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MO Calculations on Nitrosyl and Carbon Monoxyde Adducts of Haemoglobin and Coboglobin

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Bonding in the nitric oxide and carbon monoxide adducts of haemoglobin and coboglobin has been investigated using SCC–EH–MO theory. Strong pi bonding is evident in all cases and metal–ligand orbital mixing has occurred to such an extent that formal valence has little meaning. Carbon monoxide is predicted to form the stronger bond with both metals. Mössbauer and esr data have been successfully calculated in appropriate cases to test the theoretical model and predicted electron configurations.

Introduction

In an earlier paper[1] (denoted here by I) the results of SCC–EH–MO (Self Consistent Charge Extended Hückel Molecular Orbital) calculations on oxygen adducts of haemoglobin and coboglobin were reported. Comparisons with the corresponding deoxy systems were also made. Although oxygen binding to these molecules has been of prime interest because of the biological importance, interest has also centred on the nitrosyl and carbonmonoxy adducts since similar bonding might be expected to occur.[2–4] Reports on MO studies on the ligand binding to iron and cobalt porphyrins are continued in this paper where the nitrosyl and carbonmonoxy adducts are examined.

Zerner *et al.*[5] have performed a MO calculation on the carbonmonoxy adduct of haem using a more extensive basis set. This adduct was considered here using the current model so that a direct comparison with the cobalt analogue and other adducts could be made. One of the results in a previous report of MO calculations,[6] and discussed again in I for the cobalt case, was that two extra electrons must be added to the iron–oxygen moiety for a stable adduct to form. Oxycoboglobin was found not to demand these electrons. An object of the current work was therefore to investigate the possible need of extra electrons in the nitrosyl and carbonmonoxy adducts.

Method

SCC–EH–MO calculations were performed on the same model as described in I, and Table I lists the systems studied. Figures 1 and 2 respectively illustrate the metal porphyrin and geometry of the general metal adduct. For each adduct two sets of calculations were performed, one including the extra two electrons and one not. In all cases the metal was assumed to lie in the porphyrin plane and the metal–porphyrin bond lengths used in I were retained, together with the simulation of the fifth ligand. The iron–nitrogen bond length

TABLE I. Adducts studied.

System	Species Simulated[a]	Spin	Details
1	HbNO	$^1/_2$	
2	HbNO	$^1/_2$	2 extra electrons
3	CbNO	0	
4	CbNO	0	2 extra electrons
5	HbCO	0	
6	HbCO	0	2 extra electrons
7	CbCO	$^1/_2$	
8	CbCO	$^1/_2$	2 extra electrons

[a] Hb = haemoglobin; Cb = coboglobin.

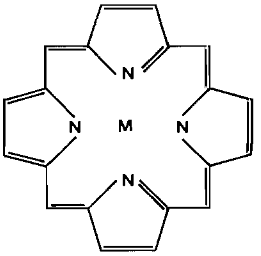

Figure 1. Metal Porphyrin.

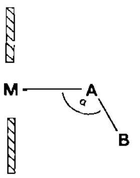

Figure 2. Geometry of General Metal Porphyrin Adduct.

was assumed to equal that observed in nitroprusside[7] and the cobalt–nitrogen distance estimated to be 0.04 Å shorter (as assumed in I for the oxygen adducts). The nitrogen–oxygen distance was taken from that observed in an iridium complex[8] where the nitrosyl group is bound in an angular fashion. Chien[3] has deduced that the Fe–N–O angle is 110° in nitrosylhaemoglobin and this angle was assumed for both iron and cobalt. Metal carbonyls are generally linear and bond lengths were taken from typical metal carbonyl complexes.[9] The iron adducts are known to be low-spin[10] and the appropriate spin was therefore imposed upon the calculations. Oxycoboglobin is observed to be low-spin[4] and from a comparison with the iron analogues, both nitrosyl and carbonmonoxycoboglobin were assumed to be low-spin also.

