Synthetic Models for the Oxygen-Binding Hemoproteins[1]

James P. Collman

Department of Chemistry, Stanford University, Stanford, California 94305

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A principal theme of bioinorganic chemistry is the design of metal complexes which mimic active sites in functional metalloproteins. The veracity of these synthetic mimics can be measured by the congruence between their spectroscopic, structural, and chemical properties and those features of the metal protein. In this Account I will describe the current status of our synthetic models for the oxygen-binding hemoproteins, hemoglobin (Hb) and myoglobin (Mb).

The respiratory proteins Hb and Mb serve to transport and to store O_2—functions essential to the life of all vertebrates.[2] The active site in Hb and Mb is a heme (Figure 1) tightly bound to a protein (globin) through about 80 hydrophobic interactions and a single coordinate bond between the imidazole of the "proximal histidine" and iron. Mb is a monomer, whereas Hb is a tetramer composed of two similar globins of unequal length. In spite of numerous differences in their amino acid sequences, all Mb and Hb globin–heme units have very similar tertiary structures[2a] consisting of eight helical regions designated by A–H. In the Mb numbering system, which is used for Mb and Hb alike, residues are numbered by their order in these segments. Thus the proximal histidine, which is common to all functional Mb's and Hb's, is invariably the eighth residue in helical region F (i.e., His-F8). The heme is wedged in a crevice between segments E and F. Oxygen binds on the E ("distal") side of the porphyrin.

The most significant property of Hb is cooperative O_2 binding: the O_2 affinity of the tetramer rises with increasing saturation.[2a,3] Cooperativity is required for the transfer of O_2 from the carrier Hb to the receptor Mb as well as for responses to other physiological requirements.

Spin-state structural relationships in heme coordination chemistry were proposed by Hoard[4] and Williams[5] and subsequently demonstrated in simple hemes by Hoard[4] and in hemoproteins by Perutz.[2a] Ferrous porphyrins have six d electrons and exhibit three spin states—each having a characteristic coordination number and structure. Six-coordinate ferrous hemes having two axial ligands, one on either side of the porphyrin, are invariably diamagnetic and low spin ($S = 0$). Its covalent radius is such that low-spin iron(II) fits into the porphyrin core without stress, as observed in carbonylmyoglobin, MbCO.[6] High-spin iron(II) porphyrins are invariably five-coordinate,[7] with the iron atom displaced well out of the porphyrin plane toward the single axial ligand, as, for example, in the deoxymyoglobin model illustrated below.[8] Iron(II) porphyrins also exhibit an intermediate spin state ($S = 1$) characterized by FeTPP.[11] In this state, which has no known biological counterpart, iron is four-coordinate and exhibits such short iron–nitrogen distances that the porphyrin develops a pronounced ruffling.[11]

The manner by which the protein in Hb and Mb regulates oxygen affinity, controls axial ligation, and provides kinetic stabilization to the iron dioxygen group, as well as the structural nature of this group, are questions which may be best addressed by the *isolation* and *full characterization* of model oxygen compounds.

Modeling the High-Spin Deoxy Stage of Mb and Hb

When we began our work, no synthetic, five-coordinate, high-spin ferrous porphyrins had been well

James. P. Collman is a native of Nebraska and received B.S. and M.S. degrees from the University of Nebraska. Following receipt of the Ph.D. from the University of Illinois in 1958, he joined the faculty of the University of North Carolina at Chapel Hill. In 1967 he moved to Stanford University, where he is Professor of Chemistry. His research interests include organotransition metal chemistry and bioinorganic chemistry. Professor Collman received the 1975 ACS Award in Inorganic Chemistry, and this Account is based in part on his award address.

(1) The following abbreviations are used throughout this article: $PP_{IX}DME$, protoporphyrinato IX dimethyl ester; TPP, *meso*-tetraphenylporphyrinato; OEP, octaethylporphyrinato; PP_D, deuteroporphyrinato; *N*-MeIm, *N*-methylimidazole; 2-MeIm, 2-methylimidazole; py, pyridine; THF, tetrahydrofuran; THT, tetrahydrothiophene; acac, acetylacetonate anion. The schematic structure 11 in Figure 5 represents an iron(II) complex of the porphyrin **10** shown in Figure 4. ΔE_Q is the Mössbauer quadrupole splitting parameter, and δ is the chemical shift from elemental iron. $p_{1/2}O_2$ represents the oxygen pressure at half saturation.

(2) (a) For an excellent review of the structure of hemoglobin and the Perutz theory of cooperativity, see M. F. Perutz, *Brit. Med. Bull.*, **32** (3), 193 (1976). For general references to Hb, Mb, and iron porphyrins, see: (b) N. W. Makinen in "Techniques and Topics in Bioinorganic Chemistry", C. A. McAuliffe, Ed., Wiley, New York, N.Y., 1975, Part 1, Chapter 2; and J. H. Pratt in "Techniques and Topics in Bioinorganic Chemistry", C. A. McAuliffe, Ed., Wiley, New York, N.Y., 1975, Part 2, Chapter 7. (c) W. S. Caughey, *Bioinorg. Chem.*, **2**, Chapter 24 (1973). (d) J. M. Rifkind, *ibid.*, **2**, Chapter 25 (1973). (e) R. E. Dickerson and I. Geis, "The Structure and Action of Proteins", Harper & Row, New York, N.Y., 1969. (f) E. Antonini and M. Brunori, "Hemoglobin and Myoglobin in Their Reactions with Ligands", Elsevier, New York, N.Y., 1971.

(3) (a) For recent discussions of cooperative oxygen binding to Hb, see J. M. Baldwin, *Brit. Med. Bull.*, **32** (3), 213 (1976); (b) M. Weissbluth, "Hemoglobin Cooperativity and Electronic Properties", Springer-Verlag, New York, N.Y., 1974.

(4) J. L. Hoard in "Porphyrins and Metalloporphyrins", K. M. Smith, Ed., Elsevier, Amsterdam, 1975, Chapter 8.

(5) (a) R. J. P. Williams, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **20**, 5 (1961); (b) R. J. P. Williams, *Chem. Rev.*, **56**, 299 (1956).

(6) (a) E. J. Heidner, R. C. Ladner, and M. F. Perutz, *J. Mol. Biol.*, **104**, 707 (1976). (b) Actually, in MbCO and related model porphyrins the iron is 0.01 Å out of the plane toward the CO.

(7) The converse, that five-coordinate iron(II) porphyrins are invariably high-spin, is not necessarily true. For example, Fe(TPP)(NO) could be considered a five-coordinate, low-spin complex: W. R. Scheidt and M. E. Frisse, *J. Am. Chem. Soc.*, **97**, 17 (1975).

(8) Hoard[4] proposed that in high-spin iron(II) porphyrins iron would protrude out of the porphyrin plane toward the single axial ligand on the grounds that the covalent radius of high-spin ferrous is too large to be accommodated by the unstrained porphyrin core. However, recent structural studies of compounds such as $Sn^{IV}Cl_2(TPP)$[9] and $Mo^{VI}(TPP)(O_2)_2$[10] exhibit an in-plane metal with a covalent radius larger than that usually found for high-spin ferrous. Furthermore, the out-of-plane character of five-coordinate iron(II) porphyrins could also arise from minimization of repulsive interactions between the axial base and the porphyrin. There is no obvious experiment to test this.

(9) D. M. Collins, W. R. Scheidt, and J. L. Hoard, *J. Am. Chem. Soc.*, **94**, 6689 (1972).

(10) B. Chevrier, Th. Diebold, and R. Weiss, *Inorg. Chim. Acta*, **19**, L57 (1976).

(11) J. P. Collman, J. L. Hoard, N. Kim, G. Lang, and C. A. Reed, *J. Am. Chem. Soc.*, **97**, 2676 (1975).

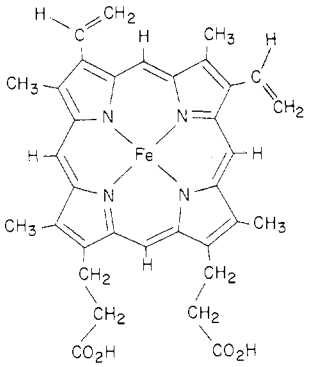


Figure 1. Iron(II) protoporphyrin IX (heme b).

