

## MO Calculations on Monomeric Oxygen Adduct Formation with Iron and Cobalt Porphyrins

MARGARET P. HALTON

*Chemistry Department, Victoria University of Wellington, New Zealand*

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*Oxygen adducts of coboglobin and haemoglobin have been studied using SCC-EH-MO theory. The proposed configurations are distinguished from alternative arrangements by the calculation of Mössbauer and esr parameters. The single unpaired electron in oxycoboglobin is calculated to have 25% 3d character, 20% O<sub>2</sub>, and 55% porphyrin pi. The effect of non-planarity in the deoxy species have been studied and a marked increase in metal-porphyrin pi bonding shown to accompany the geometry change of non-planar metal to the planar system. The consequences of this transition are discussed in terms of the co-operative effects of ligand bonding to haemoglobin and oxycoboglobin.*

### Introduction

Bonding and electron configuration in metal porphyrins have been the subject of continued interest because of their important role in biological systems. In particular, the binding of pi-type ligands to the metal is of considerable interest since the reversible oxygenation of haemoglobin has been well characterised<sup>1</sup> but not fully understood. Since 1970 there have been many reports<sup>2</sup> on the reversible formation of monomeric oxygen adducts of cobalt, including oxycoboglobin<sup>3</sup> where the coboglobin molecule is analogous to haemoglobin with the iron(II) replaced by cobalt(II). The electron arrangement in the metal-oxygen moiety is better understood for oxycoboglobin (and other cobalt-oxygen adducts) because of the presence of an unpaired electron which allows esr measurements to be made. The adduct is deduced to exist formally as Co(III)·O<sub>2</sub><sup>-</sup> with only 20% of the unpaired electron remaining on the cobalt.<sup>2,3</sup> This configuration is analogous to that proposed by Weiss<sup>4</sup> for haemoglobin (viz. Fe(III)·O<sub>2</sub><sup>-</sup>) but in the latter case a spin pairing of the odd electron on the iron with that on the oxygen must be assumed.

A molecular orbital calculation for the oxygen adduct of both iron and cobalt porphyrin was considered desirable in order to elucidate similarities and differ-

ences between the two species. Furthermore, Perutz<sup>5</sup> has proposed that a movement of the metal ion from 0.8 Å below the porphyrin plane to a planar arrangement accompanies the oxygenation process in haemoglobin, and is sufficient to trigger the well known co-operative effects or haem-haem interaction. A set of MO calculations was therefore undertaken on the deoxy iron and cobalt systems to examine the effects of such a transition on the metal orbitals, and to compare with the oxygen adducts. In view of the limitations of the theoretical model adopted the experimental parameters of the electric field gradient at the iron nucleus, determined from the Mössbauer spectrum, and the g values of the single unpaired electron in the cobalt species, determined from the esr spectrum, were employed to test the calculated electron configurations.

### Method

SCC-EH-MO (self consistent charge Extended Hückel molecular orbital) theory was applied to a theoretical model, as reported earlier<sup>6</sup> (this paper is hereafter denoted by I). This model was modified slightly in that H<sub>ii</sub> values and orbital exponents were taken from Zerner and Gouterman's<sup>7</sup> paper. Small variations in the H<sub>ii</sub> values lead to corresponding variations in the MO coefficients and energy values but comparisons of iron and cobalt systems remained unaffected in qualitative trends. Since SCC-EH-MO is inapplicable in a quantitative study, the choice of H<sub>ii</sub> values was considered satisfactory for the current investigation. The porphyrin ring (Figure 1) and fifth

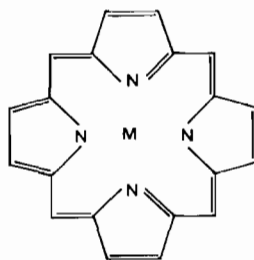


Figure 1. Metal Porphyrin.

TABLE I. Systems studied.

System	Species Simulated <sup>a</sup>	Spin	Details
1	Hb	2	Planar
2	Hb	0	Planar
3	Hb	2	Out of plane
4	Hb	0	Out of plane
5	Cb	3/2	Planar
6	Cb	1/2	Planar
7	Cb	3/2	Out of plane
8	Cb	1/2	Out of plane
9	HbO <sub>2</sub>	0	
10	HbO <sub>2</sub>	0	2 extra e's
11	CbO <sub>2</sub>	1/2	
12	CbO <sub>2</sub>	1/2	2 extra e's

<sup>a</sup> Hb = haemoglobin; Cb = coboglobin.

ligand were represented by the same basis set as in I, while all valence orbitals were used for the metal and oxygen atoms (3d, 4s, 4p; 2s, 2p respectively). This approximation for the porphyrin ring is constant, allowing comparisons between species to be made.

