

Stereochemical Trigger for Initiating Cooperative Interaction of the Subunits During the Oxygenation of Cobaltohemoglobin

(protoheme/iron porphyrin/cobalt porphyrin/high-spin/low-spin)

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ABSTRACT Relative to the standard provided by an unconstrained five-coordinate imidazoleiron(II) porphyrin, the axial separation ($N_\epsilon \cdots P_\mu$) in deoxyhemoglobin of the complexed histidine-nitrogen (N_ϵ) atom from the mean plane (P_μ) of the protoporphyrin is stretched by $> 0.30 \text{ \AA}$. Retention of the same globin framework (quaternary structure) in deoxycobaltohemoglobin implies an axial connection with the cobalt protoporphyrin that carries enhanced tension, but a somewhat smaller value of $N_\epsilon \cdots P_\mu$ than the 2.90 \AA observed in deoxyhemoglobin. Structural constraints imposed on and by the globin in support of tension in the axial linkage are conducive to doming of the entire 24-atom porphine skeleton of the protoporphyrin toward the metal (M) atom. Furthermore, the presence of an odd electron in the $3d_z$ orbital of the metal atom is responsible for an easily stretched M- N_ϵ bond. It then appears that moderate doming of the porphinato core in combination with modest stretch in the Co- N_ϵ bond can readily lead to an $N_\epsilon \cdots P_\mu$ distance approaching 2.90 \AA in deoxycobaltohemoglobin and, consequently, to compatibility with Perutz's postulated trigger for the initiation of cooperative interaction of the subunits during the reversible oxygenation of hemoglobin.

In Perutz's stereochemical mechanism (1, 2) for the reversible oxygenation of hemoglobin (Hb), the primary trigger for initiating cooperative interaction of the subunits is the large alteration in the stereochemistry of the coordination group of a protoheme that is concomitant with the transformation of any iron porphyrin from high-spin five-coordination to low-spin six-coordination (3-8). The protoheme is linked to the globin framework through the axial complexing bond formed by the iron(II) atom with the imidazole-nitrogen atom (N_ϵ) of the proximal histidine residue. A shrinkage of about 0.90 \AA in the perpendicular distance ($N_\epsilon \cdots P_\mu$) separating this axial nitrogen atom from the mean plane (P_μ) of the protoheme accompanies the transition from high-spin five-coordination in deoxy-Hb to low-spin six-coordination in oxy-Hb (6).

Hoffman *et al.* (9-11) have shown that cobaltohemoglobin (CoHb), the product of replacement in Hb of the protohemes by molecules of the corresponding low-spin ($S = 1/2$) cobalt(II) protoporphyrin (9), displays reversible uptake of oxygen that is qualitatively similar in all respects to the uptake of oxygen by hemoglobin. It appears, consequently, that the low-spin d^7 cobalt(II) atom serves as a qualitatively acceptable substitute for the d^6 iron atom both in the high-spin five-coordinate hemes of deoxy-Hb and in the low-spin oxygenated

hemes of oxy-Hb. This behavior is correlated with the electronic structures of the coordination groups in the four chemically distinctive species. In each of the unoxygenated metalloprotoporphyrins, the unpaired electron which is largely confined to the $3d_z$ orbital of the metal atom stabilizes five-coordinate geometry. The studies of Hoffman *et al.* suggest that the geometry of the oxygenated cobalt protoporphyrin is nearly identical with that of the oxygenated protoheme by reason of the transfer of the bulk of the charge associated with the odd electron from the cobalt atom to the antibonding $2p\pi^*$ orbital of the dioxygen ligand; the interactions of an effectively d^6 cobalt(III) atom with the porphinato-nitrogen (N_p) atoms and, most significantly, with the axial N_ϵ atom can then closely simulate those of the low-spin d^6 iron atom with these ligands. These considerations lead to the rational presumptions (10, 11) that deoxy-CoHb and deoxy-Hb share essentially the same, relatively compact, quaternary structure designated as type *T* (1, 2), whereas oxy-CoHb and oxy-Hb share the more open quaternary structure of type *R*, which is considered by Perutz to be adequately represented by the fully investigated structure of methemoglobin (met-Hb). These presumptions are, of course, subject to ultimate testing by x-ray structural analysis.