As in I, the electric field gradient, EFG, at the iron nucleus as observed in the Mössbauer spectrum, and the esr g parameters were calculated to test the proposed electron configurations. The parameters B and η as defined in I were again employed as the theoretical measure of the EFG, and the methods of calculating B, η, and g_{ii} from the MO coefficients is not repeated here.

Results and Discussion

Tables II–V list the pertinent results of the calculations but these are best discussed under the following subsections. For convenience, C_{2v} symmetry has been assumed in describing the molecular orbitals where the following symmetry labels apply:

$$xy \sim a_2;\ xz \sim b_1;\ yz \sim b_2;\ a_1 \sim z^2;\ a_1 \sim x^2\text{–}y^2$$

In the nitrosyl derivatives the d_π orbitals were found to mix such that

$$\Psi(b_1) = -\alpha xz + \alpha yz + \text{ligand orbitals} \quad (1)$$

$$\Psi(b_2) = \beta xz + \beta yz + \text{ligand orbitals} \quad (2)$$

where α and β are the calculated molecular orbital coefficients.

The use of the parameter B to estimate the EFG was found to give a good correlation with experimental quadrupole splittings in an earlier paper,[6] and limita-

TABLE II. Experimental and theoretical electric field gradient parameters.

System	Theory		Exptl. ΔE_Q[a] mm/sec
	B	η	
HbNO: 1	−0.231	0.004	~ 1.0
HbNO: 2	+0.349	0.00	~ 1.0
HbCO: 5	+1.260	0.00	0.36
HbCO: 6	+0.036	0.00	0.36
HbO_2[b]	−0.438	0.05	−2.25
Hb	+0.485	0.00	+2.40

[a] From Ref. No. 2. [b] Results for oxyhaemoglobin and haemoglobin are reproduced from I for comparison.

TABLE III. Calculated Δg parameters.[a]

System	Theory			Exptl.[b]		
	Δg_{xx}	Δg_{yy}	Δg_{zz}	Δg_{xx}	Δg_{yy}	Δg_{zz}
HbNO						
1	$\frac{-0.37}{\Delta_1}$ $\frac{-0.17}{\Delta_2}$	$\frac{0.18}{\Delta_3}$	$\frac{-0.065}{\Delta_4}$	+0.080	+0.023	−0.001
2[c]	$\frac{0.17}{\Delta_2}$ $\frac{+0.56}{\Delta_2'}$	$\frac{0.17}{\Delta_5}$ $\frac{-0.23}{\Delta_5'}$	$\frac{3.76}{\Delta_6}$	+0.080	+0.023	−0.001
1a[d]	$\frac{-0.54}{\Delta_1}$ $\frac{-0.18}{\Delta_2}$	$\frac{0.18}{\Delta_3}$	$\frac{0.065}{\Delta_4}$	+0.080	+0.023	−0.001
1b	$\frac{0.18}{\Delta_3}$	$\frac{-0.54}{\Delta_1}$ $\frac{-0.18}{\Delta_2}$	$\frac{0.065}{\Delta_4}$	+0.080	+0.023	−0.001
CbCO						
7	$\frac{0.15}{\Delta_1}$	$\frac{0.15}{\Delta_7}$	0	–	–	–

[a] $\Delta_1 = |E_{z2}-E_{b2}|$; $\Delta_2 = |E_{x2-y2}-E_{b2}|$; $\Delta_3 = |E_{xy}-E_{b2}|$; $\Delta_4 = |E_{b2}-E_{b1}|$; $\Delta_5 = |E_{x2-y2}-E_{b2}|$; $\Delta_6 = |E_{xy}-E_{x2-y2}|$; $\Delta_7 = |E_{z2}-E_{b1}|$. [b] Data from Ref. No. 3. [c] For system 2 there were two b_2 type MO's with significant d_π character (see Table IV). [d] See text for description of configurations 1a and 1b.

TABLE IV. Calculated molecular orbitals and energy levels.