Table I lists all of the systems studied in the current work, together with the numbering adopted. Metal porphyrin bond lengths were taken from estimates reported elsewhere<sup>7</sup> and the fifth ligand placed at 1.9 Å from the metal.<sup>5</sup> Planar and non-planar (0.8 Å below the porphyrin<sup>5</sup>) metal geometries were considered for both high and low-spin iron(II) and cobalt(II) derivatives, with no ligand in the sixth position (systems 1–8 in Table I). For the oxygen adducts the metal was assumed to lie in the porphyrin plane. Both oxygen adducts and coboglobin itself, are known to be low-spin, but haemoglobin is high-spin.<sup>3,8</sup> Differences in the deoxy forms are considered by comparing all spin states.

The geometry of the metal–oxygen moiety in oxyhaemoglobin and monomeric oxygen–cobalt adducts has been the subject of much controversy. However Rodley and Robinson<sup>9</sup> have recently reported the X-ray structure of a Co·O<sub>2</sub> adduct which reversibly binds the oxygen. The observed geometry corresponds to the Pauling<sup>10</sup> structure proposed for oxyhaemoglobin and supported by MO calculations.<sup>6,11</sup> The angular arrangement illustrated in Figure 2 was therefore adopted for both the iron and cobalt oxygen adducts. Rodley and Robinson's<sup>9</sup> measured Co–O and

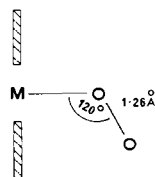


Figure 2. Geometry of Metal Oxygen Adduct.

O–O bond lengths (1.86 Å, 1.26 Å respectively) were used and the Fe–O distance estimated to be 1.90 Å from a comparison of the cobalt and iron aquo complexes.<sup>12</sup> For both adducts the M–O–O angle was idealised to 120°.

One of the conclusions reported in I was that the FeO<sub>2</sub> system required two extra electrons in order to achieve a stable adduct and prevent oxidation. Consequently for both the iron and cobalt adducts, two separate calculations were performed, one with the extra electrons and one without (systems 9–12 in Table I).

#### Calculation of g tensor

Abraham and Pryce<sup>3</sup> have given general formulae for the evaluation of the  $g_{ij}$  components of the g tensor for a single unpaired electron. Only the principal components were evaluated here and the appropriate formulae are:

$$g_{ii} = 2.0023 - 2\zeta \sum_{k=0} \langle n | l^i | k \rangle \langle k | l^i | n \rangle / (E_k - E_n) \quad (1)$$

for  $i = x, y, z$ , and the summation is over the MO's. Orbital  $k$  is either doubly occupied or empty and orbital  $n$  contains the unpaired electron. The excitation energy ( $E_k - E_n$ ) was taken as the orbital energy difference, and  $\zeta$  is the spin orbit coupling constant.

Group theory was employed to deduce the non-zero matrix elements  $\langle r | l^i | s \rangle$ , assuming  $C_{2v}$  symmetry. Only the antibonding d orbitals were considered in the summation since the values for the ligands are significantly smaller than  $\zeta_{Fe}$  or  $\zeta_{Co}$ .<sup>13</sup> Under  $C_{2v}$  symmetry the d orbitals transform as follows:

$$xy \sim a_2; \pi 1 \sim b_1; \pi 2 \sim b_2; z^2 \sim a_1; x^2 - y^2 \sim a_1$$

where the  $d_{\pi}$  molecular orbitals were found to correspond to

$$\psi(b_1) = -\alpha xz + \alpha yz + \text{ligand orbitals} \quad (2)$$

$$\psi(b_2) = -\beta xz - \beta yz + \text{ligand orbitals} \quad (3)$$

$\alpha, \beta$  being the calculated MO coefficients.