Hoffman *et al.* (10, 11) point out that the perpendicular distance ($N_\epsilon \cdots P_\epsilon$) of the axial N_ϵ atom from the mean plane (P_ϵ) of the porphinato core (or porphine skeleton) within any externally unconstrained, five-coordinate, imidazolecobalt(II) porphyrin must be substantially smaller than the 2.90 \AA estimated for the $N_\epsilon \cdots P_\mu$ distance in deoxy-Hb (1, 2)—that, indeed, it is expected to be comparable with the 2.30 \AA estimated for $N_\epsilon \cdots P_\mu$ in met-Hb. Thus, they are led to question the applicability of Perutz's stereochemical trigger for cooperative interaction to CoHb and, by extension, to Hb as well. Our proposed solution of this problem is outlined in the following paragraph and is then developed in the remainder of this paper.

Newly obtained structural data to be cited herein show that the $N_\epsilon \cdots P_\epsilon$ distance in an externally unconstrained, five-coordinate, imidazoleiron(II) porphyrin is fully 0.30 \AA shorter than the $N_\epsilon \cdots P_\mu$ distance in deoxy-Hb; this result provides the quantitative confirmation of Perutz's earlier conclusion (12) that the axial connection between the protoporphyrin and the globin is in tension. Consideration of the structural means by which the globin framework supports this tension leads to the conclusion that the framework is designed to permit significant doming of the entire porphinato core of the protoporphyrin—essentially as a flexible diaphragm—toward

Abbreviations: CoHb, cobaltohemoglobin; FeTPP and 2-Me-ImFeTPP, iron(II) and 2-methylimidazoleiron(II) derivatives, respectively, of *meso*-tetraphenylporphine.

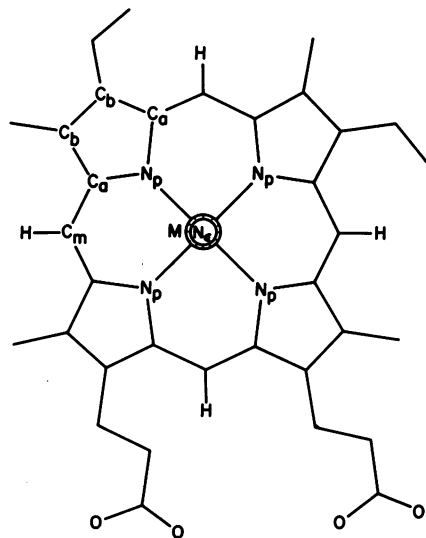


Fig. 1. Skeletal diagram in projection of a five-coordinate metalloprotoporphyrin-IX. The notation used for atoms in the porphyrinato core is displayed in one quadrant. Not shown are the 20 hydrogen atoms of the peripheral methyl, vinyl, and propionic acid substituents.

the metal atom. Structural data recently obtained for five-coordinate cobalt(II) porphyrins in which the axial ligand is either 1-methylimidazole or 1,2-dimethylimidazole lend their full support to a theoretically anticipated result: namely, that the axial Co-N_e bond is easily stretched by reason of the presence of the odd electron in the $3d_{z^2}$ orbital of the metal atom. Expansion and synthesis of the foregoing considerations then lead us to conclude that, relative to the axial connection in deoxy-Hb, the connection in deoxy-CoHb carries enhanced tension and a somewhat different distribution in strain, but is equally compatible with the stereochemical triggering of cooperative oxygenation. Although we must assume that deoxy-CoHb retains the quaternary structure established for deoxy-Hb, we need not consider Perutz's (1, 2) additional postulate of structural changes triggered in the β subunits by the steric effect of oxygen itself.

The diagram of the protoporphyrin skeleton (Fig. 1) is a useful adjunct to the discussion of the pertinent five-coordinate metalloporphyrins. The metal atom in these species always is significantly displaced from the mean plane (P_N) of the porphyrinato-nitrogen atoms (N_p) toward the axial ligand (6). The accompanying doming of the porphyrinato core or, at least, of the four N_p atoms therein toward the metal atom leads to the inequalities, $M \cdots P_N < M \cdots P_e < M \cdots P_\mu$. In each of the four known structures of high-spin iron(III) porphyrins wherein the sterically unhindered axial ligand is a methoxide (3), chloride (13, 14), or isothiocyanate ion (A. Bloom and J. L. Hoard, in preparation), the $\text{Fe} \cdots P_N$ displacement of about 0.45 \AA is accompanied by a separation of mean planes, $P_N \cdots P_e \lesssim 0.05 \text{ \AA}$. The mean plane, P_μ , of the entire porphyrin macrocycle commands interest in deoxy-Hb because it is the only such plane that can be identified and approximately positioned in the hemoprotein structure; the protoporphyrin is viewed edge-on as a rather thick band of unresolved electron density within which a rather substantial doming of the porphyrinato core toward the iron atom may be largely or wholly obscured.

