Mössbauer Spectroscopy on Oxygenated Sperm Whale Myoglobin: Evidence for an Fe^{3+}-O_2^- Coupling at the Active Center

D. Bade and F. Parak

Fachbereich Physik E 15 der Technischen Universität München

Z. Naturforsch. **33 c**, 488 – 494 (1978); received May 12, 1978

Mössbauer Spectroscopy, Oxygenated Myoglobin

^{57}Fe Mössbauer spectra of oxygenated sperm whale myoglobin (MbO_2) show a well resolved quadrupole doublet with a temperature dependent splitting. The temperature dependence of the corresponding electric field gradient tensor (EFG) can be calculated from a Fe^{3+} term scheme for the iron at the active center. The Mössbauer spectra as well as the diamagnetic character of the MbO_2-complex are then understood by an exchange coupling of the Fe^{3+}-ion with the O_2^- oxygen molecule ion. The resulting groundstate is a diamagnetic singlet. In order to keep the whole complex diamagnetic at room temperature, an exchange coupling with $|J| \geqq 300\ \mathrm{cm}^{-1}$ is necessary. As the whole model is in fair agreement with many other spectroscopic data, it is believed to be a good starting point for further detailed calculations.

Introduction

The reversible oxygen binding at the active center of haemoglobins is until today not well understood. A basic experimental information came from the magnetic susceptibility measurements of Pauling and Coryell [1]. They found that the unliganded haemoglobin, which is paramagnetic, became diamagnetic by the binding of the paramagnetic O_2 molecule. Later, two models for the spatial arrangement of the oxygen molecule bound to the iron of the haem have been developped [2 – 4]. None of these models is uniformly accepted. Both models start from the assumption, that the iron in the O_2 liganded state remains essentially ferrous as in the deoxygenated state.

Weiss [5] suggested an Fe^{3+}-O_2^--type of bonding which was supported by first molecular orbital calculations of Viale *et al.* [6]. More extensive calculations on the active center of oxygenated haemoglobins have to start with a certain assumption about the coordinates of the haem plane components. Extended Hückel calculations [7] on an O_2-liganded porphyrin complex with the oxygen molecule axis perpendicular to the haem plane proved the instability of such an axial arrangement, which should be paramagnetic, in contrast to Pauling and Coryell [1]. Further extended Hückel calculations for the iron porphyrin complex with various bond angles of the oxygen molecule were presented by Loew and Kirchner [8], who found a strong dependence of the electric field gradient tensor (EFG) on the spatial arrangement of the oxygen molecule.

Heitler London calculations were performed for two arrangements of the oxygen molecule. These calculations use a crystal field approach [9, 10] for the electronic structure of the iron as a starting point. For the oxygen molecule axis parallel to the haem plane, the diamagnetism of the system can be explained by a triplet-triplet bonding of the iron (^{3}E-state) and the oxygen molecule ($^3\Sigma$-state) [11 – 13]. In this case the iron-oxygen bond may be described as Fe^{2+}-O_2 rather than Fe^{3+}-O_2^-, because the Fe^{3+}-O_2^- states are shown to be energetically far above the groundstate. However, the extension of these calculations to the asymmetric case with the oxygen molecule axis inclined by 60° to the haem plane normal [14] yielded larger contributions of the charge transfer configurations.

Ab initio calculations on various iron porphyrin complexes were recently presented. Goddard and Olafson [15, 16] found the triplet-triplet coupling to be reasonable for the oxygen molecule axis inclined with 119° relative to the haem plane. The calculations of Dedieu *et al.* [17] on different iron porphyrin complexes also corroborate an inclined molecule axis. A comparison of various theoretical models for oxygenated haemoglobins is given in ref. 18.

Among other spectroscopic methods, ^{57}Fe Mössbauer spectroscopy presents a highly useful tool to look exclusively to the active center of haemoglobins. Mössbauer spectra of haemoglobins in deoxygenated and various differently liganded forms with the iron in the Fe^{2+} or Fe^{3+} state can be interpreted by relatively simple crystal field models for the iron at the

---

Requests for reprints should be sent to Dr. F. Parak, Physik-Department E 15, Technische Universität München, James-Franck-Straße, D-8046 Garching

active center [19 – 23]. The theory of these models was presented in ref. 24. No quantitative interpretation for the Mössbauer spectra of oxygenated haemoglobins has been given until now, although some suggestions have been made [25, 24, 8]. The large and temperature dependent quadrupole splitting in the ^{57}Fe Mössbauer spectra of oxygenated sperm whale myoglobin (MbO_2) is similar to the splitting known from deoxygenated myoglobin (deoxy-Mb) [19, 20] and in CN-liganded myoglobin (MbCN) [26]. However, these complexes are both paramagnetic, in contrast to the diamagnetic MbO_2. This diamagnetism is on the other hand similar to the diamagnetism of CO-liganded myoglobin (MbCO), which shows in contrast to MbO_2 a very small quadrupole splitting in its Mössbauer spectrum [22].

