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GROUND AND LOW LYING ELECTRONIC STATES OF OXYHEMOGLOBIN FROM TEMPERATURE DEPENDENT MÖSSBAUER AND SUSCEPTIBILITY DATA

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The temperature dependence of magnetic susceptibility and Mössbauer quadrupole splitting for oxyhemoglobin is considered to arise from contribution from a ground state singlet and two excited triplet electronic states. Using values of parameters obtained from fitting data for both measurements, the plausibility of this hypothesis is established.

1. Introduction

The Mössbauer spectra of the iron in oxyhemoglobin show a marked temperature dependence [1] and both the isomer shift and the quadrupole splitting are similar to that of ferric compounds [2], suggesting the existence of low lying paramagnetic states at energies comparable to the room temperature thermal energy. However earlier magnetic susceptibility measurements [3] had described the iron-dioxygen complex as diamagnetic at room temperature. To reconcile the susceptibility result with the Mössbauer observations it has been proposed that the oxygen molecule, bent as in the Pauling model [4], can rotate to occupy four equivalent minima separated by energy barriers [5,6]. A similar description has been proposed for model compounds, the so-called "picket fence" porphyrins [7].

However, while for the models the X-ray structure is consistent with the proposed rotations [8], it is not so for oxyhemoglobin [9] and oxymyoglobin [10], where the oxygen must be blocked in a single position. Moreover recent magnetic susceptibility data [11] are at variance with the earlier ones [3] and indicate that, in frozen solutions of HbO_2, the iron-dioxygen complex is in a thermal equilibrium between a diamagnetic singlet ground state and excited states of higher spin multiplicity. An extension of the measurements to liquid solutions at room temperature [12] shows complete agreement with the results for frozen solutions.

If the oxygen cannot rotate and the iron-dioxygen complex is not diamagnetic at room temperature, we may go back to the obvious interpretation of susceptibility and Mössbauer data in terms of states with both paired and unpaired spins. On the other hand, recently, some Fe(II) complexes have been reported, which show Mössbauer and susceptibility data very similar to those of HbO_2 and were easily interpreted on the basis of a spin equilibrium between nearly degenerate singlet and triplet ground states [13,14]. A similar model is used here to obtain a simultaneous fit of Mössbauer quadrupole splitting and magnetic susceptibility data of HbO_2.

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2. Theoretical model and calculations

As outlined in the previous section, the experimental data are consistent with a singlet ground state and low-lying levels of higher spin multiplicity. If one considers the level scheme proposed for deoxygenated hemoglobin [15,16] and previous calculations on Fe^{2+}, (d^6 configuration) complexes in C_{4v} symmetry [13], the level ^3E is the most probable candidate to approach to the ground state $^1A_1$. Therefore we assume as a model an esacoordinated Fe(II) complex with singlet and triplet levels separated by an energy comparable with the thermal one. Moreover, if the oxygen molecule cannot rotate, a rhombic component has to be included. The resulting level scheme we have considered is shown in fig. 1, where the labels of the terms are those for C_{2v} symmetry and $^3B_1$, $^3B_2$ arise from the ^3E in C_{4v} symmetry. A first assumption we made is to consider these triplet terms deriving directly from the $^3T_{1g}$ in O_h symmetry, thus neglecting the mixing with the terms at higher energy, but this assumption is not too drastic, because the next triplet states are well separated [15, 16]; in this way the two levels can be described, in the strong field configuration, as

$$|^3B_1\rangle: \quad -\tfrac{1}{2}\sqrt{3}|\zeta^2\eta^2\xi^+\theta^+\rangle - \tfrac{1}{2}|\zeta^2\eta^2\xi^+\epsilon^+\rangle,$$

$$|^3B_2\rangle: \quad \tfrac{1}{2}\sqrt{3}|\zeta^2\xi^2\eta^+\theta^+\rangle - \tfrac{1}{2}|\zeta^2\xi^2\eta^+\epsilon^+\rangle,$$

where $\xi, \eta, \zeta, \theta, \epsilon$, stand for the one-electron d levels d_{yz}, d_{xz}, d_{xy}, d_{z^2}, $d_{x^2-y^2}$, respectively.