Collman and Reed (15) have prepared well-characterized crystals of the iron(II) and the 2-methylimidazoleiron(II) derivatives of *meso*-tetraphenylporphine (FeTPP and 2-MeImFeTPP, respectively), the latter as the 1:1 ethanol solvate. Precise structure determinations for both crystals have been completed (for FeTPP by Nancy Kim and J. L. Hoard; for 2-MeImFeTPP by L. J. Radonovich and J. L. Hoard). We note that Collman and Reed (15) used the somewhat sterically hindered 2-methylimidazole, rather than unsubstituted imidazole, as the axial ligand in the high-spin five-coordinate species in order to preclude formation of the low-spin six-coordinate complex. They found also that the ethanol is needed to prevent crystallization of FeTPP in preference to a phase containing the 2-MeImFeTPP molecule. The ethanol molecule occupies a "hole" in the somewhat awkward crystalline arrangement, but is strongly hydrogen-bonded to the uncomplexed nitrogen atom of the 2-methylimidazole ligand.

The *intermediate-spin* ($S = 1$) FeTPP crystallizes in a familiar structural type (16) which is commonly assumed by four-coordinate MTPP species in which the M-N_p bond distance is $< 2.01 \text{ \AA}$ (6, 17). The iron atom is centered in the molecule with, as anticipated for intermediate-spin (3), a short Fe-N_p bond length of 1.971 \AA . It follows that the four-coordinate iron(II) and cobalt(II) protoporphyrins are isostructural and nearly isodimensional.

The geometry of the high-spin ($S = 2$), five-coordinate, 2-MeImFeTPP molecule, as observed in the crystal, is strongly modulated by the packing relations as well as by the steric interactions of the axial ligand with the porphyrinato core. The observed $\text{N}_e \cdots P_e$ value of 2.68 \AA is the sum of $P_N \cdots P_e = 0.13 \text{ \AA}$, $\text{Fe} \cdots P_N = 0.42 \text{ \AA}$, and $2.16 (\cos 10.3) \text{ \AA}$ wherein 2.16 \AA is the Fe-N_e bond length and 10.3° is the angle of tilt of this bond from the normal. Intermolecular packing in the crystal is responsible for the excessively large $P_N \cdots P_e$ separation of 0.13 \AA relative to the usual $< 0.05 \text{ \AA}$ (see above); the only short intramolecular contacts involve a pair of oppositely situated N_p atoms of the core with the hydrogen atom and the methyl substituent which are contiguous to the complexed N_e atom in the axial ligand. The 10.3° tilting of the Fe-N_e bond is directly responsible for bringing the cited hydrogen atom (which is attached to a carbon atom of the imidazole ring) to within 2.60 \AA of one N_p atom of the core; it simultaneously eases the $\text{H} \cdots N_p$ contacts between the methyl group and the other N_p atom to the point that a substantial libration of the methyl group about the bond connecting it to the imidazole ring is permitted.

We have described the foregoing intramolecular contacts in some detail because they suffice to assure us that the Fe-N_e bond length and the $\text{Fe} \cdots N_p$ displacement taken from the 2-MeImFeTPP $\cdot \text{C}_2\text{H}_5\text{OH}$ structure are maximum values for these parameters in an externally unconstrained molecule of an imidazoleiron(II) porphyrin. Given $\text{Fe} \cdots N_p > 0.30 \text{ \AA}$, steric interaction between the imidazole and the core is entirely negligible for any orientation of the ligand around the normal to P_N . Furthermore, there is no reason to suppose that $P_N \cdots P_e$ should exceed the $< 0.05 \text{ \AA}$ cited above for the high-spin iron(III) porphyrins. Consequently, the maximum value anticipated for $\text{N}_e \cdots P_e$ in the five-coordinate imidazoleiron(II) protoporphyrin that provides the system of reference for discussion of the axial connection in deoxy-Hb

is 2.63 Å. A rational estimate of the most probable value of $N_\epsilon \cdots P_\epsilon$ is 2.60 ± 0.03 Å.

