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## Model compounds for the T state of hemoglobin

(cobalt-substituted hemoproteins/cooperativity/hemoproteins/myoglobin/oxygen binding)

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Contributed by James P. Collman, November 21, 1977

**ABSTRACT** O<sub>2</sub> binding to a series of ferrous and cobaltous "picket fence" porphyrins is reported. *N*-Methylimidazole and covalently attached imidazoles give O<sub>2</sub> binding to ferrous porphyrins with  $\Delta H^\circ = -16.2$  kcal/mol ( $-67.7$  kJ/mol) and  $\Delta S^\circ = -40$  eu (standard state, 1 atmosphere O<sub>2</sub>). Similar studies with cobaltous porphyrins yield  $\Delta H^\circ = -12.8$  kcal/mol ( $-53.5$  kJ/mol) and  $\Delta S^\circ = -39$  eu. These values match well those of myoglobin and isolated subunits of hemoglobin and their cobalt reconstituted analogues. 1,2-Dimethylimidazole has been successfully used to mimic the presumed restraint of T state hemoglobin. In direct analogy to the decreased cooperativity shown by cobalt-substituted hemoglobin, model cobalt porphyrins show a smaller decrease in O<sub>2</sub> affinity than the analogous iron porphyrins when the axial base is hindered. Thermodynamic data are presented. The molecular mechanism of cooperativity in hemoglobin is discussed.

The mechanism of cooperativity in hemoglobin (Hb) is of continuing interest (1-3). Model porphyrins capable of reversible oxygen binding have contributed to a fuller understanding of myoglobin (Mb) and other monomeric hemoproteins (4-6). Very little work, however, has appeared on models for the low affinity, T state, of Hb. The effect of steric restraint built into the porphyrin (7, 8) or the axial base (9-13) has not been fully explored. We wish to report a full study of O<sub>2</sub> binding to both iron and cobalt "picket fence" porphyrins with hindered and unhindered imidazoles. In addition, we make here a preliminary report on the synthesis and characterization of "picket fence" porphyrins with covalently attached axial bases.

### MATERIALS AND METHODS

*meso*-Tetra( $\alpha,\alpha,\alpha,\alpha$ -*o*-pivalamidophenyl)porphyrin (H<sub>2</sub>T-PivPP) was prepared as described (4). Cobalt was inserted by use of anhydrous CoCl<sub>2</sub> in a solution of tetrahydrofuran with a trace of 2,6-lutidine at 50° under N<sub>2</sub>. Further purification consisted of chromatographic separation on Woelm neutral alumina. Details are presented elsewhere (14). *meso*-Tri( $\alpha,\alpha,\alpha$ -*o*-pivalamidophenyl)- $\beta$ -*o*-3-(*N*-imidazolyl)propylamidophenylporphyrin [Piv<sub>3</sub>(4CImp)Por] and *meso*-tri( $\alpha,\alpha,\alpha$ -*o*-pivalamidophenyl)- $\beta$ -*o*-4-(*N*-imidazolyl)butylamidophenylporphyrin [Piv<sub>3</sub>(5CImp)Por] were prepared by the reaction of 4-(*N*-imidazolyl)butyl chloride and 5-(*N*-imidazolyl)valeryl chloride, respectively, with *meso*-tri( $\alpha,\alpha,\alpha$ -*o*-pivalamidophenyl)- $\beta$ -*o*-aminophenylporphyrin. Extreme care must be taken to prevent exposure of the porphyrins to O<sub>2</sub> and light due to singlet molecular oxygen production catalyzed by the metal-free porphyrin (15) and trapping by the attached imidazole (16). Chemical structures of these porphyrins are

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shown in Fig. 1. The *meso*-tri( $\alpha,\alpha,\alpha$ -*o*-pivalamidophenyl)- $\beta$ -*o*-aminophenylporphyrin was prepared by the reaction of the readily available *meso*-tetra( $\alpha,\alpha,\alpha,\alpha$ -*o*-aminophenyl)porphyrin (4) with limited amounts of pivaloyl chloride [(CH<sub>3</sub>)<sub>3</sub>COCl] and isolated by column chromatography. Iron was easily inserted under inert atmosphere by use of anhydrous FeBr<sub>2</sub> in 1:1 benzene/tetrahydrofuran with a trace of 2,6-lutidine, followed by chromatographic filtration through a short plug of alumina. All intermediates and porphyrins described above have been well characterized by elemental analysis, UV/visible, nuclear magnetic resonance, magnetic circular dichroism, and Mössbauer spectroscopies, and purities demonstrated with high-pressure liquid chromatography. Details of these syntheses will be presented elsewhere.

