Anthracene Heme Cyclophanes. Steric Effects in CO, O_2, and RNC Binding†[1]

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Abstract: Equilibrium constants in benzene solution are reported for binding of imidazoles, CO, O_2, and isocyanide to two anthracene heme cyclophanes, Fe(6,6-cyclophane) and Fe(7,7-cyclophane), containing a conformationally mobile anthracene ring strapped symmetrically over the heme. The Fe(6,6-cyclophane) shows an approximately 300-fold reduction in affinity for the diatomic molecules, CO, and O_2 compared to unhindered hemes. The Fe(7,7-cyclophane) shows only slight steric effects toward CO and O_2 but larger effects with bulky isocyanides. The sizes of the cyclophane cavities are qualitatively probed with isocyanides of varying size. Kinetic data for binding of CO, O_2, and tosylmethyl isocyanide to heme cyclophanes when compared to similar data for flat analogues demonstrate that steric effects are manifested primarily in the ligand association rates. This feature of the cyclophane is very similar to observed steric effects in hemoproteins, suggesting that large conformational changes (tilting of the cyclophane cap in the models and movement of distal protein residues in hemoproteins) occur in both *prior* to the transition state for ligation. Magnitudes of steric effects in hemoproteins and cyclophanes are calculated on the basis of comparison with the unhindered chelated heme model compounds. The nature of the distal steric effect in the Fe(6,6-cyclophane) model compound suggests that the reported bending or tilting of CO in hemoglobins and myoglobin may be of minor chemical significance.

The idea that interactions with protein residues can alter the binding of ligands to hemoglobin (Hb) and myoglobin (Mb) began with the demonstration by St. George and Pauling[2] of reduced binding of isocyanides (RNC) with increasing steric bulk of the R group. X-ray crystal structures of Mb[3] and Hb[4] confirmed the Pauling proposal of "embedded hemes". Subsequent structural determinations[5-8] showed either bent or tilted binding of CO and CN^- to iron(II) and iron(III) hemoproteins, respectively, in contrast to the linear coordination of these ligands to simple metal complexes.[9] The distorted binding of CO was attributed to close contacts with distal residues (specifically His-E7 and Val-E11). These interactions are referred to as distal steric effects.

The observation that certain heme proteins which lack the usual distal histidine have higher stretching frequencies of bound carbon monoxide led to the postulates that distortion of the bound carbon monoxide bond angle is correlated with the carbon monoxide stretching frequency and with carbon monoxide affinity.[10-12]

It was further postulated that the bound carbon monoxide is bent from its normal binding position by steric interaction whereas bound dioxygen, being naturally bent,[12] does not suffer this distortion, resulting in a steric discrimination between the binding of carbon monoxide and dioxygen in hemoproteins.[10a,11a-c] We found that the simple five-coordinated heme derivatives, chelated mesoheme[13a] or protoheme,[13b] when suspended in a micellar medium having solvent polarity[14] reported to be similar to that in hemoglobin,[15] bind carbon monoxide and dioxygen with kinetic and equilibrium constants and thermodynamics which are remarkably similar to those reported for isolated hemoglobin chains or R-state hemoglobin.[13a-c] We concluded that R-state hemoglobin simply maintains the protoheme five-coordinated, soluble in water, and protected against oxidation and suffers no steric hindrance, although myoglobin and other hemoproteins having lower carbon monoxide affinities are subject to some distal side steric hindrance.[13d]

Other studies of model heme compounds have been interpreted as supporting both the general steric hindrance toward carbon monoxide and other diatomic ligands as well as the proposed steric differentiation between linearly and angularly bound diatomic ligands.[11a-d]

The heme cyclophane compounds, which we had introduced for other reasons,[16-19] seemed well suited to test these proposals directly since they provide a synthetic approach to the "heme pockets" in the active sites of hemeproteins. We therefore prepared two anthracene porphyrin cyclophanes **1** and **2**, having different pocket sizes as well as a more hindered porphyrin, **3** (Figure 1),

† Dedicated to Prof. Eraldo Antonini (1931-1983) whose publications inspired much of this work.

(1) (a) Preliminary reports of some of this work have appeared.[1b,c] (b) Traylor, T. G.; Mitchell, M. J.; Tsuchiya, S.; Campbell, D. H.; Stynes, D. V.; Koga, N. *J. Am. Chem. Soc.* **1981**, *103*, 5234. (c) Traylor, T. G.; Campbell, D. H.; Tsuchiya, S.; Stynes, D. V.; Mitchell, M. J. In "Hemoglobin and Oxygen Binding"; Ho, C., Ed.; Elsevier: North Holland, 1982; pp 425-433. (d) Abbreviations: TMIC, (*p*-toluenesulfonyl)methyl isocyanide; DCIm, 1,5-dicyclohexylimidazole; MeIm, 1-methylimidazole; DMI, 1,2-dimethylimidazole; MTAB, myristyltrimethylammonium bromide; $K_Z^{X,Y}$, equilibrium constant for the replacement of X by Y in which the ligand Z remains attached. K^Y and K_Z^Y represent equilibrium constants for addition of Y to the four- and five-coordinated hemes, respectively. A bar over the ligand as in $K_B^{\overline{CO}}$ or $K_B^{\overline{RNC}}$ signifies attachment of that ligand under the cyclophane cap. Kinetic association and dissociation rates for ligand Y are similarly represented as k_Z^Y and k_Z^{-Y}, respectively. FePiv 5CIm, see ref 11d; cyclophane nomenclature: porphyrins having groups covalently strapped over the porphyrin face are labeled *n*-, *n,n*-, *n,n,n,n*-, or *n,n,n,n*-cyclophanes to indicate a single methylene strap, or a more complex group such as an arene or adamantane group, attached to the porphyrin by two *n,n*, three *n,n,n*, or four *n,n,n,n* chains, respectively. The value of *n* represents the number of atoms between the porphyrin ring and either the other group or the other side of the porphyrin. Thus compounds with the same *n* should have pockets of similar size.

