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Biochemistry

Binding of O_2 and CO to hemes and hemoproteins

(hemoglobin/dioxygen/binding energies/model compounds)

T. G. TRAYLOR AND ALBIN P. BERZINIS

Department of Chemistry, D-006, University of California, San Diego, La Jolla, California 92093

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ABSTRACT **Enthalpies and entropies have been determined for the reversible binding of O_2 and CO to chelated protoheme, a compound having a covalently attached imidazole bound to the iron. The values, based upon 1 atm standard state, are $\Delta H_{O_2} = -14.0$ kcal (1 kcal = 4.18 kJ)/mol, $\Delta S_{O_2} = -35$ eu, $\Delta H_{CO} = -17.5$ kcal/mol, $\Delta S_{CO} = -34$ eu, $\Delta H^{\ddagger}_{O_2} = 21$ kcal/mol (dissociation), and $\Delta H^{\ddagger}_{CO} = 25$ kcal/mol (dissociation). The similarity of these values to those of high-affinity hemoproteins such as isolated hemoglobin chains or R-state hemoglobin ($\Delta H_{O_2} = -13.5$, $\Delta H_{CO} = -17.5$) show that this model compound accurately mimics the dynamic behavior of these hemoproteins, in contrast to the behavior of other, more elaborate, model compounds. The enthalpy of the replacement of O_2 by CO, ΔH_M, is 3.5 kcal/mol, about the same as that of R-state hemoglobin. This result, obtained with the model compound which most resembles the hemoglobin active site, indicates that distal side steric effects in these hemoproteins neither decrease CO affinity nor differentiate between the binding of CO and O_2. Consequences of these findings in the binding of O_2 and CO to hemoproteins are discussed.**

Structural and dynamic studies of hemoproteins have led to three distinct but not necessarily exclusive proposals for the effects of the protein on the reactivity of heme toward O_2 and CO. Two of these postulates, the proximal base tension effect (1, 2) and the proximal imidazole deprotonation effect (3–5), have recently been documented by studies of model compounds (6–11). The third postulate, of a distal side steric effect, has been the subject of some controversy in model compound studies (7, 9).

In hemoproteins studied by x-ray crystallography, the Fe^{II}—CO has the ligand "pushed" off the axis by a nearby histidine or other distal side residue (12–14), in contrast to the observations in simple complexes (15). This effect was postulated to decrease the affinity of CO (16). A corollary suggestion is that Fe^{II}—OO, being bent already, should not suffer this steric effect, and CO binding in the hemoproteins should be impaired relative to that in simple model complexes (7, 16).

In contrast to these predictions, the model compounds chelated protoheme and chelated mesoheme were reported to bind both O_2 and CO in aqueous suspension at 20°C with affinities and kinetics similar to those of the high-affinity proteins R-state hemoglobin and hemoglobin chains (8, 17, 18). A number of other simple model systems have CO affinities similar to those of the chelated hemes (6, 9, 19, 20) although the corresponding studies with O_2 have not been made.

Based upon these observations we suggested that in R-state hemoglobin or isolated hemoglobin chains the protein served only to keep the heme five-coordinated and to prevent oxidation, imparting no special effect on the heme affinity for CO or O_2. Other hemoproteins having lower CO or O_2 affinities were presumed to accomplish this alteration from the standard hemoglobin chain behavior by one of the effects mentioned above (18).

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The more elaborate iron complexes such as cyclophane heme (21, 22), capped heme (23), or picket-fence heme (7, 24, 25), all of which have bulky groups around one face of the heme, either do not mimic the hemoproteins or do so only in part. Picket-fence heme was reported to bind O_2 in toluene or in solid state (7) with an affinity similar to that of chelated heme or R-state hemoglobin but to bind CO "irreversibly" in the solid state (24) and with greatly increased affinity in solution (25). These observations led to the conclusion that model compounds bind CO more strongly than do hemoproteins (24), in contrast to the proposal discussed above.