We may now consider the critical question of how the globin framework in deoxy-Hb (or deoxy-CoHb) can support tension in the axial connection to the protoporphyrin. The axial $M-N_\epsilon$ bond between the metal atom and the proximal histidine residue provides the mechanism whereby the reaction of the globin to tension is assured at one terminus. At the other terminus, however, the required support must come from packing contacts between the globin and, at least predominantly, the peripheral substituents on the porphinato core of the protoporphyrin. The restraint thus imposed by the globin must be exerted on the side of the porphyrin from which the axial $M-N_\epsilon$ bond emerges; but the presence on this side of the histidine ligand together with the generally protective distribution of the substituents certainly limit—very probably preclude—the close approach of any part of the *restraining* globin to any part of the porphinato core.

Given the preceding analysis and the well-documented pliability of the porphinato core toward deformation normal to its mean plane (6), a doming of the *entire* 24-atom core toward the metal atom is the rational consequence of tension in the axial connection. It is illuminating to suppose that in first approximation this doming observes C_{4v} symmetry. A pyramidal core of this symmetry is permitted, but is not required, to retain planarity in each of the several trigonal bond systems centered at the N_p , C_a , and C_m atoms in the core (Fig. 1). Starting from the apex of the pyramidal configuration, the core atoms are sorted into descending parallel tiers in the order, $4N_p$, $4C_m$, $8C_a$, and $8C_b$. (The geometry is readily visualized with the aid of Fig. 1.) In the fully idealized model, the mean plane (P_ϵ) of the porphinato core lies near, but slightly below, the plane of the $8C_a$ atoms, whereas the mean plane (P_μ) of the entire protoporphyrin (metal atom always excluded) must lie still lower. The separation of P_μ from the mean plane of the peripheral substituents (which is based on the positions of 14 carbon and 4 oxygen atoms) increases monotonically with the doming of the core.

Of the 0.75 Å estimated for the $Fe \cdots P_\mu$ distance in deoxy-Hb (1, 2), perhaps as much as 0.55 Å may be attributable to the $Fe \cdots P_N$ displacement, leaving only about 0.20 Å to be assigned to doming of the core. The projection of the $Fe-N_p$ bond length onto P_N gives the radial distance ($Ct \cdots N_p$) from the center (Ct) of the square array of $4N_p$ atoms to each such atom. The $Fe-N_p$ bond length of 2.086 Å in the 2-MeIm-FeTPP molecule corresponds, of course, to the observed $Fe \cdots P_N$ of 0.42 Å and $Ct \cdots N_p$ of 2.044 Å, but equally well to $Fe \cdots P_N = 0.55$ Å and $Ct \cdots N_p = 2.010$ Å. Inasmuch as equatorial strain in a planar or quasi-planar core is minimized for $Ct \cdots N_p$ about 2.01 Å (6), it follows that the difference in the energies of the two configurations must be small. Approximate mean-plane calculations then suggest that a C_{4v} doming of the core corresponding to $P_N \cdots P_\mu$ about 0.20 Å requires the near coincidence of P_μ with the mean plane of the $8C_b$ atoms (Fig. 1). This modest doming may be compared with an S_4 (quasi- D_{2d}) ruffling of the core in a nickel porphyrin wherein two methine-carbon (C_m) atoms lie 0.52 Å above, the other two 0.52 Å below, the mean plane of the core (18).

Perutz's (1, 2) value of 2.15 Å for the $Fe-N_\epsilon$ distance in deoxy-Hb is the mean of two very rough estimates, 2.25 and 2.05 Å in the α and β subunits, of which the 2.05 Å seems im-

possibly short relative to the 2.16 Å in the 2-MeImFeTPP molecule. We expect the $Fe-N_\epsilon$ bond to be as readily extensible as the $Co-N_\epsilon$ bond (see below), and we judge that 2.20 Å is a more realistic estimate for this bond length in deoxy-Hb.

