

# THEORETICAL STUDIES OF OXYGEN BINDING\*

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## INTRODUCTION

Our objective here will be to use ideas extracted from quantitative *ab initio* calculations on model systems to develop qualitative analyses useful in examining the chemistry of classes of heme-oxygen systems. Such systems include myoglobin, hemoglobin, cytochrome P450, peroxidases, and cytochrome oxidase.

There are three important electronic configurations of ferrous Fe in the various Fe-heme proteins, the energies of which depend upon the axial ligands. These states are denoted *s*, *t*, and *q* (FIGURE 1), and correspond to  $S = 0$  (singlet),  $S = 1$  (triplet), and  $S = 2$  (quintet) spin states, respectively.<sup>1-6</sup> The intra-atomic exchange terms favor high spin, leading to *q* as the ground state. Putting the Fe into a heme destabilizes the  $x^2 - y^2$  orbital and adding the axial imidazole of Mb or Hb destabilizes the  $z^2$  orbital. The result is to decrease the spacing of the *q*, *t*, and *s* levels of the Fe atom (separated by a total of 83 kcal) down to a separation of about 30 kcal (FIGURE 2). Because of this smaller separation, these levels may be interchangeable, depending on the nature of the axial ligand at the sixth coordination site.

## BINDING OF CLOSED-SHELL LIGANDS

Adding a normal (closed-shell§) ligand such as CO or H<sub>2</sub>O to the five-coordinate Fe of Mb further destabilizes the  $z^2$  orbital, thereby stabilizing the *s* configuration with respect to the *q* and *t* configurations. There are two cases here:

1. Weak field (e.g., H<sub>2</sub>O); *q* remains the ground state.
  2. Strong field (e.g., CO); *s* becomes the ground state, as indicated in FIGURE 3.
- Note that, for closed-shell ligands, the *t* configuration is not expected to ever be the ground configuration for six-coordinate complexes.

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§No unpaired orbitals (e.g., not NO or O<sub>2</sub>).

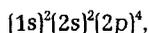
				RELATION TO HEME PLANE	RELATION TO AXIAL LIGAND	
$e_g$	$x^2-y^2$	—	—	+	PARALLEL (POINT AT N)	PERPENDICULAR
	$z^2$	—	+	+	PERPENDICULAR	PARALLEL
$t_{2g}$	$xz, yz$	++	++	++	$\pi$ -LIKE	$\pi$ -LIKE
	$xy$	++	++	+	PARALLEL (PT. BETWEEN N)	PERPENDICULAR
		s	t	q		
		(HbCO)	(HbO <sub>2</sub> )	(Hb)		

FIGURE 1. The s, t, and q configurations of ferrous Fe.

BINDING OF O<sub>2</sub>

## Preliminary Considerations

We next consider the binding of an open-shell ligand, such as the O<sub>2</sub> molecule, but we must first review some salient features of O<sub>2</sub>.<sup>6</sup> The ground state of the O atom has the configuration



with one of the three p orbitals double occupied and the other two singly occupied. This is schematically indicated in FIGURE 4a, where the 1s and 2s orbitals are ignored and the p orbitals are indicated as two-lobed figures when in the page and by a circle when perpendicular to the page. In order to form a bond between two O atoms, we must bring the atoms together so that two singly occupied orbitals overlap, as indicated in FIGURE 4b. There are two equivalent ways of doing this, and the ground state of O<sub>2</sub> is the (resonant) combination of the two. The stabilization due to a superimposition of these configurations is an important part of the bond energy of O<sub>2</sub>. Indeed, an approximate analysis of the bond energy of O<sub>2</sub> indicates that

$\sigma$ bond	47 kcal
$\pi$ bonds	<u>71 kcal</u>
total bond	118 kcal

That is, 60% of the bonding is due to the  $\pi$  system.

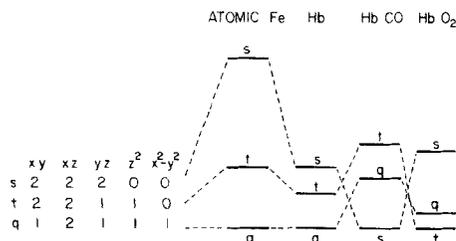
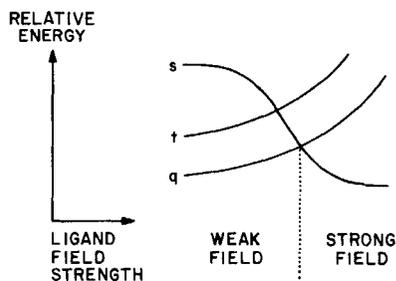


FIGURE 2. The effect of ligands on the s, t, and q configurations.

FIGURE 3. The effect of a closed-shell ligand on the states of five-coordinate ferrous Fe.