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and demonstrated that such cyclophanes provide hindrance toward the binding of CO and O_2.[1b,c,20a,b] Preliminary studies also indicated that this distal side steric hindrance is entirely due to changes in association rates.[1b,c] Studies of other cyclophane systems (compounds **3–12**) confirmed all these findings[21-24] except one—that dioxygen dissociation rates are unaffected by distal side steric effects.[22b] As a result of this single disparity, the question of steric differentiation between carbon monoxide and dioxygen seemed to remain unsettled.

Herein we describe studies of both kinetic and equilibrium measurements for the two anthracene heme cyclophanes **1** and **2**. The results illustrate the nature and relative magnitudes of distal steric effects for CO and O_2 as well as for bulky isocyanides whose steric differentiation in hemoproteins is firmly established. These results, combined with additional data on other models, allow us to test the theories concerning distal side steric effects and provide a consistent set of principles which apply to model hemes and hemoproteins for diatomic molecules as well as bulkier ligands.

Experimental Section

Chelated protoheme and chelated mesoheme were prepared as previously described.[25] The synthesis and NMR characterization of the 6,6-cyclophane **1**-P and the 7,7-cyclophane **2**-P and their iron complexes will be described in detail elsewhere.[25c] Myoglobin was a gift from R. Lumry. Tosylmethyl isocyanide (TMIC), *n*-butyl isocyanide, cyclohexyl isocyanide (Aldrich Chemical Co.), *tert*-butyl isocyanide (Fluka) were used as received.

1,5-Dicyclohexylimidazole. 1,5-Dicyclohexylimidazole (DCIm) was prepared by a modification of the method of van Leusen.[26] Cyclohexylamine, 24 mL (0.21 mol), and 10 mL of cyclohexylcarboxyaldehyde (0.085 mol) were refluxed in 80 mL of benzene over $CaCl_2$ in a Soxhlet extractor for 4 h to remove H_2O and form the Schiff base. About 50 mL of benzene was stripped off, and 100 mL of dry methanol and 16 g (0.082 mol) of tosylmethyl isocyanide were added. The solution was stirred for 20 h and then heated to reflux for 3 h. The methanol was stripped off and the residue taken up in 200 mL of saturated aqueous NaCl to give a solution and a red oil. This mixture was extracted with 400 mL of CH_2Cl_2. The CH_2Cl_2 layer was diluted with 1 L of heptane to give white needles of *p*-toluenesulfinic acid. This was filtered off and the solution concentrated to give 8 g of white crystalline product, yield 43%. The product was then boiled with an excess of K_2CO_3 (~5 g) in 50 mL of methanol for 4 h to destroy *any trace impurity of TMIC*. The methanol was stripped off, the residue taken up in heptane, the K_2CO_3 filtered off,

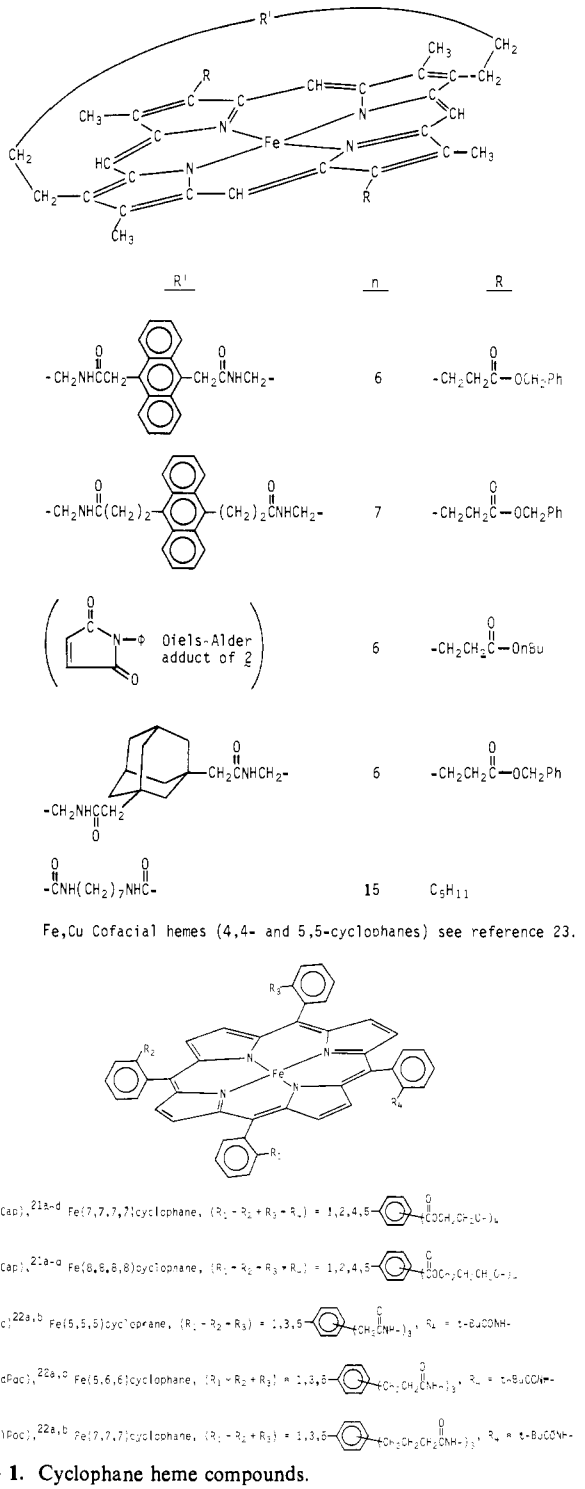

Figure 1. Cyclophane heme compounds.

