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Stereochemistry of Cooperativity Effects in the Prosthetic Group of Coboglobin*

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Based on diffraction studies of model cobalt–porphyrin systems an estimate of 0·38 Å is obtained for the maximum movement of the proximal histidine residue relative to the mean plane of the porphyrin ring on oxygenation of coboglobin, the cobalt analogue of hemoglobin. This estimate is about one-half that currently believed to exist in hemoglobin. It is suggested that an additional stereochemical basis for cooperativity of oxygenation in coboglobin may be the transition of the initially non-planar porphyrin core to essential planarity on oxygenation of the cobalt-heme.

The oxygenation of hemoglobin is a cooperative phenomenon in which the last oxygen molecule enters with less difficulty than the others because of an interaction between protein sub-units. In a series of classic studies on model systems Hoard and coworkers have provided a stereochemical explanation for the expected changes in the prosthetic group of hemoglobin upon oxygenation. [See Hoard (1971) for a recent review of the subject.] Perutz, with direct experimental results on various hemoglobins as well as Hoard's studies of model systems, has postulated a 'trigger' mechanism for this cooperativity (Perutz, 1970*a*, *b*; Perutz & TenEyck, 1971). In essence, oxygenation results in a change of the state of the iron atom of the heme group from high-spin Fe(II) to low-spin Fe(III) or low-spin Fe(II), with a concomitant movement of the Fe atom toward† the mean plane of the porphyrin ring estimated to be 0·75 to 0·95 Å. Since this Fe atom is covalently bonded to the proximal histidine residue of the globin, this residue is shifted toward the porphyrin plane with a resultant change in the structure of the protein. In this way the oxygenation of one heme unit leads to effects at the other heme units, despite the fact that the Fe atoms in the hemes are separated by more than 25 Å.

In the past few years it has been shown by a number of workers that it is possible to prepare and study complexes of the type $BCoL.O_2$, where B is a base and where L is a Schiff base (Crumbliss & Basolo, 1970; Hoffman, Diemente & Basolo, 1970) or a porphyrin (Misono, Koda & Uchida, 1969; Walker, 1970; Stynes & Ibers, 1972*a*). Following the initial studies in this area Hoffman & Petering (1970) reconstituted hemoglobin with a cobalt porphyrin. Hoffman, Spilburg & Petering (1971) showed that this material, which they called 'coboglobin', exhibits cooperative, allosteric oxygen binding. This has since been confirmed (Hsu, Spilburg, Bull & Hoffman, 1972). As Hoffman, Spilburg & Petering (1971) note, the oxygenation of coboglobin involves a change from low-spin Co(II) to low-spin Co(III). From stereochemical considerations they conclude that the cobalt ligation in coboglobin should cause a substantially smaller, perhaps negligible, motion of the proximal histidine residue relative to the mean plane of the porphyrin molecule than is postulated for hemoglobin. Perutz & TenEyck (1971) have discussed the implications of cooperative oxygen binding in coboglobin on the trigger mechanism for hemoglobin.

Here, on the basis of studies of model systems, we present a more quantitative picture of the stereochemistry of cooperative effects in the prosthetic group of

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† We choose the mean plane of the porphyrin ring (excluding substituents) as the reference point for a description of these relative motions.

coboglobin.* We predict that the movement of the proximal histidine group in coboglobin upon oxygenation is substantially smaller, perhaps only one-half, that postulated to occur in the corresponding hemoglobin system.