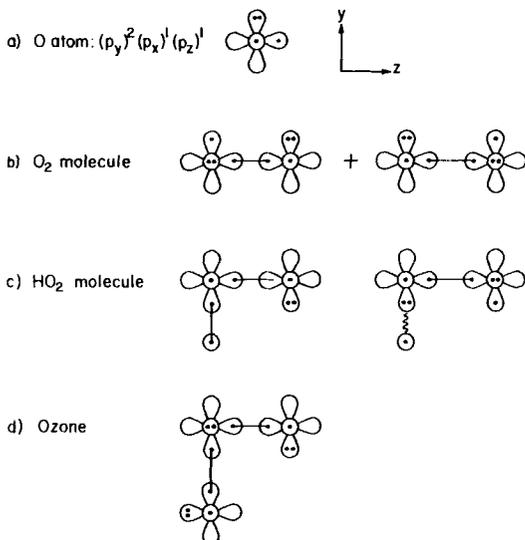


We next consider bonding  $O_2$  to a simple atom, H. Clearly, we want the (singly occupied) orbital of H to overlap a singly occupied orbital of  $O_2$  to form a  $\sigma$  bond, as in FIGURE 4c. However, the corresponding p orbital is doubly occupied in the resonant configuration of  $O_2$ , leading to a repulsive interaction. Consequently, in bonding the H to  $O_2$ , we lose most of the resonance interaction in the  $\pi$  system of  $O_2$ . The result is a much weaker H—O bond than normal:

normal H—O bond	104 kcal
loss in resonance energy	<u>57 kcal</u>
net H— $O_2$ bond	47 kcal

As a second example, consider bonding an O atom to  $O_2$ , as in FIGURE 4d. Considering only the new  $\sigma$  bond, we would estimate

gain OO $\sigma$	47 kcal
lose $O_2$ resonance	<u>57 kcal</u>
net O— $O_2$ bond	-10 kcal


 FIGURE 4. bonding O,  $O_2$ ,  $HO_2$ , and  $O_3$ .

and, hence, that ozone would be unstable. However, the new O atom has a singly occupied  $p\pi$  orbital that can also participate in bonding. If the singly occupied  $p\pi$  orbitals of the terminal O atoms in FIGURE 4d are singlet-paired, there is a net stabilization of 35 kcal, leading to an overall bond of

$$D(\text{O}-\text{O}_2) = 35 - 10 = 25 \text{ kcal.}$$

This  $\pi$  bond is not a normal covalent  $\pi$  bond but involves, in an essential way, all four electrons in the  $\pi$  system. As a result, it is referred to as the four-electron three-center bond, or, more simply, as the ozone  $\pi$  bond.

An aside concerning quantum mechanical calculations on the energies of wavefunctions such as those of ozone is relevant. Wavefunctions, such as those in FIGURE 4d, that require singly occupied orbitals to be weakly coupled cannot be accurately calculated without including electron correlation effects. Thus, although ozone is well known to be a singlet (diamagnetic) molecule, Hartree-Fock calculations on ozone lead to a triplet ground state, with the "ground"

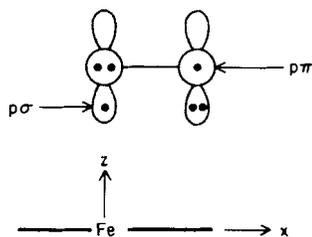


FIGURE 5. Bonding of  $\text{O}_2$  to Fe-heme.

$\sigma$ BOND: Fe  $d_{z^2}$  WITH  $\text{O}p\sigma$

$\pi$  BONDING: Fe  $d_{yz}$  WITH  $\text{O}p\pi$

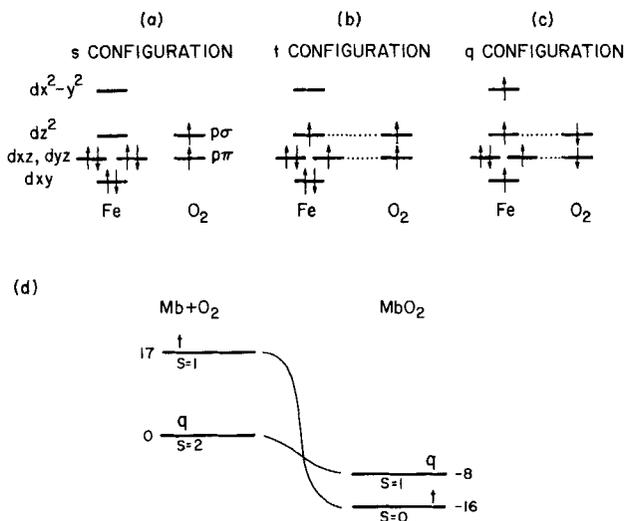
(3 CENTER, 4 ELECTRON BOND)

singlet state about 46 kcal higher!<sup>7,8</sup> Including electron correlation, as in the generalized valence bond (GVB) wavefunction,<sup>7</sup> leads to the proper singlet ground state, with the corresponding triplet about 36 kcal higher (ground state geometry).<sup>8</sup> Thus, Hartree-Fock is biased toward the triplet by about 82 kcal!