We now report the thermodynamics of the binding of O_2 and CO (Eqs. 1 and 2) and the replacement reaction (Eq. 3) which are very nearly the same as those of hemoglobin (R-state) or isolated hemoglobin chains and conclude that distal side steric effects on CO or O_2 binding in hemoglobin are not significant.

$$\mathrm{Hm} + \mathrm{O_2(gas)} \underset{k}{\overset{K}{\underset{k'}{\rightleftharpoons}}} \mathrm{Hm{-}O_2} \qquad [1]$$

$$\mathrm{Hm} + \mathrm{CO(gas)} \underset{l}{\overset{L}{\underset{l'}{\rightleftharpoons}}} \mathrm{Hm{-}CO} \qquad [2]$$

$$\mathrm{Hm{-}O_2} + \mathrm{CO(gas)} \overset{M}{\rightleftharpoons} \mathrm{Hm{-}CO} + \mathrm{O_2(gas)} \qquad [3]$$

METHODS

This synthesis and characterization of chelated protoheme (Fig. 1) are described elsewhere (18). Myristyltrimethylammonium bromide (MTAB; Aldrich) was recrystallized twice from water. Buffer solutions used in kinetic studies contained 2% MTAB (wt/wt) and were 0.05 M in sodium phosphate buffer (pH 7.3). CO (Matheson) and O_2 (Matheson) were used as received.

Kinetics were determined with a microprocessor-controlled laser flash photolysis apparatus as described (9, 26). Each kinetic

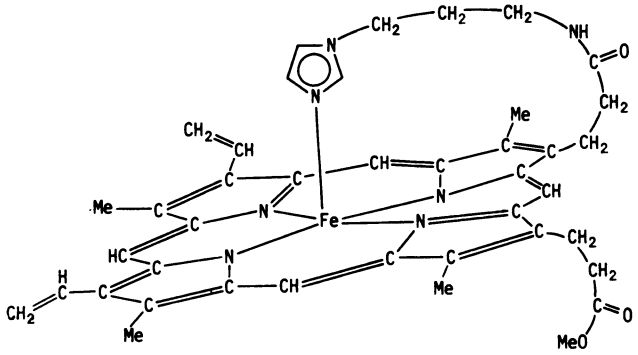


FIG. 1. Chelated protoheme.

Abbreviation: MTAB, myristyltrimethylammonium bromide.

Table 1. Kinetic and equilibrium constants for the reactions of O_2 and CO with chelated protoheme in 2% aqueous MTAB suspension at pH 7.3*

	l'			L		k'			K		
Temp., °C	10^{-6} M^{-1} sec^{-1}	$torr^{-1}$ sec^{-1}	l†, sec^{-1}	10^{-8} M^{-1}	10^{-2} $torr^{-1}$	10^{-7} M^{-1} sec^{-1}	$torr^{-1}$ sec^{-1}	k, sec^{-1}	M^{-1}	$torr^{-1}$	M‡
15.0	2.7	4.0	0.0036	7.4	11	2.0	40	23	8.8	1.8	610
20.0	3.6§	5.0	0.0089	4.0	5.6	2.6	49	47	5.5¶	1.0	560
25.0	4.7	6.0	0.014	3.4	4.3	3.9	65	93	4.2	0.7	610
29.9	6.1	7.1	0.032	1.9	2.2	5.2	80	160	3.3	0.5	440
34.8	9.2	10.1	0.067	1.4	1.5	6.5	94	260	2.5	0.36	420

* Values in $torr^{-1}$ were determined directly and those in M were calculated from appropriate solubilities from ref. 29.
† From ref. 30.
‡ $L_{torr^{-1}}/K_{torr^{-1}} = M$.
§ This rate, determined at seven different CO concentrations, was $3.57 \pm 0.07 \times 10^6$ M sec^{-1}. This is typical of the reliability of l' in this table.
¶ The slope of Eq. 4, $K/l' = 0.155 \pm 0.005$, is typical of K values in this table.

determination consisted of an accumulation of 5 to 10 runs and afforded pseudo-first-order rate constants (excess CO and O_2) with standard deviations $<\pm 1\%$ within a series of flashes and $<\pm 10\%$ between complete experiments.

