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Stereochemistry of Cobalt Porphyrins. III. The Structure of 2,3,7,8,12,13,17,18-Octaethylporphinato(1-methylimidazole)-cobalt(II). A Model for Deoxycoboglobin

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Abstract: The five-coordinate Co(II) porphyrin, Co(1-Me-Im)(OEP), has been prepared and its structure determined from three-dimensional X-ray diffraction data. The complex crystallizes with four formula units in the monoclinic space group C_{2h}^5-$P2_1/n$ with a = 14.049 (4) Å, b = 17.587 (8) Å, c = 14.331 (5) Å, and β = 95.20 (1)°. The cobalt atom is 0.16 (1) Å out of the mean plane of the porphyrin toward the imidazole ligand. The averaged Co–N(imidazole) and Co–N(porphyrin) bond lengths are 2.15 (1) and 1.96 (1) Å, respectively. The porphyrin itself is significantly nonplanar. Some of the β-C atoms of the ethyl groups are disordered. The structure suggests that the out-of-plane displacement of the cobalt atom in deoxycoboglobin is small compared with the displacement of the iron atom in hemoglobin and comparable with that in methemoglobin. In view of the fact that CoHb and met-Hb have differing quaternary structures, we conclude that structure of deoxy-CoHb is inconsistent with both the trigger mechanism of Perutz and the linear energy distribution model of Hopfield.

In an impressive series of papers[1,2a] Hoard and his coworkers have demonstrated that the porphyrin macrocycle is a remarkably flexible ligand[3-5] and that the chelated metal atom can be displaced considerably from the plane of the porphyrin.[6-9] Hoard concluded[1,6] that the displacement of the metal atom is related to its effective radius, since the size of the central "hole" in the porphyrin is relatively constant. On this basis he predicted[2] that the high-spin ferrous ion in deoxy-Hb[10] and Mb is five-coordinate and is displaced from the porphyrin plane toward the proximal histidine by 0.5–0.8 Å. Upon binding an oxygen molecule, the iron atom becomes six-coordinate and low spin, and moves back into the plane of the porphyrin. An alternative description of this process suggests that oxy-Hb can be formally described as Fe(III)–$O_2^{\cdot -}$.[12] The oxy to deoxy transition would then involve a change from high-spin Fe(II) to low-spin Fe(III) with a concomitant decrease in the radius of the iron atom.

Williams[13] and Hoard[2b] have suggested that the movement of the iron atom causes a change in the position of the imidazole group of the proximal histidine and that this leads to movements in the protein framework. On the basis of his structural studies of oxy-(met) and deoxy-Hb Perutz[14,15] has proposed that this is indeed the case and that this movement triggers conformational changes in the protein which result in an increased oxygen affinity—the so-called cooperative effect.[16] The large effective radius of a high-spin, t_{2g}^4 e_g^2, ferrous ion results mainly from the presence of electrons in the e_g orbitals. The d^7-cobaltous ion also has an unpaired electron in the e_g

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(10) The following abbreviations are used throughout: Hb, ferrous hemoglobin; Mb, ferrous myoglobin; CoHb, Co(II)-reconstituted Hb; met-Hb, ferric hemoglobin. 1-Me-Im, 1-methylimidazole; Im, imidazole; py, pyridine; 3-pic, 3-methylpyridine; pip, piperidine; TPP, *meso*-tetraphenylporphyrin dianion; OEP, 2,3,7,8,12,13,17,18-octaethylporphyrin dianion;[11] PP-IX, 2,7,12,18-tetramethyl-3,8-divinylporphine-13,17-dipropionic acid dianion; MP-IX, 2,7,12,18-tetramethyl-3,8-diethylporphine-13,17-dipropionic acid dianion; MP-IX DME, dimethyl ester of MP-IX. DMG, dimethylglyoxime dianion; salen, *N,N'*-ethylenebis(salicylidene) dianion; py-salen, α,α'-{2-(2-pyridyl)ethyl}ethylenebis(salicylidene) dianion; bae, *N,N'*-ethylenebis(benzoylacetonimine) dianion; acacen, *N,N'*-ethylenebis(acetylacetonimine) dianion; acac, acetylacetone anion; OAc, acetate anion.
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orbitals, and therefore it is expected that the Co(II) atom in deoxycoboglobin would also be displaced from the plane of the porphyrin toward the proximal histidine.[17] Indeed Walker[18] has proposed that a Co(II) atom may be out of the porphyrin plane by as much as 0.9–1.3 Å. Hoard and Scheidt[19] have argued that the Co porphyrin is easily deformed by the protein and is significantly domed. This doming, in combination with other structural changes, results in an equally large displacement of the Co atom. On the other hand, Hoffman, Spilburg, and Petering[20] have predicted that the displacement of the Co(II) atom in CoHb will be relatively small, compared with that of the high-spin Fe(II) atom in Hb, because of the smaller effective radius of a low-spin, $t_{2g}^6e_g^1$, Co(II) atom. Since CoHb exhibits a high degree of cooperativity,[17,20–23] with Hill coefficients as high as 2.3, a relatively small out-of-plane displacement of the Co atom would appear to be inconsistent with the trigger mechanism proposed by Perutz.[14,15] Obviously the structure of a five-coordinate Co(II) porphyrin is of extreme interest since it can give a realistic estimate of the displacement of the Co atom in CoHb.

Previous work in this laboratory[24] and elsewhere[18,25,26] had demonstrated that in solution Co(II) porphyrins can add one or two molecules of various nitrogenous bases to give five- or six-coordinate complexes. These complexes have been characterized by their epr and visible spectra. The stability constants of the six-coordinate species have been shown to be small compared with those of the five-coordinate species. It thus appeared feasible to prepare a five-coordinate Co(II) porphyrin complex.

In the present paper we report the preparation and structure of the five-coordinate complex, Co(1-Me-Im)(OEP). The displacement of the Co(II) atom is shown to be quite small, in agreement with the predictions of Hoffman, *et al.*[20] Furthermore, the complex is shown to be identical with the species which previous work[27] has shown is a reversible oxygen carrier.

Experimental Section

Epr[27] work has shown that imidazoles, in contrast to other nitrogenous bases, readily form five-, but not six-coordinate complexes, with Co(II) porphyrins. Because of the high solubility of the five-coordinate imidazole complexes in most solvents, including alkanes, the complex Co(1-Me-Im)(OEP) was crystallized from the pure ligand (1-methylimidazole is a liquid, mp −6°). A hot, saturated solution of the cobalt porphyrin in 1-Me-Im was prepared, under nitrogen, in a Schlenk tube and the solution was slowly cooled by placing a dewar flask, containing the Schlenk tube, in a refrigerator.

The Co(OEP) was synthesized from $Co(OAc)_2$ and H_2(OEP) by the method of Adler, *et al.*,[28] and the 1-methylimidazole (Aldrich Chemical Co.) was distilled *in vacuo* from CaH_2.

The crystals so formed are dark maroon prisms, whose large $(10\bar{1})$ face appears triangular. The crystals, which are air stable, were analyzed by Ms. Hilda Beck of the Analytical Services Laboratory of Northwestern University. *Anal.* Calcd for $CoC_{40}N_6H_{50}$: C, 71.29; H, 7.49; N, 12.55. Found: C, 71.79; H, 7.62; N, 12.44. A single-crystal epr spectrum, at liquid nitrogen temperatures, was kindly obtained by Professor B. Hoffman. It clearly shows that the compound is paramagnetic and five-coordinate.

Preliminary precession photographs, (*h*0*l*, *h*1*l*, 0*kl*, 1*kl*) showed monoclinic symmetry and systematic absences ($0k0$, $k \neq 2n$; $h0l$, $h + l \neq 2n$), consistent with space group C_2^5-$P2_1/n$. The photographs show considerable heavy streaking indicative of disorder (*vide infra*). A crystal with distances of 0.36, 1.10, and 0.39 mm between faces of the forms $\{10\bar{1}\}$, $\{101\}$, and $\{010\}$ was glued to a fine, glass fiber. The crystal was mounted with the [101] direction approximately along the spindle axis.

The lattice parameters obtained as previously described,[29,30] by hand centering of 17 reflections in the range $36 \leq 2\theta \leq 76°$ on a FACS-I diffractometer using Cu $K\alpha_1$ radiation (λ 1.540562 Å), are $a = 14.049$ (4) Å, $b = 17.587$ (8) Å, $c = 14.331$ (5) Å, and $\beta = 95.20$ (1)°. The calculated density, based on four molecules per unit cell, is 1.27 g/cm³ and agrees well with an observed value of 1.25 (1) g/cm³, as measured by flotation in aqueous zinc chloride solution.

Data were collected in shells of 2θ by the θ–2θ scan method using Cu $K\alpha$ radiation prefiltered with Ni foil. The scan range in 2θ was from 0.85° below the Cu $K\alpha_1$ peak to 0.95° above the Cu $K\alpha_2$ peak. The takeoff angle was 2.5° and the receiving counter was positioned 32 cm from the crystal with an aperture 5.5 mm high and 5.5 mm wide. The pulse height analyzer was set to admit about 90% of the Cu $K\alpha$ peak. Initially background counts of 20 sec were taken at the end of each scan range. Past a 2θ of 56° this was increased to 40 sec. A scan rate of 2° in 2θ per minute was used. Attenuators were automatically inserted if the intensity of the diffracted beam exceeded approximately 7000 counts per second during a scan. Data were collected in the range $2 < 2\theta \leq 91°$. Data collection was terminated when less than 15% of the measured reflections were statistically observable. During the course of data collection six standard reflections from diverse regions of reciprocal space were measured every 100 reflections. The deviations of these standards were all within counting statistics.