#### BONDING OF $\text{O}_2$ TO FIVE-COORDINATE FE

From the discussion of ozone, we expect the maximum bonding if both a  $\sigma$  bond and an ozone-like  $\pi$  bond can be formed. To form a  $\sigma$  bond, we require a singly occupied  $d_{z^2}$  orbital on the Fe, and, to form an ozone-like  $\pi$  bond, we require a singly occupied  $d_{yz}$  orbital (assuming that the  $\text{FeO}_2$  unit is in the  $xz$  plane), as indicated in FIGURE 5.<sup>1,8</sup>

As shown schematically in FIGURE 6, both the  $q$  and  $t$  configurations have appropriate orbitals and, hence, either one should be able to bond to  $\text{O}_2$ , with the  $q$  configuration leading to an  $S = 1$  state (triplet) for  $\text{MbO}_2$ , and the  $t$  configuration leading to an  $S = 0$  state (singlet) for  $\text{MbO}_2$ . Now we must determine which of


 FIGURE 6. Configurations for  $\text{FeO}_2$  bonding.

these two states is the ground state for  $\text{MbO}_2$ . Since the  $q$  state is 17 kcal lower for Mb and since a similar bond is formed in both cases, one might be tempted to assume that the  $q$  state ( $S = 1$ ) is lower for  $\text{MbO}_2$ . This is not the case, and the reason for this has to do with exchange interactions. Each orbital of a bond pair is equally likely to be up-spin or down-spin. As a result, pairing a  $d$  orbital with an  $\text{O}_2$  to form a bond pair reduces the intra-atomic exchange interactions with the Fe. For the  $t$  state (see TABLE 1), this results in the loss of  $\frac{1}{2}$  exchange integral ( $\sim 11$  kcal) and, hence, the bond is weaker by this amount. For the  $q$  state, however, the loss is  $2\frac{1}{2}$  exchange integrals ( $\sim 55$  kcal). Thus, the bond energy of  $\text{O}_2$  to the  $q$  configuration should be far weaker than that to the  $t$  configuration; consequently, we expect the ground state of  $\text{MbO}_2$  to be the  $S = 0$  state that arises from the  $t$  configuration.

Indeed, the actual calculations show that the ground state is the  $S = 0$  state arising from the  $t$  configuration; the  $S = 1$  state of the  $q$  configuration lies  $\sim 8$  kcal higher. In the latter state, the spin pairing of the Fe—O bonds is partially undone in order to retain the higher spin pairing of the Fe  $d$  orbitals.

 TABLE 1  
 INTRA-ATOMIC EXCHANGE TERMS FOR FERROUS FE

	Configuration		Stabilization of $q$ with respect to $t$
	$t$	$q$	
Before bond $\text{O}_2$	7	10	3
After bond $\text{O}_2$	$6\frac{1}{2}$	$7\frac{1}{2}$	1
Net loss	$\frac{1}{2}$	$2\frac{1}{2}$	
	( $\sim 11$ kcal)	( $\sim 55$ kcal)	

Using the above ideas, the net bond energy of  $O_2$  to Mb can be partitioned as follows:¶

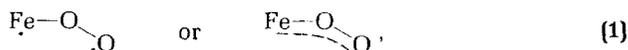
$$\begin{aligned} \text{Net bond (Mb—O}_2) &= \left( \begin{array}{l} \text{Fe—O} \\ \sigma \text{ and } \pi \\ \text{bonds} \end{array} \right) - \left( \begin{array}{l} \text{loss in} \\ \text{O}_2 \pi \\ \text{resonance} \end{array} \right) \\ &\quad - \left( \begin{array}{l} \text{excitation} \\ \text{energy} \\ q \text{ to } t \\ \text{for Mb} \end{array} \right) - \left( \begin{array}{l} \text{loss in} \\ \text{exchange} \\ \text{energy} \\ \text{of } t \text{ state} \end{array} \right) \\ &= (90 \text{ to } 105) - 57 - 17 - 11 \\ &= 5 \text{ to } 20 \text{ kcal.} \end{aligned}$$

Thus, we can view the strategy for designing a reversible oxygen carrier as follows:

1. Normally, the Fe—O  $\sigma$  and  $\pi$  bonds would be very strong, leading to irreversible bonding.
2. Thus, to obtain a weak net bond, we arrange to store most (85 kcal) of the potential bond energy as internal electronic excitation energy, which is released upon dissociation of the  $O_2$ .
  - a. Promote  $q$  to  $t$  (store 17 kcal).
  - b. Lose exchange energies in  $t$  configuration (store 11 kcal).
  - c. Lose  $\pi$  resonance of  $O_2$  (store 57 kcal).
3. Since the  $q$  configuration leads to weak binding of  $O_2$ , it can serve as an intermediate to accommodate the spin-flip needed to obtain the  $t$  configuration.
4. The destabilization of the O—O bond (loss of resonance) makes the second, distal, O atom rather reactive. Thus, to prevent dissociation pathways, one needs
  - a. to ensure that there is only one coordination site available on the Fe (accomplished by placing the Fe in the heme),
  - b. to isolate the  $FeO_2$  site from reactive species in solution (e.g., species that might protonate the outer oxygen), and
  - c. to prevent interaction with a second Fe atom (which could bond to the outer oxygen and dissociate the  $O_2$ ).