RESULTS AND DISCUSSION

We can obtain reliable equilibrium constants (K_{O_2}) for O_2 binding for almost all model compounds, protected or not, from the slope of the Gibson equation (Eq. 4) obtained with both CO and O_2 gas pressures near 1 atm (27).

$$\frac{1}{k_{obsd}} = \frac{1}{k} + \frac{K}{l'}\frac{[O_2]}{[CO]}. \qquad [4]$$

Under these conditions, many imidazole–heme–CO–O_2 mixtures are stable to oxidation, but only the chelated hemes, having five-coordinated deoxy forms, permit straightforward kinetic interpretation (17). Plotting the reciprocal of the observed rate of return from heme-O_2 to heme-CO vs. $[O_2]/[CO]$ yields a slope equal to K/l' of Eqs. 1 and 2. Because l' is determined in the same experiment at 0.5 atm (1 atm = 1.013×10^5 pascals) of CO, it can be determined accurately and independently. This method is therefore similar in reliability to a titration, especially when data for each k_{obsd} are accumulated by repeated flash photolysis until the standard deviation of the pseudo-first-order rate constant is ±0.5%. The equilibrium constants L for CO binding were determined by dividing l' from flash photolysis by l, previously obtained by the stopped-flow "CO-trap" method (28).*

The rate and equilibrium constants obtained in this way are reported in both concentration and pressure units in Table 1.

Plots of $\ln [1/P_{1/2}\,(atm)] = \ln K$ or $\ln L$ vs. the reciprocal of temperature (Fig. 2) provide the ΔH and ΔS for the equilibria of Eqs. 1–3. The values so obtained are reported along with kinetic activation energies in Table 2 and are compared with thermodynamic values from similar plots with hemoproteins and with other model systems in Table 3.

A striking fact in Table 3 is the similarity of ΔH_{O_2} (and ΔG_{O_2}) for chelated protoheme and for myoglobin, R-state hemoglobin, and three other hemoproteins. Although there is some disagreement about the hemoglobin data, the value of −14 kcal/mol for the intrinsic enthalpy of binding seems to be widely accepted (ref. 32, p. 244).

A second remarkable observation is that ΔH_{CO} for chelated protoheme also matches that for isolated chains and for R-state hemoglobin (34). This confirms our earlier conclusion, based upon similarities of L at 20°C, that this model accurately mimics R-state hemoglobin in its CO binding. We conclude that chelated protoheme in aqueous suspension mimics R-state hemoglobin (or isolated chains) in both equilibrium and kinetic properties. When this behavior is coupled to the fact that, in this medium, chelated protoheme has the same detailed structure and the same UV–visible spectra (as the deoxy, CO, and O_2 complexes) (18) as does hemoglobin, it becomes clear that this compound is an accurate model for R-state hemoglobin in this solvent system.

The data in Table 3 also afford some evidence regarding proposed influences of the protein on the relative CO and O_2 binding, the M value of Eq. 3. The discovery that the heme-CO geometry in hemoproteins differs (12–14) from that found in

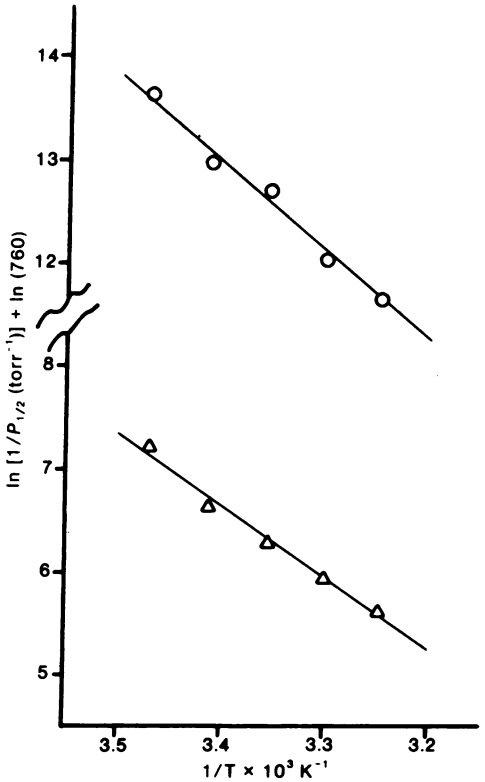


FIG. 2. Plots of the equilibrium constants $\ln (1/P_{1/2})$ for CO (○) and O_2 (△) binding versus the reciprocal of temperature. The data are from Table 1 and are converted to units of atm.