In this article a measurement of the temperature dependence of the quadrupole splitting of O_2-liganded sperm whale myoglobin is presented. An interpretation of these data with a crystal field model, following the lines of [19 – 23], is given.

Materials and Methods

The preparation of the ^{57}Fe-enriched sperm whale myoglobin was carried out according to Parak and Formanek [27]. A final concentration of the purified protein of 62.5 mg/ml was reached by dialysis. The reduction was carried out by dithionite. Pure oxygen gas was used for the oxygenation. The sample finally consisted of 25 mg myoglobin in 0.25 M NaCl-solution at pH = 7.0. The cylindrical sample holder was made from PVC and tightly closed by hostaphan windows and indium sealings. The protein solution in the sample holder was then quickly frozen in liquid propane [22]. By this method we avoid denaturation of the sample during a too slow freezing process.

The Mössbauer spectra were obtained with conventional Mössbauer spectrometers as described in ref. 28. The low temperature measurements were done with two different cryostat systems. First we used a liquid helium cryostat with an insert for the temperature variation. The whole apparatus is described in ref. 21. Additionally a vapour cryostat was used for measurements down to liquid nitrogen temperature. In this second apparatus the drive unit with the source stays permanently at room temperature and may be removed without removing the sample from the cryostat. Thus the background correction can be easily performed by introducing various additional absorbers between the source and the sample. The whole procedure is described in ref. 27. To avoid the influences of the slightly varying amplification of a proportional counter, a Si(Li) detector with an area of 3 cm² and 2 mm thickness was used for all the measurements with the vapour cryostat.

The independent temperature calibration and control of the two cryostats yielded consistent results in the whole temperature range. In both cases the voltage of a reversely biased silicon diode was used for the temperature measurement. The whole error of the temperature measurement and control was less than ± 1 K for all of our spectra.

Results

Four typical Mössbauer spectra of MbO_2 are given in Fig. 1. The temperature dependence of the quadrupole splitting is shown in Fig. 2. From the least

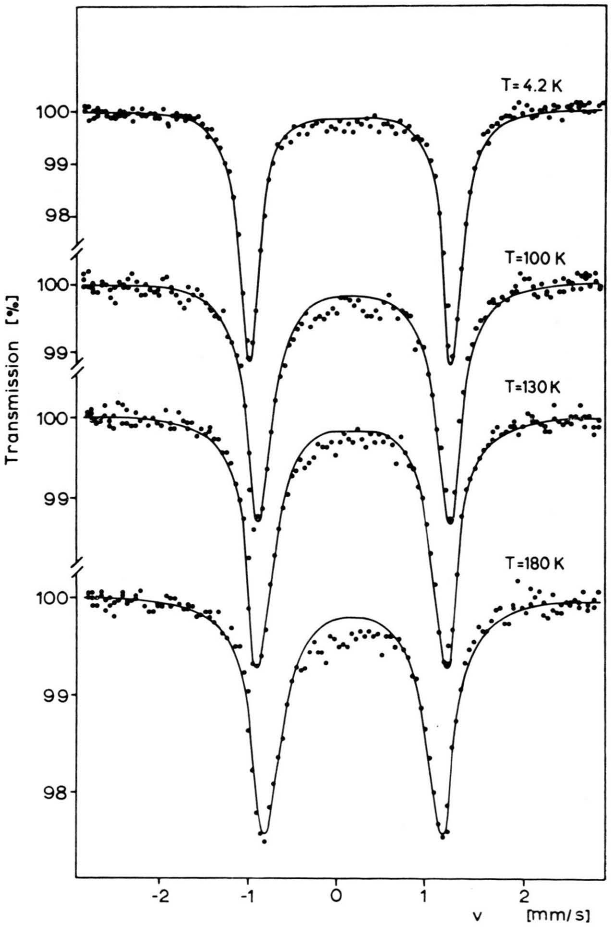

Fig. 1. Mössbauer spectra of MbO_2 at different temperatures.