The data were processed as previously described[29–31] using a value of 0.05 for p. Of the 3075 reflections measured, 2346 were unique, and of these 2019 have $F_o^2 > 3\sigma(F_o^2)$ and were used in subsequent refinements. Data were collected with $k \geq -1$ and $l \geq -1$, and the resultant 343 pairs of reflections equivalent by symmetry deviate by only 1.4% from their average values. The data were corrected for absorption, using a linear absorption coefficient of 42.56 cm⁻¹. The transmission factors ranged from 0.192 to 0.352.

A sharpened, origin removed Patterson map was calculated, and the positions of the cobalt atom and all the non-hydrogen atoms of the porphyrin, excluding the ethyl carbons, were located. Since the Patterson function is centrosymmetric, the correct coordinates of the 1-methylimidazole ligand could not be obtained. Therefore a difference Fourier map, phased on the porphyrin ring and cobalt atom was calculated. The coordinates of the axial ligand and of the α-carbon atoms of the ethyl groups were obtained from this map. The positions of the β-carbon atoms of the ethyl groups were uncertain.

Three cycles of isotropic least-squares refinement, using fixed temperature factors for all atoms, except for the cobalt, reduced R and R_w to 0.28 and 0.36. A subsequent difference Fourier map indicated possible, but disordered, positions for some of the ethyl β-carbon atoms. At this point the data were corrected for absorption.

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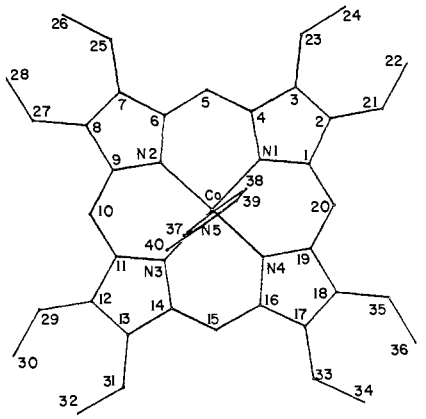

Figure 1. Drawing to indicate numbering sequence used in this paper for the 47 independent, non-hydrogen atoms.

Two further cycles of isotropic least-squares refinement were performed, with the inclusion of two disordered positions for carbon atoms, C(24), C(26), C(28), C(34), and C(36) (see Figure 1 for the numbering scheme). The occupancy of a pair of disordered atomic positions was constrained to sum to unity, and the temperature factors of the partial atoms at the two positions were required to be equal. In all, the positional and thermal parameters of 52 atoms (or partial atoms) were varied reducing R and R_w to 0.135 and 0.184, respectively. A difference Fourier map indicated considerable residual electron density around the cobalt and porphyrin atoms.

Two cycles of anisotropic, full-matrix least-squares refinement, with the constraints indicated above, and isotropic treatment of all the ethyl carbon atoms, brought R and R_w to 0.093 and 0.129. Examination of the interatomic distances and bond angles indicated that this treatment had led to chemically unreasonable bond distances within five of the eight ethyl groups. A difference Fourier map, calculated without the inclusion of the offending atoms, reasserted their presence. Examination of the refined values of the occupancy factors for the disordered atoms, C(24), C(26), C(34), and C(36), indicated more than 85% dominance of one or the other position. Therefore the minor occupant was ignored. Furthermore, since the anisotropic model had not significantly improved the agreement among the apparently equivalent bonds in the molecule, it was decided to return to an isotropic model.

One further cycle of least-squares refinement was performed in which only the cobalt atom was allowed to vibrate anisotropically. In addition, the positions of the β-carbon atoms, C(28A), C(28B), and C(34), of the porphyrin ethyl groups were idealized and only their temperature factors were refined. The rest of the obviously hopeless disorder in the molecule was ignored and none of the 50 H atoms was included. This brought R and R_w to 0.13 and 0.18.

Refinement was terminated at this point since no significant improvements could be made because of the disorder. Furthermore, none of the models tried produced any significant differences in the metal–N bond distances, the primary quantities of interest. An examination of other porphyrin structures in the literature indicates that the problems here are not unique, and that high agreement indices have been reported even with fully anisotropic models.[3,6,32]

An analysis of $\Sigma w(|F_o| - |F_c|)^2$ as a function of setting angles, magnitude of $|F_o|$, and Miller indices showed that agreement was worst for the low-angle reflections, as would be expected in view of the incomplete description of the disorder problem. The standard deviation of an observation of unit weight is 5.65 electrons.

A structure factor calculation for the 327 reflections having $F_o^2 < 3\sigma(F_o^2)$, which were omitted from the refinement, showed 55 reflections having $|F_o^2 - F_c^2| > 3\sigma(F_o^2)$ and five reflections with $\Delta F^2 \geq 10\sigma(F_o^2)$. These 327 reflections were omitted from Table I[34] where the values of $10|F_o|$ and $10|F_c|$ are given. The final atomic parameters and their errors are listed in Table II.

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Table II. Atomic Parameters for Co(1-Me-Im)(OEP)

Atom	x	y	z	B, Å^2
Co	−0.1434 (2)	0.1377 (1)	0.1780 (2)	*a*
N(1)	−0.0334 (9)	0.1216 (6)	0.1077 (9)	3.6 (3)
N(2)	−0.2300 (9)	0.1308 (6)	0.0625 (8)	3.6 (3)
N(3)	−0.2544 (8)	0.1398 (6)	0.2506 (8)	2.7 (2)
N(4)	−0.0590 (9)	0.1282 (6)	0.2941 (8)	3.3 (3)
N(5)	−0.1629 (10)	0.3811 (7)	0.1932 (9)	4.8 (3)
N(6)	−0.1318 (8)	0.2595 (6)	0.1753 (7)	3.2 (3)
C(1)	0.0632 (12)	0.1164 (9)	0.1406 (12)	4.9 (4)
C(2)	0.1225 (13)	0.1029 (11)	0.0614 (13)	6.2 (4)
C(3)	0.0617 (15)	0.1053 (12)	−0.0153 (15)	7.4 (5)
C(4)	−0.0312 (14)	0.1174 (10)	0.0113 (14)	5.6 (5)
C(5)	−0.1088 (15)	0.1142 (11)	−0.0530 (15)	7.4 (5)
C(6)	−0.2036 (13)	0.1246 (9)	−0.0265 (12)	5.1 (4)
C(7)	−0.2868 (15)	0.1217 (10)	−0.0934 (14)	6.3 (5)
C(8)	−0.3640 (14)	0.1305 (10)	−0.0462 (14)	6.4 (5)
C(9)	−0.3255 (12)	0.1343 (9)	0.0532 (12)	4.4 (4)
C(10)	−0.3855 (12)	0.1406 (9)	0.1256 (12)	4.6 (4)
C(11)	−0.3502 (11)	0.1427 (8)	0.2186 (11)	3.6 (3)
C(12)	−0.4108 (11)	0.1516 (8)	0.2950 (11)	3.6 (4)
C(13)	−0.3510 (10)	0.1519 (8)	0.3744 (11)	3.0 (3)
C(14)	−0.2570 (10)	0.1446 (8)	0.3455 (10)	3.3 (3)
C(15)	−0.1789 (12)	0.1400 (9)	0.4102 (12)	4.5 (4)
C(16)	−0.0820 (12)	0.1321 (9)	0.3847 (12)	4.8 (4)
C(17)	0.0000 (15)	0.1266 (10)	0.4516 (14)	6.2 (5)
C(18)	0.0729 (15)	0.1142 (11)	0.4003 (15)	6.7 (5)
C(19)	0.0381 (12)	0.1202 (9)	0.3039 (12)	4.5 (4)
C(20)	0.0983 (12)	0.1139 (9)	0.2321 (13)	4.8 (4)
C(21)	0.2311 (14)	0.0863 (12)	0.0713 (14)	6.6 (5)
C(22)	0.2751 (18)	0.1646 (14)	0.0684 (18)	9.3 (7)
C(23)	0.0824 (17)	0.0791 (15)	−0.1220 (17)	9.6 (7)
C(24)	0.0906 (18)	0.1513 (15)	−0.1504 (18)	9.6 (7)
C(25)	−0.2841 (14)	0.1030 (12)	−0.2010 (14)	7.5 (5)
C(26)	−0.2692 (18)	0.1809 (16)	−0.2410 (20)	10.8 (7)
C(27)	−0.4773 (19)	0.1388 (14)	−0.0836 (18)	9.7 (7)
C(28A)[b]	−0.5202	0.2122	−0.0979	13.9 (14)
C(28B)[b]	−0.5175	0.0591	−0.0746	8.8 (12)
C(29)	−0.5197 (12)	0.1608 (10)	0.2841 (12)	4.8 (4)
C(30)	−0.5688 (14)	0.0809 (11)	0.2840 (14)	7.5 (5)
C(31)	−0.3792 (12)	0.1529 (9)	0.4735 (12)	4.8 (4)
C(32)	−0.3761 (13)	0.0719 (10)	0.5178 (13)	6.5 (5)
C(33)	−0.0091 (19)	0.1396 (14)	0.5661 (19)	10.1 (7)
C(34)[b]	0.0081	0.0556	0.5931	22.3 (12)
C(35)	0.1851 (16)	0.0940 (14)	0.4423 (16)	8.5 (6)
C(36)	0.2288 (19)	0.1701 (16)	0.4497 (19)	11.3 (8)
C(37)	−0.1934 (11)	0.3082 (10)	0.2019 (11)	4.6 (4)
C(38)	−0.0544 (11)	0.3020 (11)	0.1457 (12)	5.0 (4)
C(39)	−0.0724 (13)	0.3777 (10)	0.1560 (13)	5.5 (5)
C(40)	−0.2135 (13)	0.4539 (11)	0.2172 (13)	6.7 (5)

[a] The anisotropic thermal parameters of the cobalt atom (×10^5), referred to an expression of the form $\exp[-(\beta_{11}h^2 + \beta_{22}k^2 + \beta_{33}l^2 + 2\beta_{12}hk + 2\beta_{13}hl + 2\beta_{23}kl)]$, are respectively 334 (18), 354 (12), 311 (18), −22 (11), 26 (12), and 13 (11). The corresponding rms amplitudes of vibration along the principal axes are 0.18, 0.18, and 0.24 Å. [b] Atoms C(28A), C(28B), and C(34) were idealized in the final cycle of refinement and therefore no positional errors are given. Occupancy factors of 0.5 were assigned to C(28A) and C(28B).

