ V_2(\text{eff}) &= (1 - R) e \langle r^{-3} \rangle v_2(\text{eff}) \end{aligned} \quad (2')$$

and

$$\begin{aligned} v_{zz}(\text{eff}) &= \langle v_{zz}(\text{val}) \rangle_T + v_{zz}(\text{lat}) \\ v_2(\text{eff}) &= \langle v_2(\text{val}) \rangle_T. \end{aligned} \quad (3)$$

Eq. (3) has the dimension of a c-number. Because  $v_{zz}(\text{lat})$  is correlated to the second order component of the crystalline field [4], we obtain from eq. (22) of ref. [1]

$$v_{zz}(\text{lat}) = \frac{2(1 - \gamma_\infty)(\varepsilon_3 - \varepsilon_2 - \varepsilon_1)}{[e^2(1 - R) \langle r^{-3} \rangle \langle r^2 \rangle]} \quad (4)$$

The definition (2') allows eq. (1) to be written as

$$\begin{aligned} \Delta E_Q(T) &= \frac{1}{6} e^2 Q(1 - R) \langle r^{-3} \rangle \times \\ &\times \sqrt{9 v_{zz}^2(\text{eff}) + 12 v_2^2(\text{eff})}. \end{aligned} \quad (5)$$

Unrestricted Hartree-Fock calculations [4, 5] for the free iron cation yield

$$\begin{aligned} (1 - R)_0 \langle r^{-3} \rangle_0 &= 3.3 \text{ a. u.} \\ \langle r^2 \rangle_0 &= 1.4 \text{ a. u.} \\ (1 - \gamma_\infty)_0 &= 12. \end{aligned} \quad (6)$$

The quadrupole moment  $Q$  (14.4 keV niveau  ${}^{57}\text{Fe}$ ) is taken to be 0.187 [b] [6]. Substituting these values in eq. (5) we get

$$\Delta E_Q = 2.08 \alpha^2 \sqrt{9 v_{zz}^2(\text{eff}) + 12 v_2^2(\text{eff})} [\text{mm/s}] \quad (7)$$

where the energy is given in units of the Doppler velocity and  $\alpha^2$  is the covalency factor of the  $\text{Fe}^{(2+)}$  por-

phyrin compounds. The value of  $\alpha^2$  depends on the type of bonding in the molecule and corrects the free ion matrix elements of eq. (6). The best agreement with our experiment is obtained for  $\alpha^2 = 0.9$ .

**3. Comparison with experiments.** — First experiments [1] on haemoglobin have been related earlier to the level scheme in figure 1a. No least squares fit was done at that time to determine the adjustable parameters. Although the calculated  $\Delta E_Q(T)$ -curve agreed reasonably well with the experimental data, it was obvious that the  $\Delta E_3$  value was surprisingly large. One expects that the  $\pi$ -antibonding ( $3d_{xz}, 3d_{yz} \equiv {}^5E$  orbital is raised only slightly from the  $\pi$ -antibonding  $3d_{xy} \equiv {}^5B_2$  orbital [1]. This discrepancy became still larger, when we tried to interpret our Mb data according to figure 1a. In addition the efg-tensor was found to be nearly axial symmetric ( $V_{zz} > 0, \eta \approx 0$ ) with the symmetry axis  $z$  perpendicular to the haeme plane (\*).

On the other hand, Gonser *et al.* [3] have shown that the observed asymmetry of the quadrupole doublets in the single crystal experiment of Mb at 77 K could not be understood by an axially symmetric efg along the  $z$ -axis. The assumption of an axially symmetric efg forced its principal axis  $z'$  along one of the four Fe-N directions in the haeme plane with  $V_{z'z'} > 0$ . This implies a  $n$ -fold symmetry axis ( $n > 2$ ) close to the diagonal of the haeme plane which is difficult to understand from the well-known structure of Mb. It should be noticed, however, that the results of ref. [3] remain unaffected if one redefines the right-handed set of coordinates ( $x', y', z'$ ) by the following cyclic permutation

$$\begin{aligned} x' &\rightarrow y'' \\ y' &\rightarrow z'' \\ z' &\rightarrow x'' \end{aligned} \quad (8)$$

where the  $x''$ -axis is now close to the diagonal of the haemeplane. Accordingly, the  $z''$ -axis may be chosen to be nearly perpendicular to the haeme, so that the resulting coordinate system ( $x'', y'', z''$ ) is closely related to the coordinate system ( $x, y, z$ ) generated by the rhombic symmetry of the iron site. From Laplace's equation  $\Delta V = 0$  one obtains for  $V_{z'z'} > 0$  and  $\eta' = 0$  the corresponding values in the new coordinate system:  $V_{z''z''} < 0$  and  $\eta'' = -3$ .