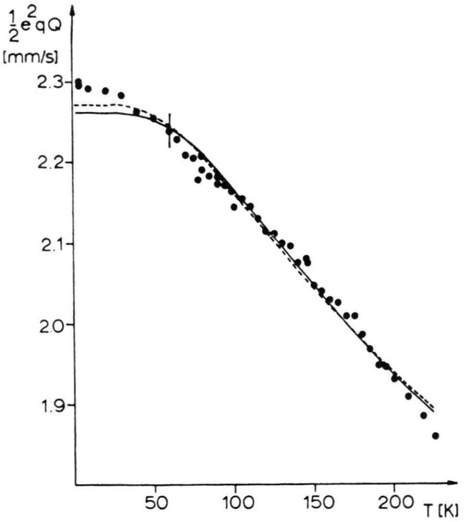

Fig. 2. Temperature dependence of the quadrupole splitting of MbO_2. Curves are calculated from the theory described in the text. Solid line corresponds to $V_{zz} < 0$, dashed line corresponds to $V_{zz} > 0$.

squares fit of Lorentzian lines to the spectra we obtained also a temperature dependence of the line width of the quadrupole doublet as shown in Fig. 3. For a series of measurements above 77 K a background correction was performed immediately after recording the spectrum. Then the Lamb-Mössbauer factors were calculated from the absorption areas of the spectra, assuming a uniform absorber thickness corresponding to 0.74×10^{18} ^{57}Fe atoms/cm^2. The f-factor of the source was taken $f = 0.785$ for ^{57}Co**Rh** [29]. The number of ^{57}Fe atoms could only be estimated with an accuracy of ±10%. In addition a frozen solution sample has certainly no uniform thickness as assumed in our evaluation. Therefore, the error of the absolute value of f' may reach 25%. The relative errors among the values at different temperature are considerably smaller. Fig. 4 shows a drawing of f' as a function of T. All results of our experiments are listed in Table I.

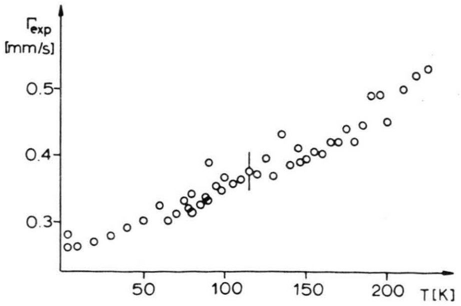

Fig. 3. Temperature dependence of the experimental linewidth Γ_{exp} received from a fit of Lorentzians to Mössbauer spectra of MbO_2.

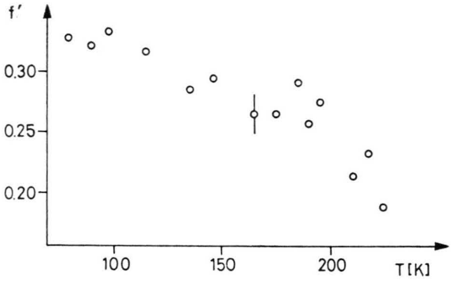

Fig. 4. Lamb-Mössbauer factor f' of the MbO_2 sample.

Discussion

The temperature dependence of the quadrupole splitting at the ^{57}Fe nucleus shown in Fig. 2 as well as the diamagnetism of the whole MbO_2 molecule can be understood by the following model. When the free O_2 molecule which is in a paramagnetic $^3\Sigma$-state [30, 31] binds to the iron of the active center of myoglobin, which is in a Fe^{2+} state with mainly ^{5}E character [20], it takes up one electron and a Fe^{3+}-O_2^- complex is formed. The groundstate of the Fe^{3+} ion becomes a ^{2}E state, while the groundstate of the O_2^- molecule becomes $^2\Pi$.

As well known from other Fe^{3+} haemoglobins [21, 32, 33], there exist several other low lying energy levels ($^6A_1$, $^4A_2$, $^2B_2$, as long as we assume a C_{4v} symmetry at the position of the iron). Taking into account a reduction of the symmetry to C_{2v} and a spin orbit coupling (compare [33]) the electronic state of the iron can be described by 8 low lying Kramers doublets. Each Kramers doublet combines with the Kramers doublet of the groundstate of the O_2^- molecule yielding one singlet and one triplet as shown in Fig. 5. The diamagnetism of the complex forces us, to assume that the singlets lie energetically below the triplets. This mechanism should be similar to the exchange coupling of transition metal ions in host crystals, as investigated by EPR [34]. The paramagnetism of the O_2^- oxygen molecule ion is well known from EPR on O_2^- in various crystals [35, 36] or adsorbed on metal surfaces [37]. The magnitude of the exchange coupling constant J may be also estimated from the literature [34]. For a distance $\leqq 3$ Å, $|J| \geqq 300\ \text{cm}^{-1}$ is reasonable, yielding an energy splitting between the singlets and the triplets

