Ozone Model for Bonding of an \( \text{O}_2 \) to Heme in Oxyhemoglobin

(electronic structure/generalized valence bond/configuration interaction/excited electronic states/electric field gradient)

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ABSTRACT Several rather different models of the Fe—O \( \text{O}_2 \) bond in oxyhemoglobin have previously been proposed, none of which provide a satisfactory explanation of several properties. We propose a new model for the bonding of an \( \text{O}_2 \) to the Fe of myoglobin and hemoglobin and report \textit{ab initio} generalized valence bond and configuration interaction calculations on FeO\( \text{O}_2 \) that corroborate this model. Our model is based closely upon the bonding in ozone which recent theoretical studies have shown to be basically a biradical with a singlet state stabilized by a three-center four-electron pi bond. In this model, the facile formation and dissociation of the Fe—O \( \text{O}_2 \) bond is easily rationalized since the \( \text{O}_2 \) always retains its triplet ground state character. The ozone model leads naturally to a large negative electric field gradient (in agreement with Mössbauer studies) and to \( \pi \)-polarized (perpendicular to the heme) charge transfer transitions. It also suggests that the 1.3 eV transition, present in Hb\( \text{O}_2 \), and absent in HbCO, is due to a porphyrin-to-Fe transition, analogous to that of ferric hemoglobinins (e.g., HbCN).

I. Introduction

There have been a number of formulations of the bonding of the \( \text{O}_2 \) to the heme in oxyhemoglobin (Hb\( \text{O}_2 \)); examples are the linear model [Fe—O—O and Fe—O=O] of Pauling and Coryell (1), the bent model

\[
\text{Fe}=\text{O} \quad \text{of Pauling (2), the ring model}
\]

\[
\text{Fe} \quad \text{O} \quad \text{Fe} \quad \text{O}
\]

\[
\text{of Griffith (3), the met-superoxide model of Weiss (4),}
\]

\[
[\text{Fe(III)}—\text{O}_2^-],
\]

and the two-electron oxidative addition model of Gray (5)

\[
\text{Fe(IV)} \quad \text{O} \quad 2^-.
\]

Recent structural studies by Collman and co-workers (6) on a model Fe—O \( \text{O}_2 \) complex strongly support the bent geometry [1], but none of these structures provides a ready explanation for the following experimental properties of oxyhemoglobin:

1. The ease of forming and breaking the bond between the Fe and \( \text{O}_2 \) (all models [1-4] suggest a drastic change in the Fe bond upon addition to the heme);

2. The large asymmetry in the charge distribution about the Fe (as measured in the Mössbauer studies, refs. 7 and 8);

3. The low-lying 1.3 eV electronic transition (9) present in Hb\( \text{O}_2 \) but absent in HbCO;

4. The strong \( \pi \)-polarized (perpendicular to the heme), visible and ultraviolet electronic transitions (9) present in Hb\( \text{O}_2 \) but absent in HbCO.

We propose a new model for the bonding of \( \text{O}_2 \) to the heme based closely upon the bonding of the \( \text{O}_2 \) to the \( \text{O} \) in ozone (10-13). This model is corroborated by extensive theoretical studies of FeO\( \text{O}_2 \) and is consistent with the above experimental observations.

II. The bonding in ozone

Recent \textit{ab initio} studies (10-13) have shown that the ground state of ozone is basically biradical in character. Ignoring the 1s and 2s electrons, the ground state of an \( \text{O} \) atom can be viewed as in Fig. 1a, where \( p \)-orbitals in the plane are indicated by two-lobed figures and the \( p \)-orbital out of the plane is indicated by a circle; the dots indicate how many electrons are in each orbital. Generalized valence bond (GVB) calcu-

Abbreviations: GVB, generalized valence bond; CI, configuration interaction; RF, Hartree-Fock.

