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SINGLE CRYSTAL STUDIES OF FERRIC MYOGLOBIN COMPOUNDS

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Abstract.- The Mössbauer measurements of single crystals of some ferric compounds have been done in the presence of a small external magnetic field perpendicular to γ-rays at the liquid He temperature. For high spin compounds, metMb and MbF, isotropic $A$-tensors have been obtained. For low spin MbN, the axis of the largest principal component of the EFG lies nearly in the heme plane and forms an angle of 32° with one of four Fe-N directions, which corresponds to the results of EPR experiments of single crystal MbN.

1. Introduction.- During the past years a large number of experimental and theoretical papers have been published concerning the determination of the electronic structure of the iron in various ferric hemoproteins. The analyses of electron paramagnetic resonance (EPR) [1-3], magnetic susceptibilities [4] and paramagnetic anisotropy measurements [5] have provided detailed knowledge of the electronic structures of high spin and low spin ions. Mössbauer studies of hemoproteins have been done with their solution or polycrystalline samples for one decade or so [6-10].

We present Mössbauer measurements of single crystals of some ferric Mb compounds in the presence of a small external magnetic field at the liquid He temperature, which contain high spin ferric metMb and MbF and low spin ferric MbN. The zero field spectra of ferric heme compounds are complicated by spin relaxation and the large amount of spin transfer to ligands [6-10]. In applied fields at 4.2 K, however, well-resolved paramagnetic hyperfine spectra were observed. We used the spin Hamiltonian formalism to analyse the Mössbauer spectra of the high spin compounds. For the low spin compound, the Mössbauer parameters and the orientations of the EFG principal axes and effective magnetic field, relative to the crystal axes, have been determined by a computer analysis of these spectra.

2. Experimental.- Single crystals of 90 % $^{57}$Fe-enriched metMb were prepared from sperm whale skeletal muscle and were grown to a size 1x1x2 mm or larger for 1 month in 75 % saturated ammonium sulfate solution containing 0.2 M phosphate buffer, pH 6.8 at 20°C. Crystallographic analysis being performed, the crystals belonged to a monoclinic system, $P_2_1_1$, with lattice constants, $a = 64.6$ Å, $b = 31.1$ Å, $c = 34.8$ Å, $\beta = 105.5^\circ$, which were identical with those of type A crystal reported by Kendrew and Parrish [11]. Single crystal samples of MbF and MbN, were prepared by putting crystals of metMb into 0.1 M NaF and NaN solution respectively, containing 75 % saturated ammonium sulfate and 0.2 M phosphate buffer pH 6.8 for a few days. The crystal was visually aligned on a lucite plate with a small lead collimator. The magnetic field was applied along the crystallographic $a$, $b$ or $c$ axis, because in this geometry the two Fe sites of the monoclinic substance are expected to show identical spectra. The Mössbauer spectra were measured at liquid He temperature with a small magnetic field (0.6 kG) perpendicular to γ-rays.

3. Results and discussions.-

3.1. High spin compounds (metMb and MbF).- The Mössbauer spectra of metMb are given in the figure 1 (left). For high spin compounds, we analysed the electronic structure with the following spin Hamiltonian,

$$H = D(S^2 - S(S+1)/3) + 2B_{\mathrm{HS}} + E[I^2 - (I+1)/3]$$

where $a_\perp$, $a$, $I$, and $S$.

We set up an $xyz$ coordinate system fixed on each of the two heme groups with different orientations, where $x$ and $y$ axes are in the heme plane and $z$ axis is perpendicular to the heme plane. We used $D$ values from the results of magnetic susceptibility of these compounds.
high spin compounds /4,5/.

Then the parameters were obtained as

\[
\text{metMb: } D = 10.0 \text{ cm}^{-1}, P = 0.55 \text{ mm/s}, A_0 = 1.31 \text{ mm/s}, \text{MbbF: } D = 7.0 \text{ cm}^{-1}, P = 0.35 \text{ mm/s}, A_0 = 1.39 \text{ mm/s}.
\]

The values of isomer shift are 0.43 ± 0.05 mm/s for metMb and 0.46 ± 0.05 mm/s for MbbF. In the figure (left), the calculated spectra of metMb are shown with solid lines. The electronic configuration of high spin ions is 6S. However, Lang et al. /6,7/ suggested that the interactions which produce non-zero D may also reduce A-tensor to axial symmetry and that \( A_0 \) is about 13% less than \( A_g \), which is estimated by the value of the quadrupole splitting of metMb. Our single crystal measurements with the magnetic field applied along the \( \alpha \) axis did not show the anisotropy of \( A \)-tensor.

3.2. Low spin compound (MbbF).- For this compound, the Mössbauer experimental spectra are shown in the figure (right). The Mössbauer effect parameters have been determined by the positions and the line intensities of the spectra /12,13/. The Hamiltonian of the hyperfine interaction written in the principal axes system of the EFG is:

\[
eQV^{zz} = -\frac{31}{41}(1+1) + \frac{31}{41}(1-I-I) + \frac{31}{41}(1-I-I) - \frac{31}{41}(1-I-I) - \frac{31}{41}(1-I-I),
\]

where \( V^{zz} \), \( V_{yy} \), \( V_{zz} \), \( \eta = \frac{1}{2} \), and \( 0 \leq \eta \leq 1 \). \( \theta \) and \( \phi \) are polar angles of the direction of the effective magnetic field \( H_{\text{eff}} \) relative to the principal axes system of the EFG. The values \( \sin^2 \theta \), \( \cos 2\phi \), and \( eQV^{zz} \) can be calculated uniquely as functions of \( \eta \) and the sign of \( eQV^{zz} \). These parameters then will result from a computer fit to the measured intensities. The EFG principal axes in the crystal system can be located from three measurements with different orientations of the crystal. The results are in detail: asymmetry parameter \( \eta = 0.4 \pm 0.1 \), quadrupole splitting \( \Delta E_0 = 2.19 \pm 0.05 \text{ mm/s} \), \( eQV^{zz} < 0 \), isomer shift = 0.30 ± 0.05 mm/s. The effective field \( H_{\text{eff}} \) is \( 184.0 \pm 5.0 \text{ kG} (H_a // a), 133.0 \pm 5.0 \text{ kG} (H_a // b), 99.0 \pm 5.0 \text{ kG} (H_a // c) \). The \( z \) axis of the EFG principal system is rather close to the heme plane and deviates by 32° from one of four Fe-N directions. In 1971, Hori /3/ measured the directions of the principal axes of the \( g \)-tensors to the heme plane. Then the direction of the \( z \) axis (principal axis of the \( g \)-tensor corresponding to the minimal \( g \)-value) is not parallel to the plane of the linked histidine ring nor to one of Fe-N directions, but is rotated about the heme normal by an angle 31°. From our single crystal experiments of low spin MbN\( _2 \), we found that the directions of the \( z \) axis for both \( g \)-tensor and EFG tensor well coincided with each other. We calculated the Mössbauer spectra of this low spin compounds with the wave functions and the energy levels which were obtained from the magnetic data and took the same method that Oosterhuis and Lang made use of to predict Mössbauer spectra of \( K_2Fe(CN)_6 \) /14/. In the figure (right), the solid lines show the calculated Mössbauer spectra. It was found that the wave functions and the energy levels obtained from EPR were well fitted to the Mössbauer spectra apart from the details.

References


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