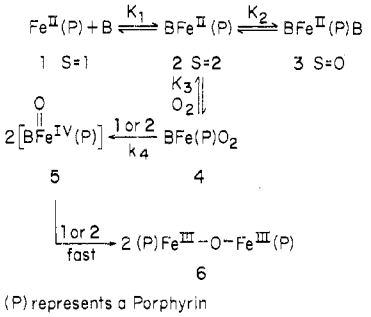


Figure 2. Axial ligation, oxygenation, and oxidation of ferrous porphyrins.

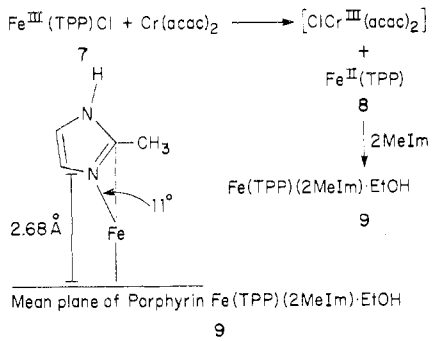


Figure 3. Preparation of deoxymyoglobin model.

characterized. The difficulty seemed to lie in the consecutive equilibria between axial bases and ferrous porphyrins characterized by K_1 and K_2 in Figure 2. We reasoned that $K_2 > K_1$, with the result that the five-coordinate complex **2** would not be the dominant form in solution. This assumption proved correct, as subsequent studies have shown K_2/K_1 to be 10–30 in aprotic solvents[12–16] at 25 °C. This ratio becomes much larger at low temperatures.[17,18]

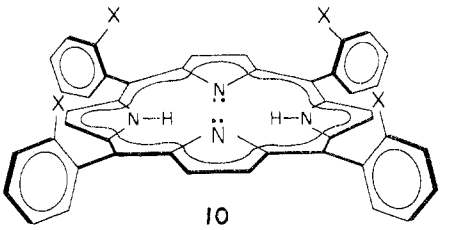


Figure 4. The Tetra-α atropisomer of ortho-substituted *meso*-tetraarylprophyrins.

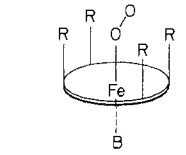
Reed proposed that the sterically hindered axial base, 2-MeIm,[1] could form a five-coordinated ferrous complex, **2**, but not a six-coordinate complex, **3**.[16] Reed assumed that the methyl group in 2-methylimidazole would experience a repulsive interaction with the porphyrin ring if iron were in the porphyrin plane. His plan worked perfectly,[19] as shown in Figure 3. Chromous reduction of the ferric complex **7** in benzene affords the sparingly soluble $Fe^{II}TPP$, **8**.[20] Treatment of FeTPP with 2-MeIm yields the five-coordinate complex **2** in solution, but isolation of this complex requires addition of ethanol to precipitate the solvate, $Fe(TPP)(2\text{-MeIm})\cdot C_2H_5OH$, **9**; otherwise the four-coordinate complex, Fe(TPP), preferentially crystallizes.[21] Hoard determined the structure of ferrous porphyrins **8** and **9**.[22] The five-coordinate complex **9** is high spin (μ(25 °C) = 5 μ_B) and exhibits Mössbauer spectra remarkably similar to those of deoxy-Hb or deoxy-Mb.[19] The structure of **9** (Figure 3) shows iron to be 0.55 Å from the mean plane of the porphyrin. The iron–N(imid) vector is tilted 11° from the normal to the porphyrin plane—a result of the predicted repulsive interaction.[23]

Modeling Oxymyoglobin

In contrast to the *reversible oxygenation* of Hb and Mb, simple ferrous porphyrins are rapidly and *irreversibly oxidized* by oxygen (Figure 2). For example, solutions of the deoxymyoglobin model **9** are rapidly oxidized in air.[24]

Wang[25] first demonstrated that simple porphyrins could be reversibly oxygenated when immobilized in *solid*[26] polymer films. Several years later while our own work was in progress, Wang's experiment was repeated and extended by Traylor who used solid films of ferrous porphyrins with appended axial ligands and demonstrated the 1:1 Fe:O_2 stoichiometry.[27] This was im-

(12) D. Brault and M. Rougee, *Biochemistry*, **13**, 4591, 4598 (1974).
(13) (a) M. Rougee and D. Brault, *Biochem. Biophys. Res. Commun.*, **55**, 1364 (1974); (b) D. Brault and M. Rougee, *ibid.*, **57**, 654 (1974).
(14) M. Rougee and D. Brault, *Biochemistry*, **14**, 4100 (1975), and references therein.
(15) J. M. Pratt in ref 2b, p 124. The change in spin state undoubtedly controls the relative magnitudes of K_1 and K_2. For example, with cobalt(II) binding the first ligand causes a change from high to low spin and $K_1 >> K_2$, whereas for iron the spin change occurs upon binding the second ligand and $K_1 < K_2$.
(16) (a) Reed's hypothesis has been verified by the solution equilibrium studies of Rogee and Brault[13b,14] who have shown that K_1 for 2-MeIm is 1.3×10^4 M^{-1} and K_2 is too small to measure at 25 °C in benzene for iron(II) deuteroporphyrin. (b) Note, however, that at low temperatures 2-MeIm forms a six-coordinate complex in solution.[17] (c) The situation is also different with ferric porphyrins.[18]
(17) G. C. Wagner and R. J. Kassner, *Biochim. Biophys. Acta*, **392**, 314 (1975).
(18) M. Momenteau, M. Rougee, and B. Loock, *Eur. J. Biochem.*, **71**, 63 (1976).
(19) J. P. Collman and C. A. Reed, *J. Am. Chem. Soc.*, **95**, 2048 (1973).
(20) This low solubility allowed us to separate pure FeTPP from the reaction mixture without chromatography. This is very important because of the extreme oxygen sensitivity of FeTPP in solution.
(21) Note that the species which crystallizes is usually determined by relative solubilities and not by solution equilibria. This significant concept controls much of what can be done synthetically as the axial ligation of porphyrins is under thermodynamic rather than kinetic control.
(22) J. L. Hoard, C. A. Reed, and J. P. Collman, paper in preparation: see also ref 4.
(23) It is possible that this interaction has slightly increased the iron to mean porphyrin plane separation from its intrinsic, unstressed value. It is, therefore, important to determine the precise structure of other, unstrained high-spin iron(II) porphyrins.
(24) Crystalline samples of **8** and **9** are stable for prolonged periods in air. The kinetic stability of crystalline samples undoubtedly depends on the porosity of the crystal rather than the intrinsic reactivity of the complex.
(25) J. H. Wang, *J. Am. Chem. Soc.*, **80**, 3168 (1958).
(26) Ferrous porphyrins in solvent-swollen, cross-linked polystyrene are irreversibly oxidized.[19]
(27) C. K. Chang and T. G. Traylor, *Proc. Natl. Acad. Sci. U.S.A.*, **70**, 2647 (1973).



11 (a) B = N-MeIm
(b) B = N-BuIm
(c) B = THF
(d) B = THT

R = NHC-C(CH$_3$)$_3$ all cases.

Figure 5. Oxygen complexes derived from the picket-fence porphyrins.

mediately followed by a series of reports describing reversible, stoichiometric oxygenation of iron(II) porphyrins[28] and macrocylic[29] complexes *in solution* at reduced temperatures. Subsequently Baldwin[30] prepared an elegant "capped" ferrous porphyrin which reversibly oxygenates in solution at room temperature. The kinetic stability of solutions containing these oxygen complexes depends on the temperature, the nature and concentration of the porphyrin and of the axial base, the partial pressure of oxygen, and the solvent polarity. The role which these factors play in kinetically stabilizing iron–dioxygen complexes can be understood (vide infra) in terms of the probable mechanism for irreversible oxygenation outlined in Figure 2. This mechanism[31] guided our own attempts which eventually led to the isolation of oxymyoglobin models.

