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Mössbauer and Molecular Orbital Study of the Myoglobin-CO Complex*

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Experimental Mössbauer spectra of the Fe^{57}-enriched CO complex of sperm whale myoglobin (MbCO) at $T = 4.2$ K with and without applied magnetic field ($H \perp \gamma$) were measured to derive the sign of the electric field gradient (EFG), the quadrupole splitting ΔE_Q, and the isomer shift δ of the heme iron. We find a positive EFG, $\Delta E_Q = 0.363$ mm/sec, and $\delta + 0.266$ mm/sec. Molecular orbital calculations were carried out to obtain theoretical estimates of EFG and ΔE_Q for several steric arrangements of the CO ligand relative to the heme group. Our results are most consistent with the conclusion that the iron is situated in the heme plane, and that a bent geometry with a Fe–C–O angle of about 135° is more favorable than a more symmetric structure with a linear Fe–C–O geometry.

Key words: Mössbauer spectra – Myoglobin-CO complex

1. Introduction

Although the study of the electronic environment of iron nuclei in heme compounds has been the subject of numerous applications of Mössbauer spectroscopy [1], a number of significant questions remain unanswered. The present work represents an attempt to use a combination of experimental and theoretical techniques to investigate the geometry of the carbon monoxide complex of myoglobin.

Our experimental work consisted of low-temperature Mössbauer spectra of the myoglobin-CO complex. By computer analysis of spectra taken with and without an applied magnetic field, we derive the isomer shift δ, the quadrupole splitting ΔE_Q, and the sign of the electric field gradient (EFG) at the iron nucleus.

Theoretical estimates of the EFG of the CO complexes of myoglobin or hemoglobin (MbCO or HbCO) have been worked out by Eicher and Trautwein [2] and by Trautwein *et al.* [3] using the crystal field approach, and by Weissbluth and Maling [4] using the results of molecular orbital (MO) calculations by Zerner,

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Gouterman and Kobayashi [5]. These MO calculations were based on an Iterative Extended Hückel Theory (IEHT) approach which was symmetry restricted, and Zerner *et al.* therefore investigated only a single MbCO geometry in which the CO axis was perpendicular to the heme "plane". In this work we present theoretical estimates of the sign and magnitude of the EFG at the iron nucleus of MbCO for various geometries, using an IEHT without symmetry restrictions [6]. From the calculations we are able to infer a probable structure for the MbCO complex.

2. Experimental

Sperm whale myoglobin enriched with Fe^{57} was prepared as described elsewhere by one of the authors [7]. A solution of metmyoglobin in 0.1 m sodium-potassium phosphate buffer at pH 7.0 was carefully purged of air and then reduced with sodium dithionite in a nitrogen stream. After reducing the ferric iron (Fe III) in metmyoglobin to the ferrous state (Fe II) in deoxymyoglobin, the nitrogen atmosphere in the myoglobin solution was replaced by carbon monoxide gas.

