Molecular description of dioxgen bonding in hemoglobin

(porphyrin/myoglobin/cooperativity/generalized valence bond/configuration interaction)

BARRY D. OLAFSON AND WILLIAM A. GODDARD III*

Arthur Amos Noyes Laboratory of Chemical Physics, California Institute of Technology, Pasadena, California 91125

Communicated by Harry B. Gray, January 31, 1977

ABSTRACT From ab initio quality calculations on model systems, we conclude that in unliganded Fe-porphyrin the Fe lies in the plane for both the high-spin (q) and intermediate-spin (t) states. Thus, the high-spin 4d4 Fe is not too big to fit into the porphyrin plane (as often suggested). We find the q state lower for a porphyrin hole radius >1.94 Å and the t state lower for smaller sizes. For the five-coordinate complex including an axial nitrogenous ligand [a model for myoglobin (Mb) and hemoglobin (Hb)], we find the ground state to be q with the Fe 0.3 Å out of the plane (recent x-ray data on deoxy Mb suggests about 0.4 Å). The origin of this out-of-plane displacement is the nonbonding repulsions between the axial ligand and porphyrin nitrogen orbitals. Pushing the Fe of the five-coordinate complex into the plane does not lead to a stable low-spin state (as usually suggested), the q and t states being the low-lying states.

Bonding the O₃⁻ form the six-coordinate complex stabilizes the t form of the Mb model, leading to a singlet state of MbO₂ with Fe in the plane. (It has often been suggested that the Fe of MbO₂ and HbO₂ is low-spin Fe²⁺; however, we find this is not to be the case.) The bonding in the MbO₂ model confirms the ozoene model of the bonding, leading to a structure consistent with the Pauling model (our calculated Fe=O bond angle is 115°). The total energy transfer to the O₂ is 0.10 electron, in disagreement with the Weiss model. Molecular orbital calculations (Har-tree-Fock) incorrectly lead to a septet ground state (S = 3) for the MbO₂ model.

The implications for the cooperative O₂ binding in hemoglobin and protein modifications of the energetics of the active site are discussed. Use of our calculated force constants for displacement of Fe perpendicular to the heme plane suggests that the movement of the Fe upon a change in the quaternary structure from the T to the R form is only about 0.04 Å toward the heme plane.

We will discuss the bonding of oxygen to hemoglobin (Hb) or myoglobin (Mb) using the results of theoretical calculations of the electronic structure of model systems representing Fe-porphyrin, Fe-porphyrin plus an additional nitrogenous axial ligand (deoxy Mb), and O₂ bound to the latter five-coordinate complex (MbO₂). In all cases the Fe is found to be high-spin or intermediate-spin (S = 2 or 1) with six electrons in the d orbitals. In no case (not even with the six-coordinate complexes) do we find a low-lying state in which the Fe is in a low-spin state (d²S = 0). We find that the properties of these model complexes are consistent with the observed properties of the active sites of Mb and Hb and that these calculations give additional insight into the protein-modified behavior of the active site of Hb. In this paper we outline the qualitative description that emerges; detailed results will appear elsewhere.

Model calculations

As a model for the theoretical study of the bonding of O₂ to Mb and Hb, we have used four NH₂ groups in a plane (D₁h symmetry) to represent the four-coordinate porphyrin ligand and an NH₃ group to represent the axial methionine ligand. Ab initio quality calculations including electron correlation (many-body) effects [generalized valence bond (26) plus configuration interaction] were carried out for a number of different molecular geometries of the four-, five-, and six-coordinate complexes.

The four-coordinate calculations were carried out with both minimal basis set and valence double zeta basis, the other calculations used only the former. An effective potential was used to replace the 18 core electrons of Fe (1-3). All of our model systems have overall charge neutrality, as is appropriate for the systems being modeled.

By representing the porphyrin ligand as four NH₂ groups, we lose the geometric stabilization resulting from the bridging methene groups. We have made no attempt to correct our calculated results for this effect in this paper, and therefore our unstrained model system will lead to optimized geometries in which the hole radius is artificially smaller than the corresponding porphyrin system. This effect appears to result in calculated porphyrin N-Fe distances that are systematically underestimated by about 0.03 Å.