Our strategy was to inhibit the inner sphere redox reaction, k_4, by preparing a new class of porphyrins which have a protective enclosure for binding oxygen on one side of the porphyrin ring. The other side was left unencumbered with the hope that it could be

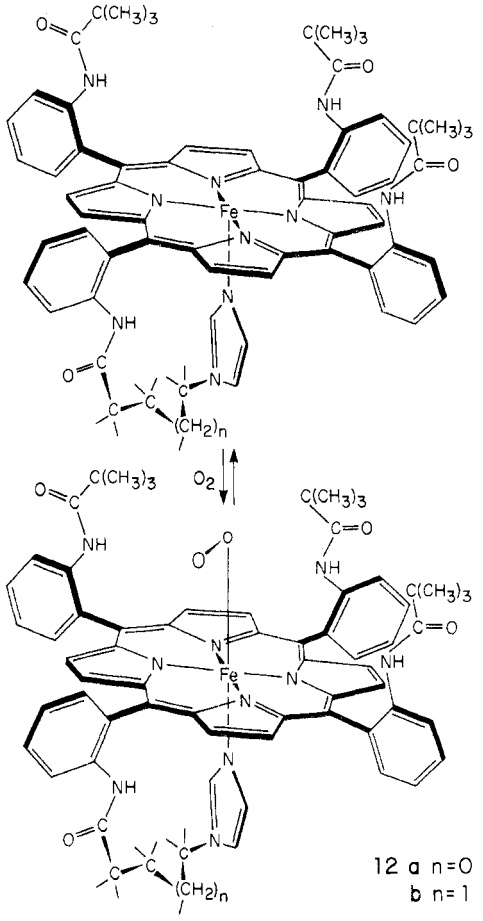


Figure 6. Picket-fence porphyrins having an appended imidazole base.

protected by a bulky axial imidazole. We called these "picket-fence porphyrins", and it was our good fortune that this approach led to the *isolation* and characterization of a series of *crystalline* dioxygen complexes.[35]

The construction of the picket-fence porphyrins (**10**, Figure 4) depends on the torsional isomerism of ortho-substituted *meso*-tetraarylporphyrins. The preparation, chromatographic separation, and elaboration of these porphyrins and the introduction of iron are described elsewhere.[35,36]

At first we were chagrined to discover that the picket fence does not strongly inhibit coordination of a second axial ligand either in solution or in the solid state.[37] Addition of various *N*-alkylimidazoles afford diamagnetic, six-coordinate complexes, Fe(TpivPP)(*N*-RIm)$_2$, which have been isolated as crystalline solids.[35] However, electronic and ^{1}H NMR spectra show that under 1 atm of O_2 at 25 °C in solution these complexes are completely oxygenated. In solution these dioxygen complexes, Fe(TpivPP)(*N*-RIm)(O_2), are kinetically stable for prolonged periods provided that 2–4 equiv of axial base are present to protect the unshielded side of

(28) (a) C. K. Chang and T. G. Traylor, *J. Am. Chem. Soc.*, **93**, 5810 (1973); (b) C. K. Chang and T. G. Traylor, *ibid.*, **95**, 8476 (1973); (c) W. S. Bringar, C. K. Chang, J. Geibel, and T. G. Traylor, *ibid.*, **96**, 5597 (1974); (d) C. K. Chang and T. G. Traylor, *Proc. Natl. Acad. Sci. U.S.A.*, **72**, 1166 (1975); (e) W. S. Bringar and C. K. Chang, *J. Am. Chem. Soc.*, **96**, 5595 (1974); (f) C. K. Chang and T. G. Traylor, *ibid.*, **95**, 8477 (1973); (g) G. C. Wagner and R. J. Kassner, *ibid.*, **96**, 5593 (1974); (h) J. Almog, J. E. Baldwin, R. L. Dyer, J. Huff, and C. J. Wilkerson, *ibid.*, **96**, 5600 (1974). (i) D. L. Anderson, C. J. Weschler, and F. Basolo, *ibid.*, **96**, 5599 (1974).

(29) J. E. Baldwin and J. Huff, *J. Am. Chem. Soc.*, **95**, 5758 (1973).

(30) (a) J. Almog, J. E. Baldwin, and J. Huff, *J. Am. Chem. Soc.*, **97**, 226 (1975); (b) J. Almog, J. E. Baldwin, R. L. Dyer, and M. Peters, *ibid.*, **97**, 227 (1975).

(31) Our plan was based on Hammond's[32] kinetic study of the autoxidation of $FeCl_2$ in aqueous ethanol. The rate-determining step was found to be second order in iron and first order in oxygen. Hammond's results also indicate that the postulated intermediate formed in the rate-determining step is not ferric ion. Thus **5** in Figure 2 has been tentatively written as an iron(IV) porphyrin analogous to "compound II" in horseradish peroxidase.[33] G. LaMar's low-temperature ^{1}H NMR results indicate a μ-peroxo ferric porphyrin intermediate (private communication). The known coordination number of deoxy-Mb and deoxy-Hb and the fact that solutions of iron(II) porphyrins are stable toward oxygen in the presence of a large excess of an axial base, such as imidazole, are also taken into account by this proposed mechanism. At the time we began our work, we were unaware of Caughey's[34] similar proposed mechanism for autoxidation of ferrous porphyrins. Caughey had recognized the inhibition of oxidation by excess pyridine as well as the role of the globin in suppressing the bimolecular step.

(32) G. S. Hammond and C.-H. S. Wu, *Adv. Chem. Ser.*, **No. 77**, 186 (1968).

(33) H. B. Dunford and J. S. Stillman, *Coord. Chem. Rev.*, **19**, 187 (1976).

(34) (a) J. O. Alben, W. H. Fuchsman, C. A. Beaudrean, and W. S. Caughey, *Biochemistry*, **7**, 624 (1968); (b) I. A. Cohen and W. S. Caughey, *ibid.*, **7**, 636 (1968). (c) N. Sadasivan, H. I. Eberspaecher, W. H. Fuchsman, and W. S. Caughey, *ibid.*, **8**, 434 (1969); (d) O. K. Kao and J. H. Wang, *ibid.*, **4**, 342 (1965); (e) I. A. Cohen and W. S. Caughey in "Hemes and Hemoproteins", B. Chance, R. E. Easterbrook, and T. Yonetani, Ed., Academic Press, New York, N.Y., 1966, pp 577–579.

(35) J. P. Collman, R. R. Gagne, C. A. Reed, T. R. Halbert, G. Lang, and W. T. Robinson, *J. Am. Chem. Soc.*, **97**, 1427 (1975); J. P. Collman, R. R. Gagne, T. R. Halbert, J-C. Marchon, and C. A. Reed, *ibid.*, **95**, 7868 (1973).

(36) Our original synthesis introduced iron in the ferric state and was followed by reduction to the ferrous state. In the case of more soluble iron porphyrins, it is more convenient to introduce iron(II) directly.

(37) This may actually be a fortunate result. Baldwin[30b] has suggested that very hindered porphyrins such as his capped porphyrin may prevent the formation of the six-coordinate **3**, thus raising the relative concentration of the very oxygen-sensitive four-coordinate complex **1** (Figure 3).