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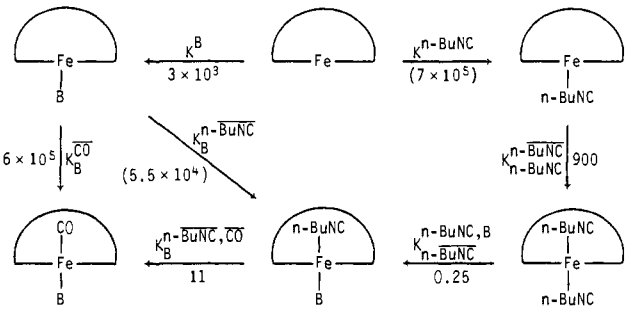

Figure 2. Equilibria for Fe(6,6-cyclophane) **1** in benzene at 25 °C. B = 1-MeIm. Values in parentheses are calculated from the alternative paths indicated. All others are from direct spectrophotometric titration. Reagents are omitted and indicated by subscripts and superscripts in the equilibrium constants which are for the directions indicated by the arrows. Equilibria are in appropriate molar concentrations, using a CO solubility of 1.0×10^{-5} M torr^{-1}.

and the solution concentrated and cooled to give a pure crystalline product: mp 113–114 °C; NMR ($CDCl_3$/Me_4Si) δ 1.2–2.0 (m, 20 H), 2.43 (m, 1 H), 3.73 (m, 1 H), 6.73 (s, 1 H), 7.47 (s, 1 H).

Anal. Calcd for $C_{15}H_{24}N_2$: C, 77.53; H, 10.4; N, 12.06. Found: C, 77.31; H, 10.51; N, 12.11.

Spectrophotometric Titrations. Solutions of reduced hemes in benzene were prepared in 1-cm-path length cuvettes described previously[25a,b] by one of two methods. (1) A benzene solution of heme obtained by adding ~5 μL of a CH_2Cl_2 solution of the Fe(III) heme to >5 mL of degassed benzene under CO was shaken with aqueous sodium dithionite. The reduced CO complex was cannulated into the cuvette under nitrogen and excess CO and H_2O removed by purging for ~30 min with argon. (2) The above method could not be used in cases where low or zero concentrations of CO were subsequently required. In these cases solutions were prepared by injecting ~5 μL of heme, reduced in methanol with a crown ether dithionite complex, as described by Mincey[27] into 5 mL of degassed benzene under argon. In a few cases, a greater amount of methanol–crown ether was required to keep the heme reduced and care was taken to ensure that the results were independent of the amount of methanol–crown ether–dithionite solution used in these cases.

Titrations were performed spectrophotometrically, following equilibration, by scanning spectra typically from 700–350 nm. Ligands were added via syringe as benzene solutions. Gases were added via gas-tight syringes to calibrated tonometers[25] and the solutions stirred by a Teflon-coated magnetic stir bar for at least 15 min to equilibrate. Clean isosbestic points were obtained in the visible spectra. Data covering saturation criteria described by Deranleu[28] were treated according to standard methods.

Kinetic Measurements. Fast rates were measured using laser flash or Sunpak 611 flashgun photolysis methods, tonometers, and data systems described previously.[25] Intermediate spectra were obtained by computer analysis of data sets for a single sample at several wavelengths (i.e., every nanometer from 390 to 440 nm). Slow kinetics ($t_{1/2}$ >10 s) for TMIC and CO dissociation rates were obtained on a Cary 15 spectrophotometer by monitoring absorbance changes at fixed wavelengths under pseudo-first-order conditions. Temperatures were maintained via water circulation through a close fitting brass block.

Results

Equilibria. Spectrophotometric titrations of the heme cyclophanes were carried out in benzene solution to maintain solvent polarity similar to the anthracene cap. This choice also allows direct comparisons with studies of the deuteroheme dimethyl ester in benzene[25,29] and picket fence[11d,22] and various heme cyclophanes in toluene. The equilibria are summarized in Figures 2 and 3.

Imidazole Binding. The binding of imidazole to simple hemes is known to give primarily the bis complex. As an example, for deuteroheme, $K^{Im} = 4 \times 10^3$ M^{-1}, $K^{Im}_{Im} = 6 \times 10^4$ M^{-1}.[29b] The Fe(6,6-cyclophane) **1** gives a clean five-coordinated heme spectrum, λ_{max} = 428 and 555 nm, on addition of **1**–MeIm, $K^{MeIm} = 3 \times 10^3$ M^{-1}, similar to the corresponding value for deuteroheme.

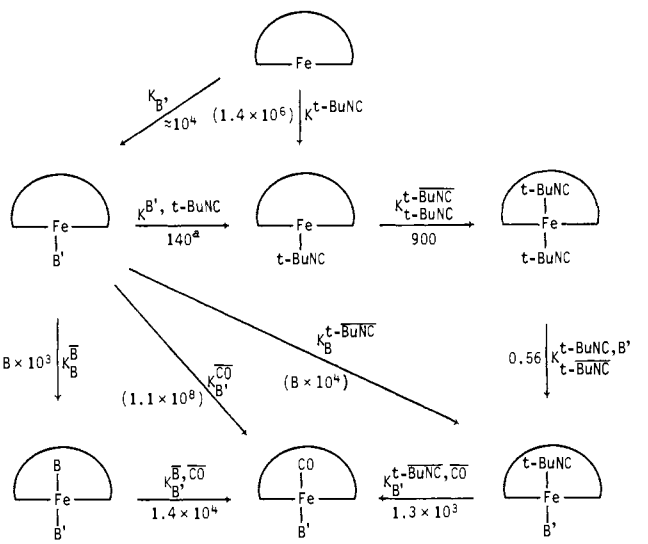

Figure 3. Equilibria for Fe(7,7-cyclophane) **2** in benzene at 25 °C: B = 1-MeIm. B′ = 1,5-dicyclohexylimidazole. See Figure 1 for other conditions. [a]This is the value for the 6,6-cyclophane **1**.