Description of the Structure

The structure consists of discrete molecules of Co(1-Me-Im)(OEP). The contents of the unit cell are shown in Figure 2, and a view of a Co(1-Me-Im)(OEP) molecule is shown in Figure 3. The numbering scheme used in this paper is illustrated in Figure 1. In Table III we present the bond distances and angles within the molecule. Table III also gives the shorter intermolecular contacts in the structure. These are largely between the ethyl β-carbon atoms, C28 and C34, of one porphyrin and pyrrole 4 of an adjacent molecule.

The Co(II) atom is five-coordinate and is out of the plane of the porphyrin toward the axially bound methyl-

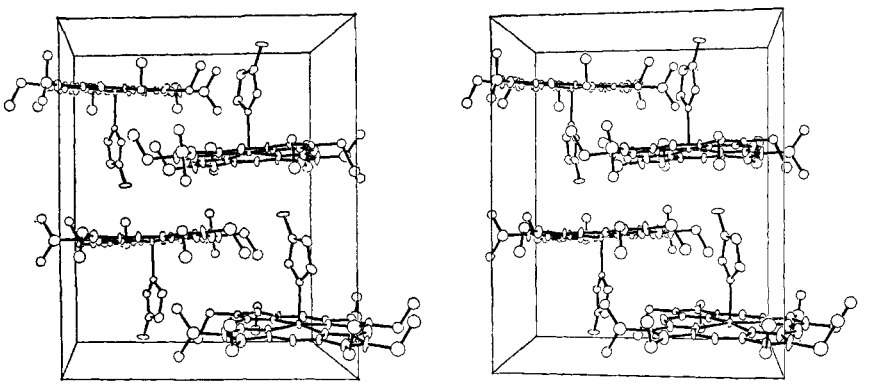

Figure 2. Stereoscopic view of the contents of one unit cell. The view is approximately along the x axis, which points toward the viewer. The positive z axis runs from right to left.

imidazole (1-Me-Im). The four, basal Co–N(porphyrin) bond distances average 1.96 (1) Å, whereas the axial Co–N(1-Me-Im) bond length is 2.15 (1) Å.

The bond distances within the porphyrin (Table III) are similar to those observed in Co(3-pic)$_2$(OEP),[32] Ni(OEP),[35] Co(1-Me-Im)(TPP),[36] and Co(pip)$_2$(TPP),[37] although the large errors preclude any detailed comparisons. The bond distances observed in the ethyl groups are especially poor. The C1(Et) carbon atoms generally appear to be displaced toward the C2(Et) carbon atoms. We attribute the observed bond distances to the inadequacy of our treatment of these disordered atoms. The bond distances and angles within the 1-methylimidazole group follow the same pattern observed in various substituted imidazoles,[38–40] and in numerous complexes containing the imidazole group.[36,41–59]

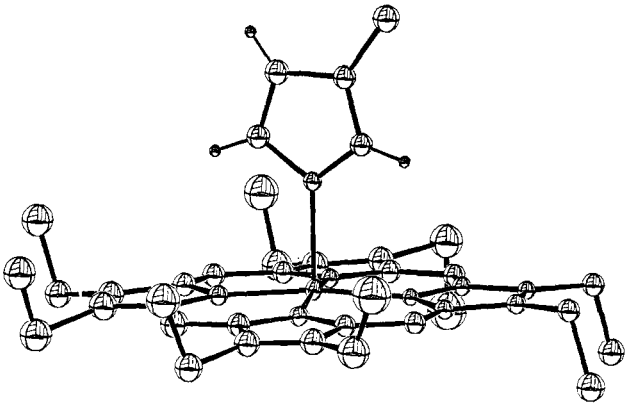

Figure 3. Drawing of the Co(1-Me-Im)(OEP) molecule. The thermal ellipsoids are drawn at the 50% probability level, except for that of C(34) which has been drawn artificially small for clarity. The calculated positions of the hydrogen atoms on the imidazole ring have been included for illustrative purposes.

The plane of the imidazole ring is tilted by 1.0° with respect to the normal to the porphyrin plane. A similar tilting of coordinated imidazole groups has been observed in Fe(Im)$_2$(DMG)$_2$ (4°),[49] [Fe(Im)$_2$(TPP)$^+$][Cl$^-$] (2.2, 2.9°),[47] [Co(Im)$_2$(TPP)$^+$][OAc$^-$] (1.4, 0.6°),[60] *trans*-Co(H$_2$O)$_2$(Im)$_2$(CO$_3$) (1.5°),[57] and in numerous other complexes. The subject has been briefly reviewed by Freeman.[61]

The plane of the imidazole ring is rotated by 10° from the N(1)–N(3) vector. See Figure 3. In metmyoglobin[62] this rotation is ~30°. Because of the orientation of the imidazole ring, HC(37) and HC(38) make nonbonded contacts of ~2.8 Å with N atoms N(1) and N(3) of the porphyrin. Very similar contacts, of 2.6–2.8 Å were observed in [Fe(Im)$_2$(TPP)$^+$][Cl$^-$].[47] It is peculiar that the imidazole ring should adopt this orientation since it is sterically the most unfavorable.[60]

The porphyrin itself is distinctly nonplanar, although the individual pyrroles are planar within the errors inherent in this determination. The relevant weighted least-squares planes are presented in Table IV, along with the dihedral angles between these planes. Adjacent pyrrole rings are tilted 1.4–4.4° with respect to each other and 1.2–3.2° with respect to the porphyrin plane. (See Table IV).

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Table III. Selected Bond Distances (Å) and Angles (deg) in Co(1-Me-Im)(OEP)[a,b]