Table I
Comparison between Key Distances (Å) in Models and Hemoproteins

	Deoxy-Mb[a]	Deoxy-Hb[a] α	Deoxy-Hb[a] β	Fe(TPP)-(2MeIm)[b]	Fe(TpivPP)-(*N*-MeIm)(O_2)[c]	Co(TPP)-(*N*-MeIm)[d]
Metal to porphyrin plane	0.55	0.60	0.63	0.55	0.03	0.14
Imidazole N to porphyrin plane	2.6	2.6	2.8	2.68	2.04	2.30
Metal to imidazole N	2.1	2.0	2.2	2.16	2.07	2.16

[a] Reference 2a. [b] Reference 22. [c] Reference 49. [d] J. L. Hoard and W. R. Scheidt, *Proc. Natl. Acad. Sci. U.S.A.*, **70**, 3919 (1973); R. G. Little and J. A. Ibers, *J. Am. Chem. Soc.*, **96**, 4452 (1974).

the porphyrin.[38] Of far greater significance is the fact that cooling or concentrating these solutions leads to analytically pure, crystalline dioxygen complexes, Fe(TpivPP)(*N*-RIm)(O_2) (R = CH_3 and *n*-Bu, **11a,b**, Figure 5).[35] Thus far we have been able to *isolate* a total of five iron–dioxygen complexes. Two of these have different axial ligands, a tetrahydrofuran, **11c**,[39] and tetrahydrothiophene, **11d**, and one has three pivalamido pickets and an appended axial imidazole **12** (Figure 6).[42]

Solutions of the "tail-base" porphyrins **12a,b** reversibly oxygenate at 25 °C as shown by ^{1}H NMR[43] and optical spectral studies. These "tail-base" porphyrins are interesting for several reasons. The oxygen binding pocket in **12a** and **12b** has low symmetry so that the dioxygen ligand may be ordered. The length and conformation of the tails can be controlled and may have a significant effect on oxygen affinities. The nature of the internal axial base can be varied[45] while its local concentration remains constant, permitting direct comparison of the relative oxygen affinities without complications from equilibria such as K_2 in Figure 2.[48]

Table II
Comparison of νO_2 For Dioxygen Complexes

Compd	νO_2,[a] cm^{-1}
Fe(TpivPP)(*N*-MeIm)(O_2)	1159[b]
Fe(TpivPP)(*N*-TrIm)(O_2)	1163[b]
Co(TpivPP)(*N*-MeIm)(O_2)	1150[b]
Cr(TPP)(py)(O_2)	1142[c]
Ti(OEP)(O_2)	898[d]
HbO_2	1107[e]
MbO_2	1103[f]
$CoHbO_2$	1106[g]

[a] Values for the uncomplexed ligand in its various oxidation states are: O_2, 1556 cm^{-1}; O_2^-, 1145 cm^{-1}; O_2^{2-}, 770 cm^{-1}. [b] Taken from ref 59. [c] Taken from ref 69. [d] Taken from ref 56. [e] Taken from ref 60. [f] Taken from ref 61. [g] Taken from ref 62.

Properties of Model Dioxygen Complexes

X-Ray Diffraction. We were fortunate to obtain single crystals of the picket-fence oxygen complex **11a**, though these turned out to be only marginally suitable for x-ray diffraction studies. In spite of poor crystal quality and limited data, Robinson has been able to determine the main structural features of **11a**.[49] Dioxygen is bound to Fe in the angular manner (**13**) first proposed by Pauling[50a] and later by Weiss,[50a] rather than the triangular structure **14** proposed by Griffith.[50b] Because of limited data, high thermal motion, and fourfold disorder[51] of the terminal oxygen in the symmetric picket-fence pocket, the two most interesting structural features cannot be accurately determined. Thus the measured O–O separation (1.16 (5) Å) is probably grossly underestimated,[52] and the Fe–O–O

(38) Under these conditions a saturated solution of the picket-fence oxygen complex undergoes about 5% oxidation at 25 °C in 5 days, which corresponds to a half-life of 2 to 3 months. Because of the complicated oxidation mechanism, it is difficult to make comparisons between the stabilities of the various iron–dioxygen complexes described in the literature, especially when critical experimental conditions such as the concentrations of iron, oxygen, and axial ligand are often not specified.

(39) The tetrahydrofuran complex, Fe(TpivPP)(THF)(O_2) cannot be isolated from solution because concentrated solutions are insufficiently stable (10^{-5} M solutions are stable for prolonged periods in THF at 25 °C). This complex was prepared by a solid gas reaction and was originally reported to be paramagnetic by direct measurement of the magnetic moment, but diamagnetic according to Mössbauer spectra determined in an applied magnetic field.[35,40] We now know that these solid samples oxygenate very slowly with simultaneous loss of a THF solvate.[41] After apparently complete oxygenation, we still observe a residual magnetic moment which slowly increases with time. Whether this moment is intrinsic, due to incomplete oxygenation, or to slow solid-state oxidation, is still not clear.

(40) J. P. Collman, R. R. Gagne, and C. A. Reed, *J. Am. Chem. Soc.*, **96**, 2629 (1974).

(41) J. P. Collman and S. Hayes, unpublished results.

(42) Halbert's and Sorrell's synthesis leading to **12** (Figure 6) deserves comment. Three amino groups in the tetraamino derivative **10** (X = NH_2) were selectively converted into pivalamido groups (~40% yield). Heating this tripivalamide derivative selectively affords two atropisomers by rotation of the phenyl having the primary amino group. The atropisomer with the amino group trans to the three pivalamides is elaborated into the tail derivatives **12a** and **12b** (the latter having four CH_2 groups).

(43) The ^{1}H NMR of **12a** at 0 °C shows two *tert*-butyl singlets in the ratio of 1:2, confirming the expected structure and the diamagnetic nature of the oxygen complex. Cycling these solutions between 0 and 90 °C does not change the 0 °C ^{1}H NMR spectrum, providing additional evidence for the solution stability of this complex. However, over prolonged periods solutions of the picket fence complex **11a** is more stable than **12a**. The metal-free derivative of **12** is very rapidly destroyed in the presence of light and oxygen—even in the solid state. We suppose that the porphyrin photosensitizes oxygen and the appended imidazole efficiently traps the singlet oxygen. Thus these tail-base porphyrins are models for the genetic disease erythropoetic protoporphyria.[44]

(44) A. A. Lamola, T. Yamane, and A. M. Trozolo, *Science*, **179**, 1133 (1973).

(45) For instance, Sorrell has characterized a derivative of **12** in which the appended axial base is a methyl thioether. Mössbauer spectra[46] show that this compound binds oxygen in solution, but the dioxygen complex is less stable than **12**. Several other groups have prepared hindered iron(II) porphyrins which nevertheless are rapidly autoxidized.[47]

(46) J. P. Collman, T. N. Sorrell, and G. Lang, unpublished results.

(47) (a) H. Dieckmann, C. K. Change, and T. G. Traylor, *J. Am. Chem. Soc.*, **93**, 4068 (1971); (b) H. Ogashi, H. Sugimoto, and Z. Yoshida, *Heterocycles*, **3**, 1146 (1973); (c) J. E. Baldwin, T. Klose, and M. Peters, *J. Chem. Soc., Chem. Commun.*, 881 (1976); (d) A. R. Battersby, D. G. Buckley, S. G. Hartley, and M. D. Turnbull, *ibid.*, 879 (1976).

(48) Many types of axial bases[28e,g] have been shown to support oxygenation of iron(II) porphyrins: imidazoles, pyridines, primary amines, an ether, a thioether, and dimethylformamide. The oxygen affinities produced by these diverse axial bases are uncertain and available data are difficult to interpret because of the competing equilibria K_1 and K_2—a problem which is aggravated by the dramatic effect which low temperatures have on these associative equilibria.[14,17,28g]

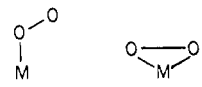
(49) (a) G. B. Jameson, G. A. Rodley, W. T. Robinson, R. R. Gagne, C. A. Reed, and J. P. Collman, *Inorg. Chem.*, submitted for publication. (b) J. P. Collman, R. R. Gagne, C. A. Reed, W. T. Robinson, and G. A. Rodley, *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 1326 (1974).

(50) (a) L. Pauling, *Stanford Med. Bull.*, **6**, 215 (1948); L. Pauling, *Nature (London)*, **203**, 182 (1964); J. J Weiss, *ibid.*, **202**, 85 (1964); (b) J. S. Griffith, *Proc. R. Soc. London, Ser. A*, **235**, 23 (1956).