Tab. I. Experimental data of the isomer shift (S referred to α-iron), line width (Γ_{exp}), Lamb-Mössbauer factor (f') and quadrupole splitting ($\frac{1}{2}e^2qQ$) of MbO_2 at various temperatures (T). For the measurements marked by ($^+$) the source was kept at room temperature.

T [K]	S [mm/s]	Γ_{exp} [mm/s]	$\frac{1}{2}e^2qQ$ [mm/s]	f'
4.2	0.138	0.280	2.296	
4.2	0.138	0.260	2.299	
10	0.138	0.261	2.291	
20	0.139	0.267	2.291	
30	0.137	0.277	2.283	
40	0.135	0.291	2.264	
50	0.135	0.300	2.256	
60	0.138	0.322	2.240	
65	0.133	0.298	2.229	
70	0.139	0.310	2.209	
75	0.136	0.330	2.206	
78	0.138	0.319	2.178	
80	0.139	0.339	2.191	
80$^+$	0.262	0.310	2.209	0.325
85	0.137	0.324	2.185	
88	0.140	0.335	2.185	
90	0.138	0.329	2.173	
90$^+$	0.262	0.391	2.181	0.319
95	0.138	0.353	2.171	
98$^+$	0.251	0.344	2.167	0.332
100	0.145	0.364	2.145	
105	0.144	0.354	2.156	
110	0.142	0.362	2.145	
115$^+$	0.241	0.374	2.129	0.316
120	0.142	0.370	2.116	
125	0.152	0.396	2.111	
130	0.149	0.366	2.100	
135$^+$	0.256	0.433	2.099	0.287
140	0.152	0.384	2.076	
145	0.156	0.411	2.081	
146$^+$	0.238	0.384	2.077	0.294
150	0.157	0.395	2.046	
155	0.160	0.405	2.041	
160	0.154	0.403	2.030	
165$^+$	0.245	0.420	2.025	0.266
170	0.157	0.417	2.009	
175$^+$	0.242	0.440	2.012	0.266
180	0.166	0.418	1.983	
185$^+$	0.219	0.445	1.969	0.293
190$^+$	0.243	0.490	1.950	0.258
195$^+$	0.233	0.490	1.944	0.275
200	0.171	0.447	1.929	
210$^+$	0.241	0.500	1.908	0.216
218$^+$	0.221	0.520	1.885	0.234
225$^+$	0.220	0.530	1.856	0.192

of about 600 cm^{-1}. In order to get a temperature dependent EFG as observed in the MbO_2 spectra, we have calculated the EFG for a ^2E groundstate and the excited states placed closely above (compare Fig. 5). The calculation of the EFG from an electronic term scheme of the Fe^{2+}-ion has been outlined in ref. 20. For the Fe^{3+}-ion the EFG is calculated in complete analogy. The total EFG at the iron nucleus is composed by a valence contribution of the electrons in the 3d-shell and a "lattice contribution" governed by the parameter $\varkappa$ (for definition see ref. 20). The temperature dependence of the EFG is then obtained by a Boltzmann average over the low lying singlets of the Fe^{3+}-O_2^- complex. These singlets are assumed to yield the same contributions to the EFG at the iron nucleus as the corresponding Kramers doublets of the Fe^{3+}, which are calculated from the terms ^2E, $^6A_1$, $^4A_2$, and $^2B_2$, taking into account a rhombic distortion of the C_{4v}-crystal field and the spin orbit coupling. In contrast to the high spin terms ^5E and $^5B_2$ of the $3d^6$ configuration, the $^6A_1$ and $^4A_2$ terms of the $3d^5$ configuration have spherical symmetry and yield no valence contribution to the EFG. However, among the low lying electronic states of the $3d^5$ configuration the terms ^2E and $^2B_2$ give large valence contributions. They are responsible for the large quadrupole splitting which is unusual for Fe^{3+}. In analogy to the terms ^5E and $^5B_2$ the charge distribution of these terms differs by one electron from spherical symmetry, and therefore also the Sternheimer factors are assumed to be the same [38].

