Spin-State/Stereochemical Relationships in Iron Porphyrins: Implications for the Hemoproteins[1]

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Received June 8, 1981

Contents

I. Introduction 543
II. Background 544
III. (Porphinato)iron Stereochemistry 545
A. (Porphinato)iron(III) Derivatives 545
B. (Porphinato)iron(II) Derivatives 548
C. Additional Comments on (Porphinato)iron Stereochemistry 548
IV. Hemoprotein Stereochemistry 550
A. General Considerations 550
B. High-Spin Ferrous Hemoproteins 550
C. High-Spin Ferric Hemoproteins 551
D. Low-Spin Hemoproteins 551
E. Hemoglobin Cooperativity 552
F. Spin-State Equilibria in Hemoproteins 552
G. Admixed Intermediate-Spin Ferric Hemoproteins 553
H. Iron–Sulfur Bonds 553
V. Conclusion 553
VI. References and Notes 553

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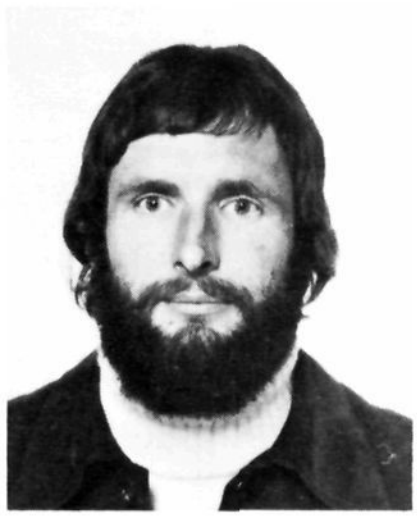

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I. Introduction

An axiom of hemoprotein biochemistry is that molecular structure provides the basis for an understanding of how these proteins work. Chemical curiosity is aroused by the commonality of the heme group to these proteins despite their impressively varied chemistry. When closely similar iron porphyrin moieties are encapulated with different polypeptides a variety of redox reactions can be performed in diverse biological systems. Hemoglobin commands special attention as an oxygen carrier, particularly because of its cooperative oxygen binding. The electron-carrying cytochromes *c* have also been intensively studied, perhaps because they are easily isolated and are conceptually the simplest of the hemoproteins. Oxygen utilization by enzymes such as the cytochromes P450 (organic substrate oxidation) and cytochrome *c* oxidase (reduction to water coupled to ATP formation) is also very important. The recurrence of the iron porphyrin active site in these hemoproteins leads to the notion that a particular physiological function is achieved by protein fine tuning of the structure around the heme. Both the axial ligation to the heme group and the nature of the immediate environment are critical variables in controlling the chemistry at the iron atom. In hemoglobin, for example, the protein provides a single axial histidine ligand and protects the vacant sixth coordination site for oxygen binding. On the other hand, the cytochromes *c* are six-coordinate with histidine and methionine ligation, a situation apparently desirable for electron transport. Such manipulation of structure at the heme group gives rise to several distinctive spin states of iron. As a corollary, therefore, a knowledge of the spin state of a hemoprotein has definite implications for structure. This was first recognized by Williams[2] and by Hoard.[3] It is this interrelationship

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of structure and spin state that we explore in detail in this review.

Apart from being the prosthetic groups of hemoproteins, iron porphyrins have a well-developed coordination chemistry. The synthetic advances of the last decade have allowed a systematic study of their X-ray crystal structures, thereby revealing a notably coherent pattern of spin-state/stereochemical relationships. So consistent are these relationships that structure can in most situations be considered the final arbiter of spin state. Similarly, given the spin state of an iron porphyrin complex in either of its commonly occurring iron(II) or iron(III) oxidation states, its structure can be predicted with a high degree of metrical accuracy. Even with incompletely characterized states such as those of iron(I) and iron(IV) confident predictions can be made.

It is in this way that iron porphyrin complexes, as models for the hemoproteins, give rise to expectations of structure and bond lengths in the hemoproteins. When the coordinate bond parameters derived from protein crystallography differ significantly from those intrinsic to model complexes, this can be taken as evidence of special, presumably important, protein constraints upon the heme. An alternative, albeit contentious, conclusion is that a lack of structural congruence between hemoproteins and model complexes points to unwitting erroneous determination of bond parameters in protein crystallography. We take up this question with respect to the out-of-plane displacement of iron in oxyhemoglobin because of its importance to the stereochemical mechanism of cooperative heme–heme interaction. In fact, it is perhaps the stereochemical "trigger mechanism" of hemoglobin cooperativity and the ensuing debate on its validity which has focused so much attention on the mutual relationship between protein constraints and the spin-state-induced change of structure at the heme group.[4] In general, the knowledge of how heme stereochemistry responds to changes in oxidation state and spin state is a prerequisite for understanding the molecular level mechanisms of hemoprotein chemistry.

By the use of tabulated data this review is comprehensive with respect to iron porphyrin stereochemistry. There is, however, an emphasis on new data reflecting the fact that in the few years since the reviews of the mid seventies[5,6] the number of reported X-ray structures of iron porphyrins has nearly tripled, several new types of coordination are now recognized, and some old beliefs have been rethought.

II. Background

Despite the varied physiological functions of the hemoproteins, almost all utilize a heme group which differs only slightly in its peripheral substituents. Figure 1 illustrates the familiar protoporphyrin IX of heme *b* which lacks any covalent linkage to the protein other than coordinate bonds to iron. Heme *c* is derived from heme *b* by the addition of cysteine thiol residues from the protein across the two vinyl groups. Heme *a* is derived from heme *b* by elaboration of one vinyl group into a polyene side chain and by conversion of one methyl group into a formyl group. An exception to these very closely related prosthetic groups is siroheme, a tetrahydroporphyrin derivative, which is used by the

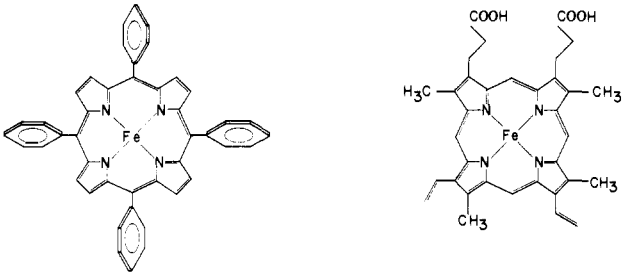

Figure 1. The heme group (right) of hemoglobin and the synthetic (*meso*-tetraphenylporphinato)iron derivative used in many model compound studies. Axial ligation occurs above and below the porphyrin plane.

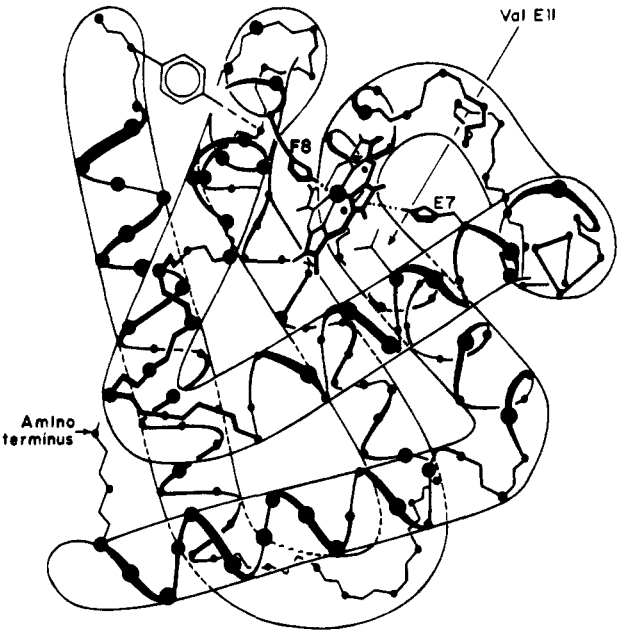

Figure 2. A schematic representation of one subunit of hemoglobin. The proximal histidine F8 and the distal Val-11 and distal histidine E7 are shown. This figure was adapted from a figure kindly provided by Dr. M. F. Perutz.

reductases. There is little evidence for more than a minor role for peripheral substituent differences in the active-site chemistry, and most studies point to axial ligand effects as a more powerful influence on heme reactivity.[7] To a large extent this legitimizes the synthetic model approach which relies heavily on derivatives of the synthetically convenient tetraphenylporphyrin (TPP) shown in Figure 1 and octaethylporphyrin (OEP).