A second approximation we introduced is to neglect completely the mixing between the levels, due to the spin–orbit interaction. This is partially justified, since the high covalency together with a likely quenching operated by the vibronic coupling [16] should strongly reduce the spin–orbit coupling.

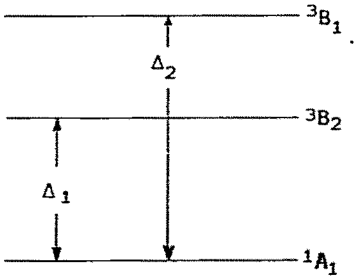

Fig. 1.

On this basis we fitted the experimental values of the magnetic susceptibility [11] by means of the van Vleck equation [17], using Δ_1 and Δ_2 as fitting parameters.

The resulting Δ_1 and Δ_2 values were then introduced in the expression (1) [18,19], which gives the temperature dependence of the quadrupole splitting ΔE_Q in C_{2v} symmetry, if lattice contributions are assumed to be negligible [16]:

$$\Delta E_Q = \tfrac{1}{2}eQ[V_{zz}^2 + \tfrac{1}{3}(V_{xx} - V_{yy})^2]^{1/2}, \tag{1}$$

where V_{kk} ($k = x, y, z$) are the components of the electric field gradient tensor and can be obtained, following the operator equivalence [19], as:

$$V_{kk} = \tfrac{4}{7}e(1 - R)\langle r^{-3}\rangle l_{kk}, \tag{2}$$

and l_{kk} is a thermal average given by:

$$l_{kk} = \sum_m \langle\psi_m|l_{kk}|\psi_m\rangle \exp(-\Delta_m/kT) \times \left[\sum_m \exp(-\Delta_m/kT)\right]^{-1}. \tag{3}$$

Here ψ_m and Δ_m are the wavefunctions and the energies, respectively of the different terms taken into account. As in a previous paper [16], the nuclear quadrupole moment Q was taken to be 0.21 b and the quantity $(1 - R)\langle r^{-3}\rangle$, where $(1 - R)$ is the Sternheimer factor for the 3d electrons, was taken to be 3.3 au, so that eq. (1) becomes:

$$\Delta E_Q = 4.1\,F \quad \text{mm/s}, \tag{4}$$

where

$$F = [l_{zz}^2 + \tfrac{1}{3}(l_{xx} - l_{yy})^2]^{1/2}. \tag{5}$$

If covalency effects are neglected, the three levels, $^1A_1$, $^3B_1$, $^3B_2$ should give no contribution to the quadrupole splitting and $\Delta E_Q = 0$. However experiments show a ΔE_Q value of 2.24 mm/s at 1.2 K for HbO_2 [1], thus indicating a strong covalent bond and an asymmetrical charge distribution.

Only a general molecular treatment could give an accurate picture of the iron-ligand covalent bond; however bonding effects may be taken into account, in a semi-empirical way, by replacing the metal d-orbitals, used in the simple point charge model, by molecular orbital combinations of the suitable symmetry [20]: indeed in a molecular orbital treatment each metal orbital is mixed with opportune ligand orbital so that the one electron levels with mainly iron character, for the different representations (we adopt the notation for C_{4v} symmetry), can be expressed as:

$$\begin{aligned}
|e_a\rangle &= \beta_1 |d_{yz}\rangle + \Sigma_i \varphi_i \\
|e_b\rangle &= \beta_2 |d_{xz}\rangle + \Sigma_i \varphi_i \\
|b_2\rangle &= \beta_3 |d_{xy}\rangle + \Sigma_i \varphi_i \qquad (6) \\
|b_1\rangle &= \alpha_1 |d_{x^2-y^2}\rangle + \Sigma_i \varphi_i \\
|a_1\rangle &= \alpha_2 |d_{z^2}\rangle + \Sigma_i \varphi_i
\end{aligned}$$

where $\Sigma_i \varphi_i$ is a short notation for the correct combination of ligand orbitals; since we are interested in the contribution of the metal d-orbitals to calculate the quadrupole splitting ΔE_Q, it is not necessary to know the values of the coefficients of the ligand orbitals, since they may contribute to ΔE_Q only to a minimum extent. Consequently if Δ_1 and Δ_2 are known, ΔE_Q becomes a function of the five parameters $\alpha_1, \alpha_2, \beta_1, \beta_2, \beta_3$; however, since $d_{x^2-y^2}$ are antibonding orbitals and are situated at energy higher than that of the ligands, we make the approximation $\alpha_1 \approx \alpha_2 \approx 1$.