(51) The four-way disorder of dioxygen and the twofold disorder of the *N*-MeIm group in **11a** give rise to three dioxygen conformers which are undoubtedly in dynamic equilibrium.

angle (131 (4)°) may be overestimated.[49] The Fe–O bond in the picket-fence dioxygen complex **11a**, 1.75 (2) Å, is slightly shorter, and the Fe–N(imid) bond, 2.07 (2) Å, is slightly longer than the expected summation of covalent radii. However, until more precise structures[53] become available, it seems foolish to ascribe any significance to these differences. The Fe in **11a** is only 0.03 Å out of the mean plane of the porphyrin toward dioxygen. In Table I these distances and angles are compared with parameters from the Mb model **9** and the hemoproteins.[54] This comparison forms the basis for estimating *l*, defined as the distance which the N^{ϵ} of the proximal imidazole moves with respect to the mean plane of the porphyrin upon oxygenation of the model ferrous porphyrins and of Hb and Mb.[2a,55] The value of *l* is important in arguments concerning cooperativity[2a] (vide infra).

Recently two metal porphyrins[10,56] which have edge-bound dioxygen groups (**14**), as in the Griffith



13 **14**

model,[50b] have been characterized structurally. On the basis of the O–O separation and νO_2 (vide infra) bands these are best described as bidentate peroxide ligands similar to Vaska's[57] famous dioxygen complex.

The Dioxygen Infrared Stretching Frequency. One of the most significant properties of coordinated dioxygen is the νO_2 frequency. At first we were unable to detect a νO_2 band in the picket-fence O_2 complexes. Then we observed a 1385-cm^{-1} band at low temperatures,[58] which turned out to be spurious.[59] Eventually Halbert and Suslick were able to identify the true νO_2 band using Fourier-transform, isotope-difference spectra.[59] This band is compared with νO_2 bands from other metal porphyrin complexes and the hemoproteins HbO_2,[60] MbO_2,[61] and $CoHbO_2$[62] (Table II). With the exception of the triangular peroxotitanium(IV) complex all νO_2 frequencies of simple macrocyclic dioxygen complexes fall in a narrow (50 cm^{-1}) range near that of free superoxide ion, 1145 cm^{-1}. Similar values, which have been reported for Co–Schiff-base–dioxygen adducts,[63] show that νO_2 is rather insensitive to the nature of the macrocyclic ligand.[64] On the basis of ESR[65] and ESCA[66] studies, the unpaired electron in the Co–dioxygen complexes is widely assigned to an oxygen π orbital,[67] suggesting that these compounds are best described as Co(III)–superoxide complexes. That the narrow range of νO_2 frequencies is close to that of free superoxide ion gives credence to the assignment of the superoxide formalism[68] to all of these *angular complexes* and of peroxide formalism to the *triangular* Ti^{IV} complex (νO_2 = 898 cm^{-1}).[70]

Table III
Zero-Field Mössbauer Parameters for Hemoglobin Model Compounds[a]

Compd	δ, mm s^{-1}		ΔE_Q, mm s^{-1}	
	4.2 K	195 K	4.2 K	195 K
Fe(TpivPP)(*N*-MeIm)(O_2)	0.28	0.24	2.11	1.44
Fe(TpivPP)(*N*-BuIm)(O_2)	0.28	0.24	2.10	1.31
Fe(TpivPP)(THF)(O_2)	0.33	0.29	2.61	2.13
Fe(TpivPP)(THT)(O_2)	0.31	0.27	2.29	1.87
HbO_2	0.24	0.20	2.24	1.89

[a] These values are taken from ref 75.

(52) This O–O distance is unreasonably short when compared with values for the three oxidation levels of the free ligand: O_2 (1.21 Å), O_2^- (~1.30 Å), and O_2^{2-} (~1.49 Å). The precise O–O separations in the related Schiff-base cobalt(III) superoxide complexes range from 1.27 to 1.30 Å: R. S. Gall, J. F. Rogers, W. P. Schaefer, and G. G. Christoph, *J. Am. Chem. Soc.*, **98**, 5135 (1976); A. Avdeef and W. P. Schaefer, *ibid.*, **98**, 5153 (1976). Apparent shortening of O–O distances due to large thermal motion and disorder is a common phenomenon: F. Halverson, *Phys. Chem. Solids*, **23**, 207 (1962); L. D. Brown and K. N. Raymond, *Inorg. Chem.*, **14**, 2595 (1975).

(53) The tetrahydrothiophene-based oxygen complex **11c** has also been structurally characterized and has features similar to those of **11a**, but the data are of even more limited quality: G. B. Jameson, W. T. Robinson, J. P. Collman, and T. N. Sorrell, unpublished results.

(54) Recent hemoprotein x-ray diffraction studies are summarized in ref 2a and 6. (a) J. C. Norvell, A. C. Nunes, and B. P. Schroenborn, *Science*, **190**, 568 (1975): neutron diffraction structure sperm whale MbCO. (b) G. Fermi, *J. Mol. Biol.*, **97**, 237 (1975): human Hb. (c) J. F. Deatheraze, K. Moffat, R. S. Loe, and C. M. Anderson, *ibid.*, **104**, 687 (1976): structure of horse met-MbCN. (d) R. Huber, O. Epp, and H. Formanek, *ibid.*, **52**, 349 (1970): structure of chironomous thumi HbCO. (e) E. A. Padlan and W. E. Love, *J. Biol. Chem.*, **249**, 4067 (1975): structure of bloodworm MbCO.

(55) Several assumptions go into the estimate of *l*. There are no structural data for MbO_2 or HbO_2, which are apparently too sensitive toward oxidation for the collection of good x-ray data.[2a] Thus values from the model compound **11a** are used. The mean plane of the porphyrin is the parameter which can be estimated from protein x-ray structures.

(56) The O–O separation in $Ti^{IV}(OEP)(O_2)$, which has the structure shown in **14**, is 1.46 Å: R. Giulard, M. Fontesse, P. Fournari, C. Lecompte, and J. Protas, *J. Chem. Soc., Chem. Commun.*, 161 (1975).

(57) L. Vaska, *Acc. Chem. Res.*, **9**, 175 (1976).

(58) J. P. Collman, R. R. Gagne, H. B. Gray, and J. W. Hare, *J. Am. Chem. Soc.*, **96**, 6522 (1974).

(59) J. P. Collman, J. I. Brauman, T. R. Halbert, and K. S. Suslick, *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 3333 (1976).

(60) C. H. Barlow, J. C. Maxwell, W. J. Wallace, and W. S. Caughey, *Biochem. Biophys. Res. Commun.*, **55**, 91 (1973).

(61) J. C. Maxwell, J. A. Volpe, C. H. Barlow, and W. S. Caughey, *Biochem. Biophys. Res. Commun.*, **58**, 166 (1974).

(62) J. C. Maxwell and W. S. Caughey, *Biochem. Biophys. Res. Commun.*, **60**, 1309 (1974).

(63) (a) A. L. Crumbliss and F. Basolo, *J. Am. Chem. Soc.*, **92**, 55 (1970); (b) J. D. Landels and G. A. Rodley, *Synth. Inorg. Met.-Org. Chem.*, **2**, 62 (1972); (c) D. A. White, A. J. Solodar, and M. M. Baizer, *Inorg. Chem.*, **11**, 2160 (1972).

(64) Unfortunately at present we have no data on the effect which the axial base has on νO_2.

(65) (a) B. M. Hoffman, T. Szymanski, and F. Basolo, *J. Am. Chem. Soc.*, **97**, 673 (1975); (b) D. Getz, E. Melamad, B. L. Silver, and Z. Dori, *ibid.*, **97**, 3846 (1975); (c) B. M. Hoffman, D. L. Diemente, and F. Basolo, *ibid.*, **92**, 61 (1970); (d) D. Diemiente, B. M. Hoffman, and F. Basolo, *Chem. Commun.*, 467 (1970); (e) E. Melamad, B. L. Silver, and Z. Dori, *J. Am. Chem. Soc.*, **96**, 4689 (1974).

(66) J. A. Lauher and J. E. Lester, *Inorg. Chem.*, **12**, 244 (1973).

(67) There is, however, a dissenting opinion: (a) B. S. Tovrog and R. S. Drago, *J. Am. Chem. Soc.*, **96**, 6765 (1974); (b) B. S. Tovrog, D. J. Kiteo, and R. S. Drago, *ibid.*, **98**, 5144 (1976).