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![Fig. 1. Orbital occupation diagrams \( \varphi \) and \( \sigma \) show the \( \sigma \) and \( \varphi \) orbitals of FeO\( \text{O}_2 \), respectively. Note that the \( d_{xz} \) orbital (lying in the porphyrin plane) is not shown.](https://example.com/fig1.png)
tions (13) on $O_2$ show that the ground state has the form in Fig. 1b that corresponds to two ground state oxygen atoms coupled together (the line indicates bond formation—singlet pairing of electrons).

Generalized valence bond calculations on ozone (10–13) show that the ground state has the form in Fig. 1c. Comparing a, b, and c of Fig. 1, we see that the bond of $O$ and $O_2$ to form ozone is made without a drastic effect upon the bonding in the $O_2$.

The doubly-occupied s-orbital (denoted as $\phi$) in Fig. 1c is shown as localized. In fact, $\phi$ delocalizes somewhat onto both terminal atoms, resulting in a significant contribution to the bonding. The configuration in Fig. 1c has two singly-occupied orbitals (denoted as $\sigma$ and $\phi$) and, hence, leads to both a singlet state and a triplet state. The requirement (Pauli principle) that both $\sigma$ and $\phi$ be orthogonal to the delocalized $\phi$, leads to an increased overlap between $\phi$ and $\phi$, and stabilizes the singlet state by about 1 eV with respect to the triplet state (comparing adiabatic energies).

Thus, we can view ozone as a biradical formed by coupling ground state O to ground state $O_2$. The singlet state of the product is stabilized by a special three-center, four-electron s-bond (10–13).

The bonding in Fig. 1c would suggest a 90° bond angle. The actual bond angle is larger (117°) due primarily to repulsive interaction between the doubly-occupied p-orbitals on the terminal atoms.

III. The bonding in FeO$_3$

Recent theoretical studies have shown that, just as in ozone, bonding of $O_2$ to $H$ (14), to $CH_4$ (15, 16), and to CHO (17) does not lead to drastic modifications of the bonding within the $O_2$. We propose that the bonding of the $O_2$ to Fe in oxyhemoglobin (HbO$_2$) and oxymyoglobin (MbO$_2$) is basically similar to the bonding of $O_2$ and $O_2$ in ozone. In order for $O_2$ to bond to Fe without a drastic modification of the $O_2$ orbitals, the following configuration of the Fe is required (see Fig. 1e and f; the z-axis is parallel to the FeO bond; the x-axis is perpendicular to the FeO$_2$ plane):

1. A singly-occupied $d_z$ orbital for forming a s-bond to the singly-occupied p$_z$ orbital of the O$_2$;
2. A singly-occupied $d_{xz}$ orbital to couple with the s system of the O$_2$ forming a three-center, four-electron s-bond (as occurs in ozone when bonding O and O$_2$);
3. No electrons in the Fe $d_{yz}$ orbital since it is highly antibonding to the nitrogens of the porphyrin (taking the Fe=O$_2$ plane to be the $yz$ plane; this means that we are implicitly considering the Fe=O$_2$ plane to pass between two of the pyrrole nitrogens as found in crystallographic studies of model compounds (6));
4. The four remaining electrons must then go into the Fe $d_x^2$ and Fe $d_y^2$ orbitals. The occupation of the Fe $d_x^2$ orbital does not disrupt the bond to the O$_2$. A doubly-occupied $d_{yz}$ orbital can be tolerated in FeO$_2$ in much the same way as the p$_z$ pair in ozone (interaction of this orbital with the p pair on the terminal O$_2$ opens up the bond angle).

We refer to this configuration of FeO$_2$ (Fig. 1e and f) as $\delta$ to indicate that there are four electrons in s orbitals (Fig. 1f). Other low-lying states have electrons excited from a s orbital (Fig. 1e) to a s orbital, leading to $\delta$ or $\delta$ states.