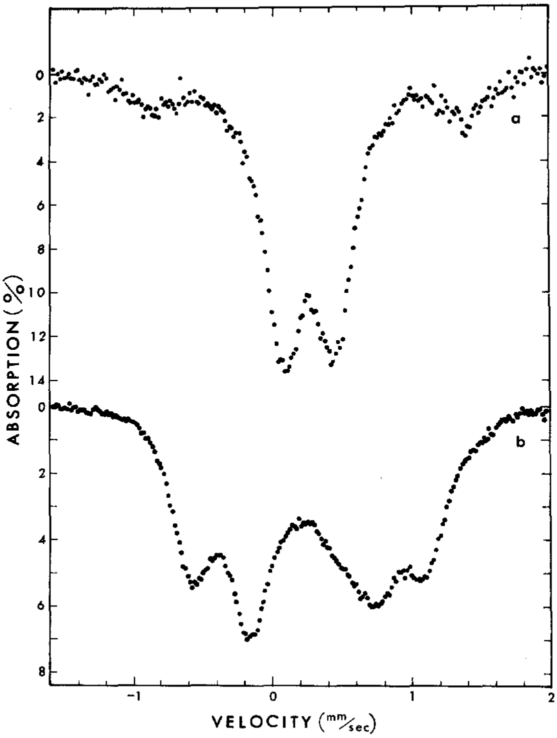

Fig. 1a and b. Experimental Mössbauer spectra of MbCO at $T=4.2\,\mathrm{K}$ with $H=0$ (a) and with $H=47$ kOe, $H \perp \gamma$ (b). The additional resonances at velocities -0.9 mm/sec and 1.33 mm/sec are due to oxygenated myoglobin (MbO_2) which remained in the sample through the preparation of MbCO; for comparison the quadruple splitting of pure MbO_2 was determined as $\Delta E_Q(77\mathrm{K}) = 2.27 \pm 0.03$ mm/sec. The broad shoulders in (b) are probably due to the MbO_2 impurity

The sample was held overnight in the CO atmosphere after which it was cooled to liquid nitrogen temperature. The MbCO sample was then vacuum sealed.

Mössbauer spectra of the sample were taken at liquid helium temperature (4.2 K) with a conventional constant-acceleration drive and a 512-channel analyzer, using a Co^{57} source of approximately 80 mC. Figure 1, Curve a shows the experimental Mössbauer spectrum in the absence of an applied magnetic field. Assuming a Lorentzian line shape, a least-squares fit to this spectrum leads to the following values for the Mössbauer parameters: Γ (line width) = 0.328 ± 0.011 mm/sec, δ (isomer shift) = 0.266 ± 0.010 mm/sec, and ΔE_Q (quadrupole splitting) = 0.363 ± 0.006 mm/sec.

Figure 1, Curve *b* shows a spectrum taken with a magnetic field of 47 kOe applied perpendicular to the γ-beam. This spectrum was analyzed by calculating a Mössbauer spectrum for polycrystalline MbCO along the lines described by Gabriel [8]. Figure 2 shows calculated spectra obtained by using the input parameters $\Gamma = 0.328$ mm/sec, $\delta = 0.266$ mm/sec, $\Delta E_Q = 0.363$ mm/sec, and a

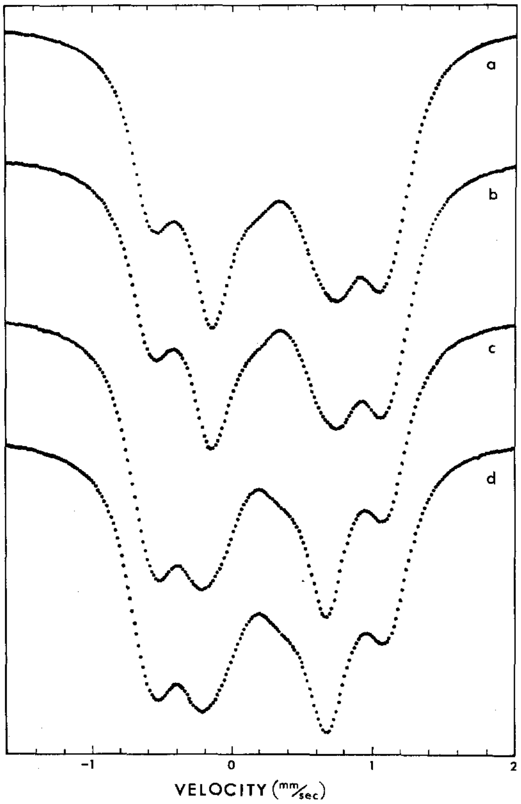

Fig. 2a – d. Theoretical Mössbauer spectra of polycrystalline MbCO, calculated with input parameters $\Gamma = 0.328$ mm/sec, $\delta = 0.266$ mm/sec, $\Delta E_Q = 0.363$ mm/sec, $H = 47$ kOe, $H \perp \gamma$, with positive EFG and $\eta = 0$ (a), with positive EFG and $\eta = 0.4$ (b), with negative EFG and $\eta = 0$ (c), and with negative EFG and $\eta = 0.4$ (d) magnetic field of 47 kOe perpendicular to the γ-beam. Curves *a* and *b* are for the EFG assumed positive, and correspond to different assumed values of the EFG asymmetry parameter $\eta = |V_{yy} - V_{xx}|/|V_{zz}|$; Curve *a* is for $\eta = 0$ and Curve *b* is for $\eta = 0.4$. The calculated spectra in Curves *c* and *d* are for the same η value, but with the assumption of negative EFG. A comparison of the experimental spectrum with the calculated spectra in Fig. 2 leads to the conclusion that the EFG at the Fe nucleus of MbCO is positive, and that a change of the asymmetry parameter in the range $0 \leqq \eta \leqq 0.4$ does not substantially affect the spectra in Fig. 2.

