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Stereochemistry of Hemes and Other Metalloporphyrins

The remarkably varied stereochemistry of the iron porphyrins is utilized in the hemoprotein function.

J. L. Hoard

A high level of interest in the porphyrins and the metalloporphyrins is fully justified by their behavior as complex physicochemical systems, and it is further enhanced by the biological circumstance that iron porphyrins serve as the hemes or prosthetic groups in the several classes of the hemoproteins (*1*). Both the chemistry and the concomitant stereochemistry (*2*) of the iron porphyrins are richly diversified, thus permitting the diversity of biological function that characterizes the distinctive families of the hemoproteins. The specificity of the function, the consequence of a severe delimitation of the reactions open to the prosthetic groups, is, of course, the primary responsibility of the protein; the constraint thus imposed is rationally presumed to be of stereochemical origin (*2*). The importance of stereochemical considerations for elucidating the behavior of the iron porphyrins and, in general terms, that of the oxygen-carrying family of the hemoproteins is the principal theme of this article. Essential to this end are the quantitatively precise descriptions of metalloporphyrin stereochemistries that are the recent products of structure analyses in which the extensive x-ray data afforded by single crystals have been utilized. Background material from earlier studies of the porphyrins and hemoproteins is introduced as needed.

Metalloporphyrins: The Porphinato Core

All porphyrins are derivatives of porphine, an aromatic molecule with the carbon-nitrogen skeleton illustrated in Fig. 1. In the porphine molecule, the

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pyrrole-carbon atoms numbered 1 to 8 and the methine-carbon atoms labeled α to δ each are attached to a hydrogen atom, as are also two of the four nitrogen atoms. Substitution for one or more of the peripheral hydrogen atoms gives rise to a porphyrin, and the further replacement of the central pair of hydrogen atoms by a metal atom yields the corresponding metalloporphyrin. This metal atom, to which the four nitrogen atoms are then complexed, assumes a coordination number $\geqq 4$ in agreement with the number ($\geqq 0$) of axial ligands that may be added. The formation of a metalloporphyrin may be otherwise described to advantage as involving the chelation of the metal cation by the doubly charged, highly aromatic, porphinato core of the porphyrin, perhaps accompanied or followed by the addition of an axial ligand or ligands.

Metalloporphyrin stereochemistry is best treated on a semiempirical basis, with the aid of such approximate theory as may be applicable. Of major significance in every metalloporphyrin are the structural properties of the chelating entity—the charged porphinato core—and the complexing properties, including the effective size, of the metal ion; the parameters employed to describe the stereochemistry are quantitatively measurable consequences of the underlying electronic structure that does not lend itself to precise theoretical discussion. Axial ligands are cast in supporting roles in a number of the metalloporphyrins, with especial effect in the iron porphyrins.

It is evident from an inspection of Fig. 1 that the metal derivatives of the synthetic porphyrins α,β,γ,δ-tetraphenylporphine and α,β,γ,δ-tetra(4-pyridyl)-porphine may retain fourfold symmetry with respect to all of their properties. This conclusion does not hold for the metal derivatives of such biologically interesting porphyrins as deuteroporphyrin-IX (the trivial name for 1,3,5,8-tetramethylporphine-6,7-dipropionic acid), protoporphyrin-IX (2,4-divinyldeuteroporphyrin-IX), or mesoporphyrin-IX (2,4-diethyldeuteroporphyrin-IX). Although several physical properties of the metalloporphyrins are known to be sensitive to the pattern of peripheral substitution (*1*), alterations in the bond parameters of the porphinato core that arise from this source are apparently too small to be certainly identified in any existing x-ray determination of crystalline structure. It is most unlikely that the length of an individual C–C bond in the core can be determined with an estimated standard deviation below 0.003 angstrom; the usual expectation is an estimated standard deviation ranging upward from 0.005 Å. Apparent differences of 0.01 to 0.02 Å in the lengths of chemically analogous bonds are attributable quite as readily to required compromises in the complicated packing of the molecules in the crystal as to the effects of asymmetric substitution on the periphery of the porphinato core. These reservations notwithstanding, the more precise determinations of metalloporphyrin structure yield quite illuminating results. It is generally observed that the mean deviation from the averaged length of each chemical type of bond is as small as, or smaller than, the estimated standard deviation of the individual bond length; it follows that averaging of the bond parameters in accordance with fourfold symmetry leads at once to the simplest, yet most generally useful, description of the stereochemistry of the porphinato core. Averaged bond lengths thus obtained for the core in various metalloporphyrins are listed in Table 1, but discussion of these data is better deferred pending consideration of the stereochemical role taken in every metalloporphyrin by an especially significant parameter of the porphinato core.