Summarizing, in order to make a strong bond between Hb and $O_2$, the configuration on the Fe must be

$$(d_{x^2-y^2})^2(d_{xy})^2(d_{xz})^1(d_{yz})^1 = (t_{1g})^4(t_{2g})^1$$

in terms of octahedral symmetry functions (note with our coordinate system $d_{x^2-y^2}$ is of $t_{2g}$ type). This configuration leads to one component of the $d$ state ($G_{4u}$ symmetry group) of Hb. Although not the ground state of deoxyhemoglobin, there is some evidence (18) that the $d$ state becomes the lowest state of Hb when the Fe is in the plane of the heme.

Most descriptions (2, 3, 7–9) of HbO$_2$ have used crystal field or ligand field concepts in which the ligand is assumed to have a closed shell configuration. Thus, it has been assumed that the $O_2$ is a singlet state and that the ferrous iron is low spin ($S = 0$).

$$(d_{xy})^2(d_{xz})^2(d_{yz})^2 = (t_{1g})^4.$$

In the GVB description, the configuration of the $O_2$ and of the iron are basically triplet states; there is then a s bond between the Fe and one O and a four-electron three-center s bond, leading to a ground singlet state despite the essentially triplet character of the Fe and of the $O_2$. Thus, the ozone model of HbO$_2$ suggests that the Fe is essentially of intermediate spin ($S = 1$), whereas ligand field descriptions usually assume either high spin ($S = 2$) or low spin ($S = 0$) ferrous iron. As shown below, only $S = 1$ iron is consistent with the Mössbauer studies.

IV. FeO$_3$ calculations

The most questionable aspect of our ozone model of the bonding in HbO$_2$ is that replacement of an O with the Fe could lead to significant changes due to the differing ionic character and overlap of various orbitals. To test the applicability of these ideas to the FeO$_3$ unit, we carried out extensive ab initio studies of the FeO$_3$ system, applying the generalized valence bond (10) and configuration interaction (CI) methods to the various possible electronic configurations (4$\sigma$, 5$\sigma$, 6$\sigma$) of FeO$_3$, using both the open geometry (4$\sigma$ Fe-OO = 136°) and the ring (Griffith) geometry.† As discussed in Section V the results are in good agreement with the model. Although the FeO$_3$ unit was treated quite well, there were serious approximations in the treatment of the rest of the protein. The porphyrin was not included explicitly; we merely excluded the $d_{yz}$ orbital (which would be highly antibonding to the pyrrole nitrogen) from occupation. The two charges formally assigned to the porphyrin ring when describing the

† (a) GVB calculations were carried out for the singlet 4$\sigma$, triplet 5$\sigma$, and triplet 6$\sigma$ states of the open geometry, and the singlet 4$\sigma$, triplet 5$\sigma$, and singlet 6$\sigma$ states for the ring geometry. An extensive CI was carried out for each set of GVB orbitals (for example, 120 spin functions or 256 determinants for the 4$\sigma$ singlets, 150 spin eigenfunctions or 180 determinants for the 5$\sigma$ triplets). (b) The geometry used was $R_{OO} = 1.75$ Å, $R_{OO} = 1.56$ Å, $\Psi_{Fe-OO} = 136°$ for the open structure; and $R_{OO} = 1.52$ Å, and $R_{OO} = 1.43$ Å for the ring structure (bond length changes between the open and ring forms were based on the differences in the corresponding states of ozone, refs. 11, 12, and 13). (c) The basis used was the (1494p4d) Gaussian basis of Wachter (1970) J. Chem. Phys. 52, 1033–1036) contracted to [4p2d10] and the Pople STO-4G basis on each oxygen (1969 J. Chem. Phys. 51, 2657–2664).
iron as ferrous are included by placing them in the 4s orbital of the iron.† We totally ignored the proximal imidazole; this is an approximation with serious consequences since it could no doubt have a strong effect upon the calculated bond energy of the Fe to the O₂ and on the oscillator strengths for transitions to the excited states. These approximations may also lead to reordering of some of the electronic states, but should not cause gross changes in the overall character of these states.