3. Molecular Orbital Calculations

Iterative extended Hückel theory calculations were carried out to estimate Mössbauer quadrupole splittings ΔE_Q and the sign of the EFG for comparison with experimental values, with the hope of obtaining some insight into the stereochemistry of MbCO[1]. The IEHT method previously described by one of the authors [6] was applied to several iron and non-iron compounds [6, 9–12] in order to find a consistent parameter set for H, C, N, O, and Fe. The calculations depend on three parameters for each type of atomic orbital: the Slater-type orbital (STO) screening parameter ζ, and the Hückel energy parameters α and $\Delta\alpha$ describing one-electron energies and their change with net atomic charge. The parameter sets we used are listed in Table 1. In all calculations iterations were performed until input and output net atomic charges were in agreement to within 0.02 electronic charge units.

Table 1. Molecular orbital parameters used in IEHT calculations (see text)

	Orbital	ζ(bohr^{-1})	α(eV)	$\Delta\alpha$(eV)
Fe	3*d*	2.87	7.0	8.0
	4*s*	1.4	7.5	8.0
	4*p*	1.4	6.5	8.0
O	2*s*	2.275	33.0	15.0
	2*p*	2.275	14.0	15.0
N	2*s*	1.95	30.0	12.0
	2*p*	1.95	11.5	12.0
C	2*s*	1.625	25.0	11.0
	2*p*	1.625	10.0	11.0
H	1*s*	1.2	11.6	14.0

The model compound which represents MbCO in our calculations is shown in Fig. 3. Figure 3a shows the heme group in the planar representation used by Zerner *et al.* Figure 3b indicates the steric arrangement in a plane perpendicular

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[1] We use the convention of positive EFG for $V_{zz} > 0$, where V_{zz} enters the Hamiltonian for the quadrupole splitting of the excited nuclear state of Fe^{57}, having quadrupole moment Q and nuclear spin I, as follows:

$$H_Q = \frac{eQV_{zz}}{4}\left[I_z^2 - \frac{5}{4} + \frac{\eta}{3}\left(I_x^2 - I_y^2\right)\right].$$

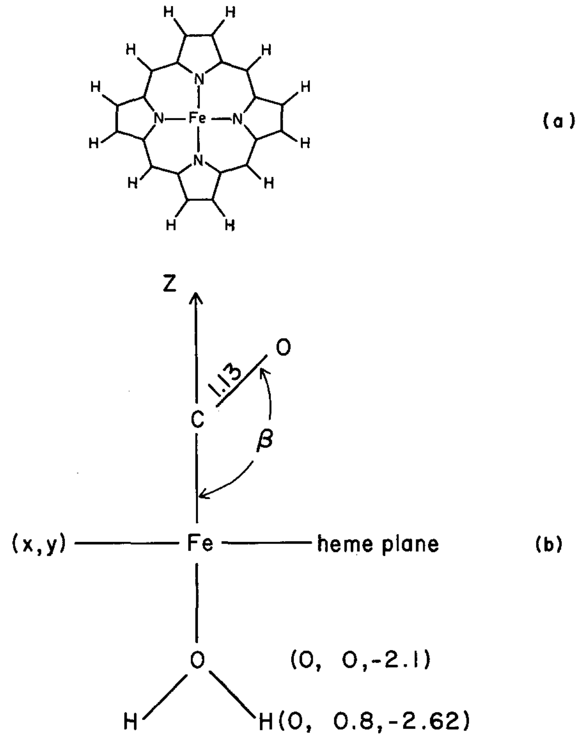

Fig. 3a and b. Molecular geometry of MbCO model used in MO calculations. Heme group (a) and steric arrangement of CO and H_2O relative to the heme plane (b). Distances in Å

to the heme of Fig. 3a. To study the influence of nonplanarity of the heme group we also used the nonplanar geometry obtained by Koenig [13] through X-ray analysis of hemin. As indicated in Fig. 3b and Table 2, various assumptions were made as to the Fe–C bond length, the Fe–C–O bond angle β, the position of the Fe atom relative to the heme group, and the occupancy of one of the two axial ligand binding sites of iron.