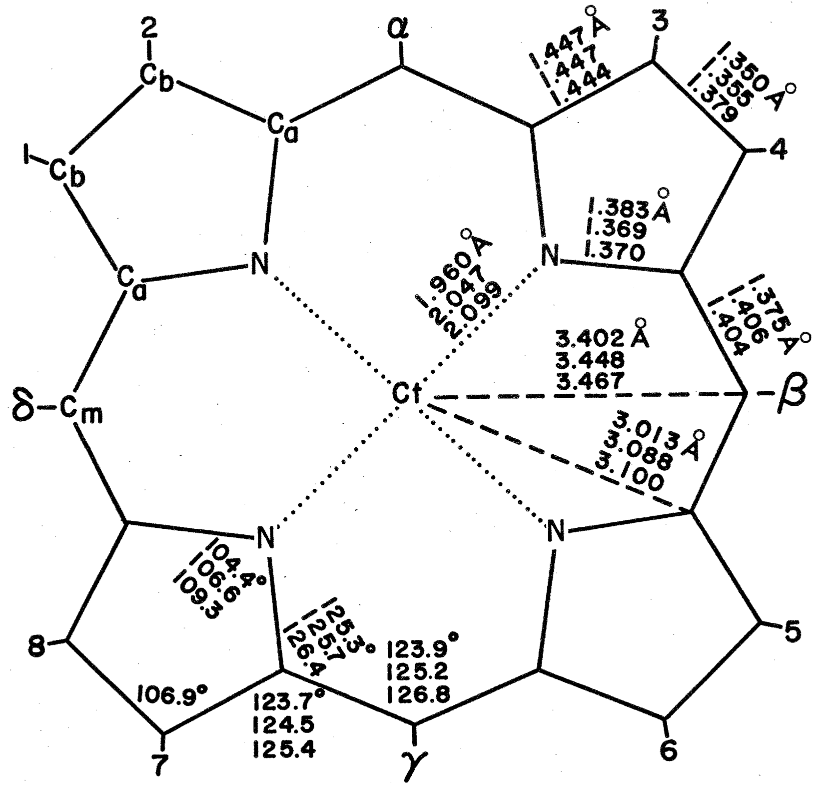

Fig. 1. Diagram of the carbon-nitrogen skeleton in the porphinato core of metalloporphyrins drawn for the real or effective retention of fourfold symmetry. The maximum symmetry is reduced from D_{4h} to C_{4v} in those metalloporphyrins wherein the metal atom is not positioned at the center (*Ct*), but is displaced out of plane along the fourfold axis. Values of the principal radii (in angstroms), bond lengths (in angstroms), and bond angles (in degrees) are entered on the diagram for three metalloporphyrins; reading downward in each tier of three, the numerical data are for the respective nickel(II), zinc(II), and tin(IV) porphyrins that are fully identified in the footnotes to Table 1. In Table 1 C_a, C_b, and C_m are the symbols used for the three chemically and structurally distinctive types of carbon atoms.

The "radius of the central hole," the Ct · · · N radius displayed in Fig. 1, is constrained by the electronic structure of the porphinato core to a rather narrow range of variation. Among the dozen or so metalloporphyrins for which the structures have been determined, the smallest value of the Ct · · · N radius, 1.960 ± 0.004 Å, occurs in the nickel(II) derivative of 2,4-diacetyldeuteroporphyrin-IX dimethyl ester (*3*) and the largest value, 2.099 ± 0.002 Å, occurs in the dichlorotin(IV) derivative of tetraphenylporphine (*4*). In each of these metalloporphyrins the metal atom M is centered in the plane, and thus the complexing M–N bond length is identified with the Ct · · · N radius. The Ni–N bond length, however, is at least 0.06 Å longer, and the Sn–N bond length is 0.05 Å shorter, than the preferred values with nitrogen atoms of monodentate ligands. By reason of its charge distribution and structure, the porphinato anion forms chelates with a variety of cations, large and small, but the resistance of the skeletal structure to undue expansion or contraction requires the range of complexing bond lengths to be much narrowed, from above and below, relative to that which applies with monodentate ligands.

Table 1. Bond lengths in the porphinato core as a function of the Ct · · · N radius (see Fig. 1). The numbers in parentheses are the weighted standard deviations of the mean in units of 0.001 Å.

Type of distance	Length (Å) in			
	NiDeut*	PyZnTPyP†	Cl_2SnTPP‡	Selected porphyrins§
Ct · · · N	1.960 (4)	2.047 (2)	2.099 (2)	2.013‖
N–C_a	1.383 (3)	1.369 (2)	1.370 (2)	1.384‖
C_a–C_b	1.447 (3)	1.447 (2)	1.444 (2)	1.449‖
C_b–C_b	1.350 (5)	1.355 (3)	1.379 (3)	1.358‖
C_a–C_m	1.375 (4)	1.406 (2)	1.404 (2)	1.389‖

* The nickel(II) derivative of 2,4-diacetyldeuteroporphyrin-IX dimethyl ester (*3*). † Tetra(4-pyridyl)porphinatomonopyridinezinc(II) (*6*). ‡ Tetraphenylporphinatodichlorotin(IV) (*4*). § Chlorohemin (*28*), the methoxyiron(III) derivative of mesoporphyrin-IX dimethyl ester (*27*), and the chloroiron(III) (*14*) and palladium(II) (*9*) derivatives of tetraphenylporphine, wherein the ostensible value of Ct···N ranges from 2.008 to 2.022 Å. ‖ Averaged value for the four porphyrins.