$\Delta g_{ii}$  values were calculated from eq.(1), as a function of ( $E_k - E_n$ ) and trends compared with experiment. EHT does not provide reliable orbital energy values and the calculated values tend to be too large. However, relative orbital energies can be estimated and contributions to  $\Delta g_{ii}$  evaluated. Comparisons of theoretical and experimental trends have been employed here to distinguish between alternative ground state configurations and to test the reliability of proposed MO explanations of bonding.

#### Calculation of Electric Field Gradient

Since the Mössbauer spectrum pertains to ground state electron configurations, the experimental Mössbauer parameters can be successfully compared with values deduced from EHT. The observed quadrupole splitting  $\Delta E_Q$  is directly proportional to the electric

field gradient (EFG) at the iron nucleus. If the cross terms of the EFG tensor,  $V$ , are neglected then:

$$\Delta E_Q \alpha V_{zz} (1 + \eta^2/3)^{1/2} \quad (4)$$

where

$$\eta = (V_{xx} - V_{yy})/V_{zz} \quad (5)$$

Clearly  $\eta$  is zero for axially symmetric molecules. As reported in I, the covalent contribution to  $V_{zz}$  and  $\eta$  can be expressed in terms of the atomic orbital populations, which in turn depend upon the MO coefficients. The following expressions can then be formulated.<sup>6,15</sup>

$$V_{ii} = V_{ii}(3d) + V_{ii}(4p) \quad (6)$$

and

$$V_{zz}(3d) = K [n_{xy} + n_{x^2-y^2} - (n_{xz} + n_{yz})/2 - n_z^2] \langle r^{-3} \rangle_{3d} 4/7$$

$$V_{zz}(4p) = K [n_x + n_y - 2n_z] \langle r^{-3} \rangle_{4p} 2/5$$

$$(V_{xx} - V_{yy})3d = K' [n_{xz} - n_{yz}] \langle r^{-3} \rangle_{3d} 6/7$$

$$(V_{xx} - V_{yy})4p = K' [n_x - n_y] \langle r^{-3} \rangle_{4p} 6/5$$

where  $K$  and  $K'$  are constants which include the Sternheimer antishielding constant,  $R$ .<sup>15</sup> If  $R$  is assumed to be the same for both 3d and 4p electrons then  $K$  and  $K'$  will be equal. The  $n_i$  values are the orbital populations as calculated from the Mulliken population analyses. Because of the more diffuse nature of the 4p orbitals,  $n_i$  was taken as the net atomic population for the  $i^{\text{th}}$  4p orbital but gross atomic population for the  $i^{\text{th}}$  3d orbital. De Vries *et al.*<sup>16</sup> have used  $\langle r^{-3} \rangle_{3d} = 32.0 \text{ \AA}^{-3}$  and  $\langle r^{-3} \rangle_{4p} = 11.6 \text{ \AA}^{-3}$ . For convenience the  $\langle r^{-3} \rangle$  values have been incorporated into the proportionality constant  $K$ , and as in I, a parameter  $B$  defined.

$$B = K^{-1} (V_{zz}(3d) + V_{zz}(4p)) \cdot 32.0/11.6 (1 + \eta^2/3)^{1/2}$$

where  $V_{zz}$  and  $\eta$  are evaluated from eqs. (5) and (6).

The calculated sign of  $B$  corresponds to the sign of the EFG, and the same sign convention used in I was adopted.

## Results and Discussion

### Oxygen Adducts

System 9 (corresponding to oxyhaemoglobin without any extra electrons) began to converge for the first nine cycles but then proceeded to diverge rapidly with a constant swapping of the orbital levels. The pseudo-minimum was examined in order to compare with the results of system 10, where the two extra electrons were included. System 9 corresponds to a  $d^4$  configuration on the iron with a calculated charge distribution of  $\text{Fe}^{0.22+} \cdot \text{O}_2^{0.72-}$ . However the orbital populations are such that a near zero electric field gradient is predicted, contrary to experiment<sup>7</sup> (Table II). Since the theoretical model was found successful in I, in interpreting quadrupole splitting for a variety of derivatives, this  $d^4$  representation for oxyhaemoglobin was assumed to be inappropriate. The alternative case, system 10

TABLE II. Experimental and theoretical ESR and Mössbauer parameters.