Two least squares fit results are given in Fig. 2. The fits with positive or negative principal components V_{zz} of the EFG are close together, because the asymetryparameter is $\eta \approx 1$. Both signs of V_{zz} give a very good agreement between theory and experiment. The crystal field parameters which are varied during the fit procedure proved to be $\varepsilon_1 = -357\,^{+650}_{-280}$ cm^{-1}, $\varepsilon_2 = 13\,037\,^{+560}_{-390}$ cm^{-1} and $\varepsilon_3 = 24\,726 \pm 150$ cm^{-1}. The values for positive and negative sign of V_{zz} are the same within the errors.

As mentioned in the introduction, the idea of a Fe^{3+}-O_2^- complex was already suggested by Weiss [5]. A detailed comparison of optical absorption spectra [39] showed a close similarity of MbO_2 to MbCN. In MbCN the iron of the haem can be treated as low spin Fe^{3+} ion [21]. Magneto optical rotation measurements [40] corroborated such a similarity. The strongest indication for a Fe^{3+}-O_2^- type of bond was given by the infrared vibration frequency of the bound oxygen molecule [41]. The frequency found is practically identical with the frequency of O_2^- and far away from the frequency of the free oxygen molecule. Moreover, O_2^- is known to reduce methaemoglobin [42], and on the other hand O_2^- is generated during the autoxidation of oxygenated haemoglobin [43] and myoglobin [44]. The Fe^{3+}-O_2^- complex seems also to be reasonable from comparison with other metal oxygen complexes [45].

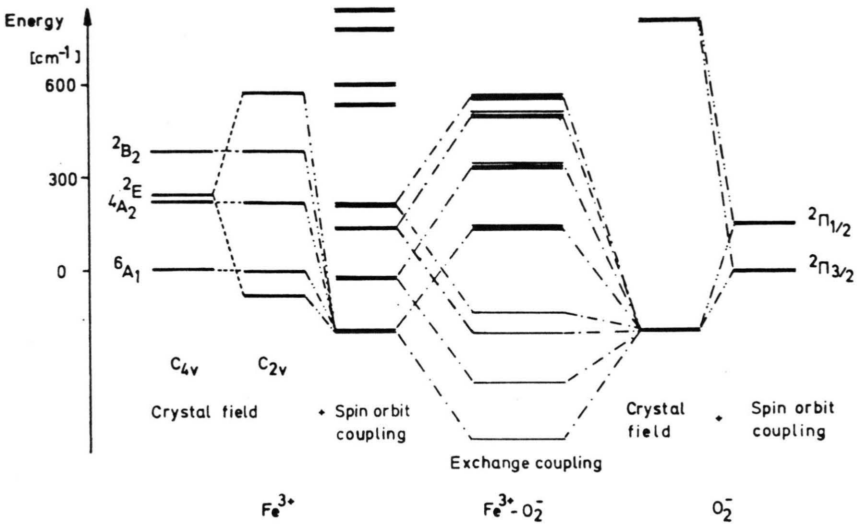

Fig. 5. Coupling scheme of the Fe^{3+}-O_2^- complex. The Kramers doublets of Fe^{3+} on the left and O_2^- on the right are coupled by exchange interaction, yielding singlet and triplet states of the Fe^{3+}-O_2^- complex.

There remains the question if the Fe^{3+}-O_2^- model is the only one which may explain the diamagnetism of the complex as well as the temperature dependence of the quadrupole splitting obtained from Mössbauer spectroscopy. In Fig. 6 various electronic states are shown for the iron atom and the oxygen molecule, respectively. There are two other combinations for the iron's and the oxygen's electronic states, which can be composed to a diamagnetic groundstate of the iron-oxygen complex.

For the free oxygen molecule, the diamagnetic $^1\Delta$ state lies roughly 8000 cm^{-1} above the $^3\Sigma$ groundstate. This $^1\Delta$ state would become the groundstate of the oxygen in an environment with a crystal field strength of 11 000 cm^{-1}, which removes the π-degeneracy of the free oxygen's electronic orbitals [46]. The diamagnetic groundstate of MbO_2 would then require a $^1A_1$ state of the iron. As already mentioned this would cause a small quadrupole splitting in the Mössbauer spectra as in MbCO, in contradiction to our experiment. The ^3E-state of the iron lies close to the ^5E-groundstate in deoxy-Mb. As often suggested [12, 24] this state could couple to the $^3\Sigma$ state of the oxygen. The EFG caused by the ^3E-state has the right order of magnitude, and therefore this triplet-triplet coupling could also be a reasonable approximative description for the iron-oxygen bond. One may therefore start with the ^3E