A. System 1: HbNO

MO	No. elects.	Energy, ev	Symmetry	Coefft. Metal Orbs.	%N_π[a]	%NO[a]
11	2	−10.935	b_1	$-0.60xz + 0.60yz$	2.5	20
12	2	−10.755	a_2	$-0.92xy + 0.23xz + 0.23yz$	–	2
13	2	−10.680	b_2	$0.38xy + 0.57xz + 0.57yz$	2.5	4
14	2	−9.934	a_1	$0.51z^2$	1.5	70
15	2	−9.695	a_1	–	25	–
16	2	−9.604	a_2	–	25	–
17	2	−9.518	b_1	$0.1xz - 0.1yz$	20	16
18	1	−9.376	b_2	$0.3xz + 0.3yz$	20	2
19	0	−9.197	a_1	$0.97(x^2 - y^2)$	–	–
20	0	−8.743	b_1	$-0.34xz + 0.34yz$	2	75
21	0	−7.251	a_1	$0.83z^2$	2	28

B. System 3: CbNO

MO	No. elects.	Energy, ev	Symmetry	Coefft. Metal Orbs.	%N_π[a]	%NO[a]
11	2	−11.213	b_1	$-0.58xz + 0.61yz$	1.5	25
12	2	−11.043		$0.68xy - 0.32xz$ $0.32yz - 0.36z^2$	–	16
13	2	−10.951		$0.72xy + 0.40xz + 0.37yz$ $+ 0.25z^2$	–	8
14	2	−10.808	b_2	$-0.2z + 0.15xy - 0.41xz$ $-0.38yz + 0.51z^2$	2	20
15	2	−9.778	a_1	–	20	20
16	2	−9.659	a_2	–	16	36
17	2	−9.640	a_2	–	25	–
18	2	−9.421	b_2	$-0.27xz - 0.27yz$	18	–
19	0	−9.334	a_1	$0.96(x^2 - y^2)$	–	–
20	0	−9.082	b_1	$-0.34xz + 0.38yz$	3	62
21	0	−8.326	a_1	$0.20z + 0.64z^2$	1.5	54

C. System 5: HbCO

MO	No. elects.	Energy, ev	Symmetry	Coefft. Metal Orbs.	%N_π[a]	%CO[a]
11	2	−11.646	b_2	$0.73xz + 0.55yz$	3	5
12	2	−11.646	b_1	$0.55yz - 0.73xz$	3	5
13	2	−11.430	a_2	$1.00xy$	–	–
14	2	−10.023	a_1	–	25	–
15	2	−9.900	a_2	–	25	–
16	2	−9.790	b_2	$0.2yz$	22.5	6
17	2	−9.790	b_1	$0.2xz$	22.5	6
18	0	−9.745	a_1	$0.97(x^2 - y^2)$	–	–
19	0	−9.127	a_1	$0.96z^2$	–	15

D. System 7: CbCO

MO	No. elecs.	Energy, ev	Symmetry	Coefft. Metal Orbs.	$\%N_\pi$[a]	%CO[a]
11	2	−10.966	b_2	0.86yz	3.5	10
12	2	−10.966	b_1	−0.86xz	3.5	10
13	2	−10.630	a_2	1.00xy	–	–
14	2	−10.375	a_1	$-0.24z + 0.58z^2$	13	–
15	2	−9.903	a_2	–	25	
16	2	−9.730	b_2	0.31yz	20	10
17	2	−9.730	b_1	−0.31xz	20	10
18	1	−9.522	a_1	$-0.73z^2$	11	6
19	0	−9.079	a_1	$-0.98(x^2-y^2)$	–	–

TABLE V. Results of Mulliken Population Analysis.

System	1 HbNO	3 CbNO	5 HbCO	7 CbCO
4s[a]	0.515	0.619	0.596	0.655
$4p_x$	0.269	0.356	0.288	0.325
$4p_y$	0.269	0.364	0.288	0.325
$4p_z$	0.170	0.311	0.279	0.429
$3d_{xy}$	2.000	1.999	2.000	2.000
$3d_{xz}$	1.730	1.792	1.806	1.711
$3d_{yz}$	1.731	1.752	1.806	1.711
$3d_{z2}$	0.895	1.216	0.392	1.390
$3d_{x2-y2}$	0.306	0.345	0.346	0.272
Metal Chge	0.113+	0.248+	0.200+	0.181+
NOP:A[b]	0.603	0.816	1.033	1.010
NOP:B[b]	0.556	0.443	0.658	0.622
Chge: A	0.245–	0.251–	0.080+	0.054–
Chge: B	0.359–	0.365–	0.120–	0.245–
Chge: N/4	0.424–	0.460–	0.596–	0.520–