Four-coordinate model complexes

First we consider the four-coordinate complex with the x and y axes along the Fe-N bonds. The two important states are high-spin Fe (denoted as q for quintet, S = 2) and intermediate-spin Fe (denoted as t for triplet, S = 1)

q: (dₓ²z²)(dₓy)(dᵧz)(dᵧz)(dₓ₋ᵧz)

and intermediate-spin Fe (denoted as t for triplet, S = 1)

t: (dₓ²z²)(dₓy)(dᵧz)(dᵧz)

A third state, corresponding to low-spin Fe (denoted as s for singlet, S = 0)

s: (dₓ²z²)(dₓy)(dᵧz)

is also considered because it has often been thought to be important for the five- and six-coordinate cases. Other states with different configurations of d electrons were calculated, but the above states were the lowest lying states of their respective spin.

Abbreviations: Hb, hemoglobin; Mb, myoglobin; q, high-spin; t, intermediate-spin; s, low-spin.

Contribution no. 5449 from the Arthur Amos Noyes Laboratory of Chemical Physics.

† To whom correspondence should be addressed.

‡ The geometry used was R₉H = 1.01 Å and R₁₁H₁₁ = 1.20 Å. The four NH₂ groups were always taken with C₅₅ symmetry.

¶ There is one correlated pair involving the porphyrin N° system in all calculations; in cases having an empty dₓ₋ᵧz orbital, one porphyrin N° pair is correlated; in the oxygenated complex, the FeO and FeO bond distances are correlated to the Fe=O bond distance (see ref. 19). An extensive configuration interaction was carried out over the doubly occupied valence orbitals, the open-shell orbitals, and the correlated orbitals (17 total orbitals), allowing up to 3056 spin eigenfunctions (4456 determinants).

The basis set used for Fe was a modification of the Gaussian basis of Wachtler ([1979] J. Chem. Phys. 52, 1033-1056) used (5d4s4p) contracted to (1d1s)₉ for the valence double zeta calculations and (2s2p)₉ for valence double zeta calculations. The Pope's Stock-4G basis was used for O, N, and H in the minimum suite of calculations ([1969] J. Chem. Phys. 51, 2657-2661) and the Pope's Stock-4 suite was used for N in the valence double zeta calculations ([1971] J. Chem. Phys. 54, 724-728).
for all the complexes we considered. Optimizing the Fe–N bond length of the four-coordinate complex, we find the optimal bond length of the q state to be 1.95 Å, which is 0.14 Å longer than the optimal bond length, 1.81 Å, found for the t state. This difference is due to the q state containing an electron in the antibonding \( d_{z^2} \) orbital; however, our calculated optimal Fe–N bond distance for high-spin Fe of 1.95 Å is far shorter than the value (approximately 2.2 Å) previously estimated (4, 5). The reason for this discrepancy is that previous estimates were based on Fe and Co complexes consisting of saturated nitrogen ligands (possessing a coordinate bond to the Fe), whereas the ligands of our complex (and porphyrin) should be viewed as partially unsaturated (two of the four Fe–N complexing bonds have significant covalent character).

Of course the constraints in the porphyrins do not easily allow radii less than 1.9 Å (free base porphyrins have hole radii of about 2.05 Å (6)); however, Baldwin and Huff (7) have made an analogous planar four-coordinate complex which has a much shorter unconstrained radius, and they find an "anomalously" short Fe–N bond of 1.84 Å (8); this is in good agreement with our value of 1.81 Å for the t state.

We find that the t state is lower than the q state for hole radii ≤1.94 Å. The calculations clearly suggest that the Baldwin–Huff compound has a triplet ground state [in agreement with the measured experimental magnetic moment of 2.8 \( \mu_B \) at 28° (8)]. Fe–porphyrin systems with Fe–N distances close to 1.94 Å should have a small difference in energy between the q and t states [e.g., Fe tetraphenylporphyrin has Fe–N = 1.972 Å and a magnetic moment of 4.4 \( \mu_B \) at 25° (9)]. We find that the s state is 2.9 eV higher than the q state (for a hole radius of 2.01 Å); consequently, geometry searches were not carried out for this state.