Experimental details

In Table 1 we list some crystallographic details for three, unpublished structures from this laboratory (Lauher & Ibers, 1974; Little & Ibers, 1974*a*,*b*), together with two other structures studied elsewhere (Scheidt, Cunningham & Hoard, 1973; Scheidt, 1973). The complexes listed in Table 1 do not represent an ideal selection for investigating the stereochemical relationship between Co(II) (PP–IX) (histidine) and Co(III) (PP–IX) (histidine) (O_2), where PP–IX symbolizes the protoporphyrin-IX dianion that is found in the heme prosthetic group of hemoglobin and myoglobin. Thus Table 1 includes no O_2 complexes. Moreover, the porphyrins utilized are $\alpha,\beta,\gamma,\delta$-tetraphenylporphyrin dianion (TPP) and 2,3,7,8,12,13,17,18-octaethylporphyrin dianion (OEP), rather than PP–IX. Finally the bases involved are as far from the imidazole (of a histidine residue) as piperidine. The reasons for the study of the compounds listed in Table 1 are purely pragmatic. Thermodynamic studies (Stynes & Ibers, 1972*a*, *b*) have shown that appreciable concentrations of BCoL $.O_2$, L = porphyrin, exist only at low temperatures and there is no evidence to date to suggest that suitable crystals of such materials are stable at ambient temperatures. With few exceptions, good crystals of metalloporphyrins have not been obtained with natural porphyrins, probably because these porphyrins lack symmetry and may be difficult to obtain pure. As a result the more symmetric, synthetic porphyrins TPP and OEP have been employed. (OEP, with substitution on the pyrrole rings, more closely resembles the natural porphyrins which are also substituted at these positions than does TPP with its phenyl substituents at the methine positions.) But even with the use of TPP and OEP it is not always possible to obtain good crystals with a specific base. Instead there has been the empirical approach of trying a number of bases, and some of the crystalline materials that resulted are listed in Table 1. It is also worth pointing out that metalloporphyrin chemistry still holds some surprises. Thus both of the Co(III) complexes (I, II) of Table 1 were prepared accidentally. Compound (I) resulted from a reaction that occurred during the evaporation of a 1:1 piperidine–chloroform solution of Co(TPP) (NO). Compound (II) was prepared from a presumed solution of Co(TPP) and imidazole in $CHCl_3$ through which O_2 was bubbled.

Although the structures listed in Table 1 were solved from data obtained by counter methods, three did not refine in a totally satisfactory way. This is the result of either poor crystal quality or of inherent difficulties with the model for X-ray scattering. In compound (I) neither the anion nor the piperidine molecules of crystallization could be well defined. In compound (II) only a limited number of data were available from a poorly scattering crystal. In compound (V) the crystals were of poor quality, as judged by obvious streaking of the X-ray photographs. Upon refinement we find that at least three of the eight ethyl groups of the OEP are disordered.

It is also apparent from Table 1 that refinement of these structures involves the determination of an unusually large number of variables. Nevertheless refinements have generally been carried out by full-matrix least-squares methods. For those structures determined in this Laboratory the calculations were performed on the CDC 7600 at Lawrence Berkeley Laboratory by remote hookup.

* We assume that the geometries of these model Co(porphyrin(base) molecules are not affected by insertion into the protein.

Table 1. *Crystallographic details on some cobalt-base-porphyrin complexes*

	Compound*	a(Å)	b(Å)	c(Å)	α(°)	β(°)	γ(°)	Z	Space group	$R(F)$†	NO‡	NV‡	Reference
(I)	Co(III) (TPP) $(pip)_2^+$ – NO_3^- .2 pip	12·049	12·911	10·405	102·73	92·04	65·24	1	$P\bar{1}$	0·102	8415	367	Scheidt *et al.* (1974)
(II)	Co(III) (TPP) $(Im)_2^+$ – OAc^- . H_2O . $CHCl_3$	13·055	17·239	11·807	104·57	91·03	107·53	2	$P\bar{1}$	0·098	1819	248	Lauher & Ibers (1974)
(III)	Co(II) (TPP) $(pip)_2$	11·503	11·830	9·934	104·99	115·64	101·49	1	$P\bar{1}$	0·043	4360	280	Scheidt (1973)
(IV)	Co(II) (OEP) $(3\text{-pic})_2$	10·187	11·258	9·753	93·10	92·32	113·28	1	$P\bar{1}$	0·036	3629	250	Little & Ibers (1974*a*)
(V)	Co(II) (OEP) (1-Me-Im)	14·049	17·587	14·331	90	95·20	90	4	$P2_1/n$	0·13§	2019	337	Little & Ibers (1974*b*)