Esr		Theory <sup>a</sup>			Exptl. <sup>b</sup>	
		$\Delta g_{xx}$	$\Delta g_{yy}$	$\Delta g_{zz}$	$\Delta g_{\perp}$	$\Delta g_{zz}$
CbO <sub>2</sub> <sup>c</sup>	11	0.25	-0.35	-0.27	-0.01	0.08
		$\Delta_1$	$\Delta_2$	$\Delta_3$		
CbO <sub>2</sub>	12	0.38	0.32	0	-0.01	0.08
		$\Delta_5$	$\Delta_2$			

Mössbauer		Theory		$\Delta E_Q$ (Exptl.) <sup>d</sup> mm/sec
		B	$\eta$	
Hb	3	+0.485	0	+2.40
HbO <sub>2</sub>	9	-0.122	0.050	-2.25
HbO <sub>2</sub>	10	-0.438	0.023	-2.25

<sup>a</sup> $\Delta_1 = |E_{xy} - E_{b1}|$ ;  $\Delta_2 = |E_{zz} - E_{b1}|$ ;  $\Delta_3 = |E_{x^2-y^2} - E_{b1}|$ ;  $\Delta_4 = |E_{b2} - E_{b1}|$ ;  $\Delta_5 = |E_{z^2} - E_{b2}|$ . <sup>b</sup>Reference no. 3  
<sup>c</sup>Cb = coboglobin; Hb = haemoglobin; <sup>d</sup>Reference no. 17.

which included the two extra electrons proposed in I, gave results similar to those reported in I. The iron is calculated to be essentially a  $d^6$ (iron II) configuration with a charge distribution  $\text{Fe}^{0.3-} \cdot \text{O}_2^{1.28-}$  while the quadrupole splitting is predicted to be large and negative in accord with experiment.<sup>17</sup> Tables II and IV include the relevant results of the MO calculation for system 10.

Both representations of oxycoboglobin converged but with vastly different results. The calculated d configurations are:

System 11:  $(a_2)^2(b_2)^2(b_1)^1(1a_1)^0(2a_1)^0$   
where the coefficients are given in Table III.

System 12:  $(a_2)^2(b_1)^2(b_2)^2(1a_1)^1(2a_1)^0$   
where the coefficient  $\alpha$  in eq. 2 is .49 and  $\beta$  is 0.55; the coefficient of the  $z^2$  orbital in  $1a_1$  is 0.67; the metal coefficients for  $a_2$  and  $2a_1$  are essentially unity.

Before oxycoboglobin can be compared with oxyhaemoglobin these two configurations must be distinguished, and the  $g$  parameters from the esr spectrum were used for this. The metal character of the unpaired electron is 40%  $z^2$  in system 12 but 25%  $d_{xy}$  in system 11. Consequently, different  $\Delta g$  values are expected. Experimental  $\Delta g$  values are available for several cobalt-oxygen adducts,<sup>2</sup> including oxycoboglobin,<sup>3</sup> and these are all very similar.  $\Delta g_{zz}$  and  $\Delta g_{\text{iso}}$  have been measured directly, and  $\Delta g_{\perp}$  inferred from these results. Table II lists both experimental and theoretical  $\Delta g$  values. System 12 is seen to predict positive  $\Delta g_{\perp}$  and zero  $\Delta g_{zz}$  in conflict with experiment. On the other hand, system 11 predicts a positive  $\Delta g_{zz}$ , small positive  $\Delta g_{xx}$  and negative  $\Delta g_{yy}$ . The relative magnitudes of the energy differences  $\Delta_2$  and  $\Delta_3$  compared with  $\Delta_1$  (see Table III) are such that the aver-

TABLE III. Calculated molecular orbitals and energy levels for oxygen adducts of iron and cobalt.

A. System 10: HbO<sub>2</sub>

MO	no. elects.	Energy, ev	Symmetry <sup>a</sup>	Coefft. metal orbs.	% N <sub>π</sub> <sup>b</sup>	% O <sub>2</sub> <sup>b</sup>
11	2	-11.615	b <sub>1</sub>	0.41xz-0.40yz	15	-
12	2	-11.607	b <sub>2</sub>	0.38xz + 0.40yz	15	-
13	2	-11.359	a <sub>1</sub>	-	21	16
14	2	-11.270	a <sub>2</sub>	-	25	-
15	2	-11.020	a <sub>2</sub>	1.00xy	-	-
16	2	-10.635	b <sub>1</sub>	-0.52xz + 0.54yz	9	8
17	2	-10.593	b <sub>2</sub>	-0.56xz-0.54yz	9	5
18	2	-10.233	b <sub>2</sub>	-0.16xz-0.16yz + 0.43z <sup>2</sup>	2	75
19	2	-9.875	b <sub>1</sub>	0.21xz-0.21yz	2	78
20	0	-9.514	a <sub>1</sub>	1.00(x <sup>2</sup> -y <sup>2</sup> )	-	-
21	0	-8.760	a <sub>1</sub>	0.88z <sup>2</sup>	1.5	32