The value of the Ct · · · N radius that corresponds more or less exactly to minimization of radial strain in the porphinato core of a metalloporphyrin must be estimated by a detailed appraisal of the stereochemical features exhibited in the spectrum of known metalloporphyrin structures. A value lying rather closer to 2.01 Å than to 2.02 Å, obtained from the first such appraisal (*5*), is strongly supported by the results of a more recent analysis (*6*) in which some newer data of special pertinence are included.

The large value of the Ct · · · N radius in the dichlorotin(IV) porphyrins (*7*) is fully attributable to the strongly complexing properties of the rather oversized tin(IV) atom. The silver(II) derivative of tetraphenylporphine provides one other authenticated structure (*8*) wherein the metal atom is centered in the porphinato core with a complexing bond length, 2.09 Å, that is greater than 2.01 Å. Although the zinc and the high-spin ferric porphyrins have M–N bond lengths of ~ 2.07 Å, the metal atom in each of these is positioned sufficiently far out of plane from the center along the unique axis as to require rather little expansion of the Ct · · · N radius above the 2.01-Å value. Other metalloporphyrins of known structure that have the metal atom centered in the core with complexing M–N bond lengths in the range from 1.98 to 2.01 Å are the copper(II) and palladium(II) derivatives of α,β,γ,δ-tetraphenylporphine (*9*) and the low-spin bis(imidazole) - α,β,γ,δ - tetraphenylporphinatoiron(III) chloride (*10*).

The dimensional data listed in Table 1 and displayed in Fig. 1 for the porphinato core in tin, zinc, and nickel porphyrins come from structure determinations in which x-ray data of particularly wide scope and high precision of intensity measurement were utilized. Inspection of Fig. 1 shows that the redistribution of strain in the porphinato skeleton which accompanies a large change in the Ct · · · N radius follows a rather complicated pattern. A specified increase in this parameter produces inhomogeneous increases of smaller magnitude in the Ct · · · C_m and Ct · · · C_a radii, the quantities that determine the radial extension of the skeleton. Bond angles, excepting only those at the pyrrole-β-carbon (C_b) atoms, are strongly affected by a changing Ct · · · N radius. The apparent insensitivity of the C_a–C_b bond length to very large variations in the Ct · · · N radius is not maintained for the other bond types. Comparison of the data for the zinc and tin porphyrins, in which both of the Ct · · · N radii are larger than the value (2.01 Å) for minimum strain, shows that only the C_b–C_b bond lengths differ significantly for the different species. But when the two radii span the value for minimum strain, as in the nickel and zinc porphyrins, it is the bridging C_a–C_m bond lengths and, to a lesser degree, the N–C_a bond lengths that differ significantly from one species to the other.

The data listed in the last column of Table 1 are averages of the results given by structure determinations for metalloporphyrins in which the Ct · · · N radii lie near to the 2.01-Å value for minimum strain. These data, with the exception of the N–C_a bond length, interpolate smoothly between those of the nickel and zinc porphyrins. The N–C_a bond length seems not to be appreciably stretched for Ct · · · N < 2.01 Å (*11*), but rather to be slightly compressed for Ct · · · N > 2.01 Å; this conclusion is supported by the structural data for metal-free porphyrins wherein the mutual repulsions of the hydrogen atoms attached to the porphinato-nitrogen atoms become important (*6*). The set of averaged bond parameters for a Ct · · · N radius of ~ 2.01 Å is most pertinent to the discussion of iron porphyrin stereochemistry (see below).

The largely empirical translation of the aromatic bond lengths that characterize the porphinato core into effective bond orders of the variously defined types is discussed elsewhere (*3, 5, 12*). Variations in bond order with bond type are substantially greater than is typical of a benzenoid hydrocarbon. In further contrast with the benzenoid hydrocarbons, the porphinato core is rather easily ruffled or domed, or both, into distinctively nonplanar configurations. Thus a ruffling of the core that requires the α- and the γ-methine carbon atoms to lie 0.38 Å above the mean plane, and the β- and the δ-atoms to lie an equal distance below, is observed in tetragonal crystals of tetraphenylporphine (*13*) and its copper(II) and palladium(II) derivatives (*9*). Less symmetrical, generally smaller departures from planarity are more usually observed for the porphinato core in crystalline metalloporphyrins; the preservation, in excellent approximation, of a localized planarity in each pyrrole ring and in each trigonal bond system emanating from a carbon atom of either the C_a or C_m type (Fig. 1) suggests that the delocalized π-bonding is little affected by the geometric undulations in the core.

Crystalline α,β,γ,δ-tetraphenylporphinatodichlorotin(IV), with the molecular configuration displayed in Fig. 2, provides the one established structure in which the equilibrium configuration of the porphinato core seems unambiguously to be planar (*4*). Exceptionally small thermal motion in a crystal of optimum dimensions made it feasible to record precise intensity data of particularly high resolution, 0.53 Å. The required symmetry of the molecule in the crystal is C_{4h}, but the precisely determined parameters are fully compatible with D_{4h} symmetry for the unconstrained molecule.