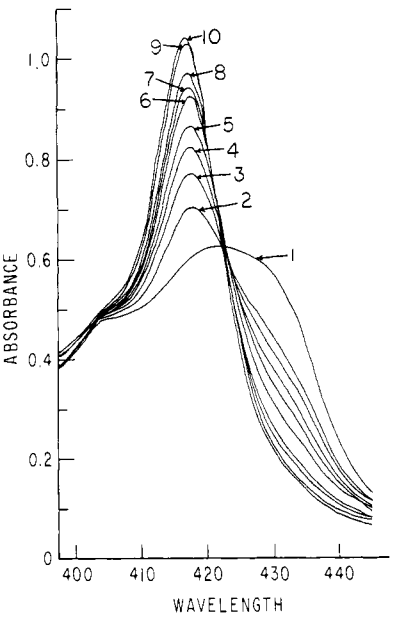

Figure 4. Spectral data for titration of Fe(7,7-cyclophane)(DCIm) with MeIm in benzene at 20 °C: [DCIm] = 0.035 M; [MeIm] × 10^3 = 0, 0.037, 0.075, 0.13, 0.22, 0.41, 0.78, 1.5, 8.8, and 16.0 M in **1**–**10**, respectively.

(A statistical factor of 2 must be taken into account for the open heme.) Only at very high **1**–MeIm concentration is there evidence for a Fe(6,6-cyclophane)$(MeIm)_2$ complex with the appearance of the characteristic α,β spectrum and Soret at 417 nm, $K^{MeIm}_{MeIm} = 5$ M^{-1}. This value is similar to that reported for the Fe(C_3cap) system.[21]

Titration of Fe(7,7-cyclophane), **2**, with **1**–MeIm shows formation of Fe(7,7-cyclophane)$(MeIm)_2$ at low [MeIm], making determination of K^{MeIm} difficult. The formation of the bis complex also complicates kinetic studies of CO binding. To circumvent this problem, we synthesized 1,5-dicyclohexylimidazole (DCIm) which is hindered with respect to getting under the anthracene cap but not with respect to the heme plane interactions as are 2-substituted imidazoles. Titration of Fe(7,7-cyclophane), **2**, with DCIm gives a clean five-coordinated visible spectrum up to 0.1 M DCIm, the largest concentration employed. DCIm is a somewhat stronger base than **1**–MeIm,[21c] and this undoubtedly accounts for the 2-fold higher $K^{DCIm} = 6 \times 10^3$ M^{-1} on **2** compared to K^{CH_3Im} on **1**. A similar difference is reported for DCIm vs. MeIm in Fe(C_2cap) and Fe($NapC_2$cap) complexes.[21c] Binding

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Table I. K_B^B for Base Binding to Hindered Hemes in Benzene or Toluene at 25 °C

	base	K^B, M^{-1}	K_B^B, M^{-1}	ref
Fe(6,6-cyclophane), **1**[a]	MeIm	3×10^3 [a]	5[a]	this work
Fe(7,7-cyclophane), **2**[a]	DCIm	8×10^3 [a]		this work
	MeIm		8×10^3 [a,b]	this work
Fe(C_2cap), **5**	MeIm	8×10^2	1	21
Fe(C_3cap), **6**	MeIm	2×10^3	5.9	21
Fe(PocPiv), **10**	MeIm		<1	22
Fe(TPP)	Im	8.8×10^3	7.9×10^4	29b
Fe(DHD)	Im	4.5×10^3	6.8×10^4	29b

[a] In benzene; all other data are for toluene. [b] For MeIm inside with DCIm outside.

of **1**–MeIm under the anthracene cap could then be obtained by titrating the Fe(7,7-cyclophane)(DCIm) complex with **1**–MeIm (Figure 4). The $K_{DCIm}^{\overline{MeIm}} = 8 \times 10^3$ M^{-1} is about 10-fold smaller than the K_{Im}^{Im} reported for deuteroheme,[29b] indicating a much smaller steric effect toward MeIm in the 7,7-cyclophane compared to 6,6-cyclophane. These equilibrium constants along with others are shown in Table I.

Carbon Monoxide Binding. The binding of CO to Fe(6,6-cyclophane)(MeIm) can be obtained through direct titration by monitoring the increase at $\lambda_{max} = 415$ nm ($K_{MeIm}^{\overline{CO}} = 6.5 \times 10^5$ M^{-1}). The corresponding Fe(7,7-cyclophane)(DCIm) binds CO too strongly to obtain $K_{DCIm}^{\overline{CO}}$ by our direct titration method. Three independent methods were used to obtain the value indirectly. The $K_{DCIm}^{\overline{CO}}$ for Fe(7,7-cyclophane) can be obtained from the ratio of rate constants described later in this paper, $k_{DCIm}^{\overline{CO}}/k_{DCIm}^{-\overline{CO}} = 6 \times 10^7$ M^{-1}, from the product $[K_{DCIm}^{\overline{MeIm}} K_{DCIm}^{\overline{MeIm,CO}}] = 1.1 \times 10^8$ M^{-1} or the product $[K^{B',t\text{-BuNC}} K_{t\text{-BuNC}}^{\overline{t\text{-BuNC}}} K_{t\text{-BuNC}}^{t\text{-BuNC,B'}} K_{B'}^{\overline{t\text{-BuNC,CO}}}] = 9 \times 10^7$ M^{-1}. This assumes that outside binding of ligands is the same on **1** and **2** (see Figure 3).