* Abbreviations used are: TPP = $\alpha,\beta,\gamma,\delta$-tetraphenylporphyrin dianion; OEP = 2,3,7,8,12,13,17,18-octaethylporphyrin dianion; pip = piperidine; Im = imidazole; 3-pic = 3-picoline = 3-methylpyridine; 1-Me-Im = 1-methylimidazole = *N*-methylimidazole; OAc^- = acetate.

† Conventional agreement index based on F.

‡ NO = number of observations; NV = number of variables.

§ At least 3 of the 8 ethyl groups are disordered.

Structural results and intercomparisons

Despite the various experimental difficulties just alluded to, it is possible to use the diverse results on the five structures (Table 2) to reach conclusions about the expected shift of the histidine residue relative to the mean plane of the porphyrin upon oxygenation of coboglobin. To estimate such a shift the only structural results needed are those for complexes of type (V), representing the deoxygenated state, and complexes of type (II), approximating the oxygenated state, if one is willing to assume that (1) the distance from the imidazole ring to the plane of the porphyrin in complexes of type (II) is representative of this same distance in the hypothetical Co(PP–IX) (histidine) (O_2) molecule and that (2) the change in porphyrin from PP–IX to OEP or TPP has a minimal effect on the stereochemistry about the cobalt atom. With these assumptions we may conclude (see below) that the shift of the proximal histidine residue relative to the mean plane of the porphyrin upon oxygenation is about 0·38 Å, perhaps one-half that postulated for hemoglobin.

The existence of structural results for the five cobalt porphyrins, rather than just for complexes(II) and (V), enables us to justify these assumptions and hence confirm this conclusion.

In order to assess the minimal approach of an imidazole ring of a histidine residue to the porphyrin plane we first examine the two Co(III) structures, (I) and (II). As pointed out by Scheidt, Cunningham & Hoard (1973) the Co–N(ax) distance of 2·060 Å in Co(TPP)–$(pip)_2^+$ is relatively long. One may ascribe this long distance to steric interactions, especially between the hydrogen atoms on the saturated piperidine molecule and the atoms of the porphyrin core. Because estimates for the van der Waals radius of hydrogen vary, it is not possible to give a rigorous value for the distance of closest approach based on such a radius. Thus there appears to be no satisfactory explanation in steric terms for the Fe–N(ax) distance of 2·127 Å in Fe(TPP) $(pip)_2$ (Radonovich, Bloom & Hoard, 1972) *vs.* the distance of 2·060 Å in the isoelectronic Co(III) complex (I). Rather it seems more fruitful to take the Co–NH_3 distance of 1·936–1·968 Å in $Co(NH_3)_6^{3+}$ as a basis for comparison (Kime & Ibers, 1969; Iwata & Saito, 1973). Since the nitrogen atom of the NH_3 ligand is tetrahedrally coordinated while that of the imidazole ligand is trigonally coordinated we estimate that the radius of the former is some 0·06 Å larger than that of the latter. Such an estimate, based on comparable differences between the sp^2/sp^3 radius of the carbon atom, ignores possible differences in pK_a, π-bonding abilities, and other electronic properties of the two bases (Little & Ibers, 1973*a*). The fact, then, that the Co–N(ax) distance is 1·93 Å in Co(TPP) $(Im)_2^+$, the same as the Co–N(sp^3) distance in $Co(NH_3)_6^{3+}$, strongly suggests that there is a steric interaction between the imidazole group and the porphyrin ring. This steric interaction further manifests itself in the staggering of the imidazole plane relative to the N_4 square of the porphyrin.† Thus, we believe that 1·93 Å represents a minimal approach distance of an imidazole ring to the porphyrin plane.