An important result from these calculations is that, even for porphyrin hole radii as small as 1.85 Å, the optimal position of the Fe is in the plane for the q state (and for the t state). That is, even for hole radii as small as 1.85 Å, the attraction of the Fe atom for the macrocycle (favoring the planar geometry) is larger than the antibonding interactions of the \( d_{z^2} \) orbital (favoring nonplanarity). This is contrary to the commonly accepted belief (13) that high-spin Fe is too large to fit into the porphyrin pocket and hence, that in order to form high-spin complexes, the Fe must move out of the porphyrin plane. However, the presence of the electron in the \( d_{z^2} \) antibonding orbital does lead to a smaller force constant for out-of-plane motion in the q state as compared with the t state. This difference in force constants is important in analyzing the cooperativity of Hb, as discussed below.

Five-coordinate model complexes

It has long been known that five-coordinate Fe–porphyrin complexes are generally high-spin (14, 15) with the Fe significantly out of the porphyrin plane (5, 16, 17). The usual explanation is that high-spin Fe is too big to fit in the plane of the porphyrin. The above calculations contradict this argument since high-spin Fe does lie in the porphyrin plane for the unliganded Fe–porphyrin complex for the range of accessible porphyrin hole radii.

To model dexeony Hb we used N1 of the fifth ligand and optimized the Fe–to-plane distance and also the Fe–N1 distance. The results are: (i) the ground state is with the Fe 0.25 Å out of the plane, (ii) the state is 20 kcal higher and only 0.13 Å out of the plane, and (iii) for the Fe 0.25 Å out of the plane, the Fe–N1 distance is 2.1 Å.

The five-coordinate Fe porphyrin systems on which crystal structure information is available all possess a domed porphyrin ring—e.g., in Fe tetraphenylporphyrin (2-methylimidazole) the porphyrin N plane is displaced 0.13 Å from the mean porphyrin plane and the pyrrole groups rotate by about 9° (18). We considered such doming in our model and find that the optimal Fe position is 0.28 Å from the N plane. In comparing with experiment, we will ignore any corrections resulting from replacing the NH3 axial ligand with imidazole.

Comparisons with experiment are difficult because no published crystal structure analysis has led to a reliable value for the distance of the Fe from the porphyrin on an analogous system. Radonovich and Hoard (18) examined Fe 2-methyl imidazole and found that the Fe is 0.42 Å out of the N plane and 0.55 Å out of the mean porphyrin plane. The methyl group leads to a short contact with the porphyrin and hence may lead to an increase over the value for unsubstituted imidazole. Recent high-resolution (2 Å) x-ray diffraction studies on Mb (T. Takano, personal communication) lead to Fe 0.55 Å from the mean porphyrin plane with the Fe about 0.42 Å from the porphyrin N plane, suggesting that the protein causes at most only a slight increase in the Fe–porphyrin separation.

Since the q state has the same electronic configuration for four- and five-coordinate complexes and since the high-spin Fe is in the plane for the four-coordinate, a natural question is, "Why does the Fe move so far out of the plane for five-coordinate complexes?" We find that the answer is nonbonded interactions between the bond pairs of the fifth ligand and the N orbitals of the porphyrin.

To test this idea we repeated the calculations of the five-coordinate complex but with the Fe atom deleted. Moving the axial ligand from infinite separation to a position 2.1 Å from the porphyrin–N plane (appropriate for a five-coordinate complex with Fe in the plane) leads to an increase of 15 kcal in the energy of the system. The origin of this energy increase is just the nonbonded repulsions (arising from the Pauli principle) between the NH3 and porphyrin electron pairs. As a result, Fe moves out of the plane until there is a balance between the five attractive Fe–N interactions and the repulsive nonbonded interactions. These calculations are a strong indication that the size of high-spin Fe is not the only major factor in determining the Fe out-of-plane distance for five-coordinate complexes.

These nonbonded interactions are sensitive to porphyrin ring size. Decreasing the porphyrin hole size from 2.01 Å to 1.85 Å leads to a doubling in the calculated distance of the Fe from the N plane. However, the calculated force constant increases by only 13%.