All hemoproteins characterized to date are globular in shape, and the heme group is tightly encapsulated by the protein (or globin). This is illustrated schematically in Figure 2 for one subunit of hemoglobin whose protein is made up of about 150 amino acids.[4] The protein can provide one or two axial ligands to iron in the fifth and sixth coordination sites above and below the porphyrin plane.

Normal hemoglobins are in the iron(II) state and are five-coordinate, having a single axial imidazole ligand from the so-called proximal histidine (labeled F8). In certain mutant hemoglobins the so-called distal residues such as histidine E7 and valine E11, which are normally noncoordinating, are replaced by ligands capable of binding to the otherwise vacant sixth site. This has dire consequences for oxygen binding. The three-dimensional structures of hemoproteins have frequently been

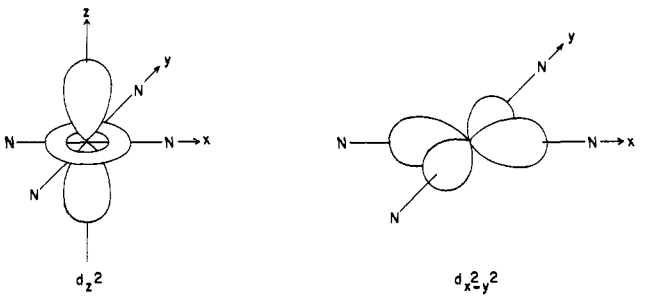

Figure 3. Stereochemically active d orbitals. These orbitals are σ antibonding and are designated e_g*. The heme group lies in the xy plane with the N_p atoms on the axes.

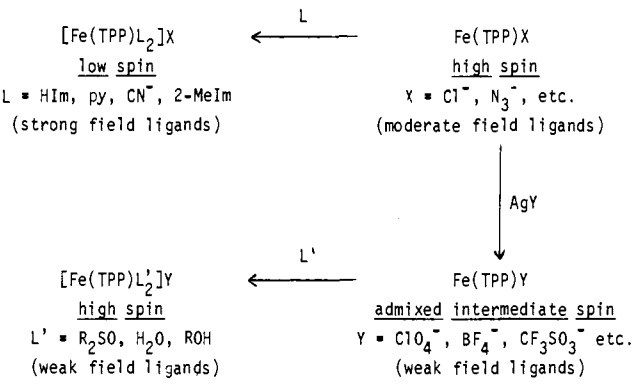

Figure 4. Scheme for the synthesis of the four major structural classes of iron(III) porphyrins.

determined by protein crystallography, and the early results on hemoglobin by Perutz mark the historic beginning of the structural approach to understanding hemoproteins.[8] From our point of view the more detailed scrutiny of the structure of the active-site components, made possible by characterizing model compounds, frequently makes the synthetic approach a useful complement to direct methods of seeking an understanding of how hemoproteins exploit fundamental coordination chemistry. For more background on particular hemoproteins the reader is referred to existing reviews.[9]

The descriptive chemistry of iron porphyrins is well suited to discussion within the oxidation state formalism. Likewise, the approximation that the valence electrons retain their essential d atomic orbital character is entirely adequate for describing the spin states arising from different energy separations of these orbitals. The qualitative ligand field theory approach to d-orbital splittings is based on the premise that those d orbitals whose lobes are directed between the ligands (d_{xz}, d_{xy}, d_{yz}) will be lower than those pointing directly at the ligands ($d_{x^2-y^2}$, d_{z^2}). In molecular orbital terms the former set (the t_{2g}) are nonbonding with respect to σ bonding while the latter set (the e_g) are antibonding. The stronger the ligand field, the greater the d-orbital splitting and the more likely a low spin state will obtain. The stereochemically active orbitals are the e_g set shown in Figure 3, and we shall see that their occupation in high-spin complexes *always* correlates with longer coordinate bond lengths compared to low-spin complexes where they are unoccupied.

III. (Porphinato)iron Stereochemistry

A. (Porphinato)iron(III) Derivatives

The ferric ion in porphinatoiron(III) complexes is invariably found to have one or two axial ligands and the complexes have tetragonal symmetry (or nearly so). In these circumstances, the five d electrons of the ferric ion can be formally arranged into three possible spin states. These are the low-spin $S = {}^1/_2$ state, the intermediate-spin $S = {}^3/_2$ state, and the high-spin $S = {}^5/_2$ state. The nature of the intermediate-spin state merits special attention because while a pure $S = {}^3/_2$ ground state would have a d orbital population $(d_{xy})^2(d_{xz},d_{yz})^2(d_{z^2})^1(d_{x^2-y^2})^0$ there is the possibility of spin–orbit coupling to a nearby $S = {}^5/_2$ state to give a new quantum mechanically admixed-intermediate state. Such an admixed $S = {}^3/_2,{}^5/_2$ state, which is quite distinct from a spin equilibrium, is conceptually difficult to visualize because the one-electron model of orbital population breaks down. However, partial occupation of the $d_{x^2-y^2}$ orbital can be envisaged as is portrayed in Table I.

The first important generalization is that the spin state and stereochemistry of the iron(III) center is controlled almost entirely by the nature and number of axial ligands. The coordination of strong field ligands leads to low-spin six-coordinate hemes, e.g., bis(imidazole)iron(III) derivatives. Weaker field ligands, typically anionic ones such as chloride, azide, etc., lead to five-coordinate high-spin derivatives. Both these types are well-known.[5,10] By systematically varying the axial ligands, we have been able to synthesize several ferric porphyrin derivatives whose spin-state/ligation modes were previously unknown. The synthetic scheme relating these derivatives is shown in Figure 4. The complete range of possibilities, as characterized by X-ray crystallography, is displayed in Table I. The effective axial ligand field of the complexes listed in this table decreases from left to right. As a consequence, the energy of the d_{z^2} orbital also decreases, giving sequentially low, high, and intermediate states. In some cases, as discussed below, the intermediate state is an admixed ($S = {}^3/_2,{}^5/_2$) state. The stereochemical consequences of the various orbital occupations can be rationalized with remarkable simplicity by considering whether or not the antibonding d orbitals, $d_{x^2-y^2}$ and d_{z^2} (Figure 3), are occupied. Occupation of $d_{x^2-y^2}$ in a high-spin derivative has one of two effects. It either gives rise to an expanded porphinato core as in the six-coordinate complexes $[Fe(TPP)((CH_2)_4SO)_2]^+$ or $[Fe(TPP)(H_2O)_2]^+$ ($Fe-N_p = 2.045$ Å) or, more commonly, extrudes the iron atom out of the porphinato plane as in the five-coordinate complexes Fe(TPP)(X) ($Fe-Ct_p = 0.51$ Å; $Fe-N_p = 2.069$ Å; mean values). In both cases, the $Fe-N_p$ distance increases markedly from that of the low-spin complexes ($Fe-N_p = 1.99$ Å). It is important to note that the larger size of the high-spin iron(III) atom does not preclude its centering in the porphinato plane. The structure of $[Fe(TPP)((CH_2)_4SO)_2]^+$, showing the in-plane iron position, is displayed in Figure 5. We note also that when high- and low-spin six-coordinate complexes are compared occupation of d_{z^2} causes extension of the axial iron–ligand bonds by ≥ 0.1 Å. This effect appears to be more pronounced in iron(II) derivatives.