Comparison of the two high-spin, six-coordinate Co(II) structures, (III) and (IV) allows us to assess approximately the effect of the porphyrin on the Co–N(ax) distance, even though neither of these structures bears any direct relation to the prosthetic group in coboglobin. The extension of the Co–N(ax) distance in these high-spin Co(II) systems results from partial oc-

† But see Ferguson, Robinson & Rodley (1972) for an argument that this staggering is electronic in origin.

Table 2. *Some details on inner coordination geometries of cobalt-base-porphyrin complexes*

		Co–N_{por} (Å)		Co–N_{ax} (Å)		Imposed
	Compound*	Ind.	Ave.	Ind.	Ave.	Symmetry
(I)	Co(TPP) $(pip)_2^+$	1·983 (3), 1·974 (3)	1·979 (5)	2·060 (3)	2·060 (3)	$\bar{1}$†
(II)	Co(TPP) $(Im)_2^+$	1·953 (14), 1·983 (15), 1·985 (14), 2·009 (15)	1·983 (11)	1·945 (16), 1·906 (15)	1·926 (20)	$\bar{1}$
(III)	Co(TPP) $(pip)_2$	1·987 (2), 1·987 (2)	1·987 (2)	2·436 (2)	2·436 (2)	$\bar{1}$
(IV)	Co(OEP) $(3\text{-pic})_2$	1·991 (1), 1·993 (1)	1·992 (1)	2·386 (2)	2·386 (2)	$\bar{1}$
(V)	Co(OEP) (1-Me-Im)	1·94 (1), 1·97 (1), 1·95 (1), 1·96 (1)	1·96 (1)	2·15 (1)	2·15 (1)	None‡

* See Table 1 for abbreviations used.

† Those complexes with imposed $\bar{1}$ symmetry necessarily have the Co atom in the mean porphyrin plane. [Compound (II) contains two independent Co(TPP) $(Im)_2^+$ ions in the cell, each with an imposed center of symmetry.]

‡ There are no constraints on the CoN_5 geometry of compound (V). The distance of the Co atom to the weighted N_4 plane of the porphyrin is 0·13 Å. The distance of the Co atom to the weighted mean plane of the porphyrin core (excluding ethyl groups) is 0·16 Å.

cupancy of the d_{z^2} orbitals directed toward the axial ligands. The porphyrin core has rigid geometrical constraints, and thus there is a considerable expansion of Co–N distances in the axial direction, the only direction not subject to such constraints. For our purposes it is sufficient to note that in structures (III) and (IV) the Co–N(ax) distances are not sterically controlled. Again if we take the sp^2/sp^3 radius change of 0·06 Å we find that the Co–N(ax) distance of 2·386 Å for Co(OEP) (3-pic)$_2$ is in excellent agreement with that of 2·436 Å for Co(TPP)(pip)$_2$. This suggests that the effect of a change from OEP to TPP is small. The equilibrium constants for the binding of NO to various iron porphyrins also bear on the question of the effects of change of porphyrin. It is known that the pressure for half nitrosylation is 0·18 torr for Fe(PP–IX) (pip)$_2$, while it is 0·40 torr for Fe(TPP) (pip)$_2$ (Stynes, Stynes, Ibers & James, 1973). In view of the presumed sensitivity of such binding constants to small electronic changes at the metal, this very small change in the pressure for half nitrosylation again suggests little effect on the stereochemistry of the metal atom upon change of porphyrin.