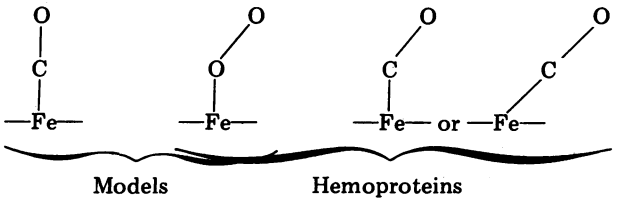
* Although reliable CO titrations for comparison are difficult to obtain because L is so large, competitive titrations of two model compounds for limited CO afford a ratio of binding constants accurately matching the same ratio determined by kinetic methods. That is, $L_1/L_2 = l_1'l_2/l_2'l_1$ in which L_1/L_2 refers to the ratio of binding to Hm_1 and Hm_2 by competitive titration. This confirms the reliability of the kinetic methods.

Table 2. Thermodynamic values for chelated heme reactions with CO and O_2 from Table 1

	Association		Dissociation		Equilibrium			
	$\Delta H^{\ddagger}$	$\Delta S^{\ddagger}$	$\Delta H^{\ddagger}$	$\Delta S^{\ddagger}$	ΔH	ΔS	ΔG_M	ΔH_M
O_2	10.3 ± 0.5	11 ± 1	21 ± .07	21 ± 1	−10.7	−10		
	(7.2)	(−13)			(−14.0 ± 0.9)	(−35)	(3.6)	(3.5)
CO	10.0 ± 0.7	6 ± 1	25	17	−14.5	−10		
	(7.2)	(−17)			(17.5 ± 1.2)	(−34)	(7.4)	

Numbers in parentheses are for l', L, k', and K in units of atm and other values for constants in terms of M. Accordingly, the numbers in parentheses refer to Eqs. 1–3. Where shown, ± is SD.

model Fe—CO systems (15) and that CO stretching frequencies vary considerably in hemoproteins (16)† suggested that steric effects on the distal side, forcing the CO off axis, might reduce its binding constant relative to the unstrained models.



A corollary to this appealing idea, suggested by Caughey (16) for low-affinity hemoproteins and extended to all hemoproteins by Collman *et al.* (24) and others (14b, 35), is that since the O_2 complex is bent in both systems, the distal side steric effect will be unimportant in O_2 binding. Thus, "linear ligands" such as CO would have decreased binding compared to "bent ligands" such as O_2 when placed in hemoproteins. R-State hemoglobin has a bent CO structure in the crystal which is presumed to result in a lowering of the CO but not O_2 affinity when compared to model systems with linear Fe—CO bonds. Tables 2 and 3 reveal ΔH_M (and ΔG_M) for this replacement of 3.5 kcal/mol for chelated protoheme and 4.0–5 kcal/mol (ref. 32, p. 263) for hemoglobin. The model has, if anything, a lower replacement energy. It is therefore unnecessary to postulate a distal side steric effect that differentiates O_2 from CO binding in hemoglobin.

Further evidence against this CO vs. O_2 differentiation is seen in the k'/l' association rate ratio. Presumably, steric effects would decrease l' more than k', thus increasing this ratio in the protein. The k'/l' ratio for the high-CO-affinity hemoproteins *Chironomus* hemoglobin, leghemoglobin, and R-state hemoglobin chains is 8–14 (ref. 31, p. 259) (35, 36) compared to 7.2 for chelated protoheme. This seems to indicate little contribution from steric effects. However, other phenomena such as imidazole deprotonation might also affect this ratio.

Other similarities of chelated protoheme and the hemoproteins are seen in the activation enthalpies. The $\Delta H^{\ddagger}_{\text{dissociation}}$ for chelated protoheme of 21 kcal/mol for O_2 and 25 kcal/mol for CO compare well with reported values of 19.5 and 23.4 kcal/mol for hemoglobin (ref. 32, p. 259) and 21 and 24 kcal/mol for hemoglobin α chains (ref. 32, p. 313).