If the prime determinant of the spin state is the axial ligand field, it should be possible to find moderate field strength axial ligands that are close to the spin crossover

TABLE I. Spin-State/Stereochemical Relationships for (Porphinato)iron(III) Complexes

	low ($S = {}^1/_2$)	high ($S = {}^5/_2$)		admixed intermed. ($S = {}^3/_2, {}^5/_2$)	intermed.[i] ($S = {}^3/_2$)
d^5 configuration[a]	$-x^2-y^2$, $-z^2$; $\uparrow\downarrow$ $\uparrow\downarrow$ $\uparrow xz,yz$	$\uparrow x^2-y^2$, $\uparrow z^2$; $\uparrow$ $\uparrow$ $\uparrow$		$\uparrow x^2-y^2$, $\uparrow z^2$; $\uparrow$ $\uparrow\downarrow$ $\uparrow$	$-x^2-y^2$, $\uparrow z^2$; $\uparrow$ $\uparrow\downarrow$ $\uparrow$
coord no.	6	5	6	5	6
examples	[Fe(TPP)(HIm)$_2$]$^+$ [11] [Fe(TPP)(PMS)$_2$]$^+$ [12] [Fe(TPP)(CN)$_2$]$^-$ [13] [Fe(proto)(1-MeIm)$_2$] [14] [Fe(TPP)(N$_3$)(Py)] [15] [Fe(TPP)(2-MeIm)$_2$]$^+$ [16] [Fe(TPP)(CN)(Py)] [17] [Fe(TPP)(NCS)(Py)] [18] [Fe(OEP)(3-ClPy)$_2$]$^+$ [19,b] [Fe(PF)(O$_2$)(1-MeIm)] [20] [Fe(PF)(O$_2$)(2-MeIm)] [21]	[Fe(meso)(OCH$_3$)] [22] [Fe(proto)(Cl)] [23] [Fe(TPP)(Cl)] [24] [Fe(TPP)]$_2$O [25] [Fe(proto)(SPhNO$_2$)] [26] [Fe(TPP)(NCS)] [27] [Fe(ODM)]$_2$O [28] [Fe(TPP)(Br)] [29] [Fe(TPP)(I)] [30] [Fe$_2$(μ-O)(FF)]·H$_2$O [31,f] [Fe(TPP)(F)] [32] [Fe(TPP)(NO$_3$)] [33,g]	or	[Fe(TPP)(OClO$_3$)] [36] [Fe(OEP)(OClO$_3$)] [37]	[Fe(TPP)C(CN)$_3$]$_n$ [38]
Fe-N$_p$, Å[c,d]	1.970 (14)-2.000 (6) [1.990]	2.060 (3)-2.087 (8) [2.069]	2.045 (8)	1.994 (29)-2.001 (5)	1.995 (3)
Fe-Ct$_p$, Å	0.0-0.11	0.39-0.62 [0.51]	0.0	0.26-0.30	0.0
Fe-Ct$_N$, Å	0.0-0.09	0.39-0.54 [0.47]	0.0	0.26-0.28	0.0
Ct$_N$-N$_p$, Å	1.97-2.00 [1.99]	[2.015]	2.045	1.981	1.995
Fe-Ax, Å[e]	1.957 (4)-2.013 (4)	-	2.08-2.316	2.029 (4)	2.317 (3)
hemoprotein examples	cyt b_5 cyt c, c_3, etc. metHb(CN)	(P-450)$_{ox}$	MetHb(H$_2$O)	cyt c'	-

[a] In this pictorial representation of the one-electron d-orbital energy levels only the relative energies of the d_{z^2} and $d_{x^2-y^2}$ levels are known with absolute certainty. [b] At 98 K. [c] N$_p$, porphinato nitrogen atom; Ct$_N$, center of the best plane of the four N$_p$; Ct$_p$, center of the best plane of the 24-atom porphinato core; Ax, axial ligand donor atom. [d] Values given are the ranges observed in the compounds listed. The number in parentheses is the estimated standard deviation for the extreme values. The values given in square brackets are the average value for all members of the given class. [e] The Fe-Ax distance will be that appropriate for Fe-N(imidazole) when this value is available. [f] FF is a confacial biporphyrin. [g] NO_3^- binds as an unsymmetrical bidentate ligand. [h] At 293 K, high-spin component of spin mixture. [i] In addition there are two complexes, of somewhat uncertain spin state, whose structure has been reported: [Fe(TPP)(EtOH)$_2$]$^+$ [39] and [Fe(OEP)(EtOH)$_2$]$^+$ [40]. These may be admixed intermediate-spin species.

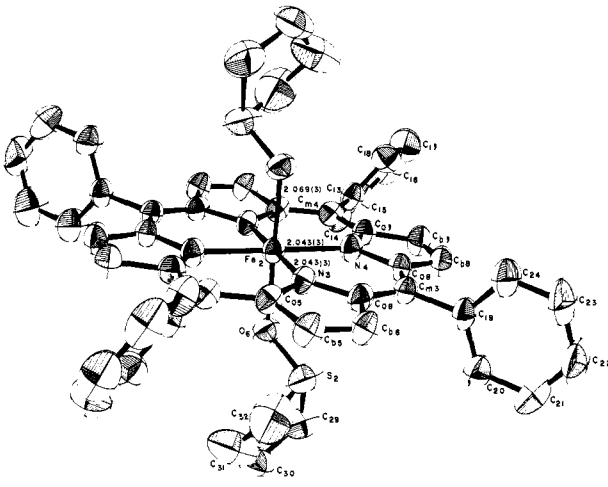

Figure 5. Computer-drawn model of $[Fe(TPP)((CH_2)_4SO)_2]^+$. Note the in-plane position of the high-spin iron(III) atom from ref 34.

point and give rise to spin isomers. Such complexes have been reported[41,42] particularly with substituted pyridine ligands and give a thermal equilibrium of high- and low-spin states. Structural characterization of $[Fe(OEP)(3\text{-}Cl(py))_2]ClO_4$ at 293 K, where the thermal equilibrium is approximately a 1:1 mixture of low- and high-spin state, yields the average structure of the two spin states.[43] The observed Fe–N_p bond distance of 2.014 (4) Å is about half way between the 1.990-Å value appropriate for the low-spin component and the 2.045-Å value for the high-spin component. The axial Fe–N(py) distance is 2.194 Å. For this compound, the obligatory structural changes accompanying the spin-state change ($S = {}^1/_2$ to ${}^5/_2$) are porphinato core expansion as well as elongation of the axial bonds. This expectation has been confirmed by recent experiments. Structure determination at 98 K yields the structure of the low-spin form of the molecule.[19] The porphinato core shows the expected radial contraction with Fe–N_p = 1.994 (6) Å; the axial Fe–N(py) distances have decreased to 2.031 (2) Å. Further work with the 293-K data to crystallographically resolve the structures of the two spin isomers was successful; the high-spin axial distance is 2.316 (1) Å and the low-spin distance is 2.043 (1) Å.[19]