For the five-coordinate complex (q state, Fe 0.3 Å out of plane) we find an optimal porphyrin hole radius 0.02 Å longer than that found for the four-coordinate q state.

O2 bonded to the five-coordinate complex

Next we consider the bonding of dioxygen to the five-coordinate complex. It has generally been assumed that pushing the Fe into the porphyrin plane (as required for the six-coordinate
oxygenated complex) stabilizes the s state of Fe, leading hence to the observed diamagnetic character. We find that the s state plays no role, lying about 2 eV above the ground state.

The current calculations including all six ligands confirm our "ozone model" for the bonding of dioxygen to Hb (19). Thus, we find that (i) a covalent sigma bond is formed between the d_{2z} orbital of Fe and an oxygen p orbital and (ii) the d_{yz} orbital is paired with the three-electron pi system of the O2 ligand. The result is that bonding O2 to the t state of Mb leads to an overall diamagnetic singlet state (S = 0) for MnO2.

\[ t: [(d_{2z})^1, (O_2\sigma)^1] \\
[(d_{yz})^1, (O_2\pi)^1] \\
(d_{xy})^2 \\
(d_{xz})^2 \]

in which orbitals in brackets correspond to bond pairs. Similarly, bonding O2 to the q state leads to an overall paramagnetic triplet state (S = 1) for MnO2:

\[ q: [(d_{2z})^1, (O_2\sigma)^1] \\
[(d_{yz})^1, (O_2\pi)^1] \\
(d_{xy})^1 \\
(d_{xz})^2 \\
(d_{z^2})^1. \]

With either state, in order for O2 to form a bond with Fe, the Fe must move back into the plane of the porphyrin to decrease the repulsive nonbonded interactions between O2 and the porphyrin nitrogens.

The question now is: "Which state, q or t, should be lower for MnO2?" We find that, even with the Fe in the plane of the five-coordinate complex, the q state is lower than the t state (for porphyrin radii >1.95 Å); hence, one might expect that the paramagnetic q state (Eq. 4) of MnO2 would be the ground state. However, our calculations lead to the diamagnetic t state as the ground state of the oxy complex (with the q state 3 kcal higher).

This result may seem mysterious. Both the q and t states can make similar bonds to O2 and the q state is lower than t for the deoxy complex; yet the t state is lower for the oxy complex. The reason behind this interchange in ordering of q and t lies in the exchange interactions. The q state is lower than the t for the deoxy complex because of the extra stabilization due to the exchange interactions of the four unpaired orbitals of the q state (six exchange terms) as compared with the t state (three exchange terms arising from the corresponding four electrons). Pairing two orbitals in each state with the O2 to form the bonds leaves two unpaired orbitals for q (decreasing the number of exchange terms by three) and no unpaired orbitals for t (decreasing the number of exchange terms by only one). Thus, the relative stabilization of q with respect to t is reduced by two exchange terms upon bonding the O2. Since each exchange term is about 1 eV, this effect is enough to interchange the ordering of q and t between deoxy Mb and MnO2.

We calculate that 0.10 electron is transferred to the O2 in agreement with our earlier calculations (19), contradicting the Weiss model (20) of the bonding. We calculate an optimal FeOo angle of 119°, in reasonable agreement with the value of 135° for model MnO2 systems (27) and 120° for model CoO2 systems (28). Indeed, our overall wavefunction and geometry are quite consistent with the early model of Pauling (21). The optimal ring size for the six-coordinate complex is 0.03 Å larger than that found for the four-coordinate complex. Again, this lengthening of the hole radius is apparently due to nonbonded repulsions between the porphyrin N and the axial ligands.

We also carried out Hartree-Fock or molecular orbital calculations on the six-coordinate complex. We found that this leads to a ground septet state (S = 3) with additional quintet (S = 2) and triplet (S = 1) states lying below the closed-shell singlet state. Thus, these calculations are not consistent with a diamagnetic ground state of MnO2. Consequently, it is essential that theoretical studies of this system include electron correlation (many-body) effects as in these generalized valence bond-configuration interaction studies. Recently, ab initio Hartree-Fock calculations (22, 23) have been reported for an Fe-porphyrin complex using an NH3 ligand and one O2 ligand. They report a lower energy for the triplet paramagnetic state than for the singlet diamagnetic state (in agreement with our finding) but did not report energies for the quintet and septet states.