The R- and T-state model systems studied by Brault and Rougee (6), Collman *et al.* (7), and in our laboratories (8, 9) reveal no differentiation of CO from O_2 by the protein during the R- to T-state change. Both the equilibria and kinetics of the T state of hemoglobin can be duplicated by model systems having proximal side iron–imidazole strain. We therefore conclude that the distal side steric effect is also unimportant in O_2 and CO binding to T-state hemoglobin as compared to model compounds for T-state hemoglobin.

The recent suggestions (14b, 23, 35, 36) that CO is bound more strongly to model compounds than to hemoproteins is investigated by comparing the available equilibrium data (Table 4).

All the model systems, in aqueous medium at pH 7, bind CO with about the same affinity as do R-state hemoglobin, leghemoglobin, and *Chironomus* hemoglobin. The possibility that MTAB or cetyltrimethylammonium bromide has some special effect on CO binding or that the deoxy form of these "flat" hemes suffers some special solvent effect (25) seems remote because chelated mesoheme in suspension and microperoxidase in aqueous solution have the same affinities at pH 7. Changing the solvent from MTAB or water to benzene lowers the $P^{CO}_{1/2}$ by about a factor of 3. Therefore, within this factor of about 3, all the unprotected model systems in all the solvents studied resemble the high-affinity hemoproteins. Note that all values of l'/l from Table 4 fall within $\pm 2 \times 10^8$ of 5.3×10^8 M^{-1}. Furthermore, this similarity extends to the association and dissociation rates as well. These data are not consistent with the theory that models bind CO stronger than do hemoproteins. In

† Other values of ΔH_{O_2} and ΔH_{CO} for hemoglobin and hemoglobin chains are higher (31) than those reported here but ΔH_M is unchanged. These higher values for the ΔH_{CO} serve only to strengthen the conclusion that models do not bind CO more strongly than do hemoproteins.

Table 3. Comparison of binding thermodynamic values

Heme	ΔH_{CO}	ΔS_{CO}	ΔH_{O_2}	ΔS_{O_2}	ΔH_M	Ref.
Chelated heme	−17.5	−34	−14.0	−35	3.5	This work
Picket-fence heme	—	—	−16.8	−42		7
Hemoglobin	−17.4		−13.5 to −14.5		4.0	31; ref. 32, pp. 244 & 263
Hemoglobin chains			−13.5			ref. 32, p. 263; 33
Myoglobin			−14 to −21*			ref. 32, pp. 221 & 354; 34
Aplysia myoglobin			−14.6			ref. 32, p. 354
Chironomus hemoglobin			−13.2			ref. 32, p. 354

All data refer to constants in units of atm^{-1}.

* Enthalpies of O_2 binding to myoglobins from different species as measured in different laboratories vary but ΔG values are constant at 4 kcal/mol compared to ΔG = 3.6 kcal/mol for chelated heme, all data at 1 atm.

Table 4. Equilibrium constants for CO binding to hemes and hemoproteins at 20°C

Compound	Solvent	l', $\times 10^{-6}$ M^{-1} sec^{-1}	l, sec^{-1}	$P_{1/2}^{CO}$	Ref.
Chelated protoheme	H_2O*	4	0.009	0.002	18
Chelated mesoheme	H_2O*	11	0.019	0.001	8, 18
	Toluene	8	≈0.05	0.0004	18
Mesoheme-1-Me-imidazole	H_2O*	6	0.008	0.001	9
Deuteroheme-1-Me-imidazole	Benzene	12	0.028	0.0002	6, 9
Pyridine-chelated mesoheme	H_2O*	12	0.035	0.002	18,
	Benzene			≈0.0003	20
Microperoxidase	H_2O, pH 9	20	0.01	0.0004	28
	H_2O, pH 7	10	0.01	0.0008	37
Leghemoglobin	H_2O, pH 7	11	0.012	0.0008	36, 38
$Hb(CO)_3$	H_2O, pH 7.3	6	0.009	0.001	39
Hb chains	H_2O, pH 7.3	4	0.016	0.003	ref. 32, p. 313
Chironomus Hb	H_2O, pH 7.3	27	0.09	0.002	40
Hb Zürich	H_2O, pH 7.3	27			41

* Suspended in 2% cetyltrimethylammonium bromide, or MTAB.

fact, only one model system, picket-fence ferroporphyrin, has been reported to have a much greater CO affinity (24, 25) than does R-state hemoglobin (7).