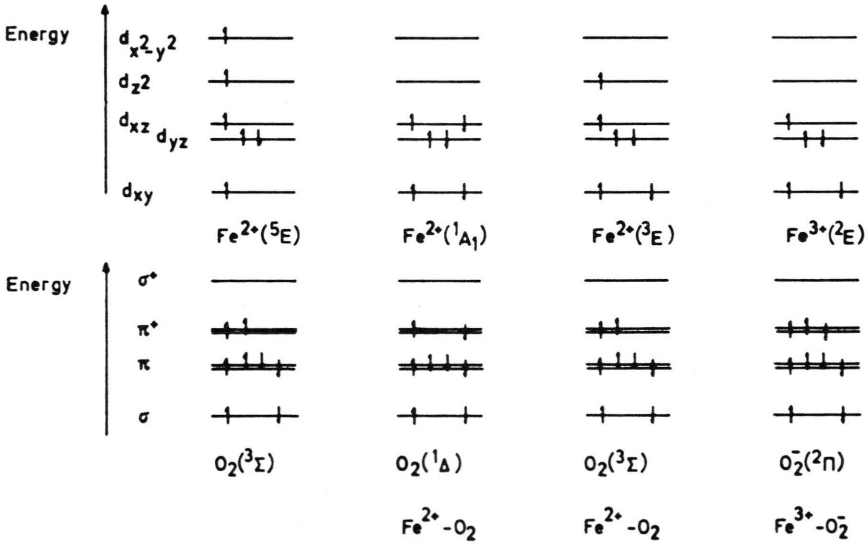

Fig. 6. Electronic states of the iron and the oxygen molecule.

term for the Fe^{2+}-ion as groundstate and then look, if there is within the crystal field model an arrangement of excited states, which give rise to the experimentally observed temperature dependent quadrupole splitting. We have performed a large number of calculations assuming the ^3E term as the groundstate. No agreement with the experimental data could be obtained by us.

No explanation is given so far for the line broadening shown in Fig. 3. Recently a week paramagnetism of oxygenated haemoglobin has been reported in ref. 47. The small contribution of paramagnetic states at higher temperatures could explain the line broadening as a magnetic relaxation phenomenon. However, the results of ref. 47 are in clear contrast to very careful susceptibility measurements on HbO_2 of Hoenig [48]. He found for the whole temperature region a susceptibility less than 0.5×10^{-3} cgs/mol. The experiment of ref. 47 is also critically discussed in a recent paper of Pauling [49].

Mössbauer spectra of the oxygenated picket fence porphyrin model compound [50] have been presented by Spartalian *et al.* [51]. Their interpretation is consistent with the X-ray structure data of the oxygenated model compound [52] showing an oxygen molecule which can move between two binding positions. If a small difference of the binding energy for these two positions is assumed, the variation of the EFG as well as the variation of the Mössbauer line width with temperature can be calculated from the relaxation theory of Blume and Tijon [53].

The different variation of the line width for oxygenated haemoglobins is discussed by Spartalian and Lang [54] assuming a solvent phase transition which yields a line broadening above 136 K. The Lamb-Mössbauer factor shown in Fig. 4 gives no hint for such a phase transition. Moreover X-ray structure data of oxygenated erythrocruorin [55] show a defined position of the O_2 molecule. No smearing of the electron density corresponding to the oxygen molecule over a large region was found. It should be mentioned that the vibration frequency of the bound oxygen molecule is also different for haemoglobin [41] and the picket fence porphyrin [56]. Therefore, conclusions for the reversible oxygen binding of the protein should not be drawn in a strict manner from the picket fence porphyrin.

The X-ray structure analysis of oxygenated erythrocruorin [55] showed a water molecule close to the bound oxygen molecule inside the haem pocket. The presence of an additional water molecule was not taken into account in all the calculations mentioned in the introduction. According to [57] an additional hydrogen bond to the oxygen molecule would stabilize an Fe^{3+}-O_2^- complex at the active center. Therefore, the additional water molecule should not be neglected in further calculations.