The excellence of the molecular packing in the tetraphenylporphinatodichlorotin(IV) crystal is further emphasized by the observation that a statistically disordered form of the crystalline arrangement is utilized in several five-coordinate metal derivatives of tetraphenylporphine. Included in this category are the chloroiron(III) (*14*), aquozinc(II) (*15*), aquomagnesium(II) (*16*), and nitrosylcobalt(II) (*17*) derivatives. The tetragonal symmetry of the crystalline arrangement is statistically preserved because the polar bond to the single axial ligand is allowed to take, with equal probability, either the parallel or antiparallel orientation relative to the unique fourfold axis of the crystal. I emphasize this point, because the statistical disorder constitutes a severe limitation on the precision with which the displacement of the metal atom from the plane of the porphinato-nitrogen atoms can be determined [see (*14–17*) for specific cases].

Zinc and Magnesium Porphyrins

Figure 3 is a diagram of the five-coordinate, square-pyramidal coordination group of C_{4v} symmetry that is basic to the stereochemical discussion of the zinc, magnesium, and high-spin iron porphyrins. Given C_{4v} symmetry, the quantitative description of the coordination group may be completed by specifying the length of the bond (M–A) from the metal to the single axial ligand A, the length of the bonds (M–N) from the metal to the porphinato-nitrogen atoms, and the value of either the Ct · · · N radius or the displacement (Ct · · · M) of the metal atom from the basal plane defined by the porphinato-nitrogen atoms. The values assumed by these parameters for the several choices of metal ion are listed in Table 2.

Nothing in the electronic structures of the d^{10} zinc and d^0 magnesium ions is suggestive of inherent preferences for square-pyramidal five-coordination. Yet studies of the complexing reactions in solution (*18*) provide equilibrium constants $> 10^3$ for the attachment of one pyridine ligand by either a zinc or a magnesium porphyrin, a constant of ~ 1 for the addition of a second pyridine ligand by a magnesium porphyrin, and no evidence whatsoever for the complexing of a second pyridine by the monopyridinezinc species. Because the stable complexing of a second axial ligand is incompatible with retention of a nontrivial out-of-plane displacement of the metal atom, the results of the equilibrium studies are simply attributable to dimensional constraints imposed by the porphinato core on these overlarge metal ions of modest complexing power (*6*). A structure determination (*19*) for crystals of the dipyridinemagnesium derivative of etioporphyrin-II yields axial bond lengths of ~ 2.34 Å; a much stronger axial bond, comparable in length with that (2.14 Å) in the zinc porphyrin, is anticipated for the five-coordinate monopyridinemagnesium species (*6*).

The structural parameters reported for the asymmetrically modified porphinato core and the coordination group in vanadyldeoxophylloerythroetioporphyrin (*20*) may be expected to apply with only minor alterations to chlorophyll. Although one of the complexing V–N bonds is significantly shorter (by ~ 0.14 Å) than the other three, the averaged values of the Ct · · · N radius (2.01 Å) and the V–N bond distances (2.065 Å), the displacement out of plane of the vanadium atom (0.48 Å), and the axial V–O bond length (1.62 Å) are strikingly similar to those of the high-spin methoxyiron(III) porphyrin (Table 2). It is likely, as a consequence, that the averaged parameters of the coordination group around the magnesium ion in chlorophyll differ little from those of a typical five-coordinate magnesium (or zinc) porphyrin. (The length of the axial bond is, of course, dependent on the choice of axial ligand.)

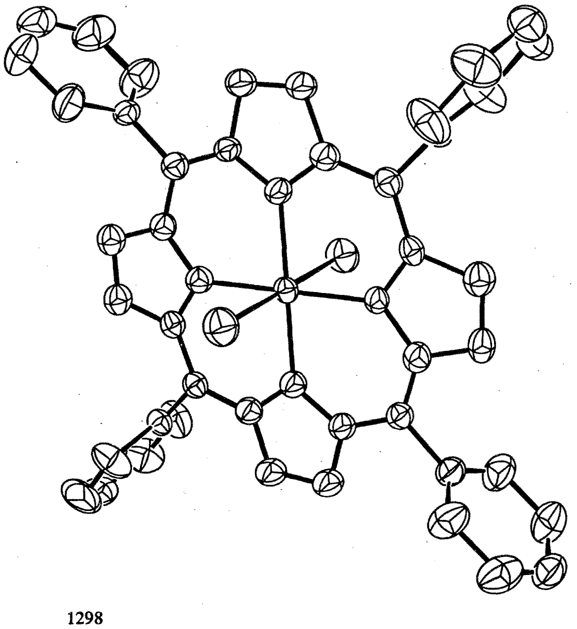

Fig. 2. Computer-drawn model, with the hydrogen atoms omitted, of the tetraphenylporphinatodichlorotin(IV) molecule of effectively D_{4h} symmetry; each atom is represented by an ellipsoid having the shape and relative size concomitant with its thermal motion in the crystal.

Iron Porphyrins

The iron porphyrins are divided quite naturally into four classes as determined by the spectroscopic ground state, either high- or low-spin, and the oxidation state, either (II) or (III), of the iron atom. The porphinato core takes a principal role in producing and enforcing the striking differences in stereochemical type that, with either iron(II) or iron(III), correlate with the spin state.