### Discussion

We will schematically represent the  $FeO_2$  bond as



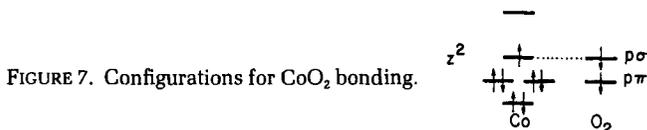
where the dotted line indicates the pairing of the radical orbitals in the  $\pi$  system.

¶Throughout this paper we quote estimates for various energy contributions. We should emphasize that such numbers are not rigorous estimates. Even in the best circumstances, there are errors in transferring bond energies from one molecule to another. These problems are magnified in the Fe-heme system of interest here. Thus, the numbers are meant to illustrate the approximate magnitudes of the various quantities.

The generalized valence bond description of the bonding suggests a strongly bent Fe—O—O bond (as first suggested by Pauling<sup>9</sup>) with *ab initio* generalized valence bond calculations leading to an optimum bond angle of 119°. <sup>1,2</sup> (In comparison, similar calculations on ozone lead to a bond angle of 116.0°, whereas the experimental value is 116.8°. <sup>8</sup>) Experimental values for MbO<sub>2</sub> and model systems have been difficult to obtain but are in reasonable agreement with the theory: 136° (1975), <sup>10</sup> ≤ 131° (1978), <sup>11</sup> ≤ 129° (1980), <sup>12</sup> 121° (1978), <sup>13</sup> and 115° (1980). <sup>14</sup>

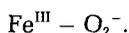
A characteristic feature of the O<sub>2</sub> unit that has a σ bond plus a singly occupied P orbital is an O—O vibrational frequency around 1100 cm<sup>-1</sup>; for example, HO<sub>2</sub> (1101 cm<sup>-1</sup>) <sup>15</sup> and O<sub>3</sub> (1042, 1103 cm<sup>-1</sup>). <sup>16</sup> In agreement with this, MbO<sub>2</sub> and model systems are observed to have vibrational frequencies in the same range. <sup>17</sup>

As pointed out above, systems with weakly overlapping orbitals, such as the π system of ozone or MbO<sub>2</sub>, require proper treatment of electron correlation to avoid serious errors in the ordering of states. Indeed, we find that Hartree-Fock calculations do not lead to the correct singlet state (*S* = 0) for the FeO<sub>2</sub> model. Rather, Hartree-Fock calculations put the *S* = 1, *S* = 2, and *S* = 3 states all below the “ground” singlet state! <sup>1,2,4</sup> Consequently, one must include electron correlation effects to stabilize such systems.



### The Superoxide Model

It is sometimes suggested that the ground state of MbO<sub>2</sub> involves a ferric Fe bonded to a superoxide ligand, <sup>18,19</sup>



Analysis of our wavefunctions shows very little charge transfer (< 0.2 *e*) to the O<sub>2</sub>, and the wavefunctions are not those of superoxide. ¶

One reason for suggesting the superoxide model is the observation that the spin on the Co of deoxycobaltoglobin shifts to the O<sub>2</sub> open oxygenation. <sup>18</sup> However, perusal of FIGURE 7 shows that this is just what is expected from a generalized valence bond model that uses a neutral O<sub>2</sub>. A second argument is that the O—O vibrational frequency of MbO<sub>2</sub> resembles that of K<sup>+</sup> O<sub>2</sub><sup>-</sup> (1145 cm<sup>-1</sup>). <sup>20</sup> However, as discussed above, this vibrational frequency also resembles that of neutral peroxy OO units such as HO<sub>2</sub> and O<sub>3</sub>.

Summarizing, there is no justification for describing the O<sub>2</sub> in HbO<sub>2</sub> as a superoxide.

¶Ignoring electron correlation leads to greater electron transfer; however, such a wavefunction does not lead to the proper spin of the system.

## SPECULATIONS ON OTHER SYSTEMS

A major objective in developing conceptual models is to be able to analyze new systems before experimental and theoretical data are available. In order to illustrate this process, we will consider some properties of iron-oxo bonds. These discussions might be relevant to compounds I and II of horseradish peroxidase (HRP), to cytochrome-c peroxidase (CCP), to the active intermediate of cytochrome P450 responsible for oxygenating substrate CH bonds, and perhaps to cytochrome oxidase.

As indicated in FIGURE 8, the oxygen atom has both  $p\sigma$  and  $p\pi$  orbitals available for making bonds. For ferrous Fe, this leads to bonding configurations, just as shown in FIGURE 6. Once again, there are two low-lying states,

$S = 0$  arising from the  $t$  configuration

and

$S = 1$  arising from the  $q$  configuration.

For  $\text{HbO}_2$ , the  $S = 0$  state ( $t$ ) was 8 kcal below the  $S = 1$  state ( $q$ ); hence, one would expect a similar result for  $\text{HbO}$ . Changing the axial ligand could

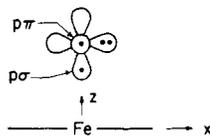
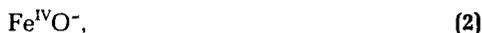


FIGURE 8. Bonding of O to Fe-heme.

interchange the levels and, indeed, indications are that the ferrous state for HRP and CCP is<sup>21</sup>  $S = 1$ .