(68) (a) This *oxidation state formalism* which is widely employed by inorganic chemists is not a physical property but merely a convenient system of grouping structurally related complexes. In spite of obvious covalency, bonding electron pairs are formally assigned to the more electronegative atom. Note that in terms of redox potentials it is reasonable that in $Cr(TPP)(py)(O_2)$[69] an electron has been transfered from the strong reductant, Cr(II), to O_2. Chemists who find fault with this formalism should attempt to prepare the analogous aluminum superoxide complex. The νO_2 value and ESR hyperfine coupling parameters for this hypothetical complex should further confirm this classification. Unfortunately there seem to be no metal–dioxygen complexes which are resonably considered to be neutral dioxygen derivatives. (b) The low-spin ferric superoxide structure for HbO_2 was first proposed by J. J. Weiss.[50a] (c) Other physical measurements which have been cited as support for this formalism are: resonance raman (Y. Yamamoto, G. Palmer, D. Gill, I. T. Salmeen, and L. Rimai, *J. Biol. Chem.*, **248**, 5211 (1973); T. G. Spiro and T. C. Strekas, *J. Am. Chem. Soc.*, **96**, 338 (1974)), K β fluorescence emission (A. S. Koster, *J. Chem. Phys.*, **63**, 3284 (1975), **56**, 3161 (1972)), and optical spectroscopy (J. B. Wittenberg, B. I. Wittenberg, J. Peisach, and W. E. Blumberg, *Proc. Natl. Acad. Sci. U.S.A.*, **67**, 1846 (1970)). (d) For a recent theoretical analysis of the bonding in oxyhemoglobin see: B. D. Olafson and W. A. Goddard III, *Proc. Natl. Acad. Sci. U.S.A.*, **72**, 2335 (1975), and references therein.

(69) S. K. Cheung, C. J. Grimes, J. Wong, and C. A. Reed, *J. Am. Chem. Soc.*, **98**, 5028 (1976).

(70) Many other triangular dioxygen complexes are known. Their νO_2 bands are grouped around 850 cm^{-1}. Vaska[57] proposed similar classifications for angular and triangular O_2 complexes.

The 50-cm^{-1} difference between the νO_2 model for porphyrins and the hemoproteins is not understood, but might be caused by polarity in the O_2 binding pocket or slight changes in the Fe–O–O angle.[59] The close correspondence between νO_2 in HbO_2 and $CoHbO_2$ again illustrates the surprising insensitivity of νO_2 to the metal.

Magnetic Properties and Mössbauer Spectra of Iron–Dioxygen Complexes. HbO_2 and MbO_2 are generally considered to be diamagnetic.[71] Very recently, however, Cerdonio[72] reported that human HbO_2 shows a magnetic susceptibility (between 25 and 250 K) consistent with a thermal equilibrium between a ground-state singlet and an excited-state triplet, the energy separation, $2J$, being 146 cm^{-1}. Our studies indicate that the model oxygen complexes **11a,b,c** are diamagnetic. For example, solutions of **11a** give sharp ^1H NMR signals. The magnetic susceptibility of crystalline **11a** between 5 and 300 K shows behavior typical of a diamagnet contaminated with <3% of a high-spin ferric impurity.[73] The susceptibilities of the THF–dioxygen complex **11d**[39] and the tail-based complexes **12a,b** are still under investigation.[74]

The Mössbauer spectra of **11a–d** under an applied magnetic field do not indicate the presence of a paramagnetic state. For example, the spectrum of **11a** at 4.2 K in a transverse magnetic field of 6 T conforms to the theoretical curve calculated for a diamagnetic material.[75] A more remarkable characteristic of the Mössbauer spectrum of HbO_2, the temperature dependence of ΔE_Q, is mirrored in the spectra of **11a–d** (Table III). For this model the temperature dependence of ΔE_Q has been interpreted in terms of an equilibrium between conformational states[75,76] of dioxygen.

The ground-state diamagnetism of both HbO_2 and the model dioxygen complexes requires spin pairing between the odd electron in the formal superoxide ligand and the single unpaired d electron of low-spin iron(III). Reed's chromium–dioxygen complex, Cr(TPP)(py)(O_2),[68] shows a magnetic susceptibility corresponding to two unpaired electrons. Chromium(III) has three unpaired 3d electrons, one of which is apparently paired with the electron on the superoxide group. Reed[69] has emphasized this apparent analogy between the iron–dioxygen complexes and his chromium–dioxygen complex.[77]

Oxidative Stability of Model Dioxygen Complexes

The FeO_2 group is thermodynamically unstable. Even Hb exists in the met (ferric) form to the extent of about 3%.[78] All of the iron–dioxygen complexes slowly decompose in solution, but the rates vary tremendously. Acids greatly accelerate this reaction.[79]

The decomposition of iron–dioxygen complexes is retarded by steric hindrance, low temperatures, low concentrations of porphyrins, polar solvents, and increased oxygen pressure. These qualitative observations can be accomodated by the hypothetical mechanism[81] shown in Figure 2. For instance the bimolecular inner sphere oxidation, k_4, should be retarded by low temperature, by steric hindrance (as in the picket fence compound 11 and Baldwin's capped porphyrin), and by the rather dilute nature ($<10^{-4}$ M) of the solutions normally employed for electronic spectral measurements. At low temperatures K_2/K_1 (Figure 2) is magnified, increasing the tendency toward coordinative saturation to the extent that even weak field ligands such as polar solvents and 2MeIm form six-coordinate complexes.[17] This should also inhibit the rate of autoxidation (step k_4, Figure 2) and may explain the reported kinetic stabilization of iron–dioxygen complexes by polar solvents.[82] At such low temperatures tail-based iron porphyrins dimerize—apparently forming six- and four-coordinate pairs.[18]

Solid samples of our oxygen complexes are stable for much longer periods.[83] Nevertheless decomposition slowly occurs, as is shown by a gradual increase in magnetic susceptibility and a growing EPR signal tentatively assigned to high-spin iron(III).

Equilibrium Studies of O_2 and CO Binding

In view of complications arising from the competing equilibria in Figure 2, it is not feasible to obtain equilibrium data for the oxygen-binding step, K_3, using the picket fence complex **11a** *in solution*. In principle this complication can be avoided by the use of a tail-based complex[84] or by an immobilized ligand.[85]

Gagné[40] discovered that the *solid* picket-fence oxygen complexes **11a–d** rapidly equilibrate with gases. For example, **11a** loses O_2 under vacuum, affording a

(71) L. Pauling and C. D. Coryell, *Proc. Natl. Acad. Sci. U.S.A.*, **22**, 210 (1936).

(72) M. Cerdonio, A. Congin-Castellano, F. Mogno, B. Pispisa, G. L. Romani, and S. Vitale, *Proc. Natl. Acad. Sci. U.S.A.*, **74**, 398 (1977).

(73) The presence of a high-spin ferric impurity is qualitatively confirmed by the EPR spectrum: J. P. Collman, T. R. Halbert, and T. N. Sorrell, unpublished results. Depending on their history, fresh samples of **11a** exhibit μ = 0.5 to 1.0 μ_B (25°), values corresponding to 0.7 to 3% high-spin ferric.

(74) For example, preliminary studies of solid **12a** show μ = 2.5 μ_B (298 K), corresponding to 18% ferric, but the ^1H NMR spectrum is sharp.

(75) K. Spartalian, G. Lang, J. P. Collman, R. R. Gagne, and C. A. Reed, *J. Chem. Phys.*, **63**, 5375 (1975).

(76) A temperature-dependent line broadening is also observed in the Mössbauer spectra **11a** and HbO_2. For the synthetic compound this has been fitted by a dynamic model[75] which considers conformational relaxation and nuclear lifetimes.

(77) C. A. Reed, *Proc. Natl. Acad. Sci. U.S.A.*, **74**, 1780 (1977).

(78) R. W. Carrell, C. C. Winterborn, and E. A. Rachmilevitz, *Brit. J. Haemotol.*, **30**, 259 (1975).

(79) This is well known in the case of HbO_2[80] and may involve proton-assisted formation of hydroperoxyl. Even metal ions such as Cu^{2+} accelerate irreversible oxidation of HbO_2. Hb mutants having either proximal or distal histidine replaced by tyrosine exist in the met state. We have found phenol to decompose **11a** and have ascribed that result to acidity rather than stabilization of the ferric state by phenoxide ligand.