With 1,2-Me_2Im as the base, direct titrations of both Fe(6,6-cyclophane)(1,2-MeIm) and Fe(7,7-cyclophane)(1,2-Me_2Im) were possible, giving $K_{1,2\text{-}Me_2Im}^{\overline{CO}} = 1.4 \times 10^4$ and 2.3×10^6 M^{-1}, respectively. The reduction in CO binding to Fe(6,6-cyclophane) compared to Fe(7,7-cyclophane) is essentially the same with 1,2-Me_2Im as with an unhindered base. Equilibrium and kinetic constants for CO binding are shown in Figures 2 and 3.

Isocyanide Binding. Addition of isocyanides to mesoheme dimethyl ester results in the appearance of a single Soret band at 423 nm due to the formation of Fe(mesoP)$(RNC)_2$ stoichiometrically, $K_{RNC}^{RNC} > K^{RNC}$ and $K^{RNC} K_{RNC}^{RNC} > 10^{11}$. In contrast, spectrophotometric titration of Fe(6,6-cyclophane) with *n*-BuNC results in the stoichiometric formation of Fe(6,6-cyclophane)(*n*-BuNC), $\lambda_{max} = 415$, $K^{BuNC} > 10^5$ M^{-1}. Only at high [*n*-BuNC] is the Fe(6,6-cyclophane)(*n*-BuNC)$_2$ complex ($\lambda_{max} = 425$) formed, $K_{n\text{-BuNC}}^{\overline{n\text{-BuNC}}} = 900$ M^{-1}. As the size of the isocyanide increases, no detectable change in K^{RNC} occurs but values of $K_{RNC}^{\overline{RNC}}$ progressively decrease (see Table II). Typical spectral data for cyclohexyl isocyanide are shown in Figure 5. Values of $K_{RNC}^{\overline{RNC}}$, therefore, primarily reflect the steric effects of the anthracene cap.

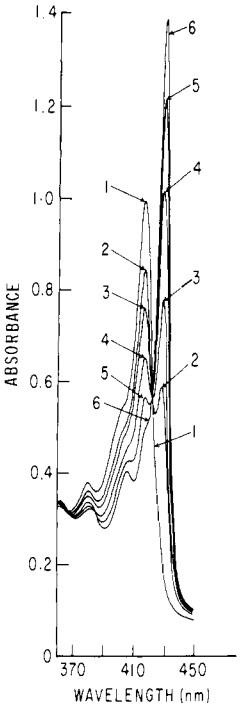


Figure 5. Spectral data for titration of Fe(6,6-cyclophane)($C_6H_{11}NC$) with $C_6H_{11}NC$ in benzene. [$C_6H_{11}NC$] $\times 10^3 = 0.07$, 2, 4, 8, 16, and 36 M in **1**–**6**, respectively.

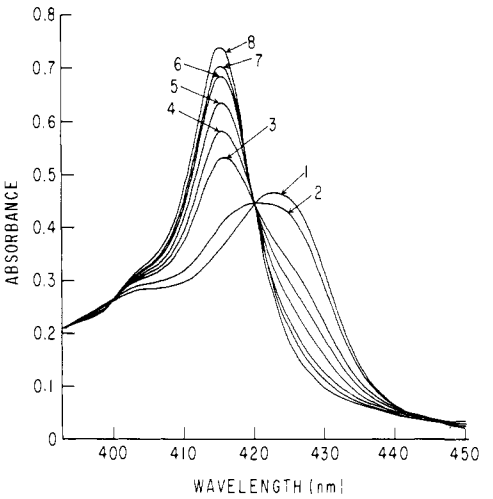


Figure 6. Spectral data for titration of Fe(6,6-cyclophane)(CH_3Im)(BuNC) with CO in benzene at [MeIm] = 0.7, [*n*-BuNC] = 7×10^{-4} M, and variable CO; [CO] $\times 10^5 = 0$, 1.68, 4.48, 8.4, 19.6, 42.98, and 650 M in **1**–**8**, respectively.

Corresponding titrations of Fe(7,7-cyclophane) show less steric hindrance, as expected. For *n*-BuNC, the titration is nearly indistinguishable from an uncapped heme, giving essentially stoichiometric formation of Fe(7,7-cyclophane)(*n*-BuNC)$_2$. With the more bulky *t*-BuNC, the steric hindrance in the 7,7 complex

Table II. Equilibrium Constants[a] for Isonitrile Binding to Hemes in Benzene at 25 °C

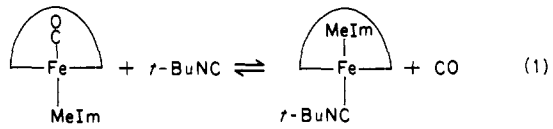
	6,6-cyclophane, **1**[b]			7,7-cyclophane, **2**[c]			chelated protoheme[d]		adamantane 6,6-cyclophane, **9**[e]		
	K_B^{RNC}	K_{RNC}^{RNC}	$K_B^{RNC,CO}$	K_B^{RNC}	K_{RNC}^{RNC}	$K_B^{RNC,CO}$	K_B^{RNC}	$K_B^{RNC,CO}$	K_B^{RNC}	K_{RNC}^{RNC}	$K_B^{RNC,CO}$
n-BuNC	5.5×10^4	900	11	4.4×10^6	$>10^5$	25	4.4×10^8	0.9			
t-BuNC	150	2	4000[f]	8×10^4	900	1280	1.7×10^8	2.4	3×10^4	1.5×10^4	5.7
TMIC	7×10^5	220	0.9	7×10^8	$>10^5$	0.16	7×10^9	0.06	2×10^5	5.0×10^3	0.9
$C_6H_{11}NC$	1.5×10^4	170	45								

[a] Estimated errors ±10% except for *t*-BuNC with 6,6-cyclophane where errors are ±30%. [b] **1**–MeIm as axial base. [c] DCIm as axial base. [d] Chelated imidazole as axial base. [e] Reference 20c. [f] Estimated from K_{RNC}^{RNC}. $K_B^{RNC,CO}$ cannot be obtained directly since *t*-BuNC competes with **1**–MeIm outside at concentrations required for inside binding.

is clearly seen with distinct formation of Fe(7,7-cyclophane)(*t*-BuNC) and Fe(7,7-cyclophane)(*t*-BuNC)$_2$ complexes. Equilibrium constants are shown in Table II along with those of reference compounds.

