Theoret. Chim. Acta (Berl.) 38, 65—69 (1975)
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Calculated Electron Densities and Experimental Isomer Shifts of Fe^{57} in the Deoxy- and CO-Compounds of Myoglobin and Hemoglobin*

Alfred Trautwein

Angewandte Physik, Universität des Saarlandes, Saarbrücken

Frank E. Harris

Department of Physics, University of Utah at Salt Lake City

Received September 26, 1974 / January 8, 1975

Relativistic calculations of various electronic configurations of the iron atom were used in conjunction with Hückel-type self consistent field molecular orbital calculations for CO-myoglobin and CO-hemoglobin (mbCO, hbCO) and with limited configuration interaction calculations for deoxy-myoglobin and deoxy-hemoglobin (mb, hb) to determine electron densities at the iron nucleus, $\varrho(0)$. The calculations included all effects of overlap of iron core and next nearest neighbour (ligand) orbitals, and the effect of potential distortion of iron core orbitals due to molecular configurations $3d^m 4s^n$. From the calculated electron densities we found that the change in the experimental Mössbauer isomer shift, $\Delta\delta = \delta_{\mathrm{mbCO,mbCO}} - \delta_{\mathrm{mb,hb}}$, was mainly due to changes in the so-called overlap distortion of iron core s orbitals. The considerably higher electron density $\varrho(0)$ in mbCO, hbCO than in mb, hb corresponds to the stronger interaction between iron and ligands in mbCO, hbCO compared to mb, hb. From the calculated values for $\varrho(0)$ and the experimental isomer shifts we derived an isomer shift calibration constant, $\alpha = \Delta\delta/\Delta\varrho(0)$, of the value $-0.242 \pm 0.039\ a_0^3$ mm sec^{-1}, which agrees reasonably well with the work of other investigators.

Key words: Myoglobin, isomer shifts of Fe^{57} in ~ – Hemoglobin, isomer shifts of Fe^{57} in ~

1. Introduction

Using molecular orbital (MO) calculations we studied several threedimensional arrangements of ligands with respect to iron in the CO- and deoxy-compounds of myoglobin and hemoglobin (mbCO, hbCO and mb, hb) in order to contribute to the understanding of the relation between molecular and electronic structure in heme compounds. From our MO interpretation of experimental quadrupole splittings and susceptibilities we previously [1, 2] derived molecular wavefunctions and energies for mbCO, hbCO and mb, hb which we used in the present work for a MO study of experimental isomer shifts of mbC, hbCO and mb, hb.

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* Supported by the European Molecular Biology Organization, the Stiftung Volkswagenwerk, the Deutsche Forschungsgemeinschaft, the National Science Foundation, and an award from the Biomedical Science Support Grant at the University of Utah.

2. Molecular Orbital Calculations

The isomer shift in Mössbauer spectroscopy, δ, depends upon the electron density at the nucleus under study, $\varrho(0)$. Values of δ are proportional to changes in $\varrho(0)$ from a reference compound: $\delta = \alpha \cdot \varrho(0)$, where α is known as the calibration constant. An estimate of $\varrho(0)$ from approximate MO calculations is based on a procedure which was described previously [3, 5], and which consists of a sequence of four steps:

1) First, we carried out an extended Hückel self-consistent-field (SCF) calculation for mbCO, hbCO. This approximation was found to be satisfactory for diamagnetic compounds like mbCO and hbCO [1, 4]. The molecular geometry involved in this calculation corresponds to geometry No. 9 of Ref. [1], which was shown to be consistent with the experimental temperature independent quadrupole splitting of mbCO and hbCO. The details of the SCF calculations have been described elsewhere [5].

In the case of mb and hb a spin-projected configuration interaction (CI) study was worked out for the valence electrons, using SCF MO's as a basis, and requiring the overall wavefunctions to be a spin eigenfunction of suitable quantum number. Since several spin-degenerate CI states lie energetically close together we took into account their mutual spin-orbit coupling (CI-LS) [2]. Applying this calculational procedure to various stereostructural models of mb and hb there was satisfactory agreement between experimental and calculated temperature-dependent quadrupole splittings and susceptibilities for model I of Ref. [2].

2) From the SCF calculations for mbCO, hbCO and from the CI-LS calculations for mb, hb we obtained the first-order density matrix of SCF and CI valence-electron wavefunctions. Letting P_{ab} be the density matrix element connecting normalized basis orbitals (AO) ψ_a and ψ_b, the valence contribution to $\varrho(0)$ takes the form

$$\varrho_{\text{valence}}(0) = \sum_{a,b} P_{ab}\, \psi_a^*(0)\, \psi_b(0)\,, \tag{1}$$

where the sum is over the entire valence basis set.