The ferrous oxo bond is sometimes written as

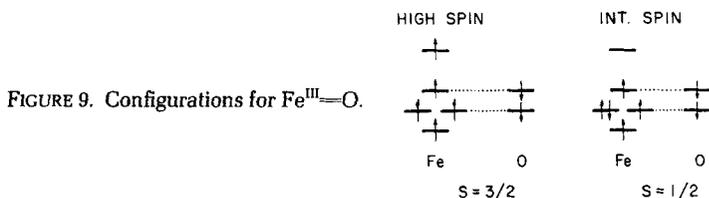


with the Fe considered to be in the ferryl oxidation state. Studies of the oxo bonds in other systems<sup>22</sup> indicate that the metal-oxo bond is rather covalent and that a diagram such as



more correctly describes the wavefunction. That is, the Fe is in a  $d^6$  configuration (ferrous or oxidation state II) but has a double bond to the oxygen atom.

The bonding of ferric Fe of an O atom is illustrated in FIGURE 9 and the intra-atomic exchange terms are analyzed in TABLE 2. Comparing TABLES 1 and 2 we see that, for ferric Fe, the high spin form is favored by one additional exchange term (~22 kcal). Consequently, we expect the high spin form ( $S = 3/2$ ) of the ferric-oxo bond to always be the ground state,<sup>20</sup> whereas, for ferrous oxo, the  $S = 0$  and  $S = 1$  states are both potential ground states (depending upon the axial ligand). As discussed above, the bonding in the ferric-oxo bond should be


 FIGURE 9. Configurations for  $\text{Fe}^{\text{III}}=\text{O}$ .

visualized as



not as

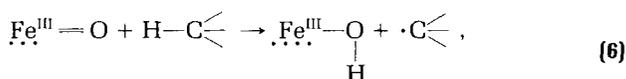


In TABLE 2, we see that the ferric-oxo bond loses  $3\frac{1}{2}$  intra-atomic exchange terms ( $\sim 77$  kcal) upon binding an O atom. Consequently, the net bond is estimated to be

$$\begin{aligned}
 (\text{Net } \text{Fe}^{\text{III}}=\text{O} \text{ bond}) &= \left( \begin{array}{c} \text{FeO} \\ \sigma \text{ and } \pi \\ \text{bond} \end{array} \right) - \left( \begin{array}{c} \text{Loss Fe} \\ \text{exchange} \end{array} \right) - \left( \begin{array}{c} \text{Loss O} \\ \text{exchange} \end{array} \right) \\
 &= (100 \text{ to } 115) - 77 - 11 \\
 &= 12 \text{ to } 27 \text{ kcal;}
 \end{aligned}$$

hence, the loss of atomic exchange energy serves as stored electronic energy that can be used to drive the chemistry of subsequent steps.

For example, in hydroxylating the CH bond of an alkane (as in cytochrome P450), the critical steps might be



followed by

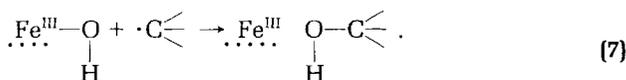
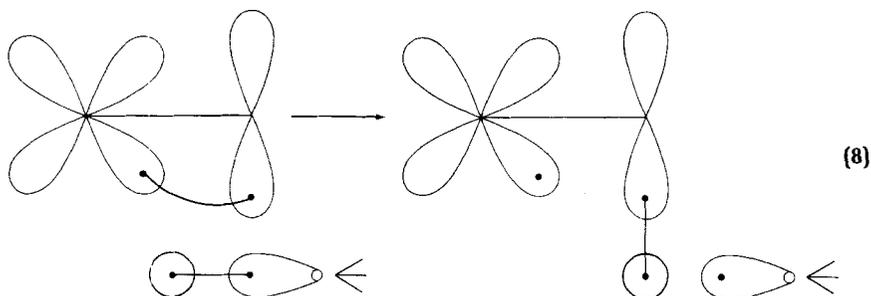


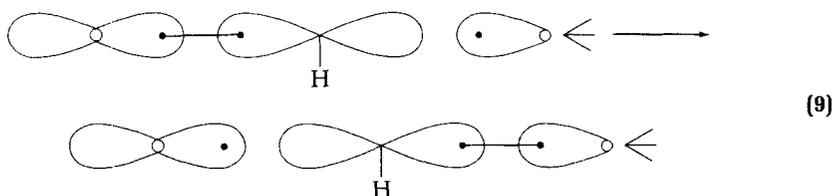
TABLE 2  
INTRA-ATOMIC EXCHANGE TERMS FOR FERRIC FE

	Configuration		Stabilization of High Spin with Respect to Intermediate Spin
	Intermediate Spin	High Spin	
Before bond O	6	10	4
After bond O	$4\frac{1}{2}$	$6\frac{1}{2}$	2
Net loss	$1\frac{1}{2}$	$3\frac{1}{2}$	