Bear in mind that our basic model of the Fe—O₂ bond rests on qualitative concepts, based on the electronic structure of ozone and of peroxyradicals. The calculations reported here serve as a first test of the model, particularly in the use of these concepts for describing the bond to a transition metal. However, the calculations do not prove the model (due to the very approximate treatment of the other ligands) nor is the model solely based on the calculations. In comparing our model with experiment, we quote quantities such as excitation energies; however, it is to be understood that these numbers are yet of only qualitative significance insofar as correspondence to oxyhemoglobin and oxyhemoglobin is concerned.

On the other hand, the FeO₂ part of the system is treated quite accurately with very extensive configuration interaction of fully *ab initio* wavefunctions. Hence, we believe that they are quite adequate for testing the ozone model of the Fe—O₂ bond in oxyhemoglobin.

V. Ground state of FeO₂ from GVB calculations

The GVB—CI calculations lead to a singlet ground state with a 4s configuration and open (Pauling) geometry, in agreement with the ozone model. The GVB orbitals† (bottom of p. 2336) for the singlet 4s open geometry ground state are shown in Fig. 2. We see that these orbitals correspond closely to the schematic description in Fig. 1e and f. The O₂ orbitals of Fig. 2 are very similar to the O₂ orbitals of free O₂, much more so than for ozone and other peroxy radicals that have been studied.

The GVB—CI wavefunction leads to Mulliken populations (on the Fe, central O, and terminal O, respectively) of 25.91, 8.09, and 8.00 electrons of which 1.14, 1.57, and 1.29 are in the σ system. Thus, there is a net transfer of 0.23 σ electrons from the Fe toward the O₂ (as expected from the ionic character of the FeO σ bond), but this is partly counterbalanced by back donation in the π system of 0.14 electrons from the O₂ toward the Fe, leading to an overall neutral system. This is contrary to the suggestion by Weiss (4) that the Fe—O₂ bond is more like Fe⁺⁺⁺O⁻⁻⁻.

These results indicate that one can quite accurately view the ground state of HbO₂ as one component of the ε state of Hb coupled with the ε of O₂ (in the xy plane) to form a singlet state.

The ozone model of HbO₂ leads to an electric field gradient (g) on the Fe that is large and negative, in agreement with the Mössbauer studies (7). In fact, when atomic orbitals (8) are used, the electric field gradient parameters are [g₀ and g₀ for Hb and HbO₂, respectively]; electronic charge e set to 1]

\[ q_0 = V_{zz} = \frac{4}{7} (1/r)^d \]

\[ \eta_{zz} = (V_{zx} - V_{zy}) = 0 \]

for Hb and

\[ q_0 = -\frac{2}{7} (1/r)^d \]

\[ \eta_{zz} = \frac{6}{7} (1/r)^d \]

for HbO₂. Thus, the quadrupole splitting energy \[ \Delta E = (Qq/2)\sqrt{1 + 1/\eta^2} \] is \[ \Delta E_D = \Delta E_0(4/7)(Q/2)(1/r)^d \] for both Hb and HbO₂. This is in excellent agreement with the results of Mössbauer experiments (7): \[ \Delta E(Hb) = 2.40 \text{ mm sec} \] and \[ \Delta E(HbO₂) = 2.24 \text{ mm sec}. \] In addition, from application of magnetic fields, it is known that the q of HbO₂ is negative (7) in agreement with our model. We consider this to be strong evidence for the ozone model of HbO₂. None of the previous models of the electronic structure of HbO₂ has been