(80) See J. M. Pratt in ref 2b, p 146.

(81) (a) A detailed kinetic study of the autoxidation of iron(II) porphyrins has not been carried out and would be challenging because of the problem of simultaneously determining the concentration and nature of the various intermediates for such a rapid reaction. However, the use of low temperatures to isolate each reaction stage[81b] and the use of tail-base porphyrins to control coordination numbers are logical tactics for such a study. (b) LaMar has used low-temperature NMR to examine this reaction: G. LaMar, private communication.

(82) At low temperatures weak-field ligands such as ethers and 2-MeIm stabilize ferrous porphyrins in the low-spin, presumably six-coordinate, state.[17]

(83) J. P. Collman, J. I. Brauman, and K. S. Suslick, *J. Am. Chem. Soc.*, **97**, 7185 (1975).

(84) Traylor employed a tail-imidazole iron(II) porphyrin and photodissociated CO in the presence of oxygen. A kinetic analysis yielded $p_{1/2}O_2$ = 0.32 Torr (20 °C), a value in good agreement with that of Mb.[28d] However, direct measurement of this equilibrium gave $p_{1/2}O_2$ = 0.2 Torr (−45 °C in CH_2Cl_2), which is an *unreasonably low affinity* at such a low temperature.[28b] See, however, ref 18.

(85) In an attempt to avoid the complication from added axial bases, Basolo employed an imidazole immobilized on silica: O. Leal, D. L. Anderson, R. G. Bowman, F. Basolo, and R. L. Burwell, Jr., *J. Am. Chem. Soc.*, **97**, 5127 (1975). However, the unreasonably low affinity obtained from this experiment, $p_{1/2}O_2$ = 230 Torr (0 °C), is comparable to the physisorption value, $p_{1/2}O_2$ = 380 (0 °C), determined for the metal-free picket fence.[83] For example, compare for Mb $p_{1/2}O_2$ = 0.08 (0 °C)!

Table IV
Thermodynamic Properties of O_2 Binding

Substance	$p_{1/2}O_2$(20 °C)[a]	ΔH[b]	ΔS[c]
Fe(TpivPP)(*N*-MeIm)[d]	0.31	−15.6	−38
OxMb[e]	0.55	−15	−37
Co(TpivPP)(*N*-MeIm)	100[f]	−12.2	−38
	44[g]	−13.1	−39
CoMb[h]	54	−12.6	−37
Co(PP_{IX}DME)(*N*-MeIm)[i]	1.4×10^4	−11.5	−45

[a] In Torr. [b] kcal mol^{-1}. [c] cal mol^{-1} deg^{-1}; standard state of O_2 partial pressure = 1 atm. [d] Reference 83. [e] Calculated from data in ref 2f. [f] Reference 94, toluene solution. [g] Reference 94, solid-gas data. [h] Average of values reported by T. Yonetani, H. Yamamoto, and G. V. Woodrow III, *J. Biol. Chem.*, 249, 682 (1974), and C. A. Spillburg, B. M. Hoffman, and D. H. Petering, *ibid.*, 247, 4219 (1972), for sperm whale CoMb. [i] Reference 87b extrapolated.

high-spin deoxy derivative, but upon exposure to oxygen this reaction is reversed (eq 1). Carbon monoxide

$$\underset{\mu = 0}{\text{Fe(TpivPP)(}N\text{-MeIm)(CO)}} \overset{\text{CO}}{\longleftarrow} \underset{\mu \sim 0}{\text{Fe(TpivPP)(}N\text{-MeIm)(O}_2\text{)}}$$

$$\overset{\text{vacuum}}{\underset{O_2}{\rightleftharpoons}} \underset{\mu = 5.2\ \mu_B}{\text{Fe(TpivPP)(}N\text{-MeIm)}} \qquad (1)$$

irreversibly forms a diamagnetic CO complex. The full implication of this latter result did not become apparent to us until recently (vide infra).

This remarkable *solid–gas equilibrium* enabled Suslick to measure the oxygen affinity of the picket-fence complex **11a** at several temperatures and thus to determine the enthalpy and entropy changes while avoiding the complications of competing equilibria.[83] Inasmuch as oxygenation of iron(II) porphyrins should result in a structural change which could be transmitted between sites, we were mildly surprised to find our solid–gas data conform to Langmuir's isotherm, eq 2, $n = 1$, indicating that all sites in the lattice are behaving

$$\frac{y}{1-y} = Kp_{O_2}^n = (p_{1/2}O_2)^{-1}p_{O_2}^n \qquad (2)^{86}$$

independently. The close correspondence between $p_{1/2}O_2$, ΔH, and ΔS values for the picket-fence complex and literature values for myoglobins[2f] (Table IV) suggests that these are characteristic of the intrinsic O_2 affinity of a ferrous porphyrin–imidazole complex. If this be true, it follows that the protein is doing little to raise or lower the O_2 affinity and that the distal residues such as His-E7, and Val-E11 have no significant effect on O_2 binding. The solid picket-fence porphyrin, like the globin tertiary structure, tends to control solvation and in that sense the former may be a good model for the latter. A major unresolved question is the possibility that the polar amide groups in the picket fence may be stabilizing the iron–oxygen group. Until we can obtain data for a model iron(II) porphyrin which does not have such a polar environment, we cannot assess this point. There are several literature reports that polar solvents increase the affinity of model porphyrins for O_2. However, reliable quantitative data are available only for the cobalt systems,[87] and these are complicated by other solvation effects.[88] Thus far we have not determined the effect of other axial bases on the oxygen affinities of the picket-fence iron(II) porphyrins.[89]

Table V
Carbon Monoxide Binding by Hemoproteins and Model Iron Porphyrins

Substance	$p_{1/2}$CO, Torr
Mb horse[a]	1.8×10^{-2}
Hb human[a]	3.5×10^{-2}
Hb Zurich[b]	$\beta < \alpha$
Fe(PP_D)(Im)	2.4×10^{-4}
Fe(PP_D)(2-MeIm)[c]	4×10^{-2}
Fe(PP_D)(THF)[c]	4×10^{-2}
Microperoxidase[d]	4×10^{-4}
Fe(TpivPP)(*N*-MeIm)[e]	"Very small"

[a] Reference 2f. [b] W. J. Wallace, J. A. Volpe, J. C. Maxwell, W. S. Caughey, and S. Charache, *Biochem. Biophys. Res. Commun.*, 68, 1379 (1976). [c] Reference 14, PP_D = deuteroporphyrinato in benzene. [d] Reference 96. [e] Reference 59.

Estimates of k_4 for binding O_2 to the fourth heme in mammalian Hb's[90] give values close to those for Mb's. However there exist several nonmammalian Hb's which have very high O_2 affinities[91] (from 50 to 70 times that of whale Mb). These high O_2 affinities are puzzling and have no clear explanation from our present knowledge of model compounds. *Compression* of the proximal His might account for this effect.

The O_2 affinities of simple cobalt(II) porphyrins have been intensively studied;[87,88] however, CoMb and our picket-fence model bind O_2 much more strongly than these simple cobalt(II) models[88] (Table IV). The difference must be caused by solvation effects.[92] In toluene solution the cobalt picket fence shows about one-half the O_2 affinity of CoMb, but the solid-state value is about the same as that of CoMb (Table V).[94]

From qualitative observations it became clear to us that our model iron(II) porphyrins bind carbon monoxide much more strongly than Mb or Hb. Good quantitative data concerning the CO affinity of a simple model heme site have been determined by Rougee.[14] Comparing CO binding constants, $(p_{1/2}CO)^{-1}$, with those of Mb and Hb (Table V) it is clear that the *N*-MeIm-based model binds CO at least 100 times more strongly than does Mb![95] We believe that this effect

(86) y = fraction of iron sites oxygenated; K = equilibrium constant in Torr^{-1}; $p_{1/2}O_2$ = partial pressure of O_2 at half-oxygenation. When $n = 1$ eq 2 corresponds to Langmuir's isotherm; when $n > 1$ eq 2 describes cooperative O_2 binding

(87) (a) R. Drago and P. Cannady, personal communication; (b) H. C. Stynes and J. A. Ibers, *J. Am. Chem. Soc.*, 94, 1559 (1972).

