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Résumé.- Les tenseurs gradient de champ électrique sur les atomes de fer dans l'oxy- et la carboxy hémoglobine sont déterminés par spectrométrie Mössbauer sur des monocristaux. Dans le composé Mb-O_2 l'interaction quadrupolaire eQV_{zz} est négative et le plus grand axe principal est pratiquement dans le plan de l'heme; par contre, dans le composé Mb-CO, eQV_{zz} est positif avec un axe pratiquement perpendiculaire au plan de l'heme.

Abstract.- The EFG tensors at the Fe atoms in myoglobin-O_2 and -CO are determined by Mössbauer experiments on single crystals. In the MbO_2 compound the quadrupole interaction (eQV_{zz}) is negative and the largest principal axis of the EFG lies nearly in the heme plane, while in the MbCO compound eQV_{zz} is positive and the axis of the EFG is nearly perpendicular to the heme plane.

Mössbauer spectroscopy has provided the most direct knowledge of the iron-oxygen binding in myoglobin (Mb) and hemoglobin (Hb). Although both the O_2 and CO compounds of Mb and Hb are diamagnetic, the quadrupole splittings in the O_2-form are quite different from those in the CO-form, and the presence of large asymmetric charge transfer is suggested in the O_2-form /1/. In order to further discuss the bonding structure of ligands to heme iron, not only the principal values of the electric field gradient (EFG) tensor, but also its orientation is strongly required to be determined for these compounds.

Single crystals were grown from a solution of sperm whale metMb, enriched with ^{57}Fe, and visually selected perfect crystals were converted into the O_2- or CO-forms. For the conversion into the O_2-form a rather troublesome procedure was needed, while the conversion into the CO-form was easily attained. The details will be reported elsewhere. The converted crystals were visually aligned, mounted on a goniometer and immediately installed into a liquid N_2 cryostat equipped with lead collimators for getting narrow γ-beams.

The measurements with single crystals were carried out at different orientations. The crystal was rotated around the *b* axis and the *b* axis was always kept to be perpendicular to the γ-rays. In figure 1(a) and (b) we present Mössbauer spectra, typical of MbO_2 and MbCO crystals. As shown in figure 2, relative intensity ratios of the quadrupole split lines of MbO_2 and MbCO are plotted as a function of the rotation around the *b* axis. The crystals belong to a monoclinic type A system and contain two hemes within one unit cell.

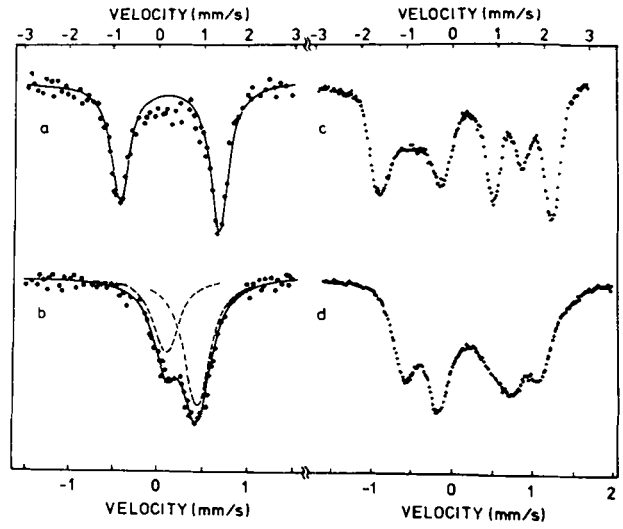

Fig. 1 : Typical Mössbauer spectra of MbO_2 (a) and MbCO (b) single crystals and magnetically perturbed Mössbauer spectra of MbO_2 (c) and MbCO (d) in the frozen solutions. The magnetic field applied perpendicular to γ-rays was 45 kOe in (c) and 47 kOe in (d).

As discussed for deoxygenated Mb in previous papers /2, 3/, manifold solutions are usually obtained in the determination of the local EFG tensor. Therefore, additional measurements of MbO_2 and MbCO in the frozen solution were carried out with applied magnetic fields to determine the sign of the quadrupole interaction and the asymmetry parameter η. The spectra are shown in figure 1 (c) and (d). From the two kinds of measurements the full knowledge of the EFG tensors has been obtained in both compounds.

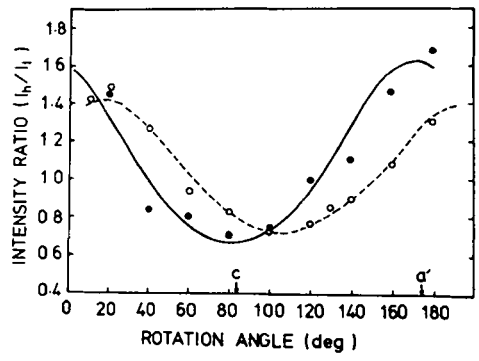

Fig. 2 : Relative intensity ratios of quadrupole split lines of MbO_2 (o---o) and MbCO (●—●) single crystals for various rotation angles around the b axis. In the positions a' and c the γ-rays are parallel to the a' and c axes, respectively. The lines were obtained from a least squares fit.

In the case of MbO_2, the fitting of the magnetically perturbed spectrum leads to : 1) the sign of the quadrupole interaction (eQV_{zz}) is negative and 2) η is limited to the range between 0.2 and 0.4. This finding reduces the manifold solutions obtained from the single crystal measurement, and the accuracy in the orientation determination of the principal axis system of the EFG tensor has been improved. From the relative line intensity of the single crystal it is found : 1) the largest principal axis of the EFG tensor is nearly in the heme plane and 2) η is limited to the range between 0.3 and 0.4. Recently several calculations have been performed in order to analyze the electronic structure of MbO_2 and HbO_2 by using the X-ray data from picket fence porphyrins /4/. Our present results would provide new experimental data to these calculations. Among them, Pariser-Parr-Pople and X_α multiple scattering calculations by Huynh *et al.* /5/ and a semiempirical calculation by Kirchner *et al.* /6/ are in good agreement with our results, apart from the details. They found that eQV_{zz} is negative and the largest principal axis of the EFG is in the heme plane. In the picket fence porphyrin the $Fe-O_2$ bonding structure is the bent Fe-O-O type proposed by Pauling /7/. For this geometry Goddard and Olafson proposed an ozone model /8/, in which the singlet state is formed by one component of ^3E state of deoxy-Mb coupled with the $^3\Sigma_g^-$ state of the O_2 molecule. In the ^3E state, however, a negative eQV_{zz} cannot be expected, although the largest principal axis of the EFG is in the heme plane.

The fitting of the magnetically perturbed spectrum in MbCO leads to : 1) eQV_{zz} is positive and 2) η is limited to the range between 0.0 and 0.4. Applying this knowledge to the analysis of the single crystal data, we find : 1) the largest principal axis of the EFG is nearly perpendicular to the heme plane and 2) η is limited to the range between 0.2 and 0.4. Recently a similar work has been performed on MbCO /9/. Although the sample preparation is quite different from ours, a nearly identical result is obtained. As MO calculations suggested /10, 11/, the positive eQV_{zz} perpendicular to the heme plane would be attributable to a relatively strong bonding of the Fe d_π's orbital with the unoccupied Π_g orbital of CO.

We are thankful for the support of the Stiftung Volgswagenwerk.

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