In order to maintain the rhombic symmetry as the underlying principle, we evaluated the efg-components rather in the system ( $x, y, z$ ) by a least square fit from the data [3], yielding

$$\begin{aligned} V_{zz}(77 \text{ K}) &< 0 \\ -16 &< \eta(77 \text{ K}) < -4 \end{aligned} \quad (9)$$

where the  $x$ -axis is nearly parallel to the NVL(416)-NPR(418) direction of these two haeme nitrogens [7].

(\*) In the remainder of the paper the direction of the  $z$ -axis is chosen to be perpendicular to the haeme plane.

Bearing these findings in mind, we started with a term scheme according to figure 1b in order to fit the measured quadrupole splittings of Mb as a function of temperature. It is obvious that the required asymmetry parameter of eq. (9) and the high-spin ground-state of the ferrous cation must be related to low-lying singlets which essentially descend from the rhombic-split  ${}^5E$ -level. Figure 3 shows the experimental data and the calculated  $\Delta E_Q(T)$ -curve which was

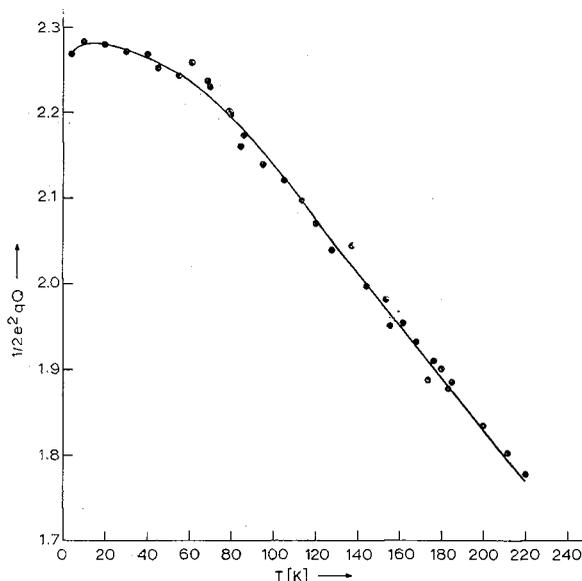


FIG. 3. — Temperature dependence of the quadrupole splitting of deoxygenated sperm whale myoglobin. The full curve is obtained by the least squares fit procedure described in the text.

obtained from eq. (7) by a five parameter least squares fit. The free parameters are the relative energies  $\Delta E_v$  of the base vectors, the rhombic perturbation  $D$  (see Fig. 1), and the lattice contribution  $v_{zz}(\text{lat})$  of eq. (3). The values of these parameters as returned by the best fit were

$$\Delta E_1 = 809 \text{ cm}^{-1}, \quad \Delta E_2 = 60 \text{ cm}^{-1}, \quad \Delta E_3 = 106 \text{ cm}^{-1}, \quad (10)$$

$$D = -298 \text{ cm}^{-1}, \quad v_{zz}(\text{lat}) = 0.14.$$

These values of the relative energies  $\Delta E_v$  correspond to the one-electron energies [1]:

$$\varepsilon_1 = 106 \text{ cm}^{-1}, \quad \varepsilon_2 = 7\,947 \text{ cm}^{-1}, \quad \varepsilon_3 = 17\,203 \text{ cm}^{-1}. \quad (10')$$

In agreement with the single crystal results of eq. (9), the efg-components at the iron nucleus are evaluated from the data to be  $\eta(77 \text{ K}) = -13.3$  and  $V_{zz}(77 \text{ K}) < 0$ .

According to eq. (4), (10') we also can estimate the lattice contribution to the total efg on the basis of the free ion matrix elements of eq. (6), yielding

$$v_{zz}(\text{lat}) = 0.22;$$

this value again is in reasonable agreement with the value obtained from the fit.

Finally, we would like to point out, that though the rhombic perturbation ( $D = -298 \text{ cm}^{-1}$ ) is much smaller than the tetragonal crystal field effects which are proportional to the  $\epsilon_v$  (eq. (22) of ref. [1]), the

charge distribution of the 3d-electrons has essentially rhombic character.

This work was supported by grants of the Deutsche Forschungsgemeinschaft.

The authors wish to thank Prof. G. M. Kalvius for his continuous support of this work.

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