CO, RNC Competition. While the binding constant $K_{RNC}^{\overline{RNC}}$ provides a measure of the steric hindrance of the cyclophane cap, the number is not directly comparable with hemoproteins where a proximal imidazole is present. Isonitriles have a smaller trans influence upon isonitrile affinity than do imidazoles.[30,31] The direct binding constant, K_B^{RNC}, is typically too large to conveniently measure in model systems. However, the competition $K_B^{CO,RNC}$ can be readily measured and the K_B^{RNC} obtained from $K_B^{RNC} = K_B^{CO,RNC}K_B^{CO}$. Typical spectral changes are shown in Figure 6. Data were analyzed at λ_{max} for the CO and RNC complexes. For Fe(6,6-cyclophane), MeIm was used as the base. Titrations were carried out at constant RNC and varying CO. Fe(7,7-cyclophane) titrations were carried out with DCIm as the base to assure that the isonitrile and not the imidazole is bound under the cap.

The need to exercise caution in the choice of the base is illustrated by the competitive titration of *t*-BuNC and CO in Fe(7,7-cyclophane). With B = MeIm a value of $K_B^{RNC,CO} = 50$ was obtained, while with B′ = DCIm, we obtained $K_{B'}^{RNC,CO} = 1.3 \times 10^3$. The latter is consistent with the general observations with butyl isocyanides that $K_{RNC}^{RNC} \times 70 \approx K_B^{RNC}$. The titration with MeIm as base is consistent with the "inside-out" equilibrium. We

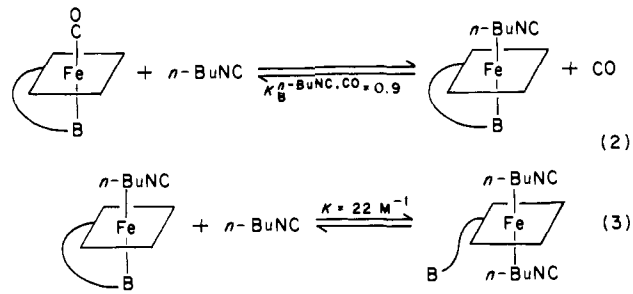


(1)

have previously seen that 1-MeIm suffers a 10-fold reduction in binding under the 7,7 cap ($K_{DCIm}^{\overline{MeIm}}$) while *t*-BuNC suffers a 2000-fold reduction ($K_{t\text{-}BuNC}^{\overline{t\text{-}BuNC}}$). Therefore, MeIm will bind under the cap in preference to *t*-BuNC.

Equally interesting are the titrations possible in the Fe(7,7-cyclophane) system with the two bulky ligands DCIm and *t*-BuNC shown in Figure 3. Starting with the five-coordinated Fe(7,7-cyclophane)(DCIm) at low base concentrations, the DCIm may be replaced by *t*-BuNC outside. (Actually this step can only be cleanly performed with the Fe(6,6-cyclophane) where no inside binding of *t*-BuNC competes. However, the outside binding characteristics of Fe(6,6-cyclophane) and Fe(7,7-cyclophane) are expected to be essentially identical.) Inside binding of *t*-BuNC trans to *t*-BuNC ($K_{t\text{-}BuNC}^{\overline{t\text{-}BuNC}}$) can also be directly determined. Finally, the external *t*-BuNC may be titrated off with DCIm, $K_{\overline{t\text{-}BuNC}}^{\overline{t\text{-}BuNC},DCIm}$. The product of these three equilibria, $K^{t\text{-}BuNC}K_{\overline{t\text{-}BuNC}}^{\overline{t\text{-}BuNC}}K_{\overline{t\text{-}BuNC}}^{\overline{t\text{-}BuNC},DCIm} = K_{DCIm}^{\overline{t\text{-}BuNC}} = 8 \times 10^4\ M^{-1}$ for Fe(7,7-cyclophane) is in excellent agreement with the independent determination of $7 \times 10^4\ M^{-1}$ based on alternative pathways shown in Figure 3.

Titrations of the unhindered chelated protoheme were carried out for comparison with results on the hindered heme cyclophanes. Addition of isocyanides to the five-coordinated chelated protoheme in benzene results in a stoichiometric titration owing to the large binding constants. However, the competition of isocyanides with CO can be conveniently studied. Additions of BuNC to chelated protoheme–CO results in two distinct spectral changes in the Soret region. At low [*n*-BuNC] the absorbance of the CO complex at 420 nm decreases and a new, less intense, peak appears at 428 nm which we assign to the six-coordinated mono-BuNC complex (eq 2). At higher [*n*-BuNC] concentrations, this peak shifts to 433 nm and becomes more intense, consistent with the removal



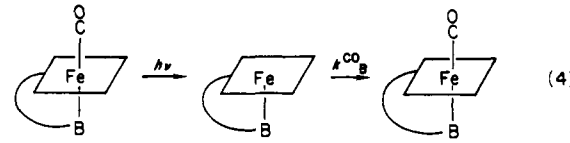
(2)

(3)

of the chelated imidazole (eq 3). Since both equilibria depend on [*n*-BuNC] but only the first is CO-dependent, the two steps may be conveniently separated by a judicious choice of CO concentration.