All solvents were distilled and stored under N<sub>2</sub>: toluene from Na metal, tetrahydrofuran from CaH<sub>2</sub>, *N*-methylimidazole (NMeIm) vacuum-distilled from KOH, 1,2-dimethylimidazole (Me<sub>2</sub>Im) vacuum-distilled from Na metal, and 2,6-lutidine passed through alumina and distilled from BF<sub>3</sub>·Et<sub>2</sub>O. Anhydrous powdered CoCl<sub>2</sub> (Alfa) was heated at 100° under reduced pressure for 30 min before use. Anhydrous FeBr<sub>2</sub> was prepared in the usual fashion (17). All experimental operations requiring an inert atmosphere were carried out in a Vacuum Atmospheres "Dri-Lab" under N<sub>2</sub>.

Oxygen binding equilibria were determined with an apparatus consisting of a 10-mm cuvette with gas inlet and outlet tubes attached to a pair of calibrated Matheson 600 rotameters which mixed pure N<sub>2</sub> with pure O<sub>2</sub> or with premixed O<sub>2</sub> in N<sub>2</sub> mixtures (Liquid Carbonic certified gas mixtures, 5.01 ± 0.20% and 0.140 ± 0.006% O<sub>2</sub> in N<sub>2</sub>). Further details are presented elsewhere (14). Concentrations of metalloporphyrins in all cases were ~50 μM. For CoTPivPP, concentrations of NMeIm and Me<sub>2</sub>Im were chosen to provide >90% five-coordinate cobalt porphyrins, based on equilibrium constants for axial base ligation determined under N<sub>2</sub> by standard spectrophotometric techniques (18).

### RESULTS

Because oxygen binding at temperatures above 0° is incomplete even at 760 torr (101 MPa) O<sub>2</sub>, a mathematical approach that does not require knowledge of the spectrum of the pure oxy-

Abbreviations: Hb, hemoglobin; Mb, myoglobin; CoHb, apohemoglobin reconstituted with Co protoporphyrinate IX; CoMb, apomyoglobin reconstituted with Co protoporphyrinate IX; Me<sub>2</sub>Im, 1,2-dimethylimidazole; NMeIm, 1-methylimidazole; TPivPP, *meso*-tetra( $\alpha,\alpha,\alpha,\alpha$ -*o*-pivalamidophenyl)porphyrinate; Piv<sub>3</sub>(4CImp)Por, *meso*-tri( $\alpha,\alpha,\alpha$ -*o*-pivalamidophenyl)- $\beta$ -*o*-3-(*N*-imidazolyl)propylamidophenylporphyrinate; Piv<sub>3</sub>(5CImp)Por, *meso*-tri( $\alpha,\alpha,\alpha$ -*o*-pivalamidophenyl)- $\beta$ -*o*-4-(*N*-imidazolyl)butylamidophenylporphyrinate; P<sub>1/2</sub>, O<sub>2</sub> pressure at half-saturation.

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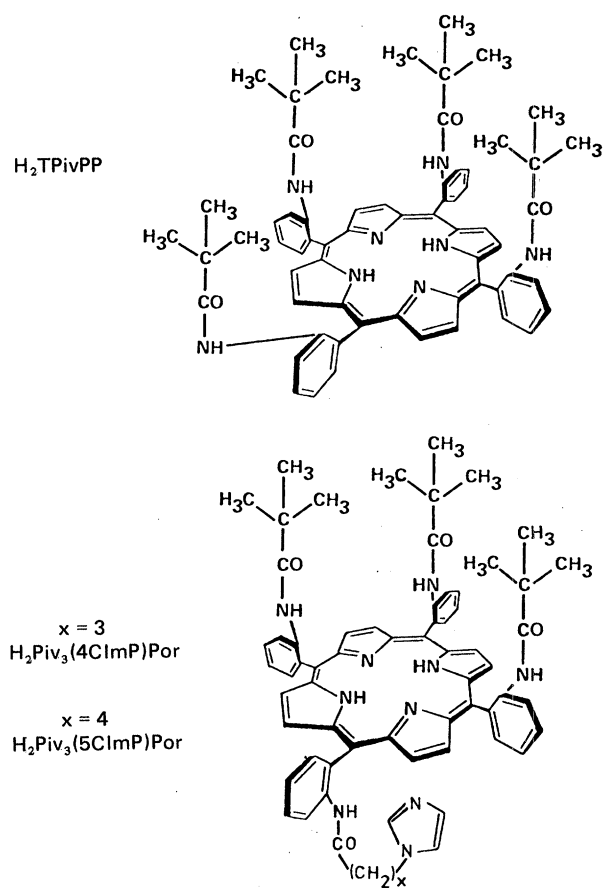