The perchlorato complex, $Fe(TPP)(OClO_3)$, which we believe is best described as a quantum admixed $S = {}^3/_2,{}^5/_2$ state,[36] illustrates an important but little appreciated point about the interdependence of the axial and equatorial ligands. Classical crystal-field theory would argue that the choice between a high- and intermediate-spin state depends solely on $d_{x^2-y^2}$ and d_{xy} separation, which has no z component. Thus, the alteration of the z-axis ligand field should not bring about a high to intermediate (or admixed) spin state change. The fallacy in this argument, when applied to real molecules which have a flexible chelate ring size, is that decreasing the z-axis ligand field brings about an increased charge attraction of the xy ligands to the iron atom. This results in further splitting of the antibonding $d_{x^2-y^2}$ orbital from the nonbonding d_{xy} and allows the intermediate spin state to become accessible (see Table I). Maltempo recognized this[44] and developed a quantum mechanical description for the situation when the energy difference between the "pure" $S = {}^5/_2$ and $S = {}^3/_2$ states is comparable in magnitude to the spin–orbit coupling constant. Under these conditions a continuum of admixed $S = {}^3/_2,{}^5/_2$ states can arise and $Fe(TPP)(OClO_3)$, which has a considerably temperature dependent magnetic moment, has been described in this fashion. $Fe(OClO_3)(OEP)$[37] is structurally and magnetically very similar to $Fe(OClO_3)(TPP)$, suggesting that it also has an admixed $S = {}^3/_2,{}^5/_2$ "intermediate" state. The distinct structural differences between the five-coordinate admixed intermediate-spin complexes and the high-spin species are shown in Figure 6.

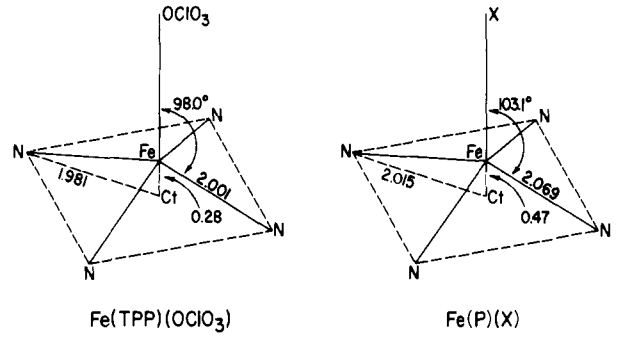

Figure 6. Schematic representations of the idealized (C_{4v} symmetry) coordination groups of high-spin [Fe(P)(X)] and [Fe(TPP)($OClO_3$)].

A logical extension of the trend toward an intermediate-spin state as the axial ligand field strength decreases is the prediction that the as yet unknown $[Fe(P)]^+$, a four-coordinate ferric porphyrin devoid of axial ligation, will have a pure $S = {}^3/_2$ spin state. For want of a truly noncoordinating anion this synthetic challenge has not yet been met. One can also envisage a range of complexes having varying ratios of $S = {}^3/_2$ and $S = {}^5/_2$ admixing since their energy separation should be extremely sensitive to the axial ligand field. In this regard the structures of $[Fe(OEP)(EtOH)_2]ClO_4$[40] and $[Fe(TPP)(EtOH)_2]BF_4$[39] are potentially very interesting. Their average coordinate bond parameters (Fe–N_p = 2.032 (9) and Fe–O = 2.138 (24) Å) are very close to those seen in the definitively high-spin $[Fe((CH_2)_4SO)_2(TPP)]ClO_4$ (Fe–N_p = 2.045 (5) and Fe–O = 2.07 and 2.09 Å).[34] Although of marginal statistical significance, the differences are in the direction (shorter Fe–N_p, longer Fe–O) expected if there was admixture of an $S = {}^3/_2$ state into the predominant $S = {}^5/_2$ state. In the case of $[Fe(TPP)(EtOH)_2]BF_4$, where some magnetic data are reported, the magnetic moment at high temperatures (5.3 μ_B) is lower than the $S = {}^5/_2$ spin only value (5.9 μ_B). It is desirable to have detailed magnetic studies on materials of known X-ray structure in search for a possible structural correlation with the degree of admixture. However, this speculation aside, the structural manifestation of an intermediate-spin state (admixed or pure) in presently known compounds is an Fe–N_p bond length similar to that of a low-spin complex. This is consistent with depopulation of the $d_{x^2-y^2}$ orbital (partial or complete). Five-coordinate intermediate-spin complexes have values for the out-of-plane iron atom displacement (Fe–Ct_p) that lie between those of low-spin complexes (<0.11 Å) and high-spin complexes (>0.39 Å). *Six*-coordinate intermediate-spin complexes have very long axial ligand bond lengths, as long as or longer than comparable high-spin derivatives. This is the structural manifestation of the high degree of tetragonal distortion to the ligand field which is obligatory for an intermediate-spin state.

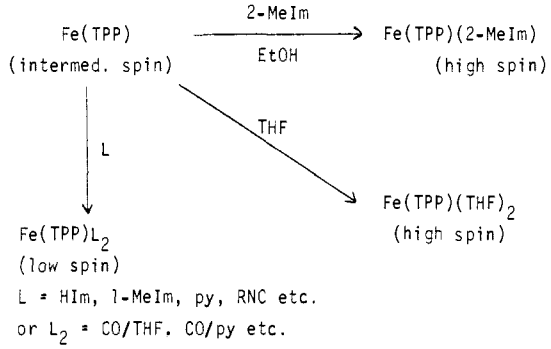

Figure 7. Scheme for the synthesis of the four major structural classes of iron(II) porphyrins.

B. (Porphinato)iron(II) Stereochemistry

The d^6 iron(II) ion can exhibit three spin states. These are the $S = 0$ low-spin state, the $S = 1$ intermediate-spin state, and the $S = 2$ high-spin state. The principles which govern iron(II) spin states and stereochemistry, many of which were predicted by Hoard's pioneering work,[3] are quite similar to those for iron(III). As a consequence of decreased charge there is a small increase in radii for iron(II) compared to iron(III). This leads to increased bond lengths in the coordination group.

The synthetic routes to the presently known range of possibilities are shown in Figure 7, and the stereochemical data are summarized in Table II. As in Table I, the effective axial ligand field decreases from left to right, giving rise to low, high, and intermediate spin states. The stereochemical consequences of populating the antibonding d orbitals, $d_{x^2-y^2}$ and d_{z^2}, are similar to those found for iron(III) except that there is one significant difference in detail. In five-coordinate high-spin complexes, the magnitude of the displacement of the iron(II) atom out of plane is no larger than that of iron(III) derivatives, despite the larger size of iron(II). However, the Fe^{II}–N_p distances are larger and the size of the central hole (Ct_N–N_p) increases by about 0.02 Å to ≥2.03 Å.

As in iron(III), it is possible to coordinate two weak-field axial ligands (THF) to yield a high-spin six-coordinate derivative. Albeit a unique example, $Fe(TPP)(THF)_2$ shows that the belief that a high-spin ferrous atom cannot fit into the porphyrinato plane is no longer correct.[56] The Ct_N–N_p distance of 2.057 Å (coincident with Fe–N_p) represents a very large amount of radial core expansion in an iron porphyrin.