Co–N(1)	1.94 (1)		
Co–N(2)	1.97 (1)	Co–N	1.96 (1)
Co–N(3)	1.95 (1)		
Co–N(4)	1.96 (1)		
Co–N(6)	2.15 (1)		
N(1)–C(1)	1.40 (2)		
N(1)–C(4)	1.39 (2)		
N(2)–C(6)	1.36 (2)		
N(2)–C(9)	1.34 (2)	N–C_a	1.37 (2)
N(3)–C(11)	1.38 (2)		
N(3)–C(14)	1.37 (2)		
N(4)–C(16)	1.37 (2)		
N(4)–C(19)	1.36 (2)		
C(1)–C(2)	1.49 (2)		
C(3)–C(4)	1.41 (2)		
C(6)–C(7)	1.44 (2)		
C(8)–C(9)	1.48 (2)		
C(12)–C(11)	1.45 (2)	C_a–C_b	1.45 (3)
C(13)–C(14)	1.43 (2)		
C(16)–C(17)	1.43 (2)		
C(18)–C(19)	1.43 (2)		
C(2)–C(3)	1.33 (2)		
C(7)–C(8)	1.34 (2)		
C(12)–C(13)	1.35 (2)	C_b–C_b	1.34 (1)
C(17)–C(18)	1.33 (2)		
C(1)–C(20)	1.36 (2)		
C(4)–C(5)	1.36 (2)		
C(5)–C(6)	1.43 (2)		
C(9)–C(10)	1.40 (2)		
C(10)–C(11)	1.38 (2)	C_a–C_m	1.39 (2)
C(14)–C(15)	1.38 (2)		
C(15)–C(16)	1.45 (2)		
C(19)–C(20)	1.39 (2)		
C(2)–C(21)	1.55 (2)		
C(3)–C(23)	1.65 (3)		
C(7)–C(25)	1.58 (3)		
C(8)–C(27)	1.64 (3)		
C(12)–C(29)	1.53 (2)	C_b–C1(Et)	1.60 (7)
C(13)–C(31)	1.51 (2)		
C(17)–C(33)	1.67 (3)		
C(18)–C(35)	1.67 (3)		
C(21)–C(22)	1.51 (3)		
C(23)–C(24)	1.34 (3)		
C(25)–C(26)	1.51 (3)		
C(27)–C(28A)	1.43 (3)		
C(27)–C(28B)	1.52 (2)	C1(Et)–C2(Et)	1.49 (7)
C(29)–C(30)	1.57 (3)		
C(31)–C(32)	1.56 (2)		
C(33)–C(34)	1.54 (2)		
C(35)–C(36)	1.47 (3)		
C(37)–N(5)	1.36 (2)		
C(37)–N(6)	1.30 (2)		
C(38)–N(6)	1.42 (2)		
C(38)–C(39)	1.37 (2)		
C(40)–N(5)	1.52 (2)		
N(1)–Co–N(3)	172.8 (4)		
N(2)–Co–N(4)	171.5 (5)		
N(1)–Co–N(2)	90.9 (5)		
N(1)–Co–N(4)	88.8 (5)		
N(2)–Co–N(3)	89.2 (5)		
N(3)–Co–N(4)	90.0 (5)		
N(1)–Co–N(6)	94.0 (4)		
N(2)–Co–N(6)	95.1 (4)		
N(2)–Co–N(6)	93.2 (4)		
N(4)–Co–N(6)	93.4 (4)		
Co–N(6)–C(37)	127 (1)		
Co–N(6)–C(38)	126 (1)		
C(1)–N(1)–C(4)	103 (1)		
C(6)–N(2)–C(9)	105 (1)		
C(11)–N(3)–C(14)	102 (1)	C_a–N–C_a	103 (1)
C(16)–N(4)–C(19)	103 (1)		
N(1)–C(1)–C(20)	126 (2)		
N(1)–C(4)–C(5)	126 (2)		
N(2)–C(6)–C(5)	128 (2)		
N(2)–C(9)–C(10)	127 (2)		
N(3)–C(11)–C(10)	125 (1)	N–C_a–C_m	126 (2)
N(3)–C(14)–C(15)	125 (1)		
N(4)–C(16)–C(15)	124 (2)		
N(4)–C(19)–C(20)	127 (2)		
C(4)–C(5)–C(6)	121 (2)		
C(9)–C(10)–C(11)	122 (2)		
C(14)–C(15)–C(16)	123 (2)	C_a–C_m–C_a	122 (1)
C(19)–C(20)–C(1)	121 (2)		
N(1)–C(1)–C(2)	110 (2)		
N(1)–C(4)–C(3)	113 (2)		
N(2)–C(6)–C(7)	111 (2)		
N(2)–C(9)–C(8)	112 (2)		
N(3)–C(11)–C(12)	112 (1)	N–C_a–C_b	112 (2)
N(3)–C(14)–C(13)	114 (1)		
N(4)–C(16)–C(17)	113 (2)		
N(4)–C(19)–C(18)	111 (1)		
C(1)–C(2)–C(3)	105 (2)		
C(4)–C(3)–C(2)	109 (2)		
C(8)–C(7)–C(6)	108 (2)		
C(7)–C(8)–C(9)	104 (2)		
C(11)–C(12)–C(13)	106 (1)	C_a–C_b–C_b	106 (2)
C(12)–C(13)–C(14)	106 (1)		
C(16)–C(17)–C(18)	105 (2)		
C(17)–C(18)–C(19)	108 (2)		
C(20)–C(1)–C(2)	123 (2)		
C(5)–C(4)–C(3)	121 (2)		
C(5)–C(6)–C(7)	122 (2)		
C(10)–C(9)–C(8)	122 (2)		
C(10)–C(11)–C(12)	123 (1)	C_m–C_a–C_b	122 (2)
C(15)–C(14)–C(13)	121 (1)		
C(15)–C(16)–C(17)	124 (2)		
C(20)–C(19)–C(18)	122 (2)		
C(1)–C(2)–C(21)	125 (2)		
C(4)–C(3)–C(23)	123 (2)		
C(6)–C(7)–C(25)	124 (2)		
C(9)–C(8)–C(27)	125 (2)		
C(11)–C(12)–C(29)	127 (1)	C_a–C_b–C1(Et)	125 (2)
C(14)–C(13)–C(31)	127 (1)		
C(16)–C(17)–C(33)	121 (2)		
C(19)–C(18)–C(35)	126 (2)		
C(3)–C(2)–C(21)	129 (2)		
C(2)–C(3)–C(23)	127 (2)		
C(8)–C(7)–C(25)	128 (2)		
C(7)–C(8)–C(27)	131 (2)		
C(13)–C(12)–C(29)	129 (1)	C_b–C_b–C1(Et)	129 (3)
C(12)–C(13)–C(31)	127 (1)		
C(17)–C(18)–C(35)	126 (2)		
C(18)–C(17)–C(33)	134 (2)		
Intermolecular Contacts			
C(28A)–C(16)			2.88
C(28A)–C(17)			2.93
C(28A)–C(18)			3.32
C(28A)–N(4)			3.23
C(28B)–C(28B)			2.99
C(34)–C(17)			3.27
C(34)–C(18)			3.20
C(34)–C(34)			3.30
C(40)–C(14)			3.49
C(40)–N(3)			3.34

[a] The figure in parentheses following an average value is the larger of that estimated for an individual value from the inverse matrix or on the assumption that the values averaged are from the same population. [b] The notation C_a, C_b, and C_m is that of Hoard.[1]

The Co(II) atom lies out of the plane of an individual pyrrole, the displacement being largest for pyrrole 3. This is similar to the situation observed in Fe(PP-IX)Cl,[33] where the Fe atom is displaced by 0.28–0.47 Å from the planes of the individual pyrroles. This may be indicative of a small amount of sp^3 character at the pyrrole nitrogen atoms.

The Co atom is displaced by 0.13 Å from the mean

Table IV. Deviations (Å $\times 10^2$) and Equations of Weighted Least-Squares Planes[a]

	Plane 1	Plane 2	Plane 3	Plane 4	Plane 5	Plane 6	Plane 7	Plane 8	Plane 9
Co	13	16	0	5	15	4	4	0 (0)	0 (0)
N(6)							0 (1)	0 (1)	1 (1)
N(5)							0 (1)		
C(37)							0 (2)		
C(38)							0 (2)		
C(39)							0 (2)		
C(40)									
N(1)	1 (1)	6 (1)	−1 (1)						1 (1)
C(1)		6 (2)	3 (2)						
C(2)		−4 (2)	0 (2)						
C(3)		−2 (2)	2 (2)						
C(4)		6 (2)	2 (2)						
C(5)		−4 (2)	−10	−6 (2)					
N(2)	−1 (1)	2 (1)		0 (1)				0 (1)	
C(6)		1 (2)		1 (2)					
C(7)		−9 (2)		−2 (2)					
C(8)		−6 (2)		2 (2)					
C(9)		−2 (2)		0 (2)					
C(10)		−4 (2)		−2	0				
N(3)	1 (1)	2 (1)			1 (1)				1 (1)
C(11)		−2 (2)			−1 (2)				
C(12)		1 (2)			1 (2)				
C(13)		3 (2)			0 (2)				
C(14)		3 (2)			0 (2)				
C(15)		0 (2)			−7	−5			
N(4)	−1 (1)	1 (1)				−1 (1)		0 (1)	
C(16)		−1 (2)				0 (2)			
C(17)		−6 (2)				3 (2)			
C(18)		−15 (2)				−4 (2)			
C(19)		−2 (2)				3 (2)			
C(20)		0 (2)				4			
C(21)		−21							
C(23)		−38							
C(25)									
C(26)									
C(27)		−3							
C(31)		−6							
C(33)									
C(35)									

Angles between Normals to the Planes

Plane A	Plane B	Angle (deg)
2	1	0.7
2	3	2.5
2	4	2.6
2	5	1.2
2	6	3.2
2	7	92.3
3	4	3.9
3	5	2.8
4	5	1.4
5	6	4.4
7	8	110.7
7	9	10.0
8	9	90.7

Coefficients of the Plane Equation $Ax + By + Cz = D$[b]

Plane	A	B	C	D	
1	1.073	17.52	−0.590	2.020	
2	1.188	17.51	−0.715	1.950	Porphyrin
3	1.710	17.42	−1.097	1.957	Pyrrole 1
4	0.771	17.51	−1.173	2.042	Pyrrole 2
5	1.034	17.51	−0.961	1.938	Pyrrole 3
6	1.797	17.44	−0.259	2.060	Pyrrole 4
7	5.177	−0.555	12.78	1.412	Imidazole
8	11.82	−1.328	−8.739	−3.434	
9	7.376	−0.434	11.46	0.922	

[a] The entries for which an error is not indicated are for atoms which were not included in the calculation of the plane. [b] The plane is in triclinic coordinates as defined by W. C. Hamilton, *Acta Crystallogr.*, **18**, 502 (1965).

plane of the pyrrole nitrogen atoms and 0.16 Å from the mean plane of the porphyrin core, toward the axial imidazole. This displacement is similar to that observed in the five-coordinate Co(II)–Schiff base com-

Table V. A Tabulation of Co(II)–N(imidazole) Bond Lengths (Å)[a]

Compound	Co–N	Ref
$Co(Im)_2(Et_2\text{-}Barb)_2$	2.022 (3)	53
trans-$Co(Im)_2(H_2O)_2(CO_3)$	2.112 (7)	57
$Co(L\text{-}Hist)_2$	2.11 (2)	56
	2.19 (2)	
Co(L-Hist)(D-Hist)	2.14 (1)	55
	2.08 (1)	
cis-$[Co(2\text{-}Me\text{-}Im)_4(NO_3)]NO_3$	2.253 (17)	54
	2.209 (17)	
	2.105 (17)	
	1.960 (13)	
$[Co(Im)_6]CO_3 \cdot 5H_2O$	2.16 (1)	58
	2.18 (1)	
$[Co(Im)_6](OAc)_2$	~2.15	59
Co(1-Me-Im)(TPP)	2.157 (3)	36

[a] The abbreviations used in this table are: Im, imidazole; Et_2-Barb, 5,5′-diethylbarbituric acid monoanion; L-Hist, L-histidine monoanion; 2-Me-Im, 2-methylimidazole.

long, axial Co(II)–N(sp^2) bonds are also observed in the five-coordinate complexes Co(salen)(py)[63] (2.10 (2)) and Co(py-salen)[64] (2.15 Å).