FIG. 1. "Picket fence" porphyrins.

genated complex was used. This approach is a modification of one by Drago (18), who has shown:

$$K^{-1} = P_{O_2} \left[ \frac{[MP]_T b \Delta \epsilon}{\Delta A} - 1 \right]$$

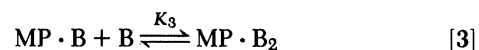
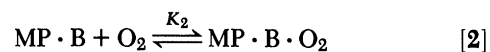
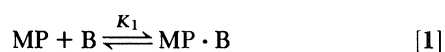
where  $K$  is the equilibrium constant for  $O_2$  binding to the  $Fe^{II}$  or  $Co^{II}$  porphyrin complex,  $[MP]_T$  is the total metalloporphyrin concentration,  $b$  is the pathlength of the cell,  $\Delta A$  is the difference between the absorbance of the solution at oxygen pressure  $P_{O_2}$  and the absorbance of the solution in the absence of oxygen, and  $\Delta \epsilon$  is the difference between molar extinction coefficients of the oxy and the deoxy complexes at the wavelength chosen. Rearranging the equation gives:

$$P_{O_2} = [MP]_T b \Delta \epsilon (P_{O_2} / \Delta A) - K^{-1}$$

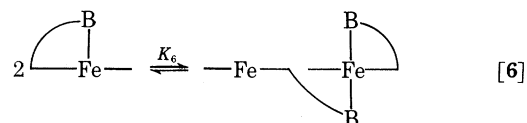
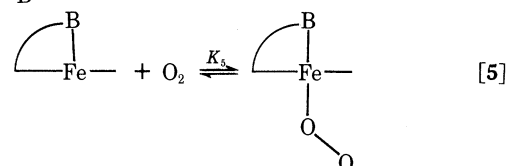
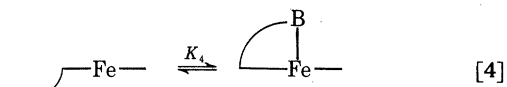
from which it is clear that, since  $[MP]_T b \Delta \epsilon$  is a constant, a plot of  $P_{O_2}$  against  $P_{O_2} / \Delta A$  should be a straight line with slope  $[MP]_T b \Delta \epsilon$  and intercept  $-K^{-1}$ .

Sets of spectra were recorded at each temperature over a wide range of  $P_{O_2}$ , and plots of  $P_{O_2}$  against  $P_{O_2} / \Delta A$  were constructed for two to four wavelengths. Straight lines were then computer fit by a linear least squares program, and equilibrium constants were determined from the intercepts (the standard deviation of these intercept values was generally less than 5%). In all cases, good isobestic points were observed.

With the simple metalloporphyrins, those without appended axial base, the major solution equilibria that must be considered between the metalloporphyrins (MP) and axial bases (B) and  $O_2$  are:



Since the reaction of interest in this study is the oxygenation reaction [2], solution conditions must be chosen under which the predominant species is  $MP \cdot B$  prior to addition of oxygen. With  $Co^{II}TPivPP$  in toluene solution under  $N_2$  at  $20^\circ C$ ,  $K_1$  is found to be  $\sim 1.7 \times 10^4 M^{-1}$  for  $NMeIm$  and  $\sim 1.4 \times 10^3 M^{-1}$  for  $Me_2Im$ . With  $Fe^{II}TPivPP$ ,  $K_1$  is  $\sim 3.7 \times 10^4 M^{-1}$  for  $Me_2Im$  under the same conditions. In no case was any evidence found for formation of six-coordinate  $MP \cdot B_2$  (reaction 3), and  $K_3$  must therefore be  $< 10$ . These observations are in keeping with previous work on the binding of hindered imidazoles to cobalt porphyrins (9) and the binding of hindered imidazoles to iron porphyrins (11–13). In these cases, since  $K_1 \gg K_3$ , solutions in which the predominant species is the desired  $MPivPP(B)$  can be prepared by judicious use of excess B. For ferrous porphyrins with unhindered imidazoles,  $K_3 > K_1$  (10, 11, 13), which precludes the ability to prepare, in solution,  $FeTPivPP(B)$ . This is the *raison d'être* of appended axial bases. With the "tailed" porphyrins, those with appended axial bases, the major equilibria are best presented in schematic form:



These are, of course, the close analogues of  $K_1$ ,  $K_2$ , and  $K_3$  of the simple metalloporphyrins. In the "tailed" porphyrins described above,  $K_4$  is very large, i.e., practically no four-coordinate iron is found in solution over the temperature range studied. The dimerization equilibrium of  $K_6$  has been well noted before (8), and is favored by low temperatures and high concentrations. In the case of our "tailed" porphyrins, such dimerization becomes appreciable at  $< -25^\circ$  and  $> 10^{-3} M$ ; however, in the temperature and concentration ranges used in these studies, this dimerization is negligible.

By the techniques already described, equilibrium constants  $K_2$  and  $K_4$  were obtained.  $\Delta H^\circ$  and  $\Delta S^\circ$  were then derived from linear least square fits to standard van't Hoff plots over as wide a temperature range as possible (generally  $> 40^\circ$ ). The data used in these plots follow as pairs of temperature ( $^\circ C$ ) and  $P_{1/2}$  (half-saturation pressure, in torr) from studies in toluene solutions:  $FePiv_3(5ClmP)Por$ , (35.8°, 1.70), (26.6°, 0.86), (25.2°, 0.60), (19.0°, 0.262), (13.0°, 0.179), (6.3°, 0.094), (1.5°, 0.0516), and ( $-5.2^\circ$ , 0.0346);  $FePiv_3(4ClmP)Por$ , (20.2°, 0.376), (19.1°, 0.328), (13.4°, 0.177), (8.5°, 0.128), (3.3°, 0.0744), (0.2°, 0.048), ( $-3.0^\circ$ , 0.0336), ( $-3.6^\circ$ , 0.0278), and ( $-3.9^\circ$ , 0.0262);  $FeTPivPP(Me_2Im)$ , (40.3°, 96.8), (34.4°, 85.4), (24.8°, 41.6), (24.1°, 34.6), (5.8°, 6.42), ( $-7.3^\circ$ , 1.90), and ( $-10.1^\circ$ , 1.49);  $CoTPivPP(NMeIm)$ , (27.0°, 179), (24.9°, 164), (24.7°, 150), (8.2°, 47.2), ( $-2.8^\circ$ , 18.7), ( $-12.6^\circ$ , 9.53), and ( $-12.8^\circ$ , 6.98); and

Table 1. Thermodynamic values for O<sub>2</sub> binding to "R" and "T" state models

System	P <sub>1/2</sub> (25°), torr*	ΔH°, kcal/mol†	ΔS°, eu‡
FeTPivPP (NMeIm) solid state§	0.49	-15.6 ± 0.2	-38 ± 1
FePiv <sub>3</sub> (4CImP)Por toluene solution	0.60	-16.8 ± 0.5	-42 ± 2
FePiv <sub>3</sub> (5CImP)Por toluene solution	0.58	-16.3 ± 0.8	-40 ± 3
FeTPivPP(Me <sub>2</sub> Im) toluene solution	38	-14.3 ± 0.5	-42 ± 2
CoTPivPP(NMeIm) solid state	61	-13.3 ± 0.9	-40 ± 3
toluene solution	140	-12.2 ± 0.3	-38 ± 1
CoTPivPP(Me <sub>2</sub> Im) toluene solution	900	-11.8 ± 0.4	-40 ± 2

\* Interpolated from ΔH° and ΔS°; estimated errors ±5%.

† Error limits given are the standard deviations of van't Hoff plots.

‡ Standard state, 1 atmosphere O<sub>2</sub>.

§ Ref. 19.