Having dismissed the effects of a change in porphyrin we are now in a position to examine complex (V), Co(OEP) (1–Me–Im), which provides the key to the discussion of the changes that occur upon oxygenation of coboglobin. Complex (V) is the model for the Co(PP–IX) prosthetic group which binds O_2 in coboglobin. In the model for Co(PP–IX) (histidine) (O_2), as we noted above, the imidazole ring of the histidine cannot approach closer than 1·93 Å to the porphyrin plane. In Co(OEP) (1–me–Im) (Complex V) the Co–N(ax) distance is 2·15 Å and the Co atom is 0·16 Å out of the mean plane of the porphyrin ring. Hence the N(of Im)-to-porphyrin plane distance is 2·30 Å. Consequently, the *maximum* movement of the imidazole group toward the mean porphyrin plane on oxygenation is 2·31–1·93 = 0·38 Å. Such a movement may indeed be somewhat less if the imidazole ligand, which is *trans* to the presumed O_2^- species, exhibits a smaller *trans* effect than O_2^-.

Based on the present estimate for the Co system and those of others for the Fe system it appears that the 'trigger' movement for oxygenation of coboglobin is substantially less than that for hemoglobin, in accordance with the prediction of Hoffman, Spilburg & Petering (1971). If one accepts that the movement of the proximal histidine group perhaps 0·3 to 0·35 Å toward the mean plane of the porphyrin ring upon oxygenation of coboglobin has an important effect on heme–heme interactions, then it is difficult to ignore the concomitant changes which occur in the movements of the atoms, particularly the peripheral atoms, of the porphyrin core itself. Upon oxygenation the distinctly non-planar porphyrin core of complex (V) moves toward the essentially planar core of complex (II). This is illustrated in Figs. 1 and 2. Movements of the peripheral atoms are, relative to the mean porphyrin plane, of the order of 0·1 to 0·15 Å. Keeping in mind that by analogy with hemoglobin one expects the atoms of the porphyrin core to be in close contact with perhaps 50 atoms of the flexible globin, we suggest that the cumulative effects of the movements of these atoms may be as important as the movement of the single, proximal histidine residue.

Perutz (1970*a*, *b*) has put forth a detailed structural model for the overall mechanism involved in the oxygenation of hemoglobin, and in doing so he has utilized the extensive structural data that exist on the oxygenated and deoxygenated forms of hemoglobin. It is impossible to pursue this course for coboglobin, as no structural data are available. It is clearly of great import to know the relationship of the quaternary and tertiary structures of oxygenated and deoxygenated coboglobin.

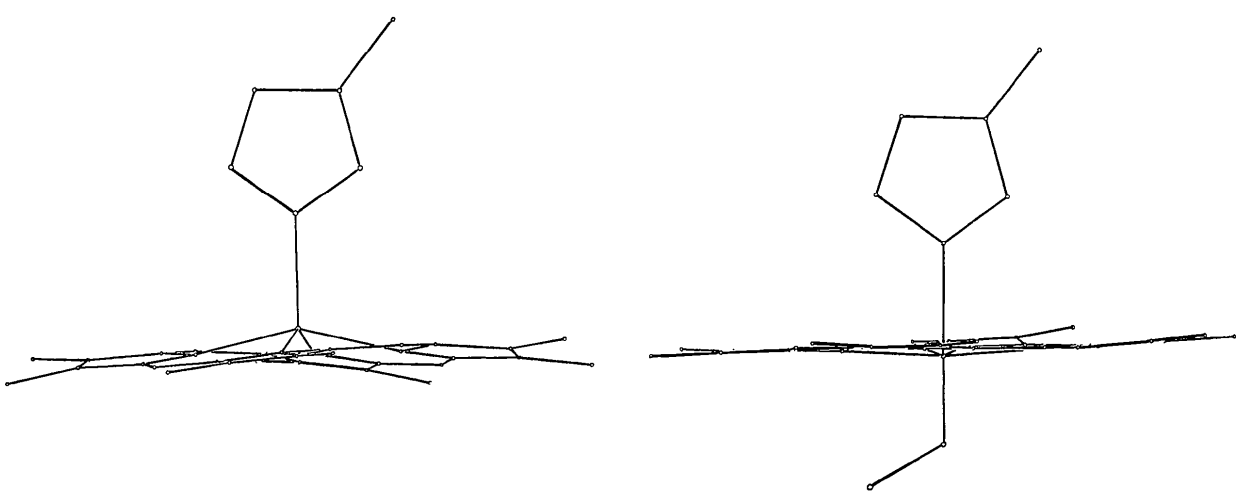

Fig. 1. Representation of the conformational changes of the porphyrin skeleton upon oxygenation. The mean plane of the porphyrin has been kept fixed to illustrate the relative movement of the imidazole ring of the proximal histidine.