Discussion

In the structure of Co(1-Me-Im)(OEP) the cobalt atom is displaced by only 0.16 (1) Å from the mean plane of the porphyrin core and 0.17 (1) Å from the plane containing the 24 atoms of the porphyrin core and the eight α-carbon atoms of the peripheral substituents. (This last parameter is equivalent to the out-of-plane displacement obtained from structural studies of proteins.) The displacement found here is clearly less than the 0.5 Å observed in the five-coordinate ferrous and ferric porphyrins listed in Table VI. It is also considerably less than the 0.75 Å displacement reported by Bolton and Perutz[66] for the ferrous ions in deoxy-Hb.

Table VI. Displacement of Metal Atom (Å) from Porphyrin Plane in Five-Coordinate Metalloporphyrins and Selected Hemoproteins

	$M\text{–}N_{porph}$[a]	$M\text{–}X_{axial}$	Δ_1[b]	Δ_2[b]	Δ_3[b]	Ref
Co(1-Me-Im)(OEP)	1.96 (1)	2.15 (1)	0.13 (1)	0.16 (1)	0.19 (1)	*c*
Co(1-Me-Im)(TPP)	1.977 (3)	2.157 (3)	0.13	0.14		*d*
Fe(Cl)(PP-IX)	2.062 (10)	2.218 (6)	0.575	0.541	0.57	*e*
μ-$[Fe(TPP)]_2$-O	2.087 (8)	1.763 (1)	0.50 (1)	0.54 (1)		*f*
$Fe(OCH_3)$(MP-IX DME)	2.073 (6)	1.842 (4)	0.456	0.483	0.50	*g*
met-Mb	2.0 (1)	2.2 (1)			0.3 (1)[n]	*h*
met-Mb					0.6	*i*
met-Ec[j]					0.3	*j*
met-Hb		1.95[α], 2.05			0.30[α,β]	*k*
Hb(deoxy)		2.25[α], 2.05[β]			0.75[α,β]	*l*
Fe(2-Me-Im)(TPP)	2.086	2.136	0.42	0.55		*m*

[a] Average of presumably equivalent bond distances. The number in parentheses is the standard deviation of the mean. The α and β superscripted entries refer to the bond lengths in the crystallographically independent α and β chains of Hb. [b] Δ_1 is the perpendicular displacement of the metal atom from the weighted, least-squares plane of the four pyrrole nitrogen atoms; Δ_2 is the displacement from the plane of the 24 atoms of the porphyrin core; Δ_3 is the displacement from the 32 atoms which comprise the porphyrin core and the α-carbon atoms of the eight peripheral substituents. Δ_3 is equal to the $M\text{–}P_\mu$ distance defined by Hoard and Scheidt,[19] and can be identified with the parameter measured in protein structure determinations. [c] This work. [d] Reference 36. [e] Reference 33. [f] Reference 9. [g] Reference 6. [h] E. A. Magnusson in "Hemoglobin and Myoglobin and their Reactions with Ligands," E. Antonnini and M. Brunori, Ed., American Elsevier, New York, N. Y., 1971, pp 85–95; ref 62. Coordinates based on 1.4-Å data for sperm whale myoglobin. [i] B. P. Schoenborn, *Cold Spring Harbor Symp. Quant. Biol.*, **36**, 569 (1971); 2.0 Å neutron diffraction data on deuterated, acid met-Mb (sperm whale). [j] R. Huber, O. Epp, and H. Formanek, *J. Mol. Biol.*, **52**, 349 (1970); R. Huber, O. Epp, W. Steigemann and H. Formanek, *Eur. J. Biochem.*, **19**, 42 (1971); 2.5 Å data. met-Ec is an abbreviation for acid meterythrocruorin; a single chain hemoglobin of the insect *Chironomous Thummi*. [k] See Table 1 of ref 15; 2.8 Å data on horse-Hb, ref 14 and 15. [l] Reference 66. See also footnote *k*, 2.8 Å data on human-Hb. [m] Reference 19. [n] Watson (footnote *h*) gives the estimated mean error in the C–C, C–N, and C–O bond lengths in met-Mb to be 0.1 Å.

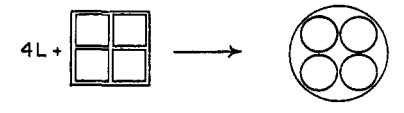
plexes, Co(py)(salen)[63] and Co(py-salen).[10,64] However, the comparison is somewhat difficult because of the distinctly unsymmetrical distortions of these Schiff bases.

The axial Co–N(6) bond length of 2.15 (1) Å is considerably longer than the equatorial Co–N bond lengths (1.96 (1) Å) and it is also significantly longer than the 2.05 (1) Å expected for an unconstrained Co(II)–N(sp^2) bond. The 2.05 (1) Å estimate is obtained if one takes the value of 2.114 (9) Å observed[65] in $Co(NH_3)_6^{2+}$ and subtracts 0.06 (1) Å, to take into account the sp^2 hybridization of the imidazole N atom.[32]

A survey of the literature (Table V) suggests that Co(II)–imidazole bond lengths are strongly influenced by the nature of the other ligands in the complex and are often a good deal longer than 2.05 Å. Similarly

This observation raises the obvious question: is such a small displacement consistent with the trigger mechanism proposed by Perutz?[14]

In the allosteric model proposed by Perutz the change in the quaternary structure of the Hb tetramer, from the unliganded conformation (represented by the structure of deoxy-Hb) to the fully liganded conformation (represented by the structure of met-Hb), is induced by changes in the tertiary structures of the individual subunits.



unliganded-Hb liganded-Hb

In this model the tertiary structure of an individual subunit changes upon ligation of that subunit. This change is triggered by a movement of the proximal histidine toward the heme plane. The movement of

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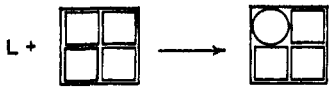
(66) W. Bolton and M. F. Perutz, *Nature (London)*, **228**, 551 (1970).



this residue is the result not only of the shift in the position of the metal atom but also of a change in the metal ligand bond lengths.

In Figure 4 we show, diagramatically, the calculation of the total movement of the proximal histidine residue for both Hb and CoHb. In deoxy-Hb we assume that the displacement of the high-spin Fe(II) ion is similar to that observed in Fe(2-Me-Im)(TPP) (*vide infra*) and that the axial Fe(II)–N(histidine) bond length is approximately equal to that observed in various high-spin Fe(II) complexes, *e.g.*, $Fe(py)_6^{2+}$,[67] Fe(naphthyridine)$_4^{2+}$,[68] and $Fe(py)_2(SCN)_4^{2-}$.[69] These Fe(II)–N(sp^2) bond lengths may be compared with the Fe(II)–O bond lengths of 2.09–2.16 Å observed in the $Fe(H_2O)_6^{2+}$ ion.[70–72] The choice of 2.27 Å represents an upper limit for the axial bond[73] and is somewhat longer than that observed in Fe(2-Me-Im)(TPP), 2.16 Å.[19] Hoard and Scheidt have independently estimated that this bond length is 2.20 Å in deoxy-Hb.[19] The values 2.27–2.16 Å define the plausible range for the Fe(II)–N_ϵ bond.

The Fe–(histidine) bond length in oxy-Hb is estimated to be 1.97 Å[47,49,74] whether the iron atom in oxy-Hb is low-spin Fe(II) or low-spin Fe(III).[12] Using these estimates a movement of ~0.8 Å is indicated for the proximal histidine in the deoxy to oxy transformation in Hb. A much larger movement (1.1 Å) would be predicted if a ~0.8 Å displacement were assumed[15] for the ferrous ion in deoxy-Hb (*vide infra*).

Similar estimates[75] can be made for CoHb using the bond lengths observed in Co(1-Me-Im)(OEP), Co(1-Me-Im)(TPP),[36] and in $Co(Im)_2(TPP)^+$.[60] On this basis the total movement of the proximal histidine in CoHb can be estimated to be ~0.4 Å, which is approximately half that predicted for Hb. In view of the structure of met-Hb (*vide infra*) it seems unlikely that such a small movement (0.4 Å) could trigger the conformational changes observed in CoHb.

Two alternative models have recently been proposed,[15,19,76] which purport to account for the apparent stereochemical differences between CoHb and Hb. In the discussion which follows, we shall describe each of these models in turn and discuss what appear to us to be their inherent shortcomings.

The first of these models may be termed the tension model and has been proposed by Perutz[15,76–79] and

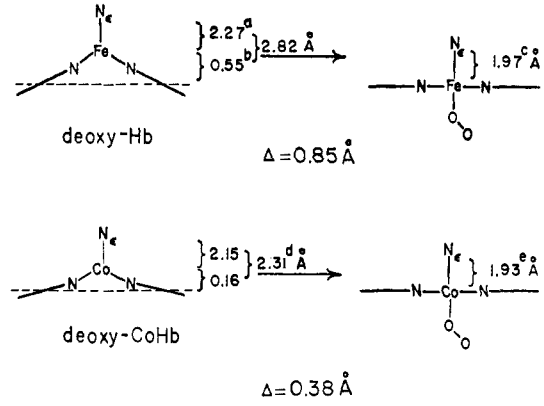
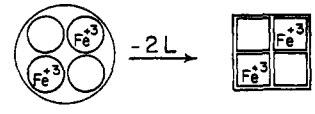


Figure 4. Illustration of the calculation of the total movement of the proximal histidine in Hb and CoHb. The bond distances and displacements of the metal atoms are those found in: (a) $Fe(py)_6^{2+}$, ref 67; (b) Fe(2-Me-Im)(TPP), ref 19; (c) $[Fe(Im)_2(TPP)]^+Cl^-$, ref 47; (d) Co(1-Me-Im)(OEP), this work; and (e) $[Co(Im)_2(TPP)]^+OAc^-$, ref 60. The displacements are taken relative to the 24-atom porphyrin core.

applied to CoHb by Hoard and Scheidt.[19] Perutz observed that in the unliganded conformation the Hb tetramer is relatively tensed or constrained compared with the liganded conformation. According to Perutz,[15] salt bridges between the C-terminal residues of the α and β chains serve the purpose of holding the molecule in this strained conformation. In this strained state the hemes are also under tension, so that presumably the ferrous ions are pulled outwards from the porphyrin planes and their oxygen affinities are diminished. To demonstrate this hypothesis, Perutz investigated the behavior of hybrid Hb's which contain a mixture of ferrous and ferric (met) subunits. In the ferric subunits the iron atoms are expected to be 0.3 Å out of the heme planes, a position intermediate between that observed in the unliganded and the liganded forms of Hb. Consequently, Perutz concluded that the tertiary structure of these met subunits would depend largely on the quaternary structure of the Hb tetramer.