CoTPivPP(Me<sub>2</sub>Im), (24.9°, 960), (24.5°, 777), (4.9°, 239), (-12.3°, 52.4), (-13.8, 50.9), and (-15.8, 33.5). These data are summarized, along with comparable data from hemoprotein systems, in Tables 1, 2, and 3. A graphic compilation of these data is presented in Fig. 2.

## DISCUSSION

The data presented in Table 1 reveal several interesting points. Clearly, the oxygen binding by the iron porphyrins with unhindered axial bases (e.g., NMeIm or our "tailed" imidazoles) are nearly identical: overall, one may say that P<sub>1/2</sub> at 25° ≈ 0.6 torr and that ΔH° = -16.2 ± 0.6 kcal/mol (-67.7 ± 2.5 kJ/mol) and ΔS° = -40 ± 2 eu (standard state 1 atmosphere O<sub>2</sub>) for all cases. These values compare well with the ΔH° of O<sub>2</sub> binding by myoglobins and isolated chain Hb, which range from -16.4 to -13.2 kcal/mol and the associated ΔS°, which range between -41 and -32 eu (27) (standard state 1 atmosphere). As noted before with the solid state binding of O<sub>2</sub> by FeTPivPP(NMeIm) (19), the intrinsic O<sub>2</sub> affinity of these simple porphyrins is at least as good as that of the native hemoproteins. These model systems do not have the possibility of hydrogen bonding or electron pair donation to the bound

Table 2. O<sub>2</sub> affinities of iron porphyrins and hemoproteins

System	Physical state	P <sub>1/2</sub> (25°), torr	Ref.
Mb, sperm whale Hb	pH 8.5	0.70	20
α chains	pH 7.5,	0.63	21, 22
β chains	0.1 M phosphate	0.25	
Hb (human, "R")*	Various†	0.15-1.5†	23
FePiv <sub>3</sub> (4CImP)Por	Toluene solution	0.60	This work
FePiv <sub>3</sub> (5CImP)Por	Toluene solution	0.58	This work
FeTPivPP(NMeIm)	Solid state	0.49	19
Hb (human, "T")*	Various†	9-160‡	23
FeTPivPP(Me <sub>2</sub> Im)	Toluene solution	38	This work

\* These are actually the first and fourth intrinsic P<sub>1/2</sub> values.

† Imai's conditions included various combinations of 0.1 M NaCl, 0.1 M phosphate, 2 mM inositol hexaphosphate, 2 mM 2,3-diphosphoglycerate, all at pH 7.4.

‡ The ratio of these "R" and "T" affinities also varied as a function of conditions, from around 40 to 500.

Table 3. O<sub>2</sub> affinities of cobalt porphyrins and cobalt hemoproteins

System	Physical state	P <sub>1/2</sub> (15°), torr	Ref.
CoMb (sperm whale)	0.1 M phosphate, pH 7	30	24, 25
CoHb (isolated chains)	0.1 M phosphate, pH 7.4	25	26
CoHb (human, "R")*	Various†	16-180‡	23
CoTPivPP(NMeIm)	Solid state Toluene solution	28 70	This work This work
CoHb (human, "T")*	Various†	120-500‡	23
CoTPivPP(Me <sub>2</sub> Im)	Toluene solution	450	This work

\* These are actually the first and fourth intrinsic P<sub>1/2</sub> values.

† Imai's conditions included various combinations of 0.1 M NaCl, 0.1 M phosphate, 2 mM inositol hexaphosphate, 2 mM 2,3-diphosphoglycerate, all at pH 7.4.

‡ The ratio of these "R" and "T" affinities also varied as a function of conditions from around 2 to 16.

oxygen, nor is the binding pocket particularly polar, nor is it shaped to fit the bound O<sub>2</sub>, nor does it have any other particularly unusual characteristics (4). To the extent that these statements are true, we may then view the oxygen affinities of both model systems and relaxed hemoproteins (e.g., Mb, isolated chain Hb, and R state Hb) as originating solely from the ferrous porphyrin imidazole system. In contrast, the carbon monoxide affinities are much lower in most hemoproteins than in the models due to the steric constraints of the hemoprotein binding pocket and the biological necessity to decrease the CO affinity relative to O<sub>2</sub> (28).