Rupture of the $Fe-N_\epsilon$ bond in deoxy-Hb must be accompanied by a relaxation of strain in the globin (an enlargement of the cavity) and, as the work of Collman and Reed (15) makes evident, the centering of the iron atom within the porphinato core. Removal of the protoheme and incorporation of the cobalt(II) protoporphyrin to give deoxy-CoHb are thereby facilitated.

The presence of an odd electron in the $3d_{z^2-y^2}$ orbital of the high-spin iron(II) atom and the absence of this electron in the low-spin cobalt(II) atom are responsible for the observation that, in the unconstrained five-coordinate porphyrins, $Fe \cdots P_N$ is about 0.28 Å larger than $Co \cdots P_N$. Relative to deoxy-Hb, the formation of the $Co-N_\epsilon$ linkage in deoxy-CoHb must give rise to enhanced tension in the axial connection, to enhanced strain in the globin framework, and, consequently, to a reduction in the value, experimentally determinable by x-ray analysis, of the $N_\epsilon \cdots P_\mu$ distance. We surmise that $N_\epsilon \cdots P_\mu$ lies within the range, 2.70–2.90 Å, but its precise value is not essential to our further analysis. It is clear that the contribution to $N_\epsilon \cdots P_\mu$ from doming of the porphinato core may exceed that in deoxy-Hb by a factor of two or three. Furthermore, as we now demonstrate, a significantly stretched $Co-N_\epsilon$ bond may also contribute to a rational value of $N_\epsilon \cdots P_\mu$. We first present the theoretical considerations that preceded the experimental study of this problem.

On either a crystal field or molecular orbital basis, the electron density associated with the odd electron in the $3d_{z^2}$ orbital of the cobalt atom is somewhat concentrated in, but is not limited to, the sixth coordination position as a "phantom" ligand. Overlap, positive or negative, of the $3d_{z^2}$ orbital with the $sp^2 \sigma$ orbital of the axial ligand is largely confined to the region of the complexing bond; the antibonding molecular orbital, wherein the unpaired electron is housed, is predominantly of $3d_{z^2}$ character. Concomitant with stretching the $Co-N_\epsilon$ bond—with, that is, decreasing overlap of metal and ligand orbitals—are movements, opposite in sign, of the energy levels associated with the bonding and antibonding molecular orbitals toward a common intermediate level. Consequently, the magnitude of the net energy stabilizing the complex may be expected to decrease rather slowly with increasing $Co-N_\epsilon$. It follows that the force constant for stretching the bond is rather small.

Determinations of crystalline structure for the 1-methylimidazolecobalt(II) derivatives of *meso*-tetraphenylporphine (W. R. Scheidt, *J. Amer. Chem. Soc.*, in press) and 1,2,8-octaethylporphine (R. Little and J. A. Ibers, private communication) yield, respectively, structural parameters as follows: $Co-N_\epsilon$, 2.16 and 2.15 Å; $Co \cdots P_N$, 0.13 and 0.14 Å; $P_N \cdots P_\epsilon$, 0.01 and 0.04 Å; $N_\epsilon \cdots P_\epsilon$, 2.30 and 2.33 Å. Structure determination for the 1,2-dimethylimidazolecobalt(II) derivative of *meso*-tetraphenylporphine (P. N. Dwyer, P. Madura, and W. R. Scheidt, in preparation) gives $Co-N_\epsilon = 2.22$, $Co \cdots P_N = 0.15$, $P_N \cdots P_\epsilon = 0.02$, and $N_\epsilon \cdots P_\epsilon = 2.38$ Å. Relative to the sterically unhindered 1-methylimidazole complexes, the 2-methyl substituent contiguous to the N_ϵ atom in the 1,2-dimethylimidazole complex is responsible for a substantial stretch of 0.08 Å in the $N_\epsilon \cdots P_N$ distance,