(88) (a) F. Basolo, H. M. Hoffman, and J. A. Ibers, *Acc. Chem. Res.*, 384 (1975). (b) D. M. Scholler, B. M. Hoffman, and D. F. Shriver, *J. Am. Chem. Soc.*, 98, 7868 (1976).

(89) There is scant information in the literature concerning this point. The reported differences between imidazole and pyridine might be caused by solvation effects, decomposition, steric effects of appended tail bases, or dimerization.[18] C. K. Chang and T. G. Traylor report R–Im to be much more effective than pyridine.[28d,f]

(90) Myoglobins from diverse sources such as ox, whale, and tuna have very similar $p_{1/2}O_2$ values. The observed scatter in these values probably represents experimental difficulties rather than intrinsic differences.

(91) (a) R. Oshino, N. Oshino, and B. Chance, *FEBS Lett.*, 19, 96 (1971); (b) J. B. Wittenberg, F. J. Bergersen, C. A. Appleby, and G. L. Turner, *J. Biol. Chem.*, 249, 4057 (1974).

(92) Earlier we found[93] that Co^{II}TPP attached to a polymeric imidazole has a much greater oxygen affinity than similar complexes in toluene—indicating that solvation is stabilizing the deoxygenated form. It is likely that simple ferrous porphyrins experience a similar effect, but this consideration has been ignored in discussions of solvent effects on their oxygen affinities.

(93) J. P. Collman, R. R. Gagne, J. Kouba, and J. Ljusberg-Wahren, *J. Am. Chem. Soc.*, 96, 6800 (1974).

(94) J. P. Collman, T. R. Halbert, S. Hayes, K. S. Suslick, and J. I. Brauman, unpublished results.

derives from structural differences. Whereas the FeCO group in the models is, as expected, linear and normal to the porphyrin plane,[98] recent structural studies[6,54] have shown that the CO group in Hb and Mb is "tilted"[99] from the porphyrin plane through steric interactions with the distal Val-E11, γ-CH_3 and the His-E7 imidazole groups. Suslick[59] proposed that this distortion lowers the CO affinity and that the role of the distal groups is to protect the heme from endogeneous CO poisoning without affecting the O_2 binding which, in its natural angular geometry, is not distorted by these groups. Mutant hemoglobins lacking one of these distal groups appear to have a higher CO affinity, but quantitative data are not available[100,101] (Table V, Hb Zurich).

Hemoglobin Cooperativity

Cooperativity during oxygenation of Hb is the result of a rather dramatic change in quaternary structure from the T ("tensed") unligated form to the R ("relaxed") ligated form.[2a] These two quaternary structures have roughly a 500-fold difference between their affinity for O_2 and for CO, which also exhibits cooperative binding. This quaternary transformation results in rearrangement of subgroups, but precise changes in the tertiary structure and the coordination environment about iron have proved difficult to ascertain. This allosteric effect is associated with rupture of salt bridges in the T form. Reagents such as phosphates which bind more strongly to the T state lower the O_2 affinity. Hb equilibrium curves are usually examined in terms of the Hill equation, eq 2 ($n \approx 3$ for mammalian Hb), which has no simple physical significance. The ligand affinities for the T and R states can be estimated from Hill plots. The intrinsic affinity of the heme units in the R form is about that of the monomer Mb, so the T state has a lower O_2 affinity than Mb or our model. The mechanism of cooperativity is controversial.[88,102] Hoard[4] and Perutz[2a] have proposed mechanisms which depend on the movement of iron through the distance l upon binding O_2.[103] It has been recently suggested that the His-F8 is restrained[104] in the T state of Hb and that this *restraint* opposes the movement of iron into the plane of the porphyrin which is needed for combination with oxygen. There is no evidence for structural differences between the coordination spheres of deoxy-Mb, the T state of Hb, and the model compound **9**. The Hoard–Perutz hypothesis is supported by at least one direct experiment.[105] Two synthetic heme polymers have been reported to exhibit weak cooperative oxygen binding.[107]

One of the most elementary questions which can be asked about cooperativity is the following: what structural features in the T state would lower the O_2 or CO affinities from the intrinsic value of an unrestrained imidazole–heme unit? There is one experimental clue whose significance has apparently not been recognized: the CO affinity of $Fe(PP_D)(2\text{-MeIm})$[1] is $^1/_{200}$th that of $Fe(PP_D)(N\text{-MeIm})$![13a,14] The former represents a heme center sterically *restrained* to the five-coordinate form—a situation which is reminiscent of the T state of Hb. Since Hb shows full cooperativity for CO binding ($n = 3$), we believe this lower CO affinity is highly significant, and we are attempting to measure the corresponding O_2 affinity using our solid–gas picket-fence technique.

The research I have discussed is the result of experimental and intellectual efforts of my students and of collaborators who are acknowledged in the references and in the text. I owe a special debt to my colleague John Brauman, who has guided the more quantitative aspects of this work, and to my student Ken Suslick, who has edited this manuscript. This research has been supported by the National Institutes of Health, Grant No. GM17880, and the National Science Foundation, Grant No. CHE75-17018.

(95) A cytochrome *c* degradation product, microperoxidase, which has an imidazole base and lacks distal groups, has been used to scavenge CO. Its recently reported CO affinity[96] corroborates the results of Rougee and Brault.[14] Note also that the low CO affinity reported for Traylor's tail model has been retracted.[97]

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(98) (a) S-M. Peng and J. A. Ibers, *J. Am. Chem. Soc.*, **98**, 8032 (1976); (b) ref. 4, p 358.

(99) Available x-ray and neutron diffraction data on carbonyl hemoproteins cannot distinguish between "bent" ($\angle$FeCO < 180°) and "tilted" ($\angle$FeCO = 180°) CO groups, but the latter seem more reasonable.[59,98a]

(100) W. S. Caughey, J. O. Alben, S. McCoy, S. H. Boyer, S. Charache, and P. Hathaway, *Biochemistry*, **8**, 59 (1969).

(101) Replacement of the distal His-E7 slightly increases the O_2 affinity.[2a] Neutron diffraction studies show that the imidazole N–H group does not hydrogen bond to CO[54] and presumably would not hydrogen bond to O_2 which would necessarily *increase* the O_2 affinity.

(102) D. M. Scholler, B. M. Hoffman, and D. F. Shriver, *J. Am. Chem. Soc.*, **98**, 7866 (1976), and references therein.

(103) (a) CoHb shows lower cooperative O_2 binding with the same T → R quaternary change. However, estimates of l for Co are 0.4 Å, compared with the present 0.6 Å for Fe. These facts have been used both to support[103b] and to attack[88] the Hoard–Perutz theories. (b) K. Imai, T. Yonetani, and J. Ikeda-Saito, *J. Mol. Biol.*, **109**, 83 (1977).

(104) Perutz proposes that in the deoxy T state the proximal His-F8 is *restrained*, not necessarily stretched from its intrinsic value.

(105) The imidazole N^{ϵ}–Fe bond in two chains of Hb is ruptured when the nitrosyl derivative is forced from the R → T state.[2a,106] There is much other indirect evidence.[2a]

(106) (a) J. C. Maxwell and W. S. Caughey, *Biochemistry*, **15**, 388 (1976); (b) M. F. Perutz, J. V. Kilmartin, K. Nagai, A. Szabo, and S. R. Simon, *ibid.*, **15**, 378 (1976).

(107) (a) E. Tsuchida, E. Hasegawa, and K. Honda, *Biochem. Biophys. Res. Commun.*, **67**, 864 (1975); (b) E. Bayer and G. Holzbach, *Angew. Chem., Int. Ed. Engl.*, **16**, 117 (1977). The cooperative oxygen binding by these polymeric hemes appears to involve competitive equilibria between five- and six-coordinate hemes (as between **2** and **3** in Figure 2), and thus differs from the cooperative effect in hemoglobin.