In summary, the population of the $d_{x^2-y^2}$ orbital in high-spin iron(II) complexes is manifest structurally in long Fe–N_p bonds. When five-coordinate, a high-spin iron(II) atom has a significant out-of-plane displacement (~0.5 Å). Comparison can be made with low-spin five-coordinate Fe(TPP)(NO)[45] having Fe–Ct_p of only 0.21 Å and the numerous low-spin six-coordinate complexes where Fe–Ct_p is always less than 0.11 Å. Both low- and intermediate-spin complexes have $d_{x^2-y^2}$ empty; the shorter Fe–N_p distances in the intermediate spin Fe(TPP) (1.97 Å) compared with low-spin $FeL_2(TPP)$ (~2.00 Å) is readily understood by considering the proportionately greater charge attraction of the ligands to the iron when the coordination number is four. This causes a severe S_4 ruffling of porphyrin core in Fe(TPP). As far as Fe–Ax distances are concerned, the population of the d_{z^2} orbital is manifest in longer axial bonds in high-spin complexes than in low-spin complexes. A useful illustration is the 2.01-Å axial distance in $Fe(TPP)(1\text{-MeIm})_2$ compared with the 2.16 Å distance in Fe(TPP)(2-MeIm). The possibility that the long Fe–N_{ax} distance in the latter compound could be accounted for by a steric effect of the 2-methyl substituent is unlikely since the low-spin Co(TPP)(1-MeIm),[59] which also has d_{z^2} occupied, has an identical distance. There remains, however, the synthetic challenge of preparing an imidazole-ligated high-spin iron(II) porphyrin complex which is unambiguously free of all steric constraints.

C. Additional Comments on (Porphinato)iron Stereochemistry

The sufficiency of the antibonding d orbital rationale for the iron atom out-of-plane displacements and the size difference between high- and low-spin iron atoms has been questioned on theoretical grounds.[60,61] Such calculations, which emphasize the importance of nonbonded repulsions between axial ligands and the porphinato core, must suffer from oversimplification since experimental observations do not support the view that steric interactions are solely responsible for large metal atom displacements. Thus, in low-spin Co(TPP)(1-MeIm)[59] (where $d_{x^2-y^2}$ is unoccupied), the metal atom displacement is only 0.13 Å, whereas in the analogous high-spin Mn(II) and Fe(II) complexes it is >0.40 Å.[62] Similar conclusions result from the comparison of the metal atom displacements in a series of five-coordinate metalloporphyrin nitrosyl complexes (M = Mn, Fe, Co).[63] These results give some idea of the relative importance of steric influences vs. the spin-state effect. Both are important in five-coordinate complexes, and the historic popularity of the d-orbital occupation theory is now seen to have arisen because of its apparent sufficiency. It remains entirely adequate to rationalize most trends in metalloporphyrin stereochemistry once considerations of effective nuclear charge, modest steric effects, and core deformation have been taken into account.[6] For example, in the series of four-coordinate M(TPP) derivatives of the first row transition metals listed in Table III the M–N_p distances show discontinuities according to $d_{x^2-y^2}$ occupation. The overall decreasing M–N_p from Cr to Ni shows the effect of increasing effective nuclear charge. The discontinuity at Mn is the result of the high-spin d^5 configuration. Similarly, the increases in the Cu–N_p and Zn–N_p distances, relative to the earlier members of the series, are again the consequence of population of the $d_{x^2-y^2}$ orbital. Another, less obvious, influence on these distances is the conformation of the porphyrin core. Deformation from planarity is frequently observed when the metal has a smaller than optimum fit to the central hole (Ct–N_p ~ 2.01 Å).[5] The effect of an S_4 ruffling is a decrease in M–N_p of about 0.02–0.03 Å and is nicely demonstrated by the two crystalline modifications of Ni(OEP). The planar form has Ni–N_p = 1.958 (2) Å[70] while the S_4 ruffled core has Ni–N_p = 1.929 (3) Å.[71]

What is quite clear from these systematic studies is the inseparability of spin state and structure. Moreover, knowledge of one allows confident prediction of the other. Some applications of this principle to as yet uncharacterized iron porphyrins is given below. The principle has also been usefully applied in other tran-

TABLE II: Spin-State/Stereochemical Relationships for (Porphinato)iron(II) Derivatives

	low ($S = 0$)		high ($S = 2$)		intermed. ($S = 1$)
d^6 configuration[a]	$-x^2-y^2$, $-z^2$; $\uparrow\downarrow xz, yz$; $\uparrow\downarrow xy$		$\uparrow x^2-y^2$, $\uparrow z^2$; $\uparrow xz, yz$; $\uparrow\downarrow xy$		$-x^2-y^2$; $\uparrow xz, yz$; $\uparrow\downarrow z^2$; $\uparrow\downarrow xy$
coord no.	5 or	6	5 or	6	4
examples	[Fe(TPP)(NO)][45] [Fe(OEP)(CS)][46]	[Fe(TPP)(Pip)$_2$][47] [Fe(TPP)(NO)(B)][48] [Fe(TPP)(CO)(Py)][49] [Fe(Deut)(CO)(THF)][50] [Fe(TPP)(THT)$_2$][12] [Fe(TPP)(*t*-BuNC)$_2$][51] [Fe(TPP)(1-MeIm)$_2$][52] [Fe(TPP)(CO)(SR)]$^-$ [53] [Fe(TPP)(CCl$_2$)(H$_2$O)][54] [Fe(C5Im)(TPP)(THT)][12]	[Fe(TPP)(2-MeIm)][55] [Fe(TPP)(SR)]$^-$ [53] [Fe(PF)(2-MeIm)][21]	[Fe(TPP)(THF)$_2$][56]	[Fe(TPP)][57]
Fe-N$_p$, Å[b,c]	1.981 (4)-2.001 (3)	1.981 (29)-2.008 (12) [2.002]	2.072 (6)-2.096 (4) [2.085]	2.057 (4)	1.972 (4)
Fe-Ct$_p$, Å	0.21-0.23	0.0-0.10	0.43-0.62 [0.53]	0.0	0.0
Fe-Ct$_N$, Å	0.21-0.23	0.0-0.11	0.40-0.52 [0.45]	0.0	0.0
Ct$_N$-N$_p$, Å	1.970-1.990	[2.000]	2.030-2.044 [2.036]	2.057	1.972
Fe-Ax, Å[d]	1.717 (7)	2.014 (5)	2.095 (6)-2.161 (5)	2.351 (3)	-
hemoprotein example	Hb(NO)·DPG[59] (T-state)	Hb(CO) cyt b_5 cyt c, c_3, etc	Hb	Hb(H$_2$O) ?	-

[a] In this pictorial representation of the one-electron d-orbital energy levels only the relative energies of the d_{z^2} and $d_{x^2-y^2}$ levels are known with absolute certainty. [b] N$_p$, porphinato nitrogen atom; Ct$_N$, center of the best plane of the four N$_p$; Ct$_p$, center of the best plane of the 24-atom porphinato core; Ax, axial ligand donor atom. [c] Values given are the ranges observed in the compounds listed. The number in parentheses is the estimated standard deviation for the extreme values. The values given in square brackets are the value for all members of the given class. [d] The Fe-Ax distance will be that appropriate for Fe-N(imidazole) when this value is available.

TABLE III. M-N_p Bond Lengths in M(TPP) Derivatives

metal	spin state, S	M-N_p,[a] Å	sym-metry[b]	ref
d^4 Cr	2	2.033 (1)	C_i	64
d^5 Mn	5/2	>2.084[c]	C_i	65
d^6 Fe	1	1.972 (4)	S_4	57
d^7 Co	1/2	1.949 (3)	S_4	66
d^8 Ni	0	1.928 (3)	S_4	67
d^9 Cu	1/2	1.981 (7)	S_4	68
d^{10} Zn	0	2.036 (6)	C_i	69

[a] The number in parentheses is the estimated standard deviation for the bond distance. [b] Symmetry required in the crystal: C_i is a planar core, S_4 is a ruffled core. [c] Crystallographic disorder sets this as a lower limit.

sition-metal porphyrin complexes such as manganese[72] where more than one spin state can exist.