Cooperative oxygen binding

We will now consider the implications of the above molecular model in understanding the nature of the heme-heme interaction leading to cooperative oxygen binding. That is, how might the protein modify the function of the heme binding site?

It is generally believed that the difference in affinity for O2 is dependent mainly on the quaternary state of Hb. Hoard (13) suggested that the large displacement of the Fe to the porphyrin plane accompanying the high-spin to low-spin transformation associated with bonding of O2 could require stereochemical alterations near the heme that would trigger the cooperative oxygenation in Hb. Perutz et al. (17, 24) provided a detailed model of these stereochemical alterations, showing how they lead to both cooperativity and the Bohr effect. However, there is still much to learn concerning the microscopic details of the difference in O2 bonding for the two quaternary forms.

We can separate the different energy (enthalpy) contributions of O2 binding in Hb as follows.

1. Starting with Hb in its optimal geometry for the q state (Fe out of the plane) and moving the Fe-imidazole group to the point where Fe is in the heme plane will change the energy of the system by

\[ \Delta E_q = -E_q (\text{Fe at equilibrium}) + E_q (\text{Fe in the plane}). \]

2. With Fe in the plane, the energy separation of the q and t states of Hb is

\[ \Delta E_{qt} = E_t (\text{Fe in the plane}) - E_q (\text{Fe in the plane}). \]

3. Given Hb in the t state with the Fe in the plane, the bond energy for bonding O2 is

\[ D_t = E_t (\text{Hb + O}_2, \text{Fe in plane}) - E_t (\text{HbO}_2, \text{Fe in plane}). \]

The total energy for binding O2 to a particular heme in deoxy Hb becomes

\[ \overline{D} = D_t - \Delta E_{qt} - \Delta E_q. \]

The difference in O2 affinity for the T and R quaternary forms of Hb (T being the tense or low-affinity form stable for Hb and R being the relaxed or high-affinity form stable for fully oxygenated HbO2) must arise from one or more of the energy terms in Eq. 5 being dependent upon the quaternary form. For example, (i) steric effects of side chains on the distal side of the

**This analysis has been simplified a bit; in the configuration interaction wavefunction a part of these intra-atomic exchange terms is retained at the sacrifice of a slightly worse pairing of the bonding orbitals.
heme could modify $D_{ij}$ between the $T$ and $R$ forms, (iii) rotation of the vinyl groups or propionic side chains into or out of the porphyrin plane could modify the conjugation of the porphyrin and thereby affect $\Delta E_{eq}$ or $\Delta E_{op}$, (iii) movement of groups such as valine E11 or histidine E7 into the porphyrin could modify the conjugation and thereby $\Delta E_{eq}$ or $\Delta E_{op}$, (iv) distortions in the protein modifying the effective size of the porphyrin hole would change $\Delta E_{eq}$ and $\Delta E_{op}$, and (v) hindered motion of the proximal imidazole could change $\Delta E_{eq}$.

Nuclear magnetic resonance evidence by Shulman et al. (25) argues against significant changes in the porphyrin conjugation and thereby against (ii) and (iii) and probably (iv) above, indicating that the difference in $O_2$ affinity between the $R$ and $T$ forms is probably due to $\Delta E_{eq}$ being different between the two forms. If this is the case, our calculated potential energy surfaces for movement of this Fe-imidazole group in the deoxy complex allows us to estimate the difference in the Fe displacement between the unliganded $T$ and $R$ forms [the analysis is similar to that of Hopfield (29)]. Earlier results from x-ray crystallographic studies indicated a very large difference in Fe position, perhaps as large as 0.3–0.4 Å, between the two forms. Our calculations indicate a much smaller change, about 0.05 Å Fe movement, as a result of the change in quaternary structure.