The reacting orbitals of (6) are



(showing the  $\pi$  bond of FeO reacting with the CH bond); the reacting orbitals of (7) are



(showing the Fe-O  $\sigma$  bond reacting with the radial orbital of the alkyl radical). In both cases, orbital changes are compatible and suggest no orbital symmetry or phase problems that might lead to a forbidden reaction. The rough energetics are as follows:

$$\begin{aligned} \Delta H_{(6)} &= \left( \begin{array}{c} \text{energy} \\ \text{to break} \\ \text{CH bond} \end{array} \right) - \left( \begin{array}{c} \text{energy} \\ \text{new} \\ \text{OH bond} \end{array} \right) + \left( \begin{array}{c} \text{energy} \\ \text{to break} \\ \text{FeO } \pi \\ \text{bond} \end{array} \right) - \left( \begin{array}{c} \text{gain in} \\ \text{Fe atomic} \\ \text{exchange} \end{array} \right) \\ &= 94 - 104 + 30 - 33 \\ &= -13 \text{ kcal,} \end{aligned}$$

$$\begin{aligned} \Delta H_{(7)} &= \left( \begin{array}{c} \text{energy} \\ \text{to break} \\ \text{Fe } \sigma \\ \text{bond} \end{array} \right) - \left( \begin{array}{c} \text{energy} \\ \text{new HO-C} \\ \text{bond} \end{array} \right) - \left( \begin{array}{c} \text{gain in} \\ \text{Fe atomic} \\ \text{exchange} \end{array} \right) \\ &= 70 - 85 - 44 \\ &= -59 \text{ kcal.} \end{aligned}$$

Without the gain in intra-atomic exchange,  $\Delta H_{(6)}$  would have been about +20 kcal endothermic. Thus, the stored atomic exchange energy in the FeO bond may play a role in facilitating the chemistry.

## SUMMARY

We discussed the bonding of O<sub>2</sub> to hemoglobin using results of ab initio calculations of idealized portions of the Hb molecule. The bond between Fe and O<sub>2</sub> is formed by coupling a triplet state (intermediate spin state) of Fe to the triplet ground state of O<sub>2</sub> (analogous to the bonding of O to O<sub>2</sub> in ozone). The coordination sphere of the Fe reduces the energy separation between the quintet, triplet, and singlet states, making an intermediate spin state accessible for bond formation. This provides the mechanism by which an O<sub>2</sub> molecule can easily and reversibly bind to Hb. Neither the diamagnetic (*t<sub>2g</sub>*)<sup>6</sup> excited state of Fe nor the excited singlet state of O<sub>2</sub> play a role in the formation of the FeO<sub>2</sub> bond. We also discussed the role of the Fe intra-atomic exchange terms and show how they serve to store electronic energy upon bond formation. An example was given, illustrating how this stored electronic energy can then be used to drive enzymatic reactions.

Metal atoms such as ferrous Fe are capable of existing in several distinct electronic configurations, depending upon the ligands. Our objective here has been to illustrate the different characteristics of these Fe configurations and to indicate why various axial ligands stabilize particular Fe configurations. In addition, we have sketched the type of orbital descriptions arising from theoretical wavefunctions and illustrated how to use these descriptions to predict chemical phenomena.

## REFERENCES

1. OLAFSON, B. D. 1978. Ph.D. Thesis, California Institute of Technology.
2. GODDARD, W. A. III & B. D. OLAFSON. 1979. In *Biochemical and Clinical Aspects of Oxygen*. W. D. Caughey, Ed.: 87-123. Academic Press. New York.
3. OLAFSON, B. D. & W. A. GODDARD III. 1981. Manuscript in preparation.
4. OLAFSON, B. D. & W. A. GODDARD III. 1977. *Proc. Nat. Acad. Sci. USA* **74**: 1315.
5. OLAFSON, B. D. & W. A. GODDARD III. 1981. In *Interaction Between Iron and Proteins in Oxygen and Electron Transport*. C. Ho., Ed. Elsevier North-Holland. New York. In press.
6. GODDARD, W. A. III & B. D. OLAFSON. 1975. *Proc. Nat. Acad. Sci. USA* **72**: 2335.
7. GODDARD, W. A. III, T. H. DUNNING, JR., W. J. HUNT & P. J. HAY. 1973. *Acc. Chem. Res.* **6**: 368.
8. HAY, P. J. & W. A. GODDARD III. 1972. *Chem. Phys. Lett.* **14**: 46; HAY, P. J., T. H. DUNNING JR. & W. A. GODDARD III. 1975. *J. Chem. Phys.* **62**: 3912.
9. PAULING L. 1949. In *Haemoglobin*. F. J. W. Roughton and J. C. Kendrew, Eds. Butterworths Scientific Publications. New York.
10. COLLMAN, J. P., F. R. GAGNÉ, C. A. REED, W. T. ROBINSON & G. A. RODLEY. 1974. *Proc. Nat. Acad. Sci. USA* **71**: 1326.
11. JAMESON, G. B., G. A. RODLEY, W. T. ROBINSON, R. R. GAGNÉ, C. A. REED & J. P. COLLMAN. 1978. *Inorg. Chem.* **17**: 850.
12. JAMESON, G. B., F. S. MOLINARO, J. A. IBERS, J. P. COLLMAN, J. I. BRAUMAN, E. ROSE & K. S. SUSLICK. 1980. *J. Am. Chem. Soc.* **102**: 3224.
13. PHILLIPS, S. E. V. 1978. *Nature (London)*. **273**: 247.
14. PHILLIPS, S. E. V. 1980. Private communication.
15. MILLIGAN, D. E. & M. E. JACOX. 1972. *J. Mol. Spectrosc.* **42**: 495.
16. HERZBERG, G. 1950. In *Molecular Spectra and Molecular Structure*, Vol. 1.: 560. D. Van Nostrand. Princeton, New Jersey.
17. COLLMAN, J. P., J. I. BRAUMAN, T. R. HALBERT & K. S. SUSLICK. 1976. *Proc. Nat. Acad.*