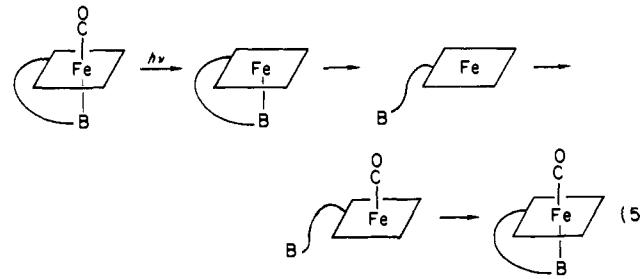
Values of $K_B^{n\text{-}BuNC,CO}$ obtained by titrating at constant CO pressure and variable [*n*-BuNC] were in good agreement with results obtained at constant [*n*-BuNC] and variable CO pressure. Titrations were carried out with [RNC] always less than 10^{-2} M to avoid complications arising from the diisocyanide complex. The results are summarized in Table II.

CO Kinetics. Flash photolysis studies of chelated hemes have been previously described[25] and are exactly analogous to corresponding methods in hemoproteins, giving a straightforward method of obtaining the CO association rate constants k_B^{CO} as well as isocyanide association rate constants. Complications can arise



(4)

if strain is introduced into the chelated arm and the reaction proceeds via the "base-off" mechanism.[25b,d]



(5)

"Base-off" effects in hemes may be anticipated whenever the CO association rate is significantly reduced or, alternatively, strain or some other effect[1b,25b,d] decreases the binding of the base in the five-coordinated complex. Photolysis of simple hemes in the presence of excess base is even more complex since a bis-base species can form and a variety of reaction modes are possible, as detailed by White.[25b] Simplifying assumptions allows the extraction of rate constants at appropriate concentrations of CO and B.[25b,32-34]

The capped hemes can eliminate some of the complexities (such as the bis-base species) but introduce new features resulting from the nonequivalence of the capped and open face of the heme. Initial flash photolysis experiments involved a study of a dichelated Fe(6,6-cyclophane) in aqueous cetyltrimethylammonium bromide (CTAB) suspension. Only one of the imidazole chelate arms can

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Table III. Rate Constants for Reaction of Hemes with Dioxygen and Carbon Monoxide

compound	solvent	k_B^{CO}, M^{-1} s^{-1}	k_B^{-CO}, s^{-1}	$k_B^{O_2}$, M^{-1} s^{-1}	$k_B^{-O_2}$, M^{-1}	$P_{1/2}^{CO}$, torr	$P_{1/2}^{O_2}$, torr	$P_{1/2}^{O_2}/P_{1/2}^{CO}$	$k_B^{O_2}/k_B^{CO}$	ref
chelated protoheme	H_2O/MTAB	3.6×10^6	0.009	2.6×10^7	47	0.0018	1.0	560	7	25a,b
	benzene	1.1×10^7	0.025	6.2×10^7	4000	0.00023	5.6	24000	6	20c, 25b
chelated mesoheme	toluene/10% CH_2Cl_2	8×10^6	0.05	5×10^7	1700	0.0005	2.8	6000	6	
	toluene	1.1×10^7	~0.05	9.0×10^7	5000	0.0005	4.9	10000	8	20c
anthracene 7,7-cyclophane, **2**	benzene	6×10^6	0.05	6.5×10^7 [a]	1000[a]	0.0009	1.4	1500	11	this work
anthracene 6,6-cyclophane, **1**	benzene	3×10^4	0.05	1×10^5	800	0.17	700[b]	4000	3.3	this work
adamantane 6,6-cyclophane, **9**	toluene	9.2×10^3	0.05	1.5×10^5	690	0.60	300	500	16	20c
15-cyclophane, **4**	benzene	9.1×10^4	0.04	1.7×10^6	250	0.05	15	300	19	34
chelated TP heme	toluene	4.2×10^6 [c]	~0.04	2.9×10^7	30000	~0.001	83	~80000	7	22b, 33b
chelated TPiv heme	toluene	3.6×10^7	0.0078	4.3×10^8	2900	2.2×10^{-5}	0.58	26600	12	22b
6,6,6-cyclophane, **11**	toluene	1.5×10^6	0.0093	1.7×10^7	71	6.5×10^{-4}	0.36	550	11	22b
5,5,5-cyclophane, **10**	toluene	5.8×10^5	0.0086	2.2×10^6	9	1.5×10^{-3}	0.36	270	3.8	22b
7,7,7,7-cyclophane, **5**	toluene	9.5×10^5	0.05			5×10^{-3}	23	4300		22b
chelated 18-cyclophane	toluene	4×10^7 [c]	0.0067	1.8×10^8 [c]	620	1.7×10^{-5}	0.29	17000	4.7	33d

[a] More recent measurements give somewhat smaller values of $k_B^{O_2}$ and $k_B^{-O_2}$ but the same value of $K_B^{O_2}$. The large value of $P_{1/2}^{O_2}$ makes this measurement somewhat inaccurate (±20%). [c] These values have been recalculated using the solubilities of 10^{-5} M $torr^{-1}$ for CO and 1.2×10^{-5} M $torr^{-1}$ for O^2 (see ref 20a).

Table IV. Kinetic Data for RNC Binding to Hemes in Benzene at 20 °C

	RNC	k_B^{RNC},[a] M^{-1} s^{-1}	k_B^{-RNC},[b] s^{-1}	k_B^{RNC},[c] M^{-1}	ref
Fe(6,6-cyclophane)MeIm	TMIC	1.4×10^4	0.020	7×10^5	this work
Fe(7,7-cyclophane)DCIm	TMIC	1.2×10^7	0.015	7×10^8	this work
chelated protoheme	TMIC	1.7×10^8	0.023	7×10^9	this work
chelated protoheme	*n*-BuNC	2.2×10^8	0.5 (0.7)	4.4×10^8	25c, 31b
Mb (SW)	*n*-BuNC	3.7×10^4 [d]	0.7[d]		
Mb (SW)[e]	TMIC	2.3×10^2 [f]	0.010	2.5×10^4	this work

[a] Calculated from k_B^{-RNC} and K_B^{RNC}. [b] By CO displacement. [c] Calculated from K_B^{CO} and $K_B^{RNC,CO}$ (Table II). [d] Data of Olson, ref 31a,c, obtained by flash photolysis. [e] Experimentally determined by direct reaction of Mb and TMIC. [f] Aqueous phosphate buffer, pH 7.3.

bind since the anthracene cap prevents chelation to the capped face. After photolysis two [CO]-dependent rate processes were detected, a fast rate with its maximum ΔOD at 409 nm and a slower rate which showed large ΔOD at 413 nm. These observations suggested complications associated with a base-off mechanism followed by CO binding to the open side of the heme (which would be expected if the rate of CO binding to the capped side were reduced).