3) The SCF and CI-LS calculations also determined the occupany of the Fe $3d$ and $4s$ atomic orbitals. These occupancies were needed to estimate the potential distortion of iron orbitals due to the shielding experienced by the electrons on the Fe atoms; thus we obtained reliable values of $\psi_{ns}(0)$ for iron.

4) The fourth step in deriving $\varrho(0)$ was to include contributions from Fe core orbitals which were omitted in the approximative MO calculations. In the procedure followed here [1, 6, 7], the Fe core orbitals are orthogonalized to the occupied valence MO's (and kept normalized and orthogonal to each other) before the charge density is calculated. If $\phi_1, \ldots, \phi_n$ are the occupied orthogonal valence orbitals to which the Fe core orbitals are to be orthogonalized, and if the orthogonalization is carried out in the order $3s$, $2s$, $1s$, we have

$$\begin{aligned}
\phi_{3s} &= N_{3s}\left(\psi_{3s} - \sum_{i=1}^{n} \langle\phi_i|\psi_{3s}\rangle\,\phi_i\right)\\
\phi_{2s} &= N_{2s}\left(\psi_{2s} - \sum_{i=1}^{n} \langle\phi_i|\psi_{2s}\rangle\,\phi_i - \langle\phi_{3s}|\psi_{2s}\rangle\,\phi_{3s}\right)\\
\phi_{1s} &= N_{1s}\left(\psi_{1s} - \sum_{i=1}^{n} \langle\phi_i|\psi_{1s}\rangle\,\phi_i - \langle\phi_{3s}|\psi_{1s}\rangle\,\phi_{3s} - \langle\phi_{2s}|\psi_{1s}\rangle\,\phi_{2s}\right),
\end{aligned} \tag{2}$$

where ψ_{3s}, ψ_{2s}, ψ_{1s} are the original Fe core orbitals and N_{3s}, N_{2s}, and N_{1s} are normalization factors. We could continue, obtaining ϕ_{3p} and ϕ_{2p}, but their contribution to $\varrho(0)$ is negligible [3]. The normalization conditions yield

$$\begin{aligned} N_{3s} &= \left(1+\sum_{i=1}^{n} |\langle\phi_i|\psi_{3s}\rangle|^2\right)^{-1/2} \\ N_{2s} &= \left(1+\sum_{i=1}^{n} |\langle\phi_i|\psi_{2s}\rangle|^2 + |\langle\phi_{3s}|\psi_{2s}\rangle|^2\right)^{-1/2} \\ N_{1s} &= \left(1+\sum_{i=1}^{n} |\langle\phi_i|\psi_{1s}\rangle|^2 + |\langle\phi_{3s}|\psi_{1s}\rangle|^2 + |\langle\phi_{2s}|\psi_{1s}\rangle|^2\right)^{-1/2} \end{aligned} \tag{3}$$

The core contribution to $\varrho(0)$ is then

$$\varrho_{\mathrm{core}}(0) = 2|\phi_{3s}(0)|^2 + 2|\phi_{2s}(0)|^2 + 2|\phi_{1s}(0)|^2 . \tag{4}$$

The appropriate relativistic values $\psi_{ns}(0)$ for iron can be derived by interpolation from the values given in Table 1 of Ref. [3]. The Fe-ligand overlap integrals were calculated using Hartree-Fock Fe and ligand orbitals appropriate to the orbital occupancy. The values $\psi(0)$ for ligand orbitals were estimated using Slater type orbitals and found to be negligible. The overlap distortion of iron core orbitals by ligands farther away from Fe than 3 Å was neglected. Further, another small effect which was not included in the study is the contribution to $\varrho(0)$ from relativistic effects upon the Fe $2p$ and Fe $3p$ orbitals. While these contributions to $\varrho(0)$ might have been individually significant, their differences are so small that they scarcely would have affected the changes $\Delta\varrho(0)$ among analoguous compounds.