- Sci. USA **73**: 3333; MAXWELL, J. C., J. A. VOLPE, C. H. BARLOW & W. S. CAUGHEY. 1974. *Biochem. Biophys. Res. Commun.* **58**: 166; BARLOW, C. H., J. C. MAXWELL, W. J. WALLACE & W. S. CAUGHEY. 1973. *Ibid.* **55**: 91.
18. BASOLO, F., B. M. HOFFMAN & J. A. IBERS. 1975. *Acc. Chem. Res.* **8**: 384.
19. WEISS J. J. 1964. *Nature* **202**: 83.
20. LEE-RUFF, E. 1977. *Chem. Soc. Rev.* **6**: 195.
21. BRILL, A. S. 1966. In *Comprehensive Biochemistry*, Vol. 14. M. Florkin and E. H. Stotz, Eds.: 447. Elsevier Publishing Company. Amsterdam.
22. RAPPE, A. K. & W. A. GODDARD, III. 1980. *J. Am. Chem. Soc.* **102**: 5114.

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#### DISCUSSION

L. ALLEN (*Princeton University, Princeton, N. J.*): You obviously have extremely interesting approaches to biochemical problems. I would suggest the following: you have a heme plane, a proximal histidine, and a six-coordinated iron with the oxygen bound on the other side. A biochemical question of the first importance would be, What happens when I take the nitrogen on the opposite side of the proximal histidine, and see what happens when that NH hydrogen bonds to the carbonyl backbone from the helix? You should do this calculation with and without that hydrogen bond. That would be an important biochemical contribution because it might well have to do with the cooperativity of the other binding properties. That will probably affect both the  $\pi$ -d back bonding as well as the  $\sigma$  bonding through the nitrogen to the iron, and you'll get a complicated effect that you can analyze the way you've done here. It's not obvious what will happen.

W. GODDARD: The point is that, if you take the other hydrogen on imidazole away and hydrogen bond it, you affect the charge and the nitrogen bond to the iron. It could, therefore, have an effect on the relative energies of these states; some people think it could be quite important.

A. VEILLARD (*Université L. Pasteur, Strasbourg, France*): In your description of the bonding of oxygen in the model, you had emphasized descriptions in terms of the  $t$  state of the iron atom. To my knowledge, there have been three CI calculations of oxygen models. Two are semiempirical CI calculations, one by Dr. Karplus in the PPP model, the second by Drs. Loew and Zerner using the ZINDO-SCF model. The third one is an *ab initio* CI calculation by Dr. Ohno. As far as I know, all CI calculations have the  $(t_{2g})^6$  configuration, namely the S state, as the leading term in the CI wavefunction. I think you are speaking in terms of a valence bond model. Others have used a molecular orbital model, and I think you emphasized one side of the description and the others have emphasized a different side. That is the first point.

Now, I have a question. Did you calculate the oxygen-oxygen stretching frequency?

GODDARD: No. The point that Dr. Veillard raises is that, for systems such as ozone, the MO description, ends up with the wrong spin state: a triplet state as

the ground state, whereas it should be one eV higher than the real singlet ground state. This problem is exacerbated in the case of the Fe—O<sub>2</sub> system. The ozone problem is still there in the MO calculation, but it's exacerbated because we have the potential of a d<sup>6</sup> high spin configuration on the iron, with all the intra-atomic exchange that that entails. So what happens when you do an MO calculation on the Fe—O<sub>2</sub> unit is that the coupling of the O<sub>2</sub> and the iron forces it to high spin. But the ground state is an overall S = 3 state, and the singlet state that you want to describe, because it's really the ground state of the system, is several eVs higher than that. So if you start with this MO wavefunction and try to fix it with the CI, it takes an extremely large order of CI, around octuples or decuples in order to fix it up. Instead of trying to solve self-consistently for the wrong state and fix it with correlation, we include the correlation by solving for the orbitals in the generalized valence bond frame. This leads to a basically correct description of the system, one which does have a fair amount of biradical character. That's still not good enough for a really quantitative discussion of bond energy and excitation energies. Having this GVB wavefunction, we did a high order CI, generally at least hexuple excitations, within in the GVB orbitals. That's to get good bond energies. Thus, our wavefunction correctly goes to the proper limit at infinity and is size-consistent or size-extensive.