Complexes formed by either the d^6 ferrous or d^5 ferric ions are usually of the strongly paramagnetic, high-spin type, wherein the distribution of electrons among the five, incompletely filled, 3*d* orbitals in the valence shell of the iron cation minimizes the number of spin pairings. With just one electron in each of these orbitals to give a half-filled subshell of spherical symmetry, the high-spin ferric ion has many of the complexing properties of a cation of intermediate radius with the inert gas configuration and displays a superior adaptability to the less usual coordination geometries. The high-spin ferrous ion, with its additional electron paired in one orbital, has a larger effective radius but is no less adaptable than the ferric ion. Neither cation seems to form stable, discrete, high-spin complexes in which the coordinated atoms are predominantly nitrogen atoms of monodentate ligands. Chelation of the ferric ion by ethylenediaminetetraacetic acid (*21*) and other closely related, multidentate, chelating agents (*22*) yields high-spin complexes wherein the Fe–N bond lengths range upward from ~2.20 Å, being always $\gtrsim$0.20 Å longer than the Fe–O bonds in the same complex.

Octahedral coordination of six, appropriately chosen, monodentate ligands forces spin pairing of the $3d$ electrons in the set of three T_{2g} orbitals (d_{xy}, d_{yz}, d_{zx}) of the iron atom; the two E_g orbitals ($d_{x^2-y^2}$, d_{z^2}), with octahedrally directed lobes, are simultaneously utilized for strong σ-bonding with the charge-donating ligands. These low-spin complexes are most effectively stabilized by ligands (such as cyanide) that are excellent σ-donors of charge density and, in addition, can accept charge in low-lying, antibonding, molecular orbitals from the T_{2g} orbitals of the iron atoms by back π-bonding. Low-spin iron(II) and iron(III) complexes are, respectively, diamagnetic and paramagnetic (one unpaired electron). The Fe–N bond lengths are predicted (*23*) to differ by only ~ 0.02 Å for chemically analogous iron(II) and iron(III) complexes with a mean value that differs insignificantly from the Co–N bond length in the analogous low-spin cobalt(III) species. The standard datum of reference in discussing the low-spin iron porphyrins is then the averaged M–N bond length, 1.955 ± 0.015 Å, given by structure determinations of cobalt(III) complexes in which the central atom forms covalent bonds with six nitrogen atoms of six monodentate ligands (*24*). This value is appreciably larger than the 1.92 Å that is given by the sum of the octahedral covalent radii (*23*) and that is observed in the low-spin ethylenediaminetetraacetatocobaltate(III) ion (*25*).

Although the electronic structure and the concomitant geometry of the quasi-rigid porphinato core in an iron porphyrin are conducive to the partial pairing of electron spins in the $3d$ subshell of the iron atom, the choice between high- and low-spin species rests ultimately with the character and number of the monodentate axial ligands (*26*). Structure analyses for the methoxyiron(III) derivative of mesoporphyrin-IX dimethyl ester (*27*), for chlorohemin (*28*), and for μ-oxo-bis[tetraphenylporphinatoiron(III)] (*29*) provide a quantitatively consistent description of the square-pyramidal five-coordination group (Fig. 3 and Table 2) that is typical for high-spin ferric porphyrins. Averaged values of the Fe–N bond length, the Ct · · · N radius, and the out-of-plane displacement of the ferric ion that are listed in Table 2 differ trivially from those observed in the methoxyiron(III) porphyrin (*27*); the length of the fifth bond is, of course, dependent on the nature of the axial ligand.

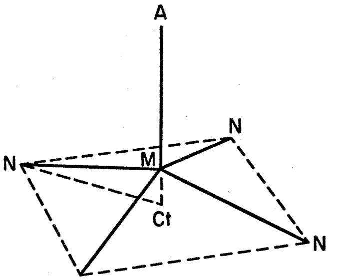

Fig. 3. The square-pyramidal (C_{4v}) geometry that characterizes the coordination groups in the zinc, magnesium, and high-spin iron porphyrins. Quantitative data for typical metalloporphyrins are listed in Table 2.

The near identity of the complexing bond lengths to porphinato-nitrogen atoms in the zinc, magnesium, and ferric porphyrins is noteworthy (Table 2), but all other comparisons of parameters reflect the superior adaptability of the high-spin d^5 ferric ion to five-coordination. Thus the Ct · · · N radius, 2.02 Å, in the iron porphyrins corresponds to trivial strain in the porphinato skeleton, and the concomitant out-of-plane displacement of the ferric ion, 0.48 Å, is so large as to allow the tight coordination of an axial ligand with little or no concern for the destabilizing effect of ligand nonbonding repulsions. In the methoxyiron(III) porphyrin, for example, the length of the axial Fe–O bond, 1.84 Å, is to be compared with the predicted value, 1.87 Å, for (low-spin) octahedral covalent bonds (*23*) and with observed values, $\gtrsim$ 2.00 Å, for the high-spin ferric iron in octahedral coordination (*21, 22*).