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† (a) We also carried out calculations with the two Fe(4s) electrons deleted. Although the character of the ground state is qualitatively unchanged, there are larger effects on the character of some excited states, especially those involving charge transfer character. (b) Most previous theoretical discussions have been in the context of molecular orbital or Hartree–Fock (HF) wavefunctions. It is particularly interesting to note that the HF method leads to a quartet ground state for FeO₂ with the singlet state only 4 eV higher. This results from intrinsic bias in the molecular orbital or HF method for high-spin states (for example, the HF method incorrectly leads to a triplet ground state for ozone with the singlet state only 2 eV higher) (10–12). It is likely that the HF method would lead to a quintet ground state of HbO₂, perhaps explaining the inability of Zerner et al. (18) in converging their approximate calculations on the axial configuration of HbO₂.
used to explain the Mössbauer results§ [low spin Fe leads to \( q = 0 \); the approximate molecular orbital calculations of Zerner et al. (18) lead to the correct magnitude (8) for \( q \) but the wrong sign (7).]

VI. Excited states of \( \text{FeO}_2 \) from GVB calculations

We find that the lowest state of the ring (Griffith) geometry is a \( 6\sigma \) state (as expected) but that it lies 1.45 eV above the lowest state of the open (Pauling) geometry. This is about the same separation as found for ozone (8-13).

As would be expected from the GVB description of the ground state, we find a number of low-lying excited states of \( \text{FeO}_2 \). There is a low-lying \( 4\pi \) triplet state at 0.37 eV corresponding to a triplet coupling of the singly-occupied orbitals of gFl. 1e and f (analogous to the low-lying triplet state of ozone). The \( 5\sigma \) configurations lead to a triplet state at 0.33 eV (20) and a singlet state at 0.36 eV; these states involve basically an \( \text{Fe} d_{z^2} \rightarrow \text{Fe} d_{z^2} \) transition (very weak). The lowest quintet state has a \( 5\sigma \) configuration and lies at 0.87 eV.

All calculated transitions of the \( \text{FeO}_2 \) unit were found to be weak. Most calculated transitions are expected to be weak because they correspond to \( d \rightarrow d \) transitions on the Fe or to triplet \( \rightarrow \) singlet transitions on the \( \text{O}_2 \).

In ozone, the lowest strong electronic transition has a peak at 4.9 eV (the Hartley band) with an oscillator strength of \( f = 0.1 \). This transition corresponds essentially to excitation of an electron from the double-occupied \( O \pi \sigma \) orbital on the center O to the single-occupied terminal \( O \pi \sigma \) orbitals (see Fig. 1c), i.e., \( \pi \)-charge transfer (11, 12). For \( \text{FeO}_2 \), we expect two analogous \( \pi \)-charge transfer transitions, each of which should have a large-\( \pi \)-polarized component. Experimentally (9), two \( \pi \)-polarized transitions have been observed in oxyhemoglobin at 3.9 eV with \( f = 0.1 \), and at 2.6 eV with \( f = 0.02 \) (whereas such transitions are not found in HbCO). We believe that the higher transition corresponds to the \( \pi \)-charge transfer (Hartley) transition of ozone; it is not clear from the model that the second expected transition would be as low as 2.6 eV. However, the calculations lead to three \( \pi \)-polarized transitions with components of charge transfer from the oxygen to the Fe in the \( \pi \) system lying at 1.8 eV, 2.0 eV, and 3.4 eV. There is also a \( \pi \)-polarized transition at 4.7 eV with a shift of \( \pi \) electron density to the outer (terminal) oxygen.

In each case, we find that the sigma system readjusts so as to keep the molecule essentially neutral, and the calculated oscillator strengths are all very small. As discussed earlier, a better treatment of the other ligands could lead to large effects upon properties such as the oscillator strength.