The cobalt porphyrins (Table 1) provide strong confirmation of the simple nature of O<sub>2</sub> binding. The models with an unhindered imidazole (i.e., NMeIm) have P<sub>1/2</sub> values virtually identical to those of the reconstituted cobalt hemoproteins (Table 3). In addition, the ΔH° and ΔS° of CoMb also compare well: -12.8 ± 0.6 kcal/mol and -39 ± 1 eu for the models compared to a range of -13.3 to -11.3 kcal/mol for ΔH° and -40 to -33 eu for ΔS° (24, 25) (standard state 1 atmosphere). In line with these ΔS° values is that predicted on statistical mechanical grounds (19) due to the loss of translational and rotational entropy of bound O<sub>2</sub>. A note should be made in comparing these studies with previous cobalt porphyrins.

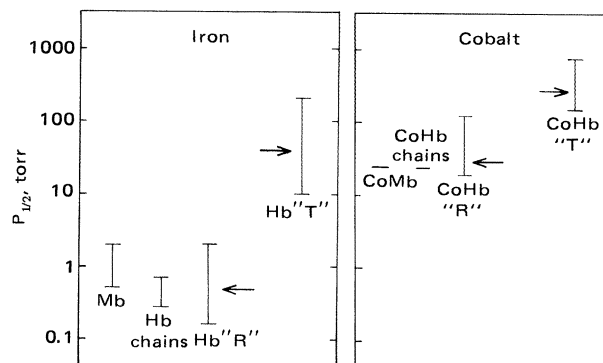


FIG. 2. Graphic compilation of O<sub>2</sub> affinities of hemoproteins and "picket fence" porphyrins. Model porphyrin affinities are shown by arrows; full numerical data appear in Tables 1, 2, and 3.

Earlier measurements of  $P_{1/2}$  of simple cobalt porphyrins such as cobalt protoporphyrin IX dimethyl ester-(NMeIm) (29), cobalt(*p*-methoxyphenyl)porphyrin-(NMeIm) (9, 30), and cobalt tetratolylporphyrins with appended bases (31) showed very low affinities, 300 times worse than our models or the hemoproteins. This appears to be due to a selective solvation of the simple flat porphyrins favoring their deoxy form. A full discussion has already been made (14).

A major point contained in Tables 2 and 3 concerns the T state of Hb and CoHb. Within the confines of the Hoard-Perutz stereochemical mechanism for cooperativity (1), Hb is viewed as having two alternative quaternary structures: the liganded, R state, whose  $O_2$  affinity is essentially that of the isolated subunits, and the deoxy, T state, whose  $O_2$  affinity is diminished. The lessened affinity of the T state is presumed to be caused by *constraint* of the proximal histidine to its unliganded equilibrium position in which the Fe is  $\sim 0.5 \text{ \AA}$  out of the mean porphyrin plane. Upon ligation of  $O_2$  (or other small molecules) to the T state form, a tension develops as the iron atom moves into the mean porphyrin plane, attempting to drag the constrained proximal histidine with it. This T quaternary state is stabilized by direct salt bridges and hydrophobic contacts between the subunits, as well as by indirect bonds between them mediated by solvent ions.

In order to probe the nature of this steric restraint experimentally, we have turned to sterically hindered axial bases. In the sense that the 2-methyl group of  $Me_2Im$  provides restraint to the motion of this axial base towards the porphyrin upon oxygenation, FeTPivPP( $Me_2Im$ ) and CoTPivPP( $Me_2Im$ ) are models for the T form of Hb and CoHb. The presence of such steric restraint with  $Me_2Im$  is obvious from the existence of 5-coordinate ferrous porphyrins (as noted earlier, with unhindered imidazoles  $K_3 > K_1$ ) and is confirmed by crystal structures of Fe tetraphenylporphyrin(2-methylimidazole) (32), FeTPivPP(2-methylimidazole) (with J. A. Ibers, unpublished data), and Co tetraphenylporphyrin( $Me_2Im$ ) (33).