Consideration of d-orbital populations allows us to predict that, contrary to literature reports of high magnetic moments,[73] iron(I) porphyrins are very unlikely to be high spin. Occupation of the $d_{x^2-y^2}$ orbital would require either extreme expansion of the porphinato core (greater than that of $Fe^{II}(TPP)(THF)_2$ or a very large out-of-plane displacement (quite unlikely because iron(I) porphyrins have low affinity for axial ligands). Similarly, iron(IV) porphyrins, where the $d_{x^2-y^2}$ orbital must be unoccupied, can be expected to have short Fe–N_p bonds (<1.990 Å) and an in-plane iron atom (or nearly so if the axial ligand field is unsymmetrical); six-coordination should be favored. The yet unknown unligated $[Fe(P)]^+$ would be expected to be a "pure" intermediate-spin complex with an in-plane Fe(III) atom and very short Fe–N_p bonds (<1.995 Å if the core is planar; <1.972 Å if the core is ruffled).

IV. Hemoprotein Stereochemistry

A. General Considerations

Before proceeding to hemoprotein stereochemistry, a few points merit discussion. First is the question of using structural data on meso-substituted tetraphenylporphinato derivatives as semiquantitative models for the naturally occurring hemes with peripheral substituents at the β-pyrrole positions (Figure 1). That the effect of different substituent patterns on metal stereochemistry is very small can be shown by direct comparison of M(TPP), M(OEP), and M(P) structures.[5]

Second, the more difficult question of the effects of protein constraints on heme stereochemistry must be addressed. Does the incorporation of a heme into the protein matrix have a significant effect on its stereochemistry? As a rule of thumb, we suspect that the magnitude of protein effects on heme stereochemistry will, at most, be comparable to the effects of crystal packing forces and ligand steric effects found in model compound structures. Support for this position comes from a number of experiments. First, cooperativity of dioxygen binding has been observed with solid picket fence porphyrin complexes[74] and is remarkably comparable in energy to hemoglobin itself. This equates the structurally propagated intermolecular packing forces in a model compound crystal with the energy of heme–heme interaction responsible for cooperativity in hemoglobin. Second, spin equilibria of the type observed in methemoglobin and cytochrome P-450 derivatives are well modeled by crystalline iron porphinato complexes.[41,42] Third, the projection of the plane of an axial imidazole onto the porphyrin plane is seen in proteins to make angles ϕ = 17–42° with the nearest N_p–Fe–N_p vector whereas in model complexes a greater range (0–45°) is found.[75,76]

Inasmuch as we equate protein constraints with packing and steric effects in model compounds, it is likely that the detailed heme stereochemistry in a specific hemoprotein will lie within the range portrayed by model compounds once sufficient compounds are known to ascertain this range. The accumulation of structures over the last few years allows us to use this working hypothesis particularly for histidine liganded hemoproteins. This position is in contrast to that taken in studies of "blue" copper proteins where evidence is becoming quite compelling for protein constraints of much greater stereochemical significance than in hemoproteins.[77]

B. High-Spin Ferrous Hemoproteins

Model compound studies place on a relatively firm basis one of the most widely used "rules" of hemoprotein stereochemistry: if high spin, a ferrous hemoprotein will be five-coordinate and will have a significant out-of-plane iron atom displacement. The variation of the axial ligands of ferrous model compounds shows that a weak axial ligand field is required for high-spin six-coordination, a circumstance unlikely to arise with naturally occurring hemoprotein ligands. In fact, every crystallographically characterized hemoprotein is ligated by imidazole from histidine; thiolate from cysteine is the probable ligand in the remainder of hemoproteins where spectroscopic probes have proved to be diagnostic. Since both imidazole and thiolate are moderately strong field ligands any significant additional interaction with a sixth ligand is likely to lead to a low-spin state.

Of all the high-spin ferrous hemoproteins, the coordination group parameters of deoxyhemoglobin (Hb) have commanded the most interest. The foregoing analysis of model compound utility allows us to give our best estimates for these parameters, assuming a relatively strain-free coordination group. This assumption seems justified in view of the small variation in the iron–imidazole stretching mode between model compounds and both T- and R-state deoxyhemoglobin. An upper limit of 0.02-Å extension of Fe–N_{Im} has been calculated as a consequence of protein strain in the T state.[78] Porphyrin core vibrational modes also show close congruence between Hb and deoxy model compounds.[79] The X-ray structure of Hb reveals an off-axis tilt of the imidazole ring of the proximal histidine, and also its orientation with respect to an N_p–Fe–N_p vector (angle ϕ) is clearly protein controlled. Curiously, these constraints are seen to some extent in Fe(TPP)(2-MeIm), making it a better model than previously recognized. With these considerations in mind our best estimates for deoxyhemoglobin are listed in the second column of Table IV. Comparison of these values with those obtained from single-crystal studies on Mb and Hb show congruence within experimental error, and limitations on the resolution of protein structures foreclose any meaningful comparison. Comparison with the Fe–N_p distance of 2.055 Å obtained by EXAFS[60]

TABLE IV: High-Spin Ferrous Coordination Parameters

	Fe(TPP)-(2-MeIm)[55]	estimate for unconstrained Hb	Hb by EXAFS[80]	Hb by X-ray[82]	Mb by X-ray[83]	erythrocruorin by X-ray[84]	μ-catena Fe(PF)[85]	[Fe(TPP)-(THF)$_2$][56]
Fe-N_p, Å	2.086 (4)	2.08	2.055	2.1	2.06	2.02	2.069 (14)	2.057 (5)
Fe-Ct_p, Å	0.55	0.50		0.60, 0.63	0.55	0.17	0.33	0.0
Fe-Ct_N, Å	0.42	0.45	0.2	-	0.42	0.23	0.28	0.0
Ct_N-N_p, Å	2.044 (4)	2.04		2.0	-	-	2.050	2.057 (5)
Fe-N_{ax}, Å	2.161 (5)	2.15		2.0, 2.2	2.1	2.2	-	-

for both Hb and a picket fence porphyrin derivative, although internally consistent, may be low by at least the absolute error of 0.01 Å. The solid-state structure of a closely related picket fence derivative[21] gives a longer Fe–N_p distance (2.072 Å). Another group of EXAFS investigators suggests an Fe–N_p distance of at least 2.064 Å.[81] Any underestimation of Fe–N_p has serious consequences for triangulation calculations leading to the out-of-plane iron displacement, a critical parameter in the trigger mechanism of Hb cooperativity discussed below.

The structure of high-spin deoxyerythrocruorin, a monomeric insect hemoglobin, presents a complication to the preceding discussion. This molecule is reported[84] to have a partially occupied water molecule in the heme pocket at the sixth coordination site which might be regarded as "semicoordinated" with Fe···O = 3.1 Å and Fe–Ct_N = 0.23 Å. The effects of a "semicoordinated" water molecule in the sixth coordination site (Fe···O = 2.9 Å) are shown by the structure of a high-spin μ-catena picket fence derivative where the fifth ligand is a carbonyl oxygen.[85] The bond parameters, particularly the decreased Fe–Ct distance, show a small but noticeable shift from those of an unambiguous five-coordinate complex toward those of six-coordinate Fe(TPP)(THF)$_2$, as displayed in Table IV. It is thus possible that the unexpectedly small iron atom displacement reported in erythrocruorin is real and could be the result of virtual six-coordination by water in the heme pocket. Such a decreased iron atom displacement should be accompanied by core expansion which may be detectable by a structure sensitive probe such as resonance Raman spectroscopy. Another unusual feature reported for erythrocruorin is the reverse doming (Fe–Ct_N > Fe–Ct_p).