Because ligation studies of picket-fence ferroporphyrin and those of chelated hemes have led to different conclusions concerning effects in hemoproteins, we compare the binding properties of these two systems. First, the ratio of CO affinity to oxygen affinity ($L/K = M$) in both systems (18, 25) is 1-2.6 $\times 10^4$ in nonpolar solvents such as toluene. In the case of chelated hemes this ratio drops to about 600 (similar to that for hemoglobin) in aqueous MTAB suspension.

Second, picket-fence heme has a 20-fold higher affinity for both O_2 and CO than does chelated heme in toluene where the O_2 (but not CO) affinity of chelated heme is greatly decreased. The consequence of this is that, in toluene, the O_2 affinity of picket-fence heme (7) and CO affinity of chelated heme (18) match those of R-state hemoglobin. In MTAB suspension, both O_2 and CO affinities of chelated protoheme as well as the thermodynamic values match those of R-state hemoglobin. Although conditions can be chosen so that picket-fence heme binds O_2 with greater, equal, or less affinity than does R-state hemoglobin, there do not seem to be any conditions under which both O_2 and CO affinities of this model compound match those of hemoglobin. This is because, under all conditions examined, picket-fence heme binds CO more strongly than does R-state hemoglobin. On the other hand, chelated hemes (unprotected hemes) seem to have CO affinities like those of R-state hemoglobin under almost all conditions examined.

Collman *et al.* (25) have suggested that the higher CO affinity of picket-fence heme is the "intrinsic affinity" of heme for CO and that our "flat" porphyrin suffers some solvent effect which happens to decrease its affinity to that of R-state hemoglobin. An alternative interpretation is that chelated hemes resemble R-state hemoglobin because the structures and environments are similar and the picket-fence ferroporphyrin behaves differently because the structure is different, just as in the case of another elaborated tetraphenyl ferroporphyrin derivative, "capped ferroporphyrin" (23). Detailed kinetic studies of the picket-fence and capped ferroporphyrins should clarify the nature of the effects which cause these two model compounds to differ from each other and from the simpler model systems.

It is possible that hemoglobin has decreased O_2 and CO affinities relative to simple hemes in the absence of solvent—e.g., in the gas phase. If this is so, then this decreased affinity is also observed with simple protoheme derivatives in solution. Any reduction in affinity of hemoglobin could, therefore, be due to a simple solvent effect and not to a steric effect peculiar to the apoprotein.

Hb Zürich has no distal imidazole (42) and it has therefore been used as an example of a hemoglobin without the distal side steric effects presumed to be present in Hb A (24, 25, 35). Having concluded that such effects are absent in Hb A, we must then explain any differences between these two proteins in some other way. If we confine our discussion to their respective R states with which we compare our model, then there does not seem to be any discrepancy. The affinity of Hb Zürich for CO is not known. However, Giacometti *et al.* (43) have concluded from studies of the kinetics of reaction of Hb Zürich and of its isolated β chain, which lacks the distal histidine, that the R states of Hb A and Hb Zürich are very similar. The replacement M value of 500 compared to 250 for Hb A (35) can be explained on the basis of a reported 50% lower affinity of Hb Zürich (R state) for O_2 (35). Finally, the geometry of the Hb Zürich CO complex was reported to be indistinguishable from that of Hb A, in which the Fe—CO is bent (42). In summary, a steric effect of the distal imidazole need not be invoked in the absence of evidence that the R states of Hb A and Hb Zürich differ greatly with regard to CO binding. It has been concluded from NMR and kinetic studies (41) that the major difference between these two proteins is in their T states, in which Hb Zürich behaves as if its β chains were in the R state.

We are not suggesting that the hemoglobin pocket has no steric effect on binding. Isonitriles bind more strongly to chelated protoheme than to hemoglobin or to any other hemoprotein (D. Stynes, personal communication), indicating that all hemoproteins are capable of differentiating ligands by distal side steric effects. Therefore, we can demonstrate such differentiation when it exists. We do not see evidence for this effect on the interaction of the small molecules CO and O_2 with hemoglobin.