The interpretation of the temperature dependence of the quadrupole splitting given in ref. 58, 59 also needs a large mobility of the O_2 between two different positions. The same mobility of the O_2 molecule is taken as an explanation for the temperature dependence of the isomer shift of the Mössbauer spectra [60]. As already mentioned, this is not supported by X-ray data [55]. The isomer shift of MbO_2 at 195 K with respect to α-iron is $S = 0.233$ mm/s. This is close to the value of HbCO ($S = 0.18$ mm/s at 195 K [26]) but also close to HbCN ($S = 0.17$ mm/s at 195 K [26]). Therefore, a decision between Fe^{2+} and Fe^{3+} is not possible from the isomer shift. It is evident, that the level scheme of the iron developped in this paper could give a temperature dependent isomer shift. Unfortunately, we see no possibility for such a calculation.

As discussed in several publications [20, 21] the crystal field parameters can be taken as a measure for the distance of the iron to it's next neighbours. From the ε_3 value obtained in this work one may conclude, that the iron lies even closer in the haemplane than in metmyoglobin [21]. A more detailed discussion of these correlations will be given elsewhere.

This work was supported by the Deutsche Forschungsgemeinschaft. We are indebted to Professors R. L. Mössbauer and G. M. Kalvius for their continuous support of this work.

[1] L. Pauling and C. D. Coryell, Proc. Nat. Acad. Sci. **22,** 210–216 (1936).
[2] L. Pauling, Haemoglobin (eds. F. J. W. Roughton, J. C. Kendrew) Butterworth's Sci. Publ. London, 57–65 (1949).
[3] J. S. Griffith, Proc. Roy. Soc. **A 235,** 23–36 (1956).
[4] L. Pauling, Nature **203,** 182–183 (1964).
[5] J. J. Weiss, Nature **202,** 83–84 (1964).
[6] R. O. Viale, G. M. Maggiora, and L. L. Ingraham, Nature **203,** 183–184 (1964).
[7] M. Zerner, M. Gouterman, and H. Kobayashi, Theoret. Chim. Acta **6,** 362–398 (1966).

[8] G. H. Loew and R. F. Kirchner, J. Am. Chem. Soc. **97,** 7388–7390 (1975).

[9] J. Otsuka, J. Phys. Soc. Japan **21,** 596–620 (1966).

[10] J. Otsuka, J. Phys. Soc. Japan **24,** 885–910 (1968).

[11] Y. Seno, J. Otsuka, O. Matsuoka, and N. Fuchikami, J. Phys. Soc. Japan **33,** 1645–1660 (1972).

[12] J. Otsuka, O. Matsuoka, N. Fuchikami, and Y. Seno, J. Phys. Soc. Japan **35,** 854–860 (1973).

[13] A. Tasaki, and J. Otsuka, IEEE Trans. Magn. **9,** 389–392 (1973).

[14] Y. Seno, J. Otsuka, O. Matsuoka, and N. Fuchikami, J. Phys. Soc. Japan **41,** 977–983 (1976).

[15] W. A. Goddard III and B. D. Olafson, Proc. Nat. Acad. Sci. USA **72,** 2335–2339 (1975).

[16] B. D. Olafson and W. A. Goddard III, Proc. Nat. Acad. Sci. USA **74,** 1315–1319 (1977).

[17] A. Dedieu, M. M. Rohmer, M. Benard, and A. Veillard, J. Am. Chem. Soc. **98,** 3717–3718 (1976).

[18] B. H. Huynh, D. A. Case, and M. Karplus, J. Am. Chem. Soc. **19,** 6103–6105 (1977).

[19] H. Eicher, F. Parak, D. Bade, and J. Tejada, J. Phys (Paris) **35,** C6, 363–366 (1974).

[20] H. Eicher, D. Bade, and F. Parak, J. Chem. Phys. **64,** 1446–1455 (1976).

[21] U. F. Thomanek, F. Parak, S. Formanek, and G. M. Kalvius, Biophys. Struct. Mech. **3,** 207–227 (1977).

[22] F. Parak, U. F. Thomanek, D. Bade, and B. Wintergerst, Z. Naturforsch. **32 c,** 507–512 (1977).

[23] A. Alfsen, D. Bade, U. van Bürck, H. Eicher, S. Formanek, G. M. Kalvius, F. Lavialle, A. Mayer, F. Parak, J. Tejada, and U. F. Thomanek, Biophys. Struct. Mech. **3,** 229–238 (1977).

[24] H. Eicher and A. Trautwein, J. Chem. Phys. **50,** 2540–2551 (1969).

[25] G. Lang, J. Appl. Phys. **38,** 915–921 (1967).

[26] G. Lang and W. Marshall, Proc. Phys. Soc. **87,** 3–34 (1966).

[27] F. Parak and H. Formanek, Acta Crystallogr. **A 27,** 573–578 (1971).