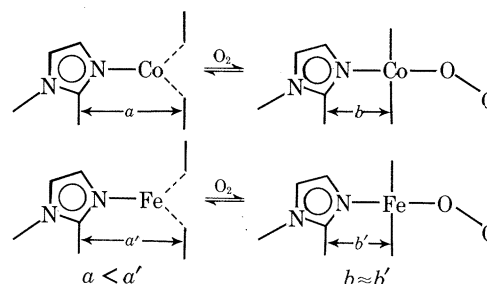
It is interesting that the hindrance offered by the 2-methyl group of  $Me_2Im$  is just that required to reduce  $O_2$  affinities to the level found in T state Hb and CoHb (Tables 2 and 3). In the iron systems, the  $\Delta\Delta G^\circ$  (the difference in  $O_2$  affinities of metalloporphyrins with NMeIm and  $Me_2Im$ ) of our model systems is, at  $25^\circ$ , 2.5 kcal/mol, which is to be compared to  $\Delta G^\circ_{41}$  of Hb (the free energy difference between the first and fourth intrinsic  $O_2$  affinities);  $\Delta G^\circ_{41}$  for Hb at  $25^\circ$  ranges from 2.1 to 3.7 kcal/mol, depending on conditions (23). In the cobalt systems,  $\Delta\Delta G^\circ$  of the model compounds is, at  $15^\circ$ , 0.8 kcal/mol, and  $\Delta G^\circ_{41}$  for CoHb, at  $15^\circ$ , is 0.4–1.6 kcal/mol, depending on conditions (23). This correspondence is happenstance, since more highly restraining bases, such as 2-isopropylimidazole or 1,2,4,5-tetramethylimidazole, should show even lower  $O_2$  affinities. In drawing comparisons between Hb and model compounds, it is important that  $\Delta G^\circ_{41}$  or, equivalently, the intrinsic equilibrium constants of Hb (as differentiated from the Adair constants, which need to be corrected for the statistics of the situation) (27) be used.

The stepwise  $O_2$  affinities of Hb shown in Table 2 have been reported by a large number of researchers (23, 34–37). The data used in this paper, those of Imai *et al.* (23), were chosen for the range of conditions and the comparable CoHb data. Generally, the data from these various groups agree well.

From Table 1, it can be noted that the restraint induced by  $Me_2Im$  in the iron porphyrins is reflected in the enthalpy of  $O_2$  binding, the entropy remaining essentially unchanged. For the cobalt analogues the differences are much smaller, but still appear to be enthalpic. This is as one would expect, since the

$\Delta S^\circ$  of binding is determined (19) almost exclusively by the loss of translational and rotational entropy of the  $O_2$ .

Before this work was completed, we made the prediction (14) that for the same hindered bases, FeTPivPP should show a much greater change in  $O_2$  affinity compared to an unhindered base than would CoTPivPP. Since the metal atom is further out of the mean porphyrin plane for five-coordinate iron(II) than cobalt(II) (32, 33, 38), one expects a greater change in the steric interaction of a bound  $Me_2Im$  for the iron(II) than cobalt(II) upon oxygenation, as illustrated below. That is to say, more



steric interaction between the hindered axial base and the porphyrin has *already* developed in the deoxy form of CoTPivPP than FeTPivPP, decreasing the stability of MTPivPP( $Me_2Im$ ) relative to MTPivPP(NMeIm) more for  $M = Co$  than  $Fe$ . Hence, we expect a greater decrease in  $O_2$  affinity for FeTPivPP (B) than for CoTPivPP(B) in changing B from NMeIm to  $Me_2Im$ . This is completely analogous to the lessened cooperativity shown by CoHb relative to native Hb. Exactly this phenomenon is observed.

This same reasoning argues that even if T state deoxyHb is restrained to its normal deoxy geometry (and hence unstrained) (1), then T state deoxyCoHb *must* be strained relative to R state deoxyCoHb or deoxyCoMb.

## CONCLUSIONS

By use of a series of iron and cobalt "picket fence" porphyrins, we have been able to reproduce the  $O_2$  affinities of Mb, Hb, CoMb, and CoHb. The basic  $O_2$  affinity shown by model ferrous and cobaltous porphyrins is the same as that of unrestrained hemoproteins (e.g., Mb, isolated chain Hb, R state Hb); special interactions between the protein and the bound oxygen are not needed to explain these hemoproteins' oxygen affinities in contrast to those for carbon monoxide (28).

By tailoring the steric interactions between the axial imidazole and the porphyrin we can mimic the decrease in ligand affinity shown in T state Hb. The restraint presumed present in the T form of Hb and CoHb has been well modeled by FeTPivPP( $Me_2Im$ ) and CoTPivPP( $Me_2Im$ ), and provides evidence on a *molecular level* that the Hoard-Perutz mechanism is viable.

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