3. Results and Discussion

The calculational methods were applied here to the two systems mbCO, hbCO and mb, hb which are in Table 1. This table also contains the experimental values of δ, the electronic configurations found for Fe in our MO calculations, and the individual values of $\varrho_{ns}(0)$. The calculated change in $\varrho(0)$ under complex-formation of mb, hb with CO may now be compared with the difference of experimental isomer shifts, $\Delta\delta = \delta_{\mathrm{mb,hb}} - \delta_{\mathrm{mbCO,hbCO}}$. Applying the equation $\Delta\delta = \alpha \cdot \Delta\varrho(0)$ it is possible to compare the ratio $\Delta\delta/\Delta\varrho(0)$ with the α-values of other authors. From the present study we found $\alpha = -0.254 \pm 0.039\ a_0^3$ mm sec^{-1}, or taking account of the second order Doppler shift correction (see Table 1, Footnote b) $\alpha = -0.242 \pm 0.039\ a_0^3$ mm sec^{-1}. This is in reasonable agreement with those values obtained in two recent calculations, namely $-0.249 \pm 0.025\ a_0^3$ mm sec^{-1} by Trautwein, Harris, Freeman, and Desclaux [3] and the value $-0.250 \pm 0.030\ a_0^3$ mm sec^{-1} by Regnard and Pelzl [9]; and it is somewhat larger than the value $-0.177 \pm 0.015\ a_0^3$ mm sec^{-1} obtained by Duff [10]. (Duff's value of $-0.230 \pm 0.020\ a^3$ mm sec^{-1} has to be corrected by the relativistic correction factor $S'(Z) = 1.3$ for iron in order to compare it with our and Regnard's *et al.* values.)

Table 1. Experimental isomer shifts δ, calculated electronic configurations $3d^m 4s^n$ for iron, and calculated relativistic electron densities $\varrho_{ns}(0)$[a] at the Fe nucleus for mbCO, hbCO and mb, hb

Compound	exp. δ[b] (mm/sec)	Configuration[c]	$\varrho_{1s}(0)$	$\varrho_{2s}(0)$	$\varrho_{3s}(0)$	$\varrho_{4s}(0)$
mbCO, hbCO	0.27 ± 0.05	$3d^{6.36}4s^{0.18}$	13600.9	1280.9	184.6	0.82
mb, hb	0.93 ± 0.05	$3d^{6.25}4s^{0.22}$	13599.9	1283.2	180.46	1.16

[a] Values of $\varrho_{ns}(0)$ in a_0^{-3} as derived from Eq. (1)–(4) of the text.

[b] Values of δ versus metallic iron at room temperature are mean values taken at 4.2 K from mbCO, hbCO and mb, hb of various species. A correction of δ due to different Debye temperatures in mbCO, hbCO and mb, hb, respectively, was omitted. Both values of δ are temperature independent up to about 150 K within the given error range. Still, however, the Debye temperatures θ_D of mbCO, hbCO and mb, hb might be different due to different bonding of iron in the two compounds. Assuming the probably unrealistic high difference of $\theta_D(\text{mbCO,hbCO}) - \theta_C(\text{mb,hb}) = 400\,\text{K} - 200\,\text{K}$ leads to second-order Doppler shift values δ_{s0} between 300 K and 77 K which differ only by about 0.03 mm/sec for the two compounds: $\delta_{s0}(\text{mb,hb}) - \delta_{s0}(\text{mbCO,hbCO}) \approx 0.03$ mm/sec.

[c] Configurations $3d^m 4s^n$ as derived from MO calculations (see Refs. [1] and [2]).

We found that the drastic decrease of δ, i.e., the drastic increase of $\varrho(0)$ during the reaction of mb or hb with CO, is mainly due to the increased overlap distortion of the iron core orbitals in mbCO, hbCO with respect to mb, hb. The valence contribution and the potential distortion effect upon $\varrho(0)$ are nearly the same for both systems. The following conclusion may be drawn from these findings: due to the stronger interaction between ligands and iron in mbCO, hbCO compared to mb, hb, the iron core contribution $\phi_{ns}(0)$ to $\varrho(0)$ is considerably higher in mbCO, hbCO than in mb, hb, and thus $\delta_{\text{mbCO,hbCO}}$ is significantly smaller than $\delta_{\text{mb,hb}}$.

The present MO interpretation of the difference between $\delta_{\text{mbCO,hbCO}}$ and $\delta_{\text{mb,hb}}$ is approximative in the sense that 1. the heme geometries for mbCO, hbCO and mb, hb used here [1, 2] are models only for the real molecular situation, 2. the MO procedure itself is approximative to the extend described in Ref. [5], and 3. the calculation of $\varrho(0)$ according to Eqs. (1)–(4) contains the main effects likely to influence the values found for $\Delta\varrho(0)$. These simplifications, however, are believed to be secondary for our attempt to derive a gross description of the relation between molecular and electronic structure of heme compounds. Therefore we were gratified to note that the molecular geometries for mbCO, hbCO and mb, hb, which we found to be consistent with experimental quadrupole splittings, $\Delta E_Q(T)$, and susceptibilities, $\chi(T)$ [1, 2], lead also to a reasonable description of the change of electron densities, $\Delta\varrho(0)$, among the similar compounds mbCO, hbCO and mb, hb.

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Prof. Dr. A. Trautwein
Universität des Saarlandes
Fachbereich 12.1
Werkstoffphysik und Werkstofftechnologie
D-6600 Saarbrücken 11
Federal Republic of Germany