As for the other three parameters, these latter are considered fitting parameters for the temperature dependence of ΔE_Q. It is evident that the fitting is significant if the values obtained are consistent with the physical sense. In fact we can expect, from semi-empirical calculations of model oxyheme [21], a value of β_3 close to unity. Moreover, if the rhombic component is small, that is a small $\Delta_2 - \Delta_1$, β_1 and β_2 are expected to be not too different one from another. Indeed, as it will be shown in the following section, the parameter values we find are in agreement with the above requirements.

3. Results and discussion

Fig. 2 shows the theoretical molar susceptibility (χ_M) versus absolute temperature, computed for $\Delta_1 = 140$ cm^{-1} and $\Delta_2 = 300$ cm^{-1}, together with the

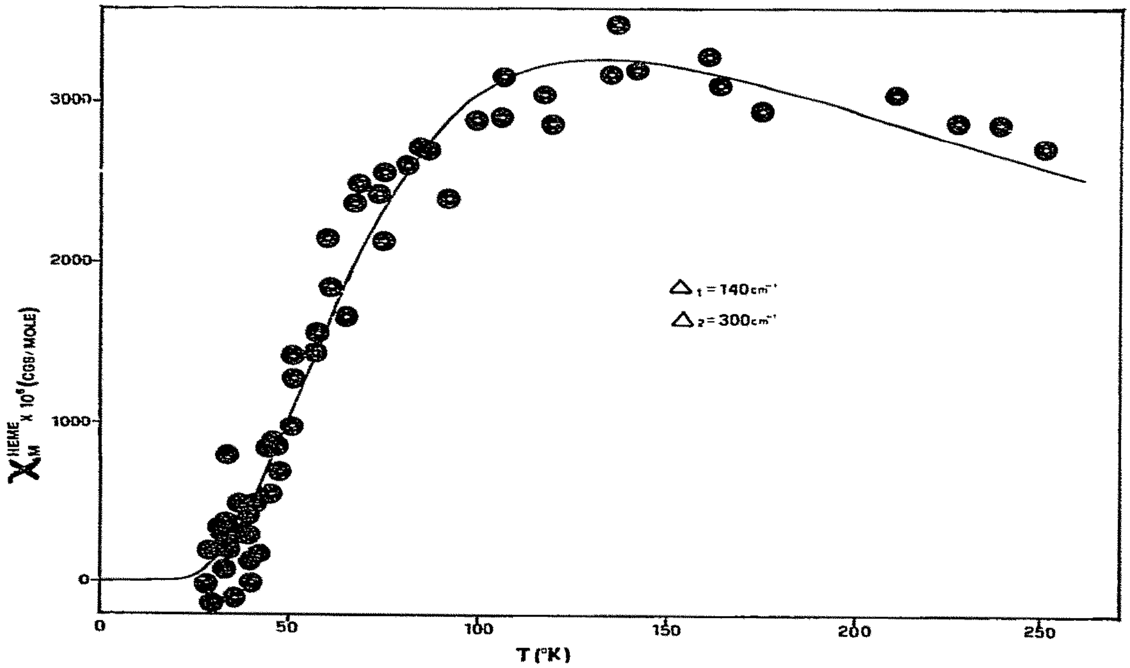

Fig. 2. Best fit of the molar susceptibility per heme versus absolute temperature as in ref. [11] with $\Delta_1 = 140$ cm^{-1} and $\Delta_2 = 300$ cm^{-1}.

experimental data [11]. Our analysis gives a satisfactory fitting also for $\Delta_1 = \Delta_2$ similarly to the previous interpretation [11], but the agreement is improved if one of the two levels, $^3B_1$ or $^3B_2$, is allowed to increase in energy; however, beyond a certain value of Δ_2, about 500 cm^{-1}, the fitting becomes worse. An analogous behaviour is observed for the theoretical temperature dependence of quadrupole splitting ΔE_Q, as shown in fig. 3.