The tightness of the unique axial bond is readily interpretable as follows (*5, 14*): the $3d_{z^2}$ orbital of the ferric ion can combine with the appropriate σ-type orbital of the axial ligand to give a strongly bonding orbital that accepts the donor electron pair and a weakly antibonding orbital that accommodates the unpaired electron; the $4s$ and $4p_z$ orbitals of the ferric ion may also contribute to the bonding. Inasmuch as the electron density associated with the antibonding orbital is more or less concentrated in the sixth octahedral position as a "phantom ligand," it enters into repulsive interaction mainly with the four complexing bonds to porphinato-nitrogen atoms. A marked enhancement of these repulsive interactions accompanies the substitution of ferrous for ferric iron in the coordination group, because the antibonding orbital must then contain a pair of electrons. The magnitudes of the concomitant lengthening of the four Fe–N bonds and of the increase in the out-of-plane displacement of the iron cation are better estimated from an alternative model (see below). The length of the unique axial bond, by contrast, should be little affected by the substitution of ferrous for ferric iron. A short bond length of ~ 1.90 Å to a neutral imidazole (or pyridine) molecule as the single axial ligand is anticipated; although inferior to methoxy or chloride as a σ-donor, the aromatic ligand can function as a π-acceptor of electron density from the d_{yz} and d_{zx} orbitals of the iron cation.

As pointed out earlier (*5, 12, 27*), the bonding interactions of the high-spin iron cation, ferrous or ferric, with the charged porphinato-nitrogen atoms are readily appreciated, and are most usefully treated, in the simple terms of crystal field theory. An x-ray structure

Table 2. Parameters of the square-pyramidal coordination groups in zinc, magnesium, and high-spin iron porphyrins (see Fig. 3). The numbers in parentheses are the estimated standard deviations of the mean in the last significant figure.

Type of distance	Length (Å) in			
	PyZnTPyP*	H_2OMgTPP†	Ferric porphyrins‡	Ferrous porphyrins§
Ct · · · M	0.33 (1)	0.27 (2)	0.48 (1)	(0.70)
Ct · · · N	2.047 (2)	2.055 (6)	2.019 (3)	(2.02)
M–N	2.073 (2)	2.072 (6)	2.074 (3)	(2.14)
M–A	2.143 (4)	2.099 (15)	1.842 (4)‖	(1.90)¶

* Tetra(4-pyridyl)porphinatomonopyridinezinc (*6*). † Tetraphenylporphinatoaquomagnesium (*16*). ‡ The methoxyiron(III) derivative of mesoporphyrin-IX dimethyl ester, chlorohemin, and μ-oxo-bis[tetraphenylporphinatoiron(III)]; listed values of the averaged parameters differ insignificantly from those reported for the methoxyiron(III) derivative of mesoporphyrin-IX dimethyl ester (*27*). § Because an x-ray analysis of structure for a high-spin ferrous porphyrin is lacking, the listings in this column are expected or most probable values of the parameters (see text). ‖ The axial ligand is methoxy. ¶ The axial ligand is imidazole or pyridine.

analysis for a high-spin ferrous porphyrin is still wanted (*30*), but upper limits for the complexing bond length to porphinato-nitrogen atoms and the out-of-plane displacement of the ferrous ion are readily estimated. Carrying over the difference of 0.12 Å between the empirical ionic radii of ferrous and ferric iron for a coordination number of six (*23*) to the high-spin ferrous porphyrin gives 2.19 Å for the length of the complexing bonds to porphinato-nitrogen atoms. Taking the Ct · · · N radius as 2.01 Å then yields a calculated out-of-plane displacement of 0.87Å for the ferrous ion. Although these values are surely overlarge (*31*), the basic merit of the model is demonstrated by Perutz's recent estimate (*32*) of 0.75 Å for the displacement of the ferrous ion from the mean plane of the heme in the structure of deoxyhemoglobin.

Published estimates of the displacement of the high-spin ferric ion from the mean plane of the protohemin in acid metmyoglobin (*33*), methemoglobin (*32*), and a meterythrocruorin (*34*), all ~ 0.30 Å, are readily interpreted by simple extension of the analysis presented earlier. The coordination group in these hemoproteins differs from that in chlorohemin or the methoxyiron(III) porphyrin (Fig. 3 and Table 2) in two respects, both of which contribute to a reduction in the out-of-plane displacement in the hemoproteins. Replacement of chloride or methoxide as the tightly bound axial ligand by the neutral imidazole (of the proximal histidine residue) allows the cation to retain a higher net charge, thus encouraging a tightening of the bonds to the negatively charged porphinato-nitrogen atoms. The modest attraction of the ferric ion for the rather distant ligand—a water molecule or a hydroxyl ion (*35*)—that occupies the sixth octahedral site must require a small expansion of the Ct · · · N radius beyond the relatively unstrained value in the strictly five-coordinate species. Thus, for example, a decrease of only ~ 0.015 Å in the bond distance to the porphinato-nitrogen atoms coupled with an equal increase in the Ct · · · N radius from the values given in Table 2 for the five-coordinate species suffice to reduce the out-of-plane displacement to 0.30 Å. In a high-spin ferrous heme, by contrast, the relatively large difference between the complexing bond length and the Ct · · · N radius requires a relatively large out-of-plane displacement of the ferrous ion that is much less sensitive to small changes in the other parameters.