From the IEHT calculations for molecular geometries 1–10 of Table 2, bond order matrix elements and atomic charges were obtained and used to evaluate quadrupole splittings by the methods described in a previous paper [9]. In Table 2 we give, for each molecular geometry, its calculated total energy E, diagonal bond order matrix elements P_{aa} of iron valence electrons, atomic charges of iron and its nearest neighbors, and quadrupole splittings ΔE_Q. Our calculations show that the original molecular geometry of Zerner *et al.* (No. 1 in Table 2) results in a ΔE_Q far from the experimental value. Adding a water molecule to the system lowers E but increases ΔE_Q (No. 2). Rotating the water around the z-axis in steps of 20° has no significant effect on E or ΔE_Q. Moving the iron into the plane of the four nitrogens reduces both E and ΔE_Q (No. 3). By taking away the water molecule and reducing the Fe–C distance to 1.64Å (No. 4) E increases but ΔE_Q decreases. The total energy is decreased by changing the heme geometry from planar (Z)

Table 2. Mössbauer and MO parameters as derived from IEHT

Structure no.		1	2	3	4
Geometry of heme[a]		Z_0	Z_0	Z_i	Z_i
H_2O[b]		no	yes	yes	no
Fe–C distance, Å[c]		1.84	1.84	1.84	1.64
β[c]		180°	180°	180°	180°
Total energy E, eV[d]		−2137.0	−2271.6	−2274.8	−2137.9
ΔE_Q calc, mm/sec[e]		1.85	2.25	1.75	1.15
P^f_{aa}	$a = d_{x^2-y^2}$	0.60	0.65	0.64	0.61
	$d_{3z^2-r^2}$	0.48	0.46	0.56	0.75
	d_{xz}, d_{yz}	1.60	1.61	1.61	1.49
	d_{xy}	2.00	2.00	2.00	2.00
	s	0.24	0.17	0.155	0.175
	p_z	0.23	0.17	0.17	0.23
	p_x, p_y	0.23	0.23	0.21	0.20
Atomic charges	Fe	0.25	0.26	0.27	0.29
	N_1–N_4	−0.11	−0.12	−0.12	−0.10
	C of CO	−0.04	−0.04	0	−0.06

[a] Z denotes the planar heme geometry described in Ref. [5]. K stands for the nonplanar heme geometry as derived by X-ray analysis of α-chlorohemin, Ref. [14]. The indices i and o indicate respectively that the iron is in the heme plane or that it is out of plane by 0.5 Å in the direction of the CO.

[b] "yes" and "no" indicates whether or not there is a water molecule bound to iron as shown in Fig. 3.

to nonplanar (K) (No. 5), and ΔE_Q was further lowered. Therefore we used for our remaining calculations molecular geometry K, without water, and iron in the plane of the four nitrogens; all three conditions have the effect of reducing ΔE_Q in the direction of the experimental value. Further reduction of ΔE_Q and E was obtained by reducing the Fe–C distance to 1.54Å. We then rotated the CO molecule from $\beta = 180°$ to 135° in steps of 15° (Nos. 6–9). The total energy increased slightly. However, our estimate of ΔE_Q indicates that a bending angle $\beta = 135°$ is more favorable than a linear geometry. A further decrease of the Fe–C distance seems not to be desirable, because both E and ΔE_Q increase again (No. 10). For all geometries, the net charge calculated for the iron atom is in agreement with our previous calculations for other-low-spin compounds.