The problem with all but one of the calculations that you mentioned is that they started with the wrong wavefunction, the Hartree-Fock function. A better way to do these calculations, which would probably result in a correct description of the order of the states, would be to solve for some of the higher spin states using UHF, and then start with those orbitals and do CI. What we do is correlate the wavefunctions while we're solving for the orbitals, and then do a CI among those correlated orbitals.

G. LOEW (*The Rockefeller University, New York*): The result from those CI calculations which use a multi-configuration ground state is not a pure S = 0 iron. In fact, we find about a 50-50 mixture of what you call the t-state and the S = 0 iron state.

I have a question. In your result, your lowest triplet state is about 8 kcal/mol, about 2400 wavenumbers, above the ground state. There is a reappearing measurement of what is supposedly paramagnetism of the myoglobin oxygen system, which would require a triplet excited state of about 200 wavenumbers. Although there is doubt about the measurement, would you be shocked if there were such a low triplet state for this system? Or is the accuracy of your calculation sufficient to exclude that?

GODDARD: I think that, at the level of these calculations, given that we do not have all the protein and that there is no proper representation of the imidazole (we use ammonia instead), it's certainly possible that this triplet state can move up or down a few tenths of an eV. I believe that the triplet state couldn't be as close as a couple of hundred wavenumbers, as is implied by some studies. But we do find a low lying triplet state, as expected from our qualitative description of the states.

M. ZERNER (*University of Guelph, Guelph, Ontario*): I'm trying to find the difference between what you're saying with the valence bond approach and

what the molecular orbital theory is saying. Insofar as both are reasonable approaches, you have to ask if there is an experiment or property that would distinguish between the two. Other properties have been calculated with success, especially by Dr. Loew. It isn't always easy to compare the GVB wavefunction with the CI wavefunction, unless the orbitals 'are localized. But, in the MO calculation on the heme complex, the MOs are not localized. You'd have to expand the molecular orbitals in terms of the GVB functions, expand those determinants, and then see whether the leading determinants that you get look like the GVB determinants or vice versa. You might say that the energy of the higher multiplicity is lower, but, after all, that's a very small fraction of the total energy, something like 0.001%. To compare those wavefunctions, you've got to do a lot of work and regroup these determinants.

GODDARD: The first point is that the MO description is a special case of the GVB description. Thus, if the MO description were best, then GVB would yield the same result. I consider the coup de grace for the MO description of the dioxygen binding in these systems to be the fact that it gets the wrong spin state. The MO description puts the triplet (and quintet and septet) below the singlet. That tells you something is very bad about your description of electronic structure. The GVB approach is the most effective approach to CI calculations in large systems. Solving self-consistently for the orbitals while building in the correlation allows you to get orbitals that are really tuned for the highly correlated wavefunction. Starting with MO wavefunctions for a system like ozone or a system with this kind of a bad description in a Hartree-Fock scheme takes incredibly high orders of CI in order to obtain the level of correlation included automatically in GVB. Even the oxy heme system, with well-correlated orbitals, requires quadruple excitations for accurate excitation energies. The particular advantage of the GVB approach, as opposed to other multi-configuration approaches or to CI, is that the process of calculating the wavefunctions requires only the same kind of quantities that were required in the Hartree-Fock calculation, so that the cost of GVB is comparable to that of the MO calculation. For systems such as  $\text{HbO}_2$ , it's really important to solve for the wavefunctions with the correlation.

LOEW: I have two comments. It seems to me that what you're implying in your analysis is that, along the way of oxygen binding, you use the fact that reactants are high spin and the ground state of the product is zero spin. You're also implying that, somewhere along the way, the oxygen stabilizes the triplet state of the iron. If you translate this into a potential surface calculation, you're implying that, somewhere along the line, there's a spin state change, somewhere intermediate between the reactants and the products. Yet, in your schematic diagram, you didn't show a transition state. What is the nature of this state? Is it an intermediate, is it a transition state? Essentially, you're saying that an oxygen binds somewhere along the line, and the system goes from a quintet to a triplet to a diamagnetic state. Your diagrams didn't describe this in any way.

GODDARD: Actually, I did have a diagram of it (FIGURE 6d). But the necessity of a spin flip is manifest even before doing any calculations whatsoever. Starting with  $S = 2$  Hb and the  $\text{O}_2$   $S = 1$  state of dioxygen, there's no way you can couple

$S = 2$  with  $S = 1$  and get  $S = 0$  for  $\text{HbO}_2$ . So you know that, somehow or other, there must be a spin flip upon binding of  $\text{O}_2$ . The point I made was that the state of Hb that has the  $t$ -configuration makes both  $\sigma$  and  $\pi$  bonds with the  $\text{O}_2$ , leading to an  $S = 0$  state that is the ground configuration of the oxy heme system. There is an excited spin one state that is actually bound with respect to dissociation. This is the triplet state that Dr. Karplus refers to in his work.