Perutz observed[76–79] that there were changes in the optical spectra of the ferric subunits, when the ferrous subunits went from a liganded to an unliganded state.



This result was interpreted as indicative of a change at the ferric subunits toward a state of higher spin. Presumably when the protein is in the unliganded conformation the ferric ions are under tension.

This description of the details of the trigger for the cooperative conformational changes in Hb differs from the previous description[14] in that instead of the five-coordinate ferrous ions holding the protein in the unliganded conformation, the protein is now holding the ferrous ions in a stressed five-coordinate state. The stability of the five-coordinate geometry provides a driving force toward the unliganded conformation,

(67) R. J. Doedens and L. F. Dahl, *J. Amer. Chem. Soc.*, **88**, 4847 (1966).
(68) P. Singh, A. Clearfield, and I. Bernal, *J. Coord. Chem.*, **1**, 29 (1971).
(69) I. Sotofte and S. E. Rasmussen, *Acta Chem. Scand.*, **21**, 2028 (1967).
(70) H. Montgomery, R. V. Chastain, J. J. Natt, and A. M. Witkowski, and E. C. Lingafelter, *Acta Crystallogr.*, **22**, 775 (1967).
(71) W. C. Hamilton, *Acta Crystallogr.*, **15**, 353 (1962).
(72) W. S. Bauer, *Acta Crystallogr.*, **17**, 1167 (1964).
(73) These estimates differ considerably from those of Perutz, who assumed unusually long Fe–N(porphyrin) bond lengths, a short Fe–N(histidine) bond length, and a much larger (0.83 Å) out-of-plane displacement of the Fe(II) ion. In his calculations Perutz[15] has incorrectly used 0.475 Å as the out-of-plane displacement of the Fe(III) ion in Fe-Cl(PP-IX).
(74) D. H. Templeton, A. Zalkin, and T. Ueki, *Inorg. Chem.*, **12**, 1641 (1973).
(75) J. A. Ibers, J. W. Lauher, and R. G. Little, *Acta Crystallogr., Sect. B*, **30**, 268 (1974).
(76) M. F. Perutz, *Nature (London)*, **237**, 995 (1972).
(77) M. F. Perutz, J. E. Ladner, S. R. Simon, and C. Ho, *J. Biol. Chem.*, in press.
(78) M. F. Perutz, A. R. Fersht, S. R. Simon and G. C. K. Roberts, *J. Biol. Chem.*, in press.
(79) M. F. Perutz, E. J. Heidner, J. E. Ladner, J. G. Bettlestone, C. Ho and E. F. Slade, *J. Biol. Chem.*, in press.

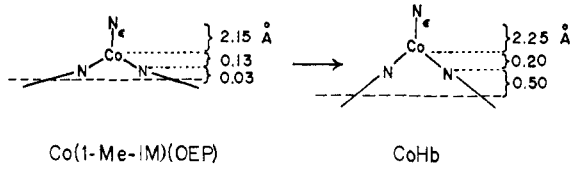


Figure 5. Illustration of the possible points of distortion of the Co(II) porphyrin prosthetic group in CoHb as proposed by Hoard and Scheidt.[19a] These authors proposed that a feasible combination of these distortions would give a total displacement of N_ϵ from the 32-atom plane of the porphyrin of 2.70–2.75 Å.[19]

which once attained is then reinforced by intramolecular interactions.

Hoard and Scheidt[19a] have applied this model to CoHb with the following modifications: (a) in the deoxy conformation the tension within the protein stretches the Co–N(proximal imidazole) bond rather than increasing the displacement of the Co atom from the plane of the porphyrin N atoms (see Figure 5); (b) because of the tension in the protein the porphyrin undergoes a substantial doming. Hoard has estimated[19b] that the distance from the imidazole nitrogen atom to the mean, 32 atom plane of the porphyrin, N_ϵ–P_μ, can be stretched from the 2.30–2.35 Å found in the five-coordinate cobalt porphyrins to 2.70–2.75 Å. The major component of this stretching is proposed to be a 0.3 Å additional doming of the porphyrin. Hoard defines doming as the symmetric distortion of a planar porphyrin molecule such that the pyrrole nitrogen atoms twist out of the mean plane of the molecule toward the five-coordinate metal atom. In this description the net movement of the proximal histidine residue in CoHb is made nearly equal to that proposed for Hb by considerably deforming the Co porphyrin geometry. As evidence in support of the tension model, Hoard and Scheidt have pointed out that the ferrous ions in deoxy-Hb[66] are reported to be 0.75 Å out of the porphyrin mean planes, P_μ, whereas model compounds, such as Fe(2-Me-Im)(TPP), show only a 0.55 Å displacement. The 0.20 Å increase is a result of the tension.

In assessing the tension mechanism it is necessary to consider first the accuracy of the protein structures and secondly the evidence (or lack thereof) for a distortion of the metalloporphyrins. Bolton and Perutz[66] give the out-of-plane displacement of the ferrous ions in both the α- and β-chains of deoxy-Hb as 0.75 Å. The Fe(II)–N(proximal imidazole) bond lengths are given as 2.25 and 2.05 Å, respectively. No error estimates are given. However, it has been indicated elsewhere[80] that the positional coordinates of the C, N, and O atoms on the periphery of a subunit carry an estimated error of ±0.5 Å. Hopfield[81] has quoted an error of ±0.1 Å in connection with the out-of-plane displacement of the Fe(II) atoms in deoxy-Hb. In the structure of met-Mb, Watson[62] attached an estimated error of ±0.1 Å to the light-atom positional coordinates. It seems likely[82] that the error in the Fe–porphyrin bond lengths in the deoxy- and met-Hb structures must be *at least* ±0.1 Å. With estimated errors of ±0.1 Å or greater it seems unjustified to claim that the out-of-plane displacement in deoxy-Hb differs significantly from the 0.55 Å observed in model compounds (see Table VI). In addition one might expect to find some further evidence in support of the tension model in the structures of the abnormal Hb's, Hb-M Milwaukee and Hb-M Iwate which both have deoxy quaternary structures. But difference Fourier syntheses on these molecules[87,88] have apparently given no indication of an increased displacement of the Fe atoms in the met subunits.

Perhaps the most convincing evidence for the tension mechanism is cited[77–79] to come from the nmr studies of normal and abnormal Hb's.[77–79,89–95] The paramagnetically shifted proton resonances of the porphyrin prosthetic groups and of those residues in the protein which are in the immediate vicinity of the hemes have been shown to be sensitive indicators of the quaternary structure of the Hb tetramer and of the state of ligation of the iron atoms.

Specifically Lindstrom, *et al.*,[89] have shown that there are large changes in the ferric heme resonances of the β subunits upon coordination of CO to the ferrous α subunits of Hb-M Milwaukee. Lindstrom, *et al.*,[89] have interpreted these shifts as indicative of structural changes being induced at the β-hemes upon ligation of the α-hemes. Similarly Ogawa, *et al.*,[90–92] have shown in their studies of the mixed valence hybrids, $(\alpha^{III}CN,\beta)_2$ and $(\alpha,\beta^{III}CN)_2$, that there are large changes in the hyperfine shifted nmr signals of the cyanomet subunits when oxygen binds to the ferrous subunits. Ogawa, *et al.*,[92] have also shown that these same changes in the nmr spectrum are induced when organic phosphates shift the quaternary structure from the oxy to the deoxy conformation. Similar shifts have been demonstrated for the ferrous hemes in various normal and abnormal Hb's.[77]

Perutz[76] has interpreted these results as a direct demonstration of the existence of tension at the heme

(80) See Table 2 of ref 15.

(81) J. J. Hopfield, *J. Mol. Biol.*, **77**, 207 (1973).

(82) The estimated errors in the Hb structure (mol wt ~64,000) may be compared with those observed in the structure of the *Chromatium* high-potential protein[83,84] (mol wt 10,000). The latter structure has been refined[84] by conventional least-squares technique using data out to 2.0 Å. The refinement included only the Fe_4S_8 cluster. The result of the refinement was to change the average Fe–Fe, Fe–S*, and Fe–S_{cys}, distances in the central core from 3.06, 2.35, and 2.01 Å to 2.72 (4), 2.26 (8), and 2.20 (2) Å, respectively. The numbers in parentheses are observed rms deviations among the presumably equivalent bond lengths. The refined values are in good agreement with those found in various model compounds.[85] Comparably large shifts in the heavy-atom bond lengths have been observed in the structures of rubredoxin[86] and ferredoxin.[84]

(83) C. W. Carter, Jr., S. T. Freer, Ng. H. Xnong, R. A. Alden, and J. Kraut, *Cold Spring Harbor Symp. Quant. Biol.*, **36**, 381 (1971).