4. Discussion

The reasonableness of our MO calculations can to some extent be assessed by examining the predicted orbital energies. In Fig. 4 we show several of the highest occupied and lowest unoccupied MO's for the symmetric Fe–C–O structure studies by Zerner *et al.* [5] (No. 1), and the bent structure best agreeing with the experimental EFG data (No. 9). As found by Zerner *et al.*, the e_gMO's which have predominantly metal character (d_{xz}, d_{yz}) lie below the MO dominated by Fe d_{xy}, because of the strong bonding of the Fe d_π orbitals with the unoccupied π_g orbitals of the CO. This situation obtains both the linear and bent geometries, but in the latter the d_{xz}–d_{yz} orbital degeneracy is lifted by about 500 cm^{-1}. The calculations for several molecular geometries of MbCO

5	6	7	8	9	10
K_i	K_i	K_i	K_i	K_i	K_i
no	no	no	no	no	no
1.64	1.54	1.54	1.54	1.54	1.40
180°	180°	165°	150°	135°	135°
−2141.4	−2141.09	−2141.07	−2141.04	−2140.5	−2139.5
0.87	0.73	0.66	0.60	0.52	0.60
0.60	0.60	0.60	0.61	0.61	0.62
0.77	0.82	0.83	0.86	0.90	0.96
1.50	1.47	1.48	1.46	1.43	1.40
1.99	1.99	1.99	1.99	1.99	1.99
0.175	0.174	0.174	0.175	0.177	0.178
0.26	0.26	0.26	0.26	0.27	0.26
0.20	0.20	0.20	0.20	0.20	0.20
0.27	0.30	0.28	0.28	0.30	0.30
−0.10	−0.10	−0.10	−0.10	−0.09	−0.10
−0.01	−0.03	−0.02	−0.02	−0.03	−0.05

[c] Fe–C distance and bond angle β are defined in Fig. 3.

[d] Total energy as derived from IEHT calculations.

[e] Calculated EFG is positive for all structures.

[f] Diagonal bond order matrix elements.

MO with mainly Fe d_{xy} remains nearly unaffected by the bending, and is essentially an occupied Fe atomic orbital at both geometries. The MO with predominantly Fe d_{z^2} character has a higher energy in structure No. 9 because the smaller Fe–C distance in that structure increases the antibonding interaction between Fe d_{z^2} and the CO orbitals. The antibonding MO with mainly Fe $d_{x^2-y^2}$ character also has a higher energy in structure No. 9, for the most part because of the lowered symmetry. For both structures we find, in agreement with Zerner *et al.*, that MO's which have predominantly Fe character lie in energy between the MO's of predominantly porphyrin character $e_g(\pi)$ and $b_{1u}(\pi)$. All in all, the MO energy levels appear reasonable for both structures.

We next examine the energies of charge-transfer and *d–d* transitions predicted for structures Nos. 1 and 9. Using an approximation introduced by Zerner *et al.*, the charge-transfer transition energies are estimated as simple orbital energy differences, while the transition energies are given (in eV) by $\varepsilon(d_{z^2}) - \varepsilon(d_{xz}, d_{yz}) - k$ and $\varepsilon(d_{x^2-y^2}) - \varepsilon(d_{xz}, d_{yz}) - k'$, with the exchange interactions $k = k(z^2, xz) = k(z^2, yz) = 0.46$ and $k' = k(x^2-y^2, xz) = k(x^2-y^2, yz) = 0.66$ for a net charge of Fe of about $= 0.30e$. The results, summarized in Table 3, show that our calculations predict significant differences between the bent and symmetric structures, with our bent-structure result in essential agreement with Zerner's result based on the symmetric structure.

An additional insight is provided by calculating term energies for lowlying states of other than singlet multiplicity. We made such calculations at a level of approximation reported in previous work [2], using both the MO's and orbital

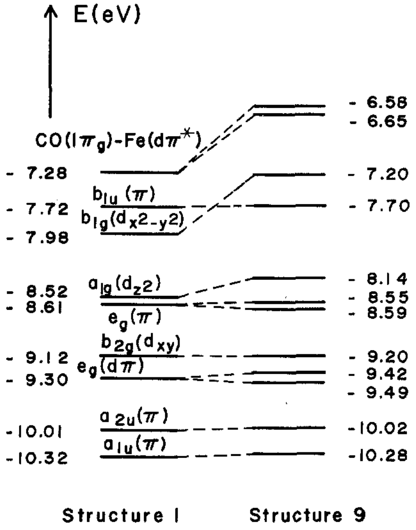

Fig. 4. IEHT energies of several highest occupied and lowest unoccupied MO's of MbCO, for geometries No. 1 and No. 9 of Table 2