B. System 11: CbO<sub>2</sub>

MO	no. elects.	Energy, ev	Symmetry	Coefft. metal orbs.	% N <sub>π</sub> <sup>b</sup>	% O <sub>2</sub> <sup>b</sup>
11	2	-11.510	b <sub>1</sub>	-0.55xz + 0.64yz	4.5	6
12	2	-11.481	b <sub>2</sub>	0.64xz + 0.55yz	5	4
13	2	-11.271	a <sub>2</sub>	1.00xy	-	-
14	2	-11.192	a <sub>1</sub>	-0.2z + 0.64z <sup>2</sup>	5	20
15	2	-10.692	b <sub>1</sub>	-0.15z <sup>2</sup>	14	40
16	2	-10.567	a <sub>2</sub>	-	24.5	2
17	2	-10.527	b <sub>1</sub>	-	5.5	78
18	2	-10.250	b <sub>2</sub>	0.34xz + 0.37yz	19	1
19	1	-10.190	b <sub>1</sub>	-0.38xz + 0.36yz	15	15
20	0	-9.919	a <sub>1</sub>	-0.65z <sup>2</sup>	2.5	50
21	0	-9.621	a <sub>1</sub>	0.98(x <sup>2</sup> -y <sup>2</sup> )	1	-

<sup>a</sup> C<sub>2v</sub> symmetry labels used. <sup>b</sup> Fraction quoted for N<sub>π</sub> is average for the four porphyrin nitrogens; total fraction quoted for O<sub>2</sub>.

age  $\Delta g_{\perp}$  value ( $\Delta g_{\perp} = (\Delta g_{xx} + \Delta g_{yy})/2$ ) is negative in agreement with experiment. Thus system 11 predicts the correct trends in g values and therefore is considered an adequate model for oxycoboglobin. Tables III and IV list the principal results of the MO calculations for system 11.

However the electron distribution in the d orbitals is contrary to that normally associated with cobalt adducts and should therefore be considered with caution, remembering the limitations of the theoretical model. In view of the earlier success of this model<sup>6</sup> it is of interest to compare the results for system 11 with those of the iron analogue.

The predicted configuration for oxycoboglobin is in marked contrast with that for oxyhaemoglobin (system 10). Inferences on the nature of bonding in the latter from interpretations of cobalt-oxygen adducts are therefore not applicable. The theory presented here is that cobalt is capable of forming stable monomeric adducts without any transfer of two extra electrons to the CoO<sub>2</sub> moiety, in contrast with iron. This may well be the explanation for the proliferation of cobalt ad-

TABLE IV. Results of Mulliken Population Analysis for oxygen adducts.

System	10	11
<sup>a</sup> 4s	0.481	0.618
4p <sub>x</sub>	0.227	0.328
4p <sub>y</sub>	0.233	0.331
4p <sub>z</sub>	0.201	0.318
3d <sub>xy</sub>	2.000	2.000
3d <sub>xz</sub>	1.999	1.869
3d <sub>yz</sub>	1.999	1.878
3d <sub>z</sub> <sup>2</sup>	0.678	1.133
3d <sub>x<sup>2</sup>-y<sup>2</sup></sub>	0.209	0.277
Metal Chge	0.026-	0.246+
<sup>b</sup> NOP, O <sup>1</sup>	0.419	0.481
<sup>c</sup> NOP, O <sup>2</sup>	0.222	0.275
Chge O <sup>1</sup>	0.429-	0.303-
Chge O <sup>2</sup>	0.852-	0.439-
Chge N/4	0.723-	0.435-

<sup>a</sup> Gross atomic populations. <sup>b</sup> Net atomic population on oxygen closest to metal. <sup>c</sup> Net atomic population on oxygen distal to metal.

ducts<sup>2</sup> compared with the few known cases of iron–oxygen bonding.