(84) C. W. Carter, Jr., J. Kraut, S. T. Freer, R. A. Alden, L. C. Sieker, A. Adman, and L. H. Jensen, *Proc. Nat. Acad. Sci. U. S.*, **69**, 3526 (1972).

(85) B. A. Averill, T. Herskovitz, R. H. Holm, and J. A. Ibers, *J. Amer. Chem. Soc.*, **95**, 3523 (1973).

(86) K. D. Watenpaugh, L. C. Sieker, J. R. Herriot, and L. H. Jensen, *Cold Spring Harbor Symp. Quant. Biol.*, **36**, 359 (1971); K. D. Watenpaugh, L. C. Sieker, J. R. Herriot, and L. H. Jensen, *Acta Crystallogr., Sect. B*, **29**, 943 (1973). In this structure all of the atoms in the protein have been refined.

(87) M. F. Perutz, P. D. Pulsinelli, and H. M. Ranney, *Nature (London), New Biol.*, **237**, 259 (1972).

(88) J. Greer, *J. Mol. Biol.*, **59**, 107 (1971).

(89) T. R. Lindstrom, C. Ho, and A. V. Pisciotta, *Nature (London), New Biol.*, **237**, 263 (1972).

(90) S. Ogawa, R. G. Shulman, and T. Yamane, *J. Mol. Biol.*, **70**, 291 (1972).

(91) S. Ogawa, R. G. Shulman, M. Fujiwara, and T. Yamane, *J. Mol. Biol.*, **70**, 301 (1972).

(92) S. Ogawa and R. G. Shulman, *J. Mol. Biol.*, 70, 315 (1972).

(93) K. H. Winterhalter and K. Wüthrich, *J. Mol. Biol.*, **63**, 477 (1972).

(94) D. G. Davis, T. R. Lindstrom, N. H. Mock, J. J. Baldassare, S. Charache, R. T. Jones, and C. Ho, *J. Mol. Biol.*, **60**, 101 (1971).

(95) R. Hershberg and T. Asakura, *J. Mol. Biol.*, 735 (1972).

groups when the protein is in the deoxy conformation. However, it appears that the observed nmr shifts are very sensitive indicators of changes in the heme environment and that these shifts are reflecting much smaller perturbations of the hemes than the tension mechanism implies. This can best be illustrated by noting that in the nmr experiments with met-Hb the α- and β-hemes are seen to be nonequivalent, yet the structural studies show no differences in the iron porphyrin geometry. Similarly the nmr experiments[95] show that the spectra of the α- and β-hemes in met-Hb(CN) are different from the spectra of the hemes in the isolated α or β chains. This result, if interpreted in terms of the tension model, would indicate that the hemes in met-Hb(CN) are under tension relative to the hemes in the isolated subunits.

An indication of the sensitivity of the heme nmr spectra to relatively minor changes is given by the work of Hill and Morallee.[96] They demonstrated that changes in the para substituent of the bis-pyridine complexes, $[Fe(py\text{-}X)_2(PP\text{-}IX)]^+Cl^-$, caused shifts as great as 8 ppm in the porphyrin methyl resonances. Kurland, *et al.*,[97] have shown that changing the axial ligand from cyanide to imidazole produces similarly large shifts. Shulman, *et al.*,[98] have reported large shifts in the position of a heme methyl group on the addition of cyclopropane to met-Mb(CN). Crystallographic studies[99] had previously demonstrated that the cyclopropane molecule sits above the pyrrole upon which that methyl group is a substituent. The crystallographic studies showed no changes in the heme coordinates upon coordination of the cyclopropane molecule.

In view of these results it is unjustified to conclude that the nmr experiments have demonstrated the existence of appreciable tension in the Fe–proximal histidine bond. The changes in the nmr spectra of Hb-M Milwaukee and in the cyanomet hybrids can be most easily explained if one looks at the nature of the sixth ligand, that which is coordinated at the distal side of the porphyrin. In Hb-M Milwaukee crystallographic studies[87] have shown that the sixth ligand is the γ-carboxyl group of the aberrant glutamic acid at position E11(67)β. Previous structural studies[14,15] have shown that residue E11(67)β undergoes significant shifts when the protein changes its quaternary structure. Indeed the movement of valine E11(67)β in normal Hb-A is central to the stereochemical explanation of the increase in the oxygen affinities of the β-hemes.[14] Therefore it seems certain that the γ-carboxyl group moves during the quaternary conformational change and that this movement is reflected in the β-heme resonances. It is important to note that ferric hemes with weak-field sixth ligands are in equilibrium between high- and low-spin forms. Smith and Williams[100] have shown that an understanding of this equilibrium is central to the interpretation of the visible spectra of hemeproteins. They have shown that this equilibrium is very sensitive to the protein conformation and to the nature of the sixth ligand. Thus, one expects that the ferric hemes with weak-field axial ligands could change spin states as the quaternary structure of the protein changes. This shift will involve appreciable changes in the bond lengths at these hemes.[101] In the mixed-valence cyanomet hybrids[90-92] similarly large peturbations of the axial sixth ligand (cyanide ion) are also likely. There is a considerable body of evidence which indicates that the distal histidine in Hb-A interacts with most ligands coordinated to the hemes.[102-111] Stryer, *et al.*,[107] have shown this in the structure of met-Mb(N_3). Similarly Hendrickson, *et al.*,[108] have shown that the cyanide ion in met-Hb(CN) (Lamprey) is in a position to hydrogen bond to the distal histidine. Hendrickson, *et al.*, found that the cyanide ion appeared to be coordinated in a *bent* fashion, and that it was considerably displaced from the iron–heme axis.

We conclude that the nmr experiments are very sensitive to small changes in heme and the orientation of the fifth and sixth axial ligands.[112,113] The results of the nmr experiments on Hb do not require changes in the metalloporphyrin geometry as great as those proposed to occur in Hb or CoHb by Hoard and Scheidt.[19]

Direct evidence against the tension model in CoHb comes from resonance Raman studies[114,115] of Hb and CoHb and from epr experiments with CoHb and model systems. In their studies of CoHb Woodruff, *et al.*,[115] found that the shifts in the structure-sensitive Raman bands indicate that the Co(II) atom in deoxy-CoHb cannot be out of the porphyrin plane by more than 0.2 Å and more importantly that the porphyrin has essentially the same conformation as is observed in Co(1-Me-Im)(OEP). The Raman experiment is particularly sensitive to the type of distortions of the porphyrin core proposed by Hoard and Scheidt.[19a]

Similarly, epr experiments[116,117a] show that the spectrum of deoxy-CoHb is essentially identical with that of Co(1-Me-Im)(OEP), while the spectra of Co(II)

(96) H. A. O. Hill and D. G. Morallee, *J. Amer. Chem. Soc.*, **94**, 731 (1972).
(97) R. J. Kurland, R. G. Little, D. G. Davis, and C. Ho, *Biochemistry*, **10**, 2237 (1971).
(98) R. G. Shulman, S. Ogawa, K. Wüthrich, T. Yamane, J. Peisach, and W. E. Blumberg, *Science*, **165**, 251 (1969).
(99) B. P. Schoenborn, *Nature (London)*, **214**, 1120 (1967).
(100) D. W. Smith and R. J. P. Williams, *Struct. Bonding (Berlin)*, **7**, 1 (1970).
(101) E. Konig and K. J. Watson, *Chem. Phys. Lett.*, 6, 457 (1970); H. C. Stynes and J. A. Ibers, *Inorg. Chem.*, **10**, 2306 (1971); J. G. Leipoldt and P. Coppens, *Inorg. Chem.*, **12**, 2269 (1973).
(102) T. Yonetani, H. Yamamoto, F. Kayne, and G. Woodrow, *Biochem. Soc. Trans.*, **1**, 44 (1973); T. Yonetani, H. Yamamoto, and T. Iizuka, *J. Biol. Chem.*, **249**, 2168 (1974).
(103) J. O. Alben and W. S. Caughey, *Biochemistry*, **7**, 175 (1968).
(104) W. S. Caughey, J. O. Alben, S. McCoy, S. H. Boyer, S. Charache, and P. Hathaway, *Biochemistry*, **8**, 59 (1969).
(105) S. McCoy and W. S. Caughey, *Biochemistry*, **9**, 2387 (1970).
(106) R. Huber, O. Epp, and H. Formanek, *J. Mol. Biol.*, **52**, 349 (1970).
(107) L. Stryer, J. C. Kendrew, and H. C. Watson, *J. Mol. Biol.*, **8**, 96 (1964).
(108) W. A. Hendrickson and W. E. Love, *Nature, (London), New Biol.*, **232**, 197 (1971).
(109) E. Bayer and P. Schretzman, *Struct. Bonding (Berlin)*, **2**, 181 (1967).
(110) J. E. Bailey, J. G. Bettlestone, and D. H. Irvine, *J. Chem. Soc. A*, 2913 (1968); J. G. Beetlestone, R. O. Ige, and D. H. Irvine, *ibid.*, 1368 (1970).
(111) C. K. Chang and T. G. Traylor, *J. Amer. Chem. Soc.*, **95**, 8477 (1973).
(112) H. Rein, O. Ristau, and W. Scheler, *FEBS (Fed. Eur. Biochem. Soc.) Lett.*, 24, 24 (1972).
(113) Y. Henry and R. Banerjee, *J. Mol. Biol.*, **73**, 469 (1973).
(114) H. Brunner and H. Sussner, *Biochim. Biophys. Acta*, **310**, 20 (1973).
(115) W. H. Woodruff, T. G. Spiro, and T. Yonetani, *Proc. Nat. Acad. Sci. U. S.*, **71**, 1065 (1974).
(116) B. M. Hoffman and D. H. Petering, *Proc. Nat. Acad. Sci. U. S.* **67**, 637 (1970).
(117) (a) L. Marchant and B. M. Hoffman, personal communication. (b) A referee has pointed out that the differences in the epr spectra could reflect the reduced symmetry and canting of the axial Co–Im bond (as observed in Fe(2-Me-Im)(TPP)[19]) and not a stretching of this axial bond.

porphyrin complexes with sterically hindered imidazoles are quantitatively different.[117b] Furthermore it has been shown[118] that sterically hindered bases, such as 2,6-dimethylpyridine, do not bind to Co(II) porphyrins. These bases would be expected to bind if the Co atom were free to move out of the porphyrin plane or if the porphyrin were free to undergo a substantial doming.