mostly taken in the $\text{Co} \cdots \text{N}_e$ bond. Inasmuch as the plane of the imidazole ring is rotated 20° from coincidence with a coordinate plane, the accompanying tilt of the $\text{Co}-\text{N}_e$ bond by 5.2° from the normal requires the 4-hydrogen atom of the ligand to approach rather closely to a pair of adjacent core atoms: $\text{H} \cdots \text{N}_p = 2.65 \text{ \AA}$ and $\text{H} \cdots \text{C}_a = 2.57 \text{ \AA}$. The most favorable orientation of the 2-methyl substituent, which represents the equilibrium configuration of the librating methyl group, corresponds to intramolecular contacts of $\text{H} \cdots \text{N}_p = \text{H} \cdots \text{C}_m = 2.83 \text{ \AA}$. Intermolecular packing is largely responsible for the observed asymmetric conformation of the porphinato core; the axial bonding in the molecule is not sufficiently assertive to require a commonly observed ruffling of the core that would eliminate any need for the short $\text{H} \cdots \text{C}_a$ contact of 2.57 \AA . Although some stretch in the $\text{N}_e \cdots \text{P}_N$ distance is surely required by the steric interactions, we judge that the magnitude of the observed stretch is in agreement with the theoretical expectation of a readily extensible $\text{Co}-\text{N}_e$ bond.

Further study of this interesting phenomenon (W. R. Scheidt) involves the structural characterization of a 2,4,5-trimethylimidazolecobalt(II) porphyrin. Steric interactions of the ligand with the core are expected to conform to 2-fold symmetry, to require a substantial ruffling or folding of the core, and to have $\text{N}_e \cdots \text{P}_N$ about 2.5 \AA . That a complex with so much stretch in $\text{N}_e \cdots \text{P}_N$ should be stable is suggested by completed structural studies of the *bis*(piperidine)cobalt(II) derivative of *meso*-tetraphenylporphine (W. R. Scheidt, *J. Amer. Chem. Soc.*, in press) and the *bis*(3-picoline)cobalt(II) derivative of 1,2, . . . 8-octaethylporphine (R. Little and J. A. Ibers, private communication). The respective $\text{Co}-\text{N}_e$ bond lengths of 2.44 and 2.39 \AA that are observed in these centrosymmetric six-coordinate species display the full effect of the odd electron in the $3d_{z^2}$ orbital of the cobalt atom. Equilibrium measurements of the complexing reactions in toluene solution show that most of the enthalpy change favoring the formation of the six-coordinate species is, in fact, associated with the initial formation of the five-coordinate complex (19); the decrease in enthalpy accompanying the complexing of the second axial ligand is apparently ≈ 2 kcal/mol. Thus we anticipate that the marked change in electronic structure that must attend the excision of one axial ligand from the six-coordinate cobalt porphyrin will suffice to stabilize the five-coordinate species in which the $\text{N}_e \cdots \text{P}_N$ distance is stretched to at least 2.40 \AA . It is clear, moreover, that a five-coordinate imidazolecobalt porphyrin is best suited to tolerate such stretch.

It is probable that a *quasi- C_{4v}* doming of the entire porphinato core is conducive to a larger than normal value of the $\text{Co} \cdots \text{P}_N$ displacement. In the markedly pyramidal configuration reported (20) for the nitrosyl*bis*-(dimethyl-dithiocarbamate)cobalt(II) molecule, the cobalt atom at the apex of the pyramid lies 0.52 \AA out-of-plane from the plane of the four sulfur ligands. The doming of the core in

deoxy-CoHb cannot be so sharply pyramidal and a $\text{Co} \cdots \text{P}_N$ displacement $\leq 0.25 \text{ \AA}$ is anticipated.

The several additive components that contribute to a rational value of $\text{N}_e \cdots \text{P}_N$ in deoxy-CoHb must each support the same tension. This tension, though larger than that in deoxy-Hb, must be rather small. We judge that a doming of the core as measured by a $\text{P}_N \cdots \text{P}_\mu$ separation of about 0.50 \AA , a $\text{Co} \cdots \text{P}_N$ displacement of about 0.20 \AA , and a $\text{Co}-\text{N}_e$ bond length of about 2.25 \AA are feasible values for the individual components. The sum of these individually estimated components, 2.95 \AA , is unnecessarily large. It is, consequently, the more probable that some combination of the three components does suffice to put—or keep—deoxy-CoHb on the same basis as deoxy-Hb in respect to the stereochemical triggering of cooperative oxygenation. A *posteriori* support of the initial assumption that deoxy-CoHb and deoxy-Hb share a common quaternary structure is evidently provided by our analysis.

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