Both adducts are predicted to have strong pi bonding of metal to the oxygen, and a loss of formal valence state on the metal due to considerable back bonding. Clearly, any substituent on the porphyrin ring which increases pi electron donation to the metal is expected to enhance that metal's ability to form adducts, and this is indeed observed.<sup>2,8</sup>

Formally, the metals exist as iron(II) and cobalt(IV) but formal valence has little meaning in these systems where the metal orbitals are so mixed with those of the ligands (see Table III). The theoretical model used here predicts a similar charge distribution for oxyhaemoglobin to that reported in I, and comparisons with Weiss's<sup>4</sup> proposed configuration  $\text{Fe(III)} \cdot \text{O}_2^-$  are not repeated here. Now, the calculated electron configuration for oxycoboglobin is not only distinct from that in the iron analogue, but also somewhat different from that which has been deduced by esr workers.<sup>2</sup> The unpaired electron has been estimated from esr data to be only 20% metal character, and the 25% calculated here agrees well. However, most authors have assumed that the unpaired electron character not on the metal must belong to the oxygen molecule, leading to the assumed configuration  $\text{Co(III)} \cdot \text{O}_2^-$ .

The MO calculation shows a different electron configuration. Because there is strong pi bonding between the 3d and oxygen  $\pi$  orbitals the associated antibonding  $d_\pi$  orbitals are raised in energy such that formally a  $d^5$  configuration results. However the effective charge is reduced to 0.22+ by the significant covalent mixing. The unpaired electron is 25%  $d_\pi$ , 20%  $\text{O}_2$ , and 55%  $\text{N}_\pi$  in character, contrary to the 20% d, 80%  $\text{O}_2$  inferred from esr.<sup>2</sup> The  $\Delta g$  values can be accounted for by considering the  $d_\pi$  contribution alone in eq. (1), since  $\zeta_{\text{Co}}$  is much greater than  $\zeta_{\text{N}}$  and  $\zeta_{\text{O}}$ .<sup>14</sup> A series of calculations of  $\Delta g_{ii}$  using all molecular orbitals 11–21 (see Table III) were performed to confirm this. System 11 leads to an effective charge of 0.74– on the oxygen molecule which is significantly less than the assumed 1.0–. The oxygen–oxygen bond length in Rodley and Robinson's adduct is 1.26 Å, compared with 1.28 Å in the superoxide and 1.208 Å in neutral oxygen.<sup>9</sup> This intermediate distance can be interpreted as corresponding to an effective charge of 0.78–, assuming linear interpolation, and this is in close agreement with that charge calculated here.

Inspection of Tables III and IV allows the following comparisons between oxycoboglobin and oxyhaemoglobin to be made. In system 10 the  $z^2$  antibonding orbital is of higher energy than the  $x^2-y^2$ , while the reverse holds for system 11. If metal–porphyrin sigma bonding is assumed to be comparable in the two adducts, the metal–oxygen bonding through the  $z^2$  orbital is stronger in oxyhaemoglobin than oxycoboglobin. Conversely, the relative energies of the antibonding

$d_\pi$  orbitals imply greater metal–oxygen pi bonding in oxycoboglobin than oxyhaemoglobin. The oxygen molecule is calculated to bear a greater negative charge in oxyhaemoglobin than in oxycoboglobin but this is largely due to the extra two electrons included in the calculation for system 10. A measure of the relative bond strengths of the metal– $\text{O}_2$  bond can be made by comparing the net overlap populations for the two adducts. Cobalt is seen to form the stronger bond.

#### Deoxy-derivatives and Adduct Formation

All of systems 1–8 converged within a satisfactory tolerance by the twelfth cycle. Since haemoglobin is known to be high-spin and coboglobin low-spin<sup>3</sup> these cases were of particular interest, but for completeness all eight cases were studied. A full tabulation of results is not presented, only those pertinent to a discussion on metal geometry being given. Table V gives the calculated energy and d electron configuration for systems 1–8.

The calculated total energies can be used to predict the most stable configuration of the four cases for each metal. System 3, which corresponds to high-spin non-planar iron(II) porphyrin is predicted to be the most stable iron species, while system 8, corresponding to low-spin non-planar cobalt(II), is predicted to be the most stable cobalt species. In spite of the neglect of Coulomb repulsion terms in EHT, these calculations have predicted the correct spin state for both coboglobin and haemoglobin. More importantly, both metals are expected to lie out of the porphyrin plane, in agreement with Perutz's<sup>5</sup> deductions for haemoglobin.