The stereochemistry of the octahedral coordination group in the low-spin bis(imidazole)tetraphenylporphinatoiron(III) cation [conveniently written as $(Im_2FeTPP)^+$], as given in a recent x-ray analysis of crystalline structure for the $(Im_2FeTPP)^+Cl^-$ salt (*10*), conforms rather closely to the a priori description that was freely employed in earlier discussions of iron porphyrin stereochemistry (*5, 12, 27*). No symmetry is required of the $(Im_2FeTPP)^+$ cation in the monoclinic crystal, but neither the porphinato core nor the equatorial bonding in the coordination group is appreciably affected by the complicated packing relations that characterize the crystalline arrangement. The four complexing bonds to porphinato-nitrogen atoms have the averaged length of 1.989 ± 0.003 Å, not unexpectedly nearer to the Ct · · · N radius of ~ 2.01 Å for minimum radial strain in the porphinato core than to the 1.955 ± 0.015 Å anticipated for low-spin covalent bonds to six monodentate ligands. The orientation around an axial Fe–N bond of a flat imidazole ligand that minimizes nonbonding repulsions with the porphinato-nitrogen atoms (*36*) is such that the plane of the imidazole molecule intersects the equatorial plane in a line that makes angles of ± 45 and ± 135 degrees with the equatorial complexing bonds. This specification is nearly met by one imidazole ligand in the observed configuration; the length of the associated Fe–N bond is 1.957 ± 0.004 Å. Misorientation of the other imidazole ligand by ~ 27 degrees, the presumed consequence of packing compromises in the crystalline arrangement, leads to an Fe–N bond length of 1.991 ± 0.005 Å. That even the shorter axial bond is slightly stretched is suggested by the apparent displacement, 0.009 ± 0.003 Å, of the iron atom from the equatorial plane toward the more distant of the complexed imidazole-nitrogen atoms. Thus it appears that an Fe–N distance of 1.95 to 1.96 Å is appropriate for both axial bonds in the unconstrained species.

An x-ray analysis of the structure of crystalline bis(piperidine)tetraphenylporphinatoiron(II) (*37*) gives complexing Fe–N distances to the porphinato-nitrogen atoms of 2.004 ± 0.004 Å, in excellent agreement with expectation, and Fe–N bond lengths to the piperidine-nitrogen atoms of 2.126 ± 0.005 Å. The apparently excessive length of the axial bonds is primarily attributable to pronounced nonbonding repulsions between piperidine-hydrogen and porphinato-nitrogen atoms that retain their vigor for all orientations of the piperidine ligand around the complexing bond (*37*). If we compare this axial bond length with that in the $(Im_2FeTPP)^+$ species, it may be noted that the incapacity of the piperidine ligands for entering into π-bonding is conducive to some lengthening of the complexing bonds with either iron(II) or iron(III), but rather more so with the former; iron(II) is generally a better π-donor, but a poorer σ-acceptor, of electron density than iron(III).

The recognition in 1965 (*27*) of the pervasive constraints imposed by the porphinato core on the coordination geometry led to the overall pattern for iron porphyrin stereochemistry that was freely employed in discussions of the role taken by heme stereochemistry (*5, 12, 27*) in such hemoproteins as myoglobin and hemoglobin. Quantitative refinement of the earlier formulation has been provided by recent structural data (*10, 32, 36, 37*), as detailed herein.

Stereochemistry of the Protoheme in Hemoglobin

Concomitant with the diverse nature of their biological functions, the several families of the hemoproteins carry hemes with distinctive coordination groups and characteristic patterns of peripheral substitution on the porphinato core. Each of the four possible combinations of ferrous or ferric iron in a high- or a low-spin ground state is realized in one or more of the naturally occurring hemes, but not one of these latter has an exact chemical duplicate among the few iron porphyrins that have lent themselves to precise x-ray analyses of structure (*30*) during recent years. Consequently, it is the more important to recognize that the stereochemistry of the coordination group does follow well-defined structural principles that have passed quite exacting tests. The currently most cogent application of these principles is to the configurational and conformational changes that accompany the complexing of molecular oxygen by the protohemes of hemoglobin (*5, 12, 27, 32*). [The protoheme is the iron(II) derivative of protoporphyrin-IX].