In brief, we conclude from the available evidence that the Co atom in CoHb is ≈ 0.2 Å out of the mean plane, P_μ, of the porphyrin. There is no convincing evidence that the globin can or does exert a force which increases this out-of-plane displacement of the Co atom or stretches the Co–N(imidazole) bond in CoHb.

A second mechanism for the cooperative effects observed in Hb is that of Hopfield.[81] In this mechanism it is proposed that the conformation of the protein responds *linearly* to changes in the distance between the porphyrin plane and the metal atom. In this *linearly distributed energy model* the free energy of cooperativity is stored as small amounts of strain in many bonds. The model relates the total energy change to small structural changes at the metalloporphyrin.

Woodruff, *et al.*,[115] have used this model to relate the lowered cooperativity in CoHb (Hill coefficient, $n \geq 2.3$), as compared with Hb ($n = 2.8$), to a smaller (0.4 Å) movement of the proximal histidine in CoHb. Both they and Hopfield[81] have used Perutz's structural parameters[14,15] as a basis for their description of the ferroporphyrin geometry in deoxy-Hb. We have indicated (*vide supra*) that those parameters are subject to considerable error. Woodruff, *et al.*,[115] have assumed that in CoHb there is no deformation of the metalloporphyrin, such as that proposed by Hoard and Scheidt.[19]

The linearly distributed energy model suffers from one major fault: *it cannot account for the differing quaternary structures of deoxy-CoHb and met-Hb.* In order to make this point clear, it will first be necessary to describe the structure of met-Hb.[119] The structure usually referred to as that of oxy-Hb is actually not the dioxygen complex at all but rather the oxidized product, met-Hb, in which all four of the Fe atoms are in the ferric state. The Fe atoms are purportedly six-coordinate. The sixth ligand is apparently a water molecule, and since water is a weak-field ligand the Fe atoms are considerably out of the plane of the porphyrins toward the N atom of the proximal histidines (see Table VI). The Fe(III)–H_2O bond would have to be very long, but this does not appear to be unusual, *viz.*, $Mn(N_3)(TPP)(CH_3OH)$ (2.3 Å).[120] The structure of met-Hb has been used[14,15] to describe the structure of oxy-Hb, the fully liganded Hb in which the Fe atoms are six-coordinate and are roughly centered in the porphyrin planes.[121]

The important facet of the structure which we wish to emphasize here is that the geometries of the metalloporphyrins in both met-Hb and deoxy-CoHb are approximately the same. The N atoms of the proximal histidine residues in both met-Hb and deoxy-CoHb are apparently[123] both ~2.3 Å from the mean plane of the porphyrins, and yet the two molecules have different quaternary and tertiary structures (*vide infra*). A linear strain model would predict that they would have the same structures.

The tension model of Perutz avoids this problem by suggesting[19] that the structure of the metalloporphyrin in CoHb is different from that in met-Hb. However, it is difficult to see why the cobaltous porphyrin distorts and drives the protein to the deoxy conformation and yet the ferric porphyrin behaves differently.

Kilmartin[122] has recently shown that, in the presence of the allosteric inhibitor inositol hexaphosphate (IHP), met-Hb can in fact adopt the deoxy conformation. This result has been interpreted by Perutz[77–79] to indicate that, because of the intermediate position of the Fe atom in met-Hb, solutions of aquomet-Hb should contain a significant fraction of molecules in the deoxy conformation in dynamic equilibrium with molecules in the oxy conformation. In contrast, kinetic studies by Hoffman, *et al.*,[124] have shown that the CoHb tetramer is in the deoxy conformation and have given no evidence for an equilibrium mixture of two conformers. Furthermore IHP causes no changes in the epr spectrum of deoxy-CoHb.[124]

In view of the preceding discussion we conclude that the nature of the trigger mechanism is still unexplained. The evidence so far obtained about the structures of CoHb and met-Hb is contradictory so far as the trigger mechanism is concerned. If the out-of-plane displacement of the Co atom in CoHb is sufficient to put the protein in the deoxy conformation, then met-Hb should have a deoxy conformation, since the Fe atom is considerably out of the plane of the porphyrin. Numerous experiments[122] have demonstrated that met-Hb can adopt the deoxy conformation in solution. Furthermore, it has recently been reported[125] that crystals of met-Hb[126] with the deoxy quaternary structure can in fact be prepared. What remains to be demonstrated is the magnitude of the out-of-plane displacement of the Fe atom in met-Hb in solution.

One significant difference between met-Hb and deoxy-CoHb is that the ferric atom has a sixth ligand and the cobaltous ion does not. We suggest that the interaction of the distal histidine with this ligand and the constraints of the crystalline environment in the structure of met-Hb[118] might be sufficient to keep the protein in the oxy conformation, even though the heme geometry favors a deoxy conformation.

If it could be shown that the small out-of-plane displacements expected in met-Hb and deoxy-CoHb are

(118) R. G. Little, B. M. Hoffman, and J. A. Ibers, *Bioinorg. Chem.*, 3, 207 (1974).

(119) M. F. Perutz, H. Muirhead, J. M. Cox, and L. G. C. Goaman, *Nature (London)*, 219, 131 (1968).

(120) V. W. Day, B. R. Stults, E. L. Tasset, R. O. Day, and R. S. Marianelli, *J. Amer. Chem. Soc.*, 96, 2650 (1974).

(121) Perutz has cited[15,78,79] unpublished difference Fourier maps which show that the quaternary structures of high-spin aquomet-Hb and low-spin oxy-Hb, carbonyl-Hb, and cyanomet-Hb are the same. On the other hand Kilmarten[122] has referred to similar work which shows that there are differences in the tertiary structure of the subunits.

(122) J. V. Kilmarten, *Biochem. J.*, 133, 725 (1973).

(123) In met-Hb the distance of the proximal histidine from the mean plane of the porphyrin is 2.3 Å or *greater*. The ~0.3 Å displacement reported in *met*-Hb is ~0.2 Å less than would be expected on the basis of a comparison with the structures of a number of simple ferric porphyrins. (See Table VI.) We have explicitly assumed that the porphyrin prosthetic group in CoHb has a structure similar to that of Co(1-Me-Im)(OEP) (*vide supra*).

(124) B. M. Hoffman, Q. Gibson, and C. Bull, personal communication.

(125) L. Anderson, *J. Mol. Biol.*, 79, 495 (1973); experimental section part g.

(126) The "met-Hb" crystals appear to be incompletely oxidized material. The degree of oxidation may be more important, with respect to protein conformation, than the article indicates.[125]

sufficient to cause the quaternary structural change, then it seems clear that the trigger mechanism must be explained in terms of a number of factors not previously[14,15] emphasized. Foremost among these are the effect of the doming of the porphyrin core on the adjacent protein[75,127] and the interaction of the distal histidine[102-111] with the sixth ligand. It has been suggested[128,129] that a hydrophobic, nonpolar environment of the distal side of the heme prevents irreversible oxidation of the heme and hence accounts for the stability of oxy-Hb and oxy-Mb. We wish to suggest that the presence of a strategically placed, highly polar group (the distal histidine) is important to the stabilization of coordinated dioxygen. Studies of a simple model system support this conclusion.[130]

Conclusion

This series of papers on the structures of Co porphyrin complexes provides firm evidence that compounds of the type Co^{II}(base)(porphyrin) have the Co(II) atom about 0.16 (1) Å out of the mean plane of the nonplanar porphyrin, with a Co–N(imidazole) bond length of 2.15 (1) Å. Evidence has also been presented from the structure of $Co^{III}(Im)_2(TPP)^+$ that the Co is in the center of the planar porphyrin skeleton and that the Co–N(imidazole) bond length is 1.93 (2) Å. If the former complex is taken as a model for the prosthetic group in deoxy-CoHb and the latter as a model for that group in oxy-CoHb then the maximum movement of the proximal histidine relative to the mean plane of the porphyrin core on oxygenation of CoHb is less than 0.4 Å.

Evidence has been presented for and against the two models that have been proposed which purportedly account for the apparent differences between the structures of CoHb and Hb. We conclude that there are inherent shortcomings, or at least unresolved difficulties, both in the trigger mechanism of Perutz[14,15,76-79] and in the linearly distributed energy model of Hopfield.[81] We believe that studies of model systems, such as reported in this series of papers, in conjunction with studies of the natural systems themselves, will ultimately provide an explanation of the allosteric mechanism in hemoglobin.

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Supplementary Material Available. A listing of structure factor amplitudes will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St. N.W., Washington, D. C. 20036. Remit check or money order for $4.00 for photocopy or $2.00 for microfiche, referring to code number JACS-74-4452.

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