[28] G. M. Kalvius and E. Kankeleit, Mössbauerspectroscopy and its Applications pp. 169–196, Int. Atomic Energy Agency Panel Proceeding Series STI/PUB/ 304, Wien 1972.

[29] W. A. Steyert and R. D. Taylor, Phys. Rev. **134,** A716–A722 (1964).

[30] W. Moffitt, Proc. Roy. Soc. **A 210,** 245–268 (1951).

[31] J. S. Griffith, J. Chem. Phys. **40,** 2899–2900 (1964).

[32] U. F. Thomanek, F. Parak, and B. Wintergerst, Z. Naturforsch. **32 c,** 11–19 (1977).

[33] H. Eicher, Z. Naturforsch. **30 c,** 701–710 (1975).

[34] J. Owen and E. A. Harris, Electron Paramagnetic Resonance (ed. S. Geschwind) Pergamon Press N. Y. 1972.

[35] W. Känzig and M. H. Cohen, Phys. Rev. Letters **3,** 509–510 (1959).

[36] T. Ichikawa, M. Iwasaki, and K. Kuwata, J. Chem. Phys. **44,** 2979–2980 (1966).

[37] M. Setaka and T. Kwan, Bull. Chem. Soc. Japan **43,** 2727–2731 (1970).

[38] G. Harris, Theoret. Chim. Acta **10,** 155–180 (1968).

[39] J. B. Wittenberg, B. A. Wittenberg, J. Peisach, and W. E. Blumberg, Proc. Nat. Acad. Sci. **67,** 1846–1853 (1970).

[40] V. E. Shashoua, Symp. Farad. Soc. **3,** 61–69 (1970).

[41] C. H. Barlow, J. C. Maxwell, W. J. Wallace, and W. S. Caughey, Bioch. Biophys. Res. Commun. **55,** 91–95 (1973).

[42] H. C. Sutton, P. B. Roberts, and C. C. Winterbourn, Bioch. J. **155,** 503–510 (1976).

[43] R. Wever, B. Oudega, and B. F. van Gelder, Biochim. Biophys. Acta **302,** 475–478 (1973).

[44] T. Gotoh and K. Shikama, J. Biochem. **80,** 397–399 (1976).

[45] C. A. Reed and S. K. Cheung, Proc. Nat. Acad. Sci. USA **74,** 1780–1784 (1977).

[46] J. S. Griffith, Oxygen in the Animal Organism (ed. F. Dickens, E. Neil) Pergamon Press, Oxford 1964.

[47] M. Cerdonio, A. Congiu-Castellano, F. Mogno, B. Pispisa, G. L. Romani, and S. Vitale, Proc. Nat. Acad. Sci. USA **74,** 398–400 (1977).

[48] H. E. Hoenig, private communication.

[49] L. Pauling, Proc. Nat. Acad. Sci. USA **74,** 2612–2613 (1977).

[50] J. P. Collman, R. R. Gagne, C. A. Reed, T. R. Halbert, G. Lang, and W. T. Robinson, J. Am. Chem. Soc. **97,** 1427–1439 (1975).

[51] K. Spartalian, G. Lang, J. P. Collman, R. R. Gagne, and C. A. Reed, J. Chem. Phys. **63,** 5375–5382 (1975).

[52] J. P. Collman, R. R. Gagne, C. A. Reed, W. T. Robinson, and G. A. Rodley, Proc. Nat. Acad. Sci. USA **71,** 1326–1329 (1974).

[53] J. A. Tijon and M. Blume, Phys. Rev. **165,** 456–461 (1968).

[54] K. Spartalian and G. Lang, J. Phys. (Paris) **37,** C6, 195–197 (1976).

[55] E. Weber, W. Steigemann, T. A. Jones, and R. Huber, J. Mol. Biol. **120,** 327–336 (1978).

[56] J. P. Collman, R. R. Gagne, H. B. Gray, and J. W. Hare, J. Am. Chem. Soc. **96,** 6522–6524 (1974).

[57] E. Bayer and P. Schretzmann, Structure and Bonding **2,** 181–250 (1967).

[58] L. Cianchi, M. Mancini, and G. Spina, Lett. Nuov. Cim. **16,** 505–508 (1976).

[59] L. Cianchi, M. Mancini, G. Spina, and R. Cappelletti, J. Theor. Biol. **67,** 757–763 (1977).

[60] L. Cianchi, M. Mancini, G. Spina, and R. Cappelletti, J. Theor. Biol. **67,** 765–768 (1977).