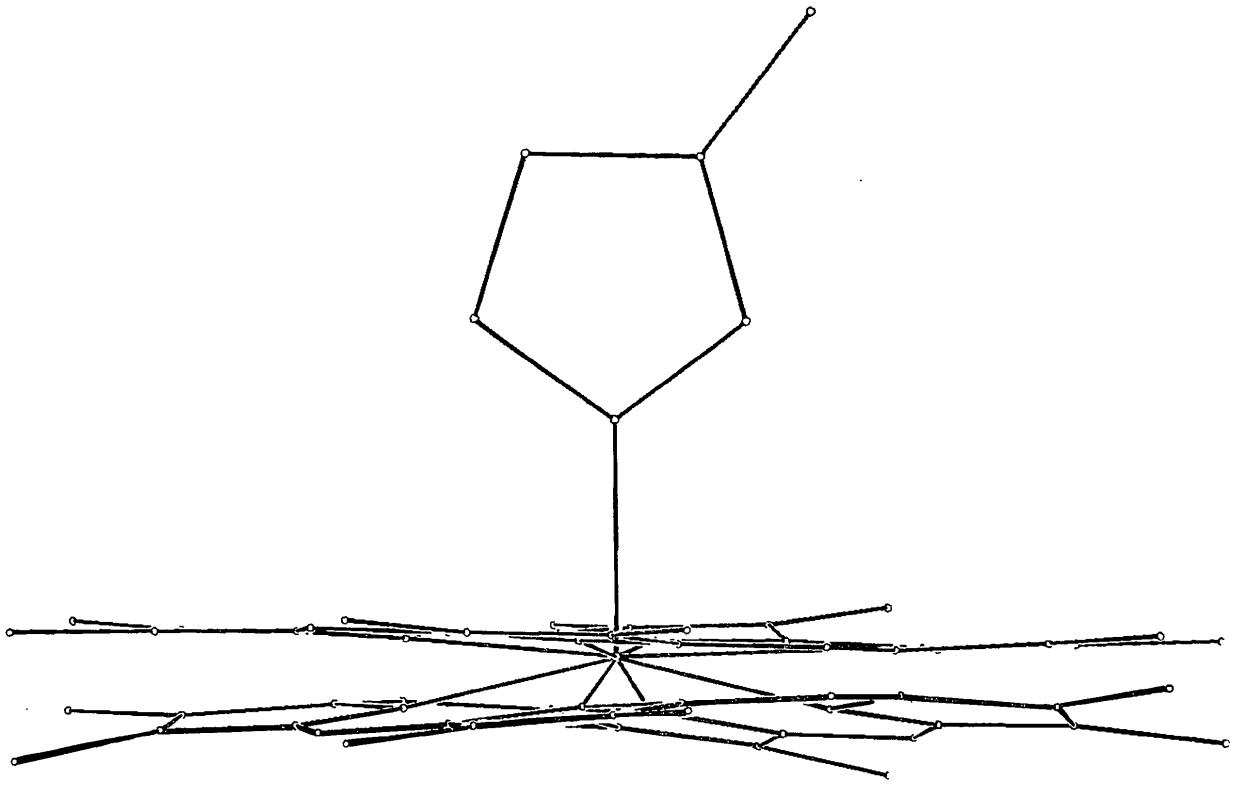

Fig. 2. The two conformations of the porphyrin skeleton illustrated in Fig. 1 superimposed to illustrate the movement of the porphyrin relative to a fixed globin framework.

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