These findings are consistent with a little rhombic component, $\Delta_2 - \Delta_1 \approx 100–300\ cm^{-1}$, as it is expected on the basis of the stereochemistry of the chromophore and of recent theoretical results for deoxyhemoglobin and myoglobin [16,22], for which negligible or very small rhombic components are required.

As for the parameters β_1, β_2 and β_3, we have found that β_3 must be very close to 1, $0.95 \leqslant \beta_3 \leqslant 1.0$, while the best values for β_1 and β_2 are comprised in the range 0.6–0.7: the fitting of fig. 3 was obtained setting $\beta_3 = 0.98$ and $\beta_1 = \beta_2 = 0.69$. The condition $\beta_1 = \beta_2$ is not strictly necessary, because little difference between the two parameters do not alter appreciably the values of ΔE_Q.

The strong reduction of the values of β_1 and β_2 with respect to unity is expected, because the orbitals d_{xz} and d_{yz} of the iron are mainly involved in the covalent bonding with the oxygen molecule, when this is situated in a bent configuration [9,10].

From our calculations the asymmetry parameter $\eta = (V_{xx} - V_{yy})/V_{zz}$ can be evaluated, and the result $\eta \lesssim 0.3$ in the range 0–300 K, is consistent with those obtained for model compounds, $\eta \lesssim 0.5$ [21,23].

4. Conclusions

In conclusion, we note that, in spite of the crude approximations made, our model accounts very well for both Mössbauer and magnetic data, using a set of reasonable values for the parameter. As it is evident from fig. 3, the hypothesis of a singlet–triplet spin-equilibrium works as well as the rotating oxygen model in explaining the temperature dependence of the quadrupole splitting. On the other hand, the "oxygen rotating model", in our opinion, can interpret with difficulty the dependence of the magnetic susceptibility with temperature. Therefore, we may conclude that the "spin equilibrium" hypothesis appears the most likely in those systems, like oxyhemoglobin and oxymyoglobin, where the oxygen is blocked in a single position, although it is possible that both restricted rotation in the protein and a singlet–triplet equilibrium contribute to the temperature dependence of the Mössbauer spectra. At last we would like to remark that our simplified hypothesis works very well, but, on the other hand, more accurate molecular orbital calculations on explicit HbO_2 models are needed for further confirmations.

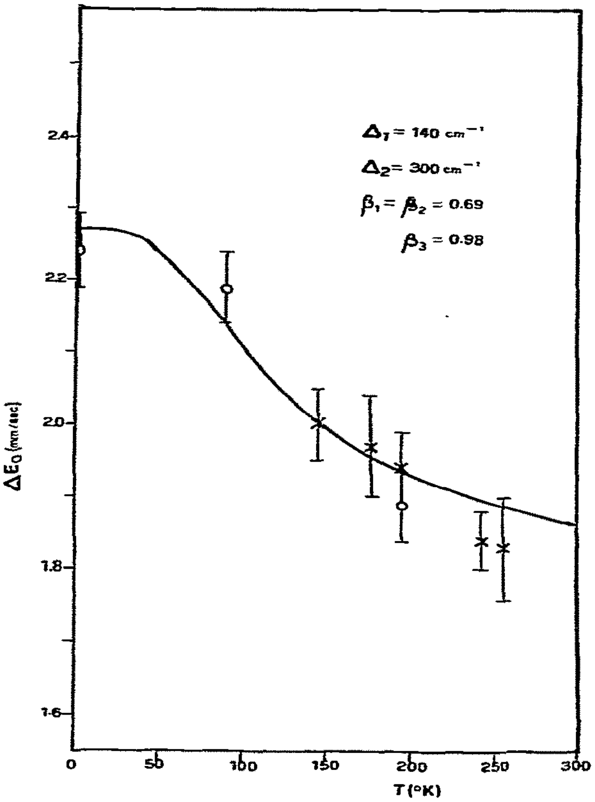

Fig. 3. Best fit of the quadrupole splitting versus absolute temperature. Experimental data from ref. [1] and [6].

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