VII. \( \text{CoO}_2 \)

From the above model of the \( \text{Fe} - \text{O}_2 \) bond, we expect analogous Co systems to involve essentially the same \( \text{O}_2 \) configuration but with an additional electron in the Co \( d_{z^2} \) orbital [Co configuration \( (d_{x^2-y^2})^2(d_{z^2})^2(d_{x^2})^2(d_{xy})^2 \)]. Thus, the deoxy case has a singly-occupied \( d_{z^2} \) orbital on the Co, whereas the CoO2 has all orbitals paired except for the single-occupied (antibonding) \( \pi \) orbital on the \( \text{O}_2 \) (the \( d_{z^2} \) orbital of Fig. 2d is doubly-occupied, leaving the antibonding orbital on the \( \text{O}_2 \) as singly-occupied).

Electron spin resonance studies (20) of Co(II) Schiff base compounds (exhibiting reversible \( \text{O}_2 \) bonding) show that the five-coordinate deoxy complex's low spin with the unpaired electron in a \( d_{z^2} \) orbital, whereas the CoO2 compound has spin \( \frac{1}{2} \) with the spin density on the \( \text{O}_2 \). This is in excellent agreement with the GVB model. One would expect a comparable but slightly smaller bond angle for \( \text{CoO}_2 \) than for \( \text{FeO}_2 \) (due to the loss of the three-center \( \pi \) bond) as observed (21) (\( \langle \xi \text{CoO} \rangle = 126^\circ, \langle \xi \text{FeO} \rangle = 136^\circ \)).

In the usual ligand field models, it is assumed that an \( \text{O}_2 \) ligand is in its singlet state, in disagreement with the electron spin resonance data for Co(II) \( \text{O}_2 \) (20). As a result it was concluded (20) that the CoO2 bond is Co(III) \( \text{O}_2^- \) [analogous to the Weiss formulation (4) of FeO2]. The GVB model leads to a simple explanation of the experimental results for Co(II), without such assumptions.

VIII. Other comments

Stephens and Eaton (22) studied ferric hemoglobins with magnetic circular dichroism and concluded that the 1.3 eV absorption corresponds to a porphyrin \( \rightarrow \) Fe \( t_{2g} \) transition (there is at least one \( t_{2g} \) hole for ferric compounds). In the ozone model of HbO2, there is also one \( t_{2g} \) hole on the Fe so that a similar transition is expected, as observed (9). In HbCO where all the \( t_{2g} \) orbitals are double-occupied, no corresponding transition is found. Also, for CoO2 such a transition would not be expected.

The upper state of the \( \pi \)-charge transfer transition in ozone dissociates to \( \text{O}(1\text{D}) \) plus \( \text{O}_2(1\Delta_g) \) with a quantum yield of unity. Similar photodissociation may occur for the \( \pi \)-charge transfer states of HbO2 leading then to either Hb in a singlet state plus \( \text{O}_2(1\Delta_g) \) or to Hb=O in a singlet state plus \( \text{O}(1\Sigma) \). In addition to the usual state of the Hb, this photodissociation leads to a highly reactive state of the dissociated species \( \text{O}(1\Sigma) \) or \( \text{O}_2(1\Delta_g) \) which may well attack molecules of the protein defining the cavity around the heme. The interesting possibility here is that particular photon energies would lead to characteristic damage of the protein due to the specific chemistry of the excited dissociation products.

IX. Conclusions

We believe that the ozone model for the bonding of the \( \text{O}_2 \) to a heme provides a useful model for understanding the properties of oxyhemoglobin. The validity of this model is corroborated by \textit{ab initio} studies of the FeO2 system and by the agreement with experimental properties.

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§ Eicher, H. & Trautwein, A. ([1969] \textit{J. Chem. Phys.} 50, 2540-2551) attempted to explain the Mössbauer data of Hb and HbO2 in terms of interactions between the \( \text{Fe}(d) \), \( \text{O}(d) \), \( \text{Hb}(d) \), and \( \text{Co}(d) \) states of Fe(\( d \)) induced by spin-orbit coupling. They concluded that only \( \text{Fe}(d) \) could explain the Mössbauer data for HbO2. (Their calculated \( \Delta E \) due to \( \text{Fe}(d) \) was a factor of 50% too large.)