[a] Gross Atomic Populations. [b] Ligand atoms A and B as in Figure 2.

tions of its use are not repeated here. However, the calculated B values reported in I for haemoglobin, where the same theoretical model was employed as in the present work, are included in Table II for comparison. Because EHT does not provide reliable absolute orbital energies, Δg_{ii} values were calculated as a function of orbital energy differences, Δ, and trends compared with experiment (see Table III).

Nitrosyl adducts

Both iron–nitrosyl systems converged satisfactorily but with very different results. System 1 predicts a configuration which can be described formally as either d^3 or d^5 but the d_π orbitals are so mixed with the ligand pi system that formal valence has little meaning. The effective charge distribution is found to be $Fe^{0.113+}$ $N^{0.245-}$ $O^{0.359-}$. In contrast system 2 has a formal configuration of d^7 and effective charge distribution of $Fe^{0.117-}$ $N^{0.390-}$ $O^{0.553-}$. This significantly negative charge on the iron infers that the extra two electrons in system 2 exaggerates any electron donation not described by the theoretical model. However, these two configurations can be more rigorously distinguished from a comparison of Δg_{ii} and B values (see Tables II and III).

A small quadrupole splitting is evident in the Mössbauer spectrum[2] of nitrosylhaemoglobin but the sign of the EFG has not been determined. The small negative EFG calculated for system 1 can account for the observed quadrupole splitting but the large positive EFG predicted from system 2 cannot be reconciled with experiment. It is interesting to note that for both systems, in spite of the asymmetric geometry assumed, the asymmetry parameter η is calculated to be negligible. An analogous result was found for oxyhaemoglobin.[1,6] The unpaired electron is predicted to have 10% d_π, and 90% porphyrin nitrogen pi character in system 1, but 90% x^2-y^2 and 40% NO in system 2. Different Δg_{ii} values are therefore expected.

Experimental Δg_{ii} values for nitrosylhaemoglobin are positive for Δg_{xx} and Δg_{yy} but negative for Δg_{zz}[3] and neither system 1 nor 2 predicts all three Δg_{ii} values correctly. However, Δg_{zz} is generally the most accurately determined from the esr spectrum, and if the correct prediction of Δg_{zz} is employed as a criterion, system 1 is seen to be the better description. For completeness though, two other probable configurations were considered independently of the MO calculation, viz

1a: $(xy)^2(\pi b_1)^2(\pi b_2)^1(z^2)^0(x^2-y^2)^0$
1b: $(xy)^2(\pi b_2)^2(\pi b_1)^1(z^2)^0(x^2-y^2)^0$

and Δg_{ii} values calculated (see Table III). The orbitals πb_1 and πb_2 are as defined by eqs.1 and 2 with the MO coefficients α and β guessed at 0.3, but the other 3d orbitals assumed to be pure metal in character. Since trends of Δg_{ii} values are of interest these assumptions are not critical. In both cases only one Δg_{ii} value is correctly predicted and in neither case is it Δg_{zz}.

In view of the Mössbauer and esr results system 1 is considered to be the best representation of the bonding in nitrosylhaemoglobin.

Of the two simulations of nitrosylcoboglobin only system 3 converged. This was taken to infer that two extra electrons are not required by the Co–NO moiety to form a stable adduct. System 3 predicts a formal configuration of either d^4 or d^6 but as with the iron analogue the pi bonding is too marked for formal valence to be useful. Results for system 3 are presented in Tables IV and V; Mössbauer and esr parameters are inappropriate for nitrosylcoboglobin (cobalt is not a Mössbauer nucleus and this adduct was assumed to be diamagnetic). A comparison with the other adducts is delayed until after the section on carbonmonoxy derivatives.