C. High-Spin Ferric Hemoproteins

The high-spin state of a ferric hemoprotein is easily established by a variety of physical techniques. The structural implications of a high-spin state are that a moderate axial ligand field is present and that the coordination number may be either five or six. For example, oxidized, substrate-bound P-450 is safely assumed to be five-coordinate with an axial thiolate[86] and aquomethemoglobin is six-coordinate with water and imidazole as the axial ligands. Slightly higher ligand fields such as in the resting state of P-450 or azidomethemoglobin give rise to low-spin states or spin equilibria. Slightly lower ligand fields such as in cytochrome *c'* yield the unusual admixed intermediate-spin state. These cases are discussed below.

We expect *five*-coordinate high-spin ferric hemoproteins to have Fe–N_p distances and Fe–Ct displacements within the range of those found for the 12 known five-coordinate complexes (Table I). In six-coordinate species, the iron atom will move toward the porphyrin plane by an amount dependent on the relative importance of bonding to the two, usually dissimilar, axial ligands. A concomitant decrease in Fe–N_p and an increase in core size can be expected. Consistent with this expected pattern, the resonance Raman frequency sensitive to core expansion[87] (the A_{2g} mode near 1550–1570 cm^{-1}) for aquometHb is much closer to that of [Fe(proto)(Me_2SO)$_2$]$^+$ than that of Fe(proto)(Cl).[90] The structure of [Fe($(CH_2)_4SO$)$_2$(TPP)]$^+$ shows a significantly expanded core compared with FeCl(TPP). These structural implications for the hemoproteins are borne out in the X-ray results reported on horse aquometHb whose dimensions (Fe–Ct_p = 0.07 (6) and 0.21 (6) Å; Fe–N_p = 2.04 Å)[88] approach those of the characterized six-coordinate complexes in Table I.

A somewhat puzzling result is the reported 0.40-Å iron atom displacement in sperm whale aquometmyglobin.[89] Since the resonance Raman spectra of myoglobin and hemoglobin are identical,[90] it is likely that the degree of core expansion, i.e., the value of Ct–N_p, is almost the same (~2.04 Å). The interplay of core expansion vs. iron atom displacement in model compounds suggests that the 0.40-Å displacement of metMb(H_2O) may be an overestimate. The issue is, however, complicated by possible ruffling and doming effects of the porphyrin core and the presently incomplete understanding of the quantitative treatment of resonance Raman frequencies.

D. Low-Spin Hemoproteins

While a few *five*-coordinate low-spin model complexes such as Fe(P)(NO) and Fe(P)(CO) are known and others may exist in certain Fe(P)(X) derivatives such as X = CN or R, their only probable existence in hemoproteins is in certain in vitro nitrosyl derivatives or as short-lived reaction intermediates. Thus, it is widely accepted that a low-spin state is diagnostic of six-coordination in both ferrous and ferric states. For example, in the low-spin oxidized (resting) state of P-450 an unknown ligand is assumed to coordinate trans to the thiolate.[86] Similarly, the low-spin heme *a* of cytochrome oxidase is assumed to be six-coordinate.[91]

The stereochemistry expected for low-spin hemoproteins comes from the range of bonding parameters for the numerous model compounds listed in Tables I and II. The range of values is relatively small. The Fe–N_p distances are close to 2.00 Å and are slightly larger for iron(II) than for iron(III). Progressive shortening below the average distances listed in Tables I and II is likely to cause an increasing amount of ruffling of the porphinato core. Such ruffling can also be influenced by steric effects from the axial ligands

whose approach to the low-spin iron atom is relatively close.

The out-of-plane displacement of the iron atom has not been observed to be greater than 0.11 Å in any six-coordinate low-spin model complex, even when greatly dissimilar or sterically demanding axial ligands are present: 0.10 Å in Fe(DP)(CO)(THF),[50] 0.086 Å in Fe(PF)(O_2)(2-MeHIm),[21] and 0.11 Å in Fe(TPP)-(NO)(4-Me-Pip).[48] This leads us to consider seriously the recent reports of a 0.22 (3) Å displacement of iron in oxymyoglobin[92] and 0.30 Å in oxyerythrocruorin.[93] The erythrocruorin case is especially peculiar since the protein crystallography suggests that the iron atom actually moves *further* out of plane toward the proximal histidine upon binding dioxygen, a feature quite discordant with model compound structures. In fact, many of the structural features reported for liganded erythrocruorins challenge the validity of the present cause-and-effect theory of spin state and stereochemistry. The obvious question is "are these parameters correct and what are the real error limits?" Recent work comparing two refined structures of bovine trypsin[94] suggests that the errors in atomic positions can be relatively large even in substantially refined protein structures. The recent comparison of two hemerythrin structures[95] gives an interesting analysis of the difficulties that can arise in protein crystallography. Historically, the overinterpretation of protein crystallographic results has led to serious mistakes. Moreover, the unconsidered use of tabulated bond distances and angles, without regard to error limits, has been a common practice. At this point we can only urge considered caution in interpreting hemoprotein structural results that are in serious disagreement with reliable model compound results.

E. Hemoglobin Cooperativity

A molecular level explanation of the cooperativity of dioxygen binding to Hb must address two questions: How is the O_2 affinity of the T state lowered relative to the R state? How does a ligation change at one heme propagate a change of affinity at another?

Considering the first question, there now seems to be general adherence to the concept that protein constraints in the T state which lead to strain in the coordination group upon oxygenation will lower the O_2 affinity.[96-98] In other words, protein constraints which favor the high-spin, five-coordinate geometry of the heme group and prevent it from attaining its optimal low-spin, six-coordinate geometry will lower the affinity. The early idea of a tense, stretched iron–histidine linkage in the deoxy T state has been largely replaced by the idea of a tethered histidine which cannot follow the iron to its in-plane position in oxyHb without steric strain. It may be a mistake to focus on a single cause of lowered O_2 affinity since *any* protein constraint in the T state which favors the high-spin deoxy five-coordinate geometry over the low-spin liganded geometry will contribute. A number of suggestions, many supported by model compound experiments, have been made for mechanism(s) by which the oxygen affinity is lowered in the T state. These include proximal histidine constraints,[96,99,100] distal site blocking,[101] proximal histidine deprotonation,[102] porphyrin ring effects,[103] and "environment" effects of dipoles and electrostatics.[104] The current resolution of hemoglobin X-ray crystal structures suggests that both asymmetric tilting of the tethered proximal histidine as well as partial steric blocking of the O_2 binding site are the most important factors.[101]

Considering the second question of the mechanism of heme–heme interaction, it is clear that coordinate bond changes accompanying O_2 binding in the T state must induce steric and electrostatic strains which eventually cause the protein configuration to adopt the high-affinity R state. Model compounds tell us that coordinate geometry changes will involve (a) ~0.5-Å iron atom movement toward the heme plane, (b) ~0.6-Å movement of the histidyl imidazole toward the heme group, (c) a small but significant contraction of the porphyrin core with possible loss of doming and the onset of ruffling, (d) the decreased opportunity for asymmetric tilting of the proximal histidine, and (e) the possibility of the O_2 ligand displacing distal residues from the O_2 binding pocket. As more O_2 binding to the T state proceeds, the cumulative steric and electrostatic strain from these changes triggers tertiary and quaternary structure changes (particularly in the F helix, at the FG corner, and the $\alpha_1\beta_1/\alpha_2\alpha_\beta$ interface)[101] which lead to the R configuration. Upon switching to the R state there is no longer any strain induced by the ligand binding and the affinity is essentially that intrinsic to the iron.