CONCLUSIONS

Because we have used a structurally and spectroscopically accurate model compound, chelated protoheme, and have chosen a solvent system in which this model compound mimics the l', l, k', k, K, L, M, ΔH_{O_2}, ΔH_{CO}, and ΔH_M values, we suggest that our results are consistent with the following conclusions.

i. Protoheme model compounds bind O_2 and CO with affinities similar to those of high-affinity hemoproteins (e.g., hemoglobin chains).

ii. Distal side steric effects neither decrease CO affinity nor differentiate CO and O_2 affinities in high-affinity hemoproteins.

iii. Bending of the Fe—CO bond does not correlate with CO affinities.

It is not yet clear which of the effects mentioned in the introductory section are responsible for the decreased CO affinity in low-affinity hemoproteins, nor is it clear whether steric effects can differentiate CO and O_2 in these hemoproteins (e.g., myoglobin, horseradish peroxidase). We have prepared a sterically hindered cyclophane heme (22), a deprotonated imidazole model compound (10), and a model having proximal side tension (9), all of which have decreased CO affinities.

Note Added in Proof. Romberg and Kassner (44) recently reported a ratio of equilibrium constants for NO and CO binding to 1-methylimidazole–protoheme of 2000 compared to 1700 for hemoglobin and 15,000 for myoglogin. This confirms the proposal (8) that, whereas hemoglobin shows no preferential steric effect to CO binding, myoglobin is subject to a small steric effect. This result also is evidence for steric differentiation of CO and NO in myoglobin.