Table 3. Energies (eV) estimated for charge transfer and *d–d* transitions in MbCO (see text)[a]

MbCO structure[b]	1[c]	1[d]	9[d]	
charge transfer				
$a_{2u}(\pi) \rightarrow a_{1g}(d_{z^2})$	1.7	1.43	1.88	
d–d				
$^1A_1 \rightarrow {}^3E, d_{xz}, d_{yz} \rightarrow d_{z^2}$	1.85	1.24	1.74[e]	1.81[f]
$^1A_1 \rightarrow {}^3E, d_{xz}, d_{yz} \rightarrow d_{x^2-y^2}$	2.85	2.08	2.88[e]	2.95[f]

[a] Symmetry labels refer to structure No. 1, and are only approximate for structure No. 9; MO labels refer to predominant atomic-orbital contributions.

[b] See Table 2.

[c] Calculated by Zerner *et al.* (Ref. [5]).

[d] This work.

[e] Transition from the d_{xz} orbital.

[f] Transition from the d_{yz} orbital.

energies found in the present work and the corresponding quantities as reported by Zerner *et al.* These calculations are presented in Table 4. We find that the bent and symmetric structures as described by our calculations have significantly different term energies, with the bent structure predicted to have the experimentally observed singlet ground state. The symmetric structure is predicted with our calculations to have a quintet ground state. Our bent-structure term energies, moreover, are in close correspondence with those obtainable from Zerner's study of the symmetric structure.

Table 4. Energies (eV) of many-electron terms $^3A_2$, $^5B_2$, ^3E of MbCO relative to $^1A_1$ (see text)[a]

MbCO structure[b]	1[c]	1[d]	9[d]
$^3A_2$	1.99	0.75	1.71
$^5B_2$	0.55	−0.74	0.46
^3E	0.56	−0.06	0.42
$^1A_1$	0	0	0

[a] Symmetry labels refer to structure No. 1; for structure No. 9 the spin multiplicity continues to be a precise description but the geometrical symmetry is only approximate.

[b] See Table 2.

[c] Calculated by us from data of Zerner *et al.* (Ref. [5]).

[d] This work.

The EFG data and the calculated spin multiplicity of the ground electronic state both favor a bent geometry (No. 9) for Fe–C–O in MbCO, in opposition to the fact that the linear geometry has a slightly lower calculated total energy. The indicators for the bent geometry are probably more reliable than the total energy, as simple approximate MO studies are known to be far better at describing gross charge distributions than they are at predicting electron repulsion energies. Incidentally, we note that our present results suggest that it might be unwise to attach too much significance to the energy minimum found recently by Halton [14] for bent Fe–O–O in oxyhemoglobin, and which we were able to reproduce [15].

Overall, our results for the bent geometry are remarkably close to those found for the linear geometry by Zerner *et al.* This near-coincidence is due to the fact that their study used different atomic parameters than ours. Zerner's parameters were chosen to fit transition energies of the porphyrin system itself, while ours were based on a total of approximately 60 molecules of diverse structure, none heme compounds, and not all containing iron. We therefore argue that our parameter set may be the more reliable for discussing changes in predicted spectral properties with changes in geometry. A bent structure is also consistent with X-ray studies on the carbon monoxide complexes of erythrocruorine [16] and glyceryhemoglobin [17] which indicate Fe–C–O angles of 145 ± 15°. However, note that our calculations do not take into account any side chains which might stabilize the CO in a bent geometry.

Our final conclusions, then, are that in MbCO, the bonding between the heme iron and the proximal histidine is very weak; the iron is situated in or nearly in the heme plane, in agreement with recent findings [16], the Fe–C distance is somewhat smaller than the typical iron-carbonyl distance of 1.84Å; and a Fe–C–O bond angle of $<180°$ is more likely than a linear geometry.

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