Since cooperativity is nature's device for *lowering* O_2 affinity, it is instructive to think of the reverse mechanism. The production of deoxy high-spin sites in R-state oxyHb must induce strain which triggers the R- to T-state switch.

In summary, the present analysis of spin-state/stereochemical relationships lends credence to the theory that a spin-state change can provide the stereochemical trigger for cooperativity. The remaining difficulty with the theory is to determine the relative importance of such stereochemical forces via-â-vis the electronically induced forces resulting from electronic changes in the coordination group upon ligation change.

F. Spin-State Equilibria in Hemoproteins

Thermal spin-state equilibria of a number of ferric hemoproteins have been observed.[105] The results of the variable-temperature structural studies on [Fe-(OEP)(3-Cl(py))$_2$]$^{+}$[19] make evident that the obligatory structural changes accompanying the low-spin → high-spin transition in the hemoproteins are porphinato core expansion and elongation of the axial bonds. Some difference in the position of the iron atom with respect to the porphinato plane may occur and would be dependent on the relative differences in bonding of the dissimilar axial ligands.

A feature of particular interest is the protein dependence of the thermal spin-state equilibria[105-107] for hemoprotein species with nominal parity of axial ligation. These subtle differences suggest protein modification of coordination geometry. Consistent with the idea that the magnitudes of protein effects are comparable to the effects of crystal packing forces is the observation[108] that two crystalline forms of [Fe(OEP)(3-Cl(py))$_2$]ClO_4 show significantly different temperature-dependent magnetic susceptibilities in the solid state. The triclinic form[19,43] shows susceptibilities consistent with the $S = {}^5/_2 \rightarrow S$

= $^1/_2$ transition; the monoclinic modification has susceptibilities that are significantly higher. Both crystalline forms have identical composition. Results from model compounds should provide clarification of possible protein effects on spin equilibria.

G. Admixed Intermediate-Spin Ferric Hemoproteins

The close similarity of the magnetic parameters of Fe(TPP)($OClO_3$)[36] with those of the proposed admixed intermediate-spin component[44] of oxdized cytochrome *c'* have put this spin state on a firm basis for heme proteins. The structural implications for this hemoglobin-like cytochrome are that there is a greater degree of tetragonal distortion in the ligand field than in high-spin metHb(H_2O), presumably arising from a slight weakening of the axial ligand field. A number of possibilities for this have been discussed.[36] The two most likely explanations are that the sixth ligand site (H_2O) is vacant or that the imidazole ligation is weakened by a protein effect. Comparison of the structures of metHb(H_2O) and cyt *c'*[109] suggests an origin for this latter effect. In metHb(H_2O) the exo NH of the ligating imidazole is specifically H-bonded to a nearby peptide residue. This is expected to induce a partial imidazol*ate* character to the proximal histidine, thereby increasing its ligand field slightly above that of "free" imidazole.[72] This is the case for all structurally characterized hemoproteins except cytochrome *c'* whose exo-NH is solvent exposed.[109] Thus, the lack of specific H-bonding in cytochrome *c'* may result in an imidazole of slightly weaker ligand field.

Regardless of the precise cause, any effect which weakens the axial ligand field of a high-spin ferric hemoprotein may bring the spin state into the admixed $S = {}^3/_2, {}^5/_2$ spin regime.

H. Iron–Sulfur Bonds

Structures for a total of seven different (porphinato)iron complexes containing a sulfur ligand have been reported. Three have thioether ligands,[12] three have thiolate ligands,[26,53] and the last has thiol and thiolate ligands.[42] Excluding the last compound, which is disordered, the observed Fe–S bond distances fall within the range 2.324–2.360 Å. This very narrow range is observed despite the fact that these compounds differ in the nature of the sulfur ligand, spin state of iron, oxidation state of iron, and coordination number of the complex. We have noted that the Fe–S distances in the six-coordinate low-spin thioether derivatives were essentially unchanged on redox (Fe^{II}–S = 2.336 (3), Fe^{III}–S = 2.341 (17) Å).[12] Slightly larger differences are observed for the high-spin five-coordinate thiolate complexes with Fe^{II}–S = 2.360 (2) and Fe^{III}–S = 2.324 (2) Å.[26,53] A change in spin state *and* coordination number for the iron(II) thiolate leads to essentially no change in Fe–S (low-spin complex = 2.352 (2) Å).[53] These differences are very much smaller than Fe–N_{ax} distance changes in corresponding N-ligated (porphinato)iron derivatives. They are also much smaller than the 0.09-Å change in bond length in the tetrahedral [Fe(S_2-*o*-xyl)$_2$]$^{1-/2-}$ structural pair.[110] While there are too few data regarding Fe–S bond lengths in model complexes to be certain, the data do suggest that unconstrained hemoproteins will have Fe–S distances falling close to this range. This is the case, within experimental error, for cytochrome *c*.[111]

V. Conclusion

By using the current accumulation of structural data to set range expectations for the bonding parameters in iron porphyrins and hemoproteins we bring into focus both the utility and problems arising from attempting to reconcile model complex data with that from the hemoproteins. The use of model compound structures to establish expectations for hemoprotein structure is subject to the same caveats as the common practice of using bond lengths from X-ray crystal structures for those present in solution (e.g., in Resonance Raman spectroscopy[87]). Thus, in realistically applying model compound structures to hemoprotein structure (a) a spread of distances must be considered, (b) realistic consideration must be given to error limits, and (c) a sufficient and appropriate data base must be available. Whether protein constraints upon the heme like those reported for erythrocruorin[84] are strong enough to thwart the intrinsic stereochemistry dictated by spin state remains to be seen. Filling the gaps in our knowledge to better establish the parameter ranges is limited only, on one hand, by the skill of synthetic chemists in preparing the requisite model compounds for structure analysis, and, on the other hand, by the ability of protein crystallographers to extract more reliable data from frequently unobliging proteins. The prospects are good that an eventual, satisfying union of these approaches will achieve the ultimate goal of understanding the detailed molecular mechanisms of action of the hemoproteins.

Acknowledgments. We are most pleased to acknowledge the contributions of our co-workers in the cited references and the financial support of the National Institutes of Health and the National Science Foundation.

VI. References and Notes

(1) Abbreviations used in this article: P, dianion of a generalized porphyrin; TPP, dianion of *meso*-tetraphenylporphyrin; OEP, dianion of octaethylporphyrin; meso, dianion of mesoporphrin IX dimethyl ester; deut, dianion of deuteroporphyrin IX dimethyl ester; proto, anion of protoporphyrin IX; PF, dianion of "picket fence" porphyrin; HIm, imidazole; 1-MeIm, 1-methylimidazole; 2-MeIm, 2-methylimidazole; py, pyridine; 3-Cl(py), 3-chloropyridine; THF, tetrahydrofuran; $(CH_2)_4SO$, tetramethylene sulfoxide; Hb, hemoglobin; cyt, cytochrome; PMS, pentamethylene sulfide; THT, tetrahydrothiophene; Pip, piperidine; *t*-BuNC, *tert*-butyl isocyanide; IHP, inositol hexaphosphate; Me_2SO, dimethyl sulfoxide.
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