The hemoproteins that reversibly store or transport molecular oxygen include monomeric species, such as myoglobin, that carry just one bound protoheme each, and the "tetrameric" hemoglobin molecule in which each of the four subunits (two α-chains and two β-chains) carries a protoheme. With molecular weights of $\sim$17,000 each, myoglobin and the α- and β-monomers are chemically and structurally rather closely related to one another. The ferrous porphyrin occupies a pocket in the protein framework, and it is directly attached to the framework through an axial complexing bond from the iron atom to an imidazole group of a histidine residue (*32–34*). Molecular oxygen is the other axial ligand of the iron(II) atom in the low-spin oxygenated (oxy) form of the hemoprotein; the sixth coordination position around the ferrous iron is, of course, unoccupied in the high-spin reduced (deoxy) form. Modulation of the reversible oxygenation of hemoglobin is the direct consequence of cooperative action among the four closely associated subunits in the molecule—the classic "heme-heme interaction" (*38*). The equilibrium uptake of oxygen by deoxyhemoglobin is described approximately by the Hill equation (*38*),

$$y/(1-y) = KP^n$$

where y is the fractional saturation, K is a constant, P is the partial pressure of oxygen, and n is an empirical measure of the cooperative interaction; for the monomeric species of the hemoproteins n is unity, whereas for a normal hemoglobin n is 2.7 to 2.9. The cooperative interactions of the subunits simultaneously give rise to the Bohr effect, a physiologically significant alteration of the *p*H of the system that is linked with the varying oxygen affinity (*38*).

As early as 1963, low-resolution x-ray analyses of the structures of two hemoglobins (*39*) had shown that the iron atoms are spaced so far apart in the molecule, >25 Å for every pairing, that direct heme-heme interactions could be dismissed as a plausible source of the cooperative phenomena; it was further reported (*39*) that the quaternary structures of deoxy- and oxyhemoglobin differ substantially, a conclusion that was subsequently verified (*32*). Wyman's estimate, published in 1964, of 10 to 12 kilocalories per mole of hemoglobin for the decrease in the Gibbs (free) energy attributable solely to cooperative interactions during oxygenation is notable in two respects: although it represents only about 10 percent of the overall decrease in the Gibbs energy that accompanies the oxygenation of hemoglobin in solution, it is predominantly or wholly of entropic origin (*38*). Wyman suggested accordingly that the cooperative interactions are probably to be identified with an entropy-dominated conformational change. But if the conformation of the heme, apart from the presence or absence of a sixth ligand, were otherwise invariant to change in either the spin state or the oxidation state of the iron atom—as was reported to be the case in a number of hemoprotein structure analyses dating from 1964 onward (*40*)—the only obvious source of a conformational disturbance that could be propagated to the regions of subunit interaction and amplified therein would be eliminated. Hoard and his co-workers noted (*12, 27*), however, that these structure analyses (*40*) were based upon a special version of Fourier difference synthesis in its lowest order of approximation, a procedure wherein the critical uncertainties in the complex phases of the derived quasi-isomorphous structure are altogether ignored (*41*). They concluded, consequently, that the results of these difference syntheses should be largely discounted in favor of the physically rational expectation that the structural principles governing iron porphyrin stereochemistry would apply with, at most, trivial variations to heme stereochemistry in the hemoproteins (*5, 12, 27*).

Thus Hoard *et al.* pointed out (*27*) that addition to the five-coordinate, high-spin ferrous ion in the protoheme of any sixth ligand effecting the transition to the low-spin state should simultaneously bring the iron and the four porphinato-nitrogen atoms into virtual coplanarity with much shortened bonds, and that the amplitude of the relative motion (along the normal to the heme plane) should exceed 0.5 Å. For the specific case of oxygenation, they noted further that, if the two atoms of the oxygen molecule were symmetrically attached to the iron atom (giving a sterically crowded seven-coordinate geometry), the iron atom would need to be displaced from the plane of the nitrogen atoms toward the oxygen molecule in order to achieve suitable overlap with the electron cloud of the latter (*42–44*). It was then evident that a movement $\geq$0.5 Å of the iron atom relative to the porphinato core, concomitant with oxygenation of the heme, could be expected to require cooperative movements (translations and rotations of groups) in the protein framework and thus provide the starting point for a mechanism to account for the cooperative interactions in hemoglobin (*12*).

That the structural principles governing iron porphyrin stereochemistry are equally applicable to the protohemes in the hemoglobins is fully supported by the results of recent analyses of hemoprotein structure (*45*) in which, by contrast with the earlier, zeroth-order, difference syntheses (*40*), the complex phases appropriate to a specific structure were determined *ab initio*. Thus Love reports (*45*) that both of the low-spin carboxyhemoglobin and cyanomethemoglobin molecules (from lamprey) display, as predicted (*27*), precise centering of the respective iron(II) and iron(III) atoms within the hemes. And, as noted earlier, Perutz's recent examination (*32*) of his deoxyhemoglobin structure has revealed that the ferrous ion is displaced $\sim$0.75 Å from the mean plane of the heme. Perutz, indeed, has put forward a boldly conceived and rather detailed structural model for the overall mechanism of oxygenation in which the conformational change attending the oxygenation of a single heme suffices to initiate cooperative interactions in the hemoglobin molecule. The marked differences in the quaternary structures of the deoxy- and oxy- species is recognized in all approaches to the mechanism of cooperative interaction (*46*), but Perutz proposes to trace the course of the conformational changes from initiation at a heme to completion. That his initial model will be subject to major amplification and modification in the light of other physicochemical studies is scarcely to be doubted.

Conclusion

Metalloporphyrins, most notably the iron porphyrins, observe clearly defined, internally consistent, structural principles that promise to be fully applicable to the hemes in the several families of the hemoproteins.

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