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1. Perutz, M. F., Heidner, E. J., Ladner, J. E., Bettlestone, J. G., Ho, C. & Slade, F. (1974) *Biochemistry* **13,** 2187–2200.
2. Hoard, J. L. (1966) in *Hemes and Hemoproteins,* eds., Chance, B., Estabrook, R. W. & Yonetani, T. (Academic, New York), pp. 9–24.
3. Morrison, M. & Schonbaum, R. (1976) *Annu. Rev. Biochem.* **45,** 861–888.
4. Peisach, J. (1975) *Ann. N.Y. Acad. Sci.* **244,** 187–203.
5. Dunford, H. B. (1974) *Physiol. Vég.* **12,** 13–23.
6. Rougee, M. & Brault, D. (1975) *Biochemistry* **14,** 4100–4106.
7. Collman, J. P., Brauman, J. I., Doxsee, K. M., Halbert, T. R. & Suslick, K. S. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 564–568.
8. Geibel, J., Cannon, J., Campbell, D. & Traylor, T. G. (1978) *J. Am. Chem. Soc.* **100,** 3575–3585.
9. White, D. K., Cannon, J. B. & Traylor, T. G. (1979) *J. Am. Chem. Soc.* **101,** 2443–2454.
10. Mincey, T. & Traylor, T. G. (1979) *J. Am. Chem. Soc.* **101,** 765–766.
11. Schwartz, J. C., Stanford, M. A., Moy, J. N., Hoffman, B. M. & Valentine, J. S. (1979) *J. Am. Chem. Soc.* **101,** 3398–3399.
12. Heidner, E. J., Ladner, R. C. & Perutz, M. F. (1976) *J. Mol. Biol.* **104,** 707–722.
13. Norvell, J. C., Nunes, A. C. & Schoenborn, B. P. (1975) *Science* **190,** 568–569.
14. Steigemann, W. & Weber, E. (1979) *J. Mol. Biol.* **127,** 309–320.
15. Peng, S. M. & Ibers, J. A. (1976) *J. Am. Chem. Soc.* **98,** 8032–8036.
16. Caughey, W. S. (1970) *Ann. N.Y. Acad. Sci.* **174,** 148–153.
17. Chang, C. K. & Traylor, T. G. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 1166–1170.
18. Traylor, T. G., Chang, C. K., Geibel, J., Berzinis, A., Mincey, T. & Cannon, J. (1979) *J. Am. Chem. Soc.* **101,** 6716–6731.
19. James, B. R., Reimer, K. M. & Wong, T. C. T. (1977) *J. Am. Chem. Soc.* **99,** 4815–4820.
20. Momenteau, M., Rougee, M. & Loock, B. (1976) *Eur. J. Biochem.* **71,** 63–76.
21. Diekmann, H., Chang, C. K. & Traylor, T. G. (1971) *J. Am. Chem. Soc.* **93,** 4068–4070.
22. Traylor, T. G., Campbell, D. & Tsuchiya, S. (1979) *J. Am. Chem. Soc.* **101,** 4748–4749.
23. Budge, J. R., Ellis, P. E., Jr., Jones, R. D., Linard, J. E., Szymanski, T., Basolo, F., Baldwin, J. E. & Dyer, R. L. (1979) *J. Am. Chem. Soc.* **101,** 4762–4763.
24. Collman, J. P., Brauman, J. I., Doxsee, K. M., Halbert, T. R., Hayes, S. E. & Suslick, K. S. (1978) *J. Am. Chem. Soc.* **100,** 2761–2766.
25. Collman, J. P., Brauman, J. I. & Doxsee, K. M. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4035–4039.
26. Berzinis, A. (1979) Dissertation (Univ. of California, San Diego, CA).
27. Noble, R. W. & Gibson, Q. H. (1969) *J. Biol. Chem.* **244,** 3905–3908.
28. Sharma, W. S., Ranney, H. M., Geibel, J. F. & Traylor, T. G. (1975) *Biochem. Biophys. Res. Commun.* **66,** 1301–1306.
29. Schafer, K. & Lax, E. (1962) in *Landolt-Bornstein Tables* (Springer, Berlin), Vol. 2, Part 2, pp. 1–89.
30. Traylor, T. G., Campbell, D., Sharma, V. & Geibel, J. (1979) *J. Am. Chem. Soc.* **101,** 5376–5383.
31. Gaud, H. T., Barisas, B. G. & Gill, S. J. (1974) *Biochem. Biophys. Res. Commun.* **59,** 1389–1394.
32. Antonini, E. & Brunori, M. (1971) *Hemoglobin and Myoglobin in Their Reactions with Ligands* (North Holland, Amsterdam).
33. De Renzo, E., Ioppolo, C., Amiconi, G., Antonini, E. & Wyman, J. (1967) *J. Biol. Chem.* **242,** 4850–4854.
34. Wang, M. R., Hoffman, B. M., Shire, S. J. & Gurd, F. R. N. (1979) *J. Am. Chem. Soc.* **101,** 7394–7398.
35. Tucker, P. W., Phillips, S. E. U., Perutz, M. F., Houtchens, R. A. & Caughey, W. S. (1978) *Biochemical and Clinical Aspects of Hemoglobin Abnormalities,* ed. Caughey, W. S. (Academic, New York), pp. 1–15.
36. Caughey, W. S., Houtchens, R. A., Lanir, A., Maxwell, J. C. & Charache, S. (1978) in *Biochemical and Clinical Aspects of Hemoglobin Abnormalities,* ed. Caughey, W. S. (Academic, New York), pp. 29–56.
37. Wittenberg, J., Appleby, C. A. & Wittenberg, B. A. (1972) *J. Biol. Chem.* **247,** 527–530.
38. Moffatt, K., Deatherage, J. F. & Seybert, D. W. (1979) *Science* **206,** 1035–1042.
39. Imamura, T., Riggs, A. & Gibson, Q. (1971) *J. Biol. Chem.* **247,** 521–526.
40. Geibel, J. (1976) Dissertation (Univ. of California, San Diego, CA).
41. Sharma, V. S., Schmidt, M. R. & Ranney, H. M. (1976) *J. Biol. Chem.* **251,** 4267–4270.
42. Amiconi, G., Antonini, E., Brunori, M., Formanek, H. & Huber, R. (1972) *Eur. J. Biochem.* **31,** 52–55.
43. Giacometti, G. M., DiOrio, E. E., Antonini, E., Brunori, M. & Winterhalter, K. H. (1977) *Eur. J. Biochem.* **75,** 267–273.
44. Romberg, R. W. & Kassner, R. J. (1979) *Biochemistry,* **18,** 5387–5392.