The calculated EFG for system 3 is of the correct sign and relative magnitude (see Table II), providing the support for this model. It is interesting to note that the strong  $d_\pi$ -porphyrin  $\pi$  bonding in planar coboglobin is sufficient to raise the antibonding  $d_\pi$  orbitals to a higher energy than the  $z^2$ . The consequent  $\Delta g$  values for system 6 do not agree with experiment, demonstrating the similar geometries of coboglobin and haemoglobin, viz both have out of plane metal ions.

TABLE V. Resultant configurations for deoxy metal porphyrins.

System	Configuration	Total Energy
1	$(xy)^2(d_\pi)^2(z^2)^1(x^2-y^2)^1$	-332.62
2	$(xy)^2(d_\pi)^4$	-320.30
3	$(xy)^2(d_\pi)^2(z^2)^1(x^2-y^2)^1$	-332.93
4	$(xy)^2(d_\pi)^4$	-331.39
5	$(xy)^2(z^2)^2(d_\pi)^2(x^2-y^2)^1$	-324.98
6	$(xy)^2(z^2)^2(d_\pi)^3$	-324.62
7	$(xy)^2(d_\pi)^3(z^2)^1(x^2-y^2)^1$	-330.10
8	$(xy)^2(d_\pi)^4(z^2)^1$	-359.28

Oxyhaemoglobin has been assumed to have near planar iron<sup>5</sup> and the reported structure<sup>9</sup> of the cobalt–oxygen adduct does indeed show a planar arrangement. Since pi bonding has been deduced to be important in oxygen binding, the effect on the  $3d_{\pi}$  orbitals of the change in metal geometry is of particular interest. Cobalt is low-spin in both the deoxy- and oxy-forms<sup>3</sup> so results for systems 6 and 8 only are pertinent. The calculated metal character in the antibonding  $3d_{\pi}$  orbitals changes from 88% to 65% with the movement of the cobalt into the porphyrin plane, with a further decrease to 25% in the oxygen adduct. Clearly, the transition from non-planarity to planarity leads to a marked increase in pi bonding, prior to oxygenation.

Similarly, the iron porphyrin is calculated to exhibit a decrease in  $d_{\pi}$  character. In non-planar high-spin haem the  $d_{\pi}$  orbitals have 80% metal character and this reduces to 20% in the planar low-spin deoxy system.

The metal–fifth ligand distance was maintained at 1.9 Å for all calculations so the effect on the  $z^2$  orbital is essentially constant. However the total movement of the metal–imidazole unit must influence the protein chain and has been postulated to be the origin of the co-operative effects.<sup>5</sup> The results presented here infer that the transition of the metal into the porphyrin plane is prompted by the need for substantial pi-bonding in order to achieve oxygen binding.

## Conclusions

The MO calculations reported here predict distinctly different electron configurations in the respective oxygen adducts of haemoglobin and coboglobin. Both systems have a large negative charge on the oxygen molecule but the iron exists formally as  $d^6$  while the cobalt as  $d^5$ . The unpaired electron is calculated to have 25%  $3d_{\pi}$ , 20%  $O_2$ , and 55% porphyrin pi character. ESR and Mössbauer data have been employed to support the proposed configurations. The extra two electrons proposed earlier (1) as a necessary requirement for the formation of a stable iron–oxygen adduct are confirmed, but coboglobin does not need them. In a subsequent paper it will be shown that this two electron donation is unique to the iron–oxygen system; the apparently analogous nitroso and carbon-monoxide adducts of both coboglobin and haemoglobin do not demand this donation.

Perutz<sup>5</sup> has suggested that the 0.8 Å displacement of the iron in the haemoglobin–oxyhaemoglobin reaction is sufficient to trigger the well known co-operative effects which accompany ligand binding. The calculations reported here demonstrate that strong pi bonding of metal to oxygen, and metal to porphyrin is associated

with the change in metal position. This pi bonding may directly influence the protein structure through the interaction of the globin with porphyrin substituents and/or cause the transition of the metal into the porphyrin plane which in turn triggers the co-operative effects.

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