Carbonmonoxy adducts

As with the nitrosyl adducts, both iron cases converged. System 5 predicts a formally d^6 configuration and charge distribution of $Fe^{0.200+}$ $C^{0.080+}$ $O^{0.120-}$ while system 6 predicts a d^8 configuration and charge distribution of $Fe^{0.183-}$ $C^{0.350-}$ $O^{0.447-}$. Again the negative charge on the iron in the latter case infers an exaggeration of any electron donation. However, the Mössbauer data can be employed to confirm the choice of system 5 (esr is not applicable). The experimental quadrupole splitting is very small and system 5 successfully predicts a small positive EFG. On the other hand, system 6 predicts an abnormally large EFG which cannot be reconciled with experiment. Thus system 5 is considered to be an appropriate representation of carbonmonoxy haemoglobin.

Of the two cobalt cases, only system 7 converged, and to a (formal) d^7 configuration. The unpaired electron is calculated to have 53% d_{z^2}, 44% porphyrin nitrogen pi and 3% NO orbital character. The Δg_{ii} values were calculated but no experimental data is yet available for comparison. Results for oxycoboglobin were presented in I and comparison with these shows that the esr spectra of the two adducts are predicted to be quite different.

Comparison of pi bonded metal adducts

The choice of suitable representations of the nitrosyl and carbonmonoxy adducts has been reported above. A comparison of general features is now made and reference to the oxygen adducts discussed in I is made for completeness.

An important result of these calculations is that only oxyhaemoglobin demands two extra electrons to be added to the metal sixth ligand system in order to form a stable adduct. The theory of oxygen binding to haem presented earlier[1,6] is therefore unique to the haemoxygen system. However the discussion reported in I on the significance of the movement of the metal ion into the porphyrin plane, upon ligand binding, is common to all three ligands (O_2, NO, CO). The MO's reported here and in I all show considerable mixing of the $3d_\pi$ orbitals with both the sixth ligand and porphyrin pi system. Strong bonding through the z^2 orbital also occurs, particularly for the Co systems since the z^2 MO coefficient is markedly less than unity.

Both nitrosyl adducts are calculated to bear a greater negative charge than the corresponding carbonmonoxy derivatives, but this is not paralleled by an increased positive charge on the metal. Instead, the porphyrin nitrogens show a corresponding decrease in negative charge in the nitrosyl adducts, inferring that an electron donation to the metal has occurred to compensate for the transfer to the sixth ligand. This suggests that substituents on the porphyrin ring with an increase in pi electron donating power will leads to a greater increase in NO affinity than for CO.

A comparison of the total net overlap populations for the general ligand AB shows that, for both iron and cobalt, the ligands are bound with increased strength in the order $O_2 > NO > CO$. The greater affinity for carbonmonoxide than molecular oxygen by haemoglobin is well established[11] and of biological importance. However, attempts to observe carbonmonoxycoboglobin have not been successful[4] although these MO results predict a stable adduct can form. No attempts to prepare nitrosylcoboglobin have been reported but this too is predicted to be stable.

Conclusion

MO calculations on these systems has led to a description of their bonding and electron distribution, and Mössbauer and esr data have been satisfactorily accounted for. Bonding in both carbonmonoxy derivatives is much as would be expected from simple bonding concepts;[9] the iron and cobalt adducts are unambiguously d^6 and d^7 respectively while significant pi bonding is evident in both cases. However the carbonmonoxide ligand bears only a small negative charge which is concentrated on the oxygen atom. In contrast, the nitrosyl derivatives show no obvious formal configuration because of the extent of metal–ligand orbital mixing. There is a greater net electron transfer to the nitrosyl group than in the carbon monoxide analogues but the total bonding to the metal is stronger with the latter ligand.

Although molecular oxygen, nitric oxide and carbon monoxide might have been expected to exhibit similar bonding, the current MO results indicate significant differences. However in all cases strong pi bonding is involved. The discussions reported in I on the implications of this pi bonding in relation to the cooperative effects is appropriate for the nitrosyl and carbon monoxide ligands also.

Acknowledgements

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