From our calculations we find the optimal Fe out-of-plane distance for the five-coordinate $q$ state to be 0.28 Å with $\Delta E_{eq} = 7.8$ kcal/mol. We calculate a force constant for moving the Fe-imidazole unit toward the porphyrin plane of $k = 200$ kcal/Å:

$$E_{eq} = 7.8 \text{ kcal}$$

$$x = 0.28 \text{ Å}$$

$$k = 200 \text{ kcal/Å.}$$

The crystal structure data (T. Takano, personal communication) on Mb lead to $x = 0.42$ Å, whereas we calculate $x = 0.28$ Å for the free complex. Such a difference could be real; however, considering the uncertainties in both experimental and theoretical values, we will assume that the forces on the Fe for Mb and for the $R$ form of Hb are much the same as in the free complex:

$$x_R = 0.42 \text{ Å}$$

$$k_R = 200 \text{ kcal/Å.}$$

Assuming the cooperative effect to be due to $\Delta E_{eq}$ then requires that

$$\Delta E_{eq}^T = \Delta E_{eq}^R + 3.6 \text{ kcal.}$$

We assume that this increase in $\Delta E_{eq}$ is due to the increased rigidity of the tertiary structure in the $T$ form of the protein (e.g., due to the extra salt bridges). Pulling the Fe into the plane must act against this additional protein force, $F_p$, increasing $\Delta E_{eq}$. This force will act upon the unliganded Fe to distort it to a new position, $x^T$. Assuming $F_p$ is constant, $x_T$ and $x_R$ are related by

$$3.6 \text{ kcal} = \frac{1}{2} k(x_T^2 - x_R^2)$$

leading to

$$x_T - x_R = 0.041 \text{ Å.}$$

Thus, the force of the protein on the Fe-imidazole group merely shifts the energy surface for pulling Fe-imidazole into the plane by an amount such that $\Delta E_{eq}$ is increased by 3.6 kcal. This small displacement corresponds to $F_p = 8.2$ kcal/Å.

The most critical assumption in the above analysis is that the Fe-imidazole group is constrained by the protein in the $T$ form and is essentially free of protein forces in the $R$ form. The presence of additional protein forces in the $R$ form will increase the displacement of the Fe between the two forms. Experimental data consistent with such a small change are now emerging. Recent crystal structure studies (T. Takano, personal communication) of deoxy Hb and deoxy Mb suggest a displacement $x_T - x_R = 0.05$ Å.

**Summary**

On the basis of model calculations we conclude the following.

1. In unliganded Fe-porphyrin, the ground state is $q$ or $t$, depending upon the radius of the porphyrin hole.

2. The optimal position of the four-coordinate Fe is in the plane, even for the $q$ state.

3. Addition of an axial ligand leads to an optimal Fe position out of the plane due to a balance between attractive interactions of the Fe to the axial and porphyrin ligands and repulsive nonbonded repulsions of the axial and porphyrin nitrogens. For five-coordinate complexes these effects can serve to stabilize the $q$ state, even for porphyrin radii for which the $t$ state is lower at the Fe in-plane geometry.

4. It was previously assumed that pushing five-coordinate Fe into the plane stabilizes the $q$ state of Fe. We find that, even with Fe in the plane, both high-spin Fe and intermediate-spin Fe are significantly better than low-spin. In addition, for porphyrin hole radii typical of MoO2, the $q$ state is the lowest (even with the Fe in the plane).

5. Bonding an $O_2$ to the five-coordinate complex pairs two of the Fe orbitals and moves the Fe back into the plane, leading to an overall bond much as in oxygen and a geometry close to that suggested by Pauling. This stabilizes the $t$ state of the Fe, leading correctly to a diamagnetic ground state of MoO2. The Fe of FeO2 is not in the $s$ state as is usually assumed. The charge transfer from Fe to $O_2$ (0.10 electron) is in disagreement with the Weiss model.

6. Electron correlation effects are of great importance in the oxygenated complex; Hartree-Fock (or molecular orbital) calculations lead to a septet (paramagnetic) ground state with quintet and triplet states also lying below the diamagnetic closed-shell singlet state.

7. Considering this model allows us to partition the $O_2$ affinity into three logically distinct terms that can be affected differently by the change from the $T$ to $R$ quaternary forms. Using our calculated force constant of the five-coordinate complex, we predict that in the $T$ quaternary form of Hb the Fe is displaced only about 0.04 Å farther from the N plane than in the $R$ form.

**References**