Studies of the di-*n*-butyl ester of Fe(6,6-cyclophane) in the presence of external **1**-MeIm in benzene also gave two rate processes which depended on both [CO] and [MeIm]. Spectral intermediate studies show that the fast rate involves the conversion of Fe(6,6-cyclophane)(MeIm) λ_{max} = 426 nm to a species with λ_{max} = 406 nm. Subsequent conversion of this species to Fe(6,6-cyclophane)(MeIm)(CO), λ_{max} = 415, then occurs at a slower rate.[20d] The intermediate species λ_{max} = 406 nm is assigned to a complex with CO bound outside and methanol (introduced with the reducing agent) bound inside the anthracene cap. At high [MeIm] ≥ 0.25 M, essentially only a single rate is observed which is largely independent of λ. Intermediate spectra show that the initially formed species after the flash is a mixture of Fe(6,6-cyclophane)(MeIm) λ_{max} = 425 nm and Fe(6,6-cyclophane)-(MeIm)$_2$ λ_{max} = 418 nm as expected from K_{MeIm}^{MeIm} = 5 M^{-1}, and the observed rate must be corrected accordingly. This correction involves an equilibrium and no interfering rate processes since both association and dissociation rates for MeIm are faster than the process being observed.

If DCIm is used as the base, the binding of base under the cap is eliminated for both the 6,6- and 7,7-cyclophanes even at the high concentrations required to diminish the base-off mechanisms. When DCIm was used, a single, pseudo-first-order rate constant was obtained which is proportional to [CO] and largely independent of DCIm at concentrations from 0.2 to 1 M at the usual measuring wavelength (417 nm) but a somewhat faster rate occurred at 428 nm. This rate is almost wavelength-independent at 1.0 M DCIm.

When 1,2-Me_2Im was used as the base, it was impossible to completely eliminate the base-off mechanism for the Fe(6,6-cyclophane) complex (**1**–1,2-Me_2Im) due to the very small value of k_B^{CO}. For the 7,7-cyclophane **2**, the base-off mechanism was not a problem at [1,2-Me_2Im] > 0.2 M, and $k_{1,2\text{-}Me_2Im}^{CO}$ was obtained. Kinetic constants are summarized in Table III.

Dissociation Rates. Direct determination of k_B^{-CO}, B = **1**–MeIm or DCIm, was made by displacement with TMIC. In the absence of the base-off path ([B] large) and neglecting the reverse reaction,

$$k_{obsd} = \frac{k_B^{-CO} k_B^{TMIC}[TMIC]}{k_B^{TMIC}[TMIC] + k_B^{CO}[CO]} \quad (6)$$

Experiments were carried out where $k_B^{TMIC}[TMIC] \gg k_B^{CO}[CO]$ such that $k_{obsd} = k_B^{-CO}$. Rates were independent of base or TMIC concentrations in this range. The observed k_B^{-CO} are in reasonable agreement with those calculated from $k_B^{-CO} = k_B^{CO}/K_B^{CO}$.

Of the isocyanides studied, only TMIC had sufficiently slow dissociation rates for study by slow mixing methods. Displacement of TMIC by CO at 1.0 M [MeIm] or [DCIm] was carried out at high [CO] and low [TMIC]. First-order rate constants were independent of a 3-fold variation in [base] or [CO], identifying $k_{obsd} = k_B^{-TMIC}$ in the manner described by Olson and White for other isonitriles.[31b] Data for myoglobin was obtained in aqueous solution for comparison. These data are given in Table IV.

O_2 Kinetics. Oxygen binding kinetics were measured by using the Gibson technique previously described for hemoglobin, myoglobin,[35,36] and chelated hemes.[13a,25a] The technique takes advantage of the approximately 10-fold faster rate of O_2 addition to heme compounds compared to CO and the much greater binding constant of CO to hemes.

Photolysis of BHmCO in the presence of CO and O_2 gives the five-coordinated heme which subsequently may add either CO or O_2 in a fast step. Owing to the greater $k_B^{O_2}$, conditions can typically be obtained where 20–90% of the BHm is trapped as HmO_2. Subsequent return of the $BHmO_2$ to BHmCO in a slow

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step gives the oxygen binding constants.

$$\mathrm{BHmCO} \underset{k_B^{CO}}{\overset{k_B^{-CO}}{\rightleftharpoons}} \mathrm{BHm} \underset{k_B^{-O_2}}{\overset{k_B^{O_2}}{\rightleftharpoons}} \mathrm{BHmO_2} \qquad (7)$$

Treating this system as an approach to equilibrium after BHmCO photolysis the first observed fast step is given by

$$k_{fast} = k_B^{O_2}(O_2) + k^{-O_2} + k_B^{CO}(CO) + k_B^{-CO} \qquad (8)$$

Heme compounds almost always have much higher association and dissociation rates for O_2 than for CO, and therefore conditions are easily obtained in which $k_B^{CO}(CO)$ and k^{-CO} are negligible, allowing direct determination of $k_B^{O_2}$ from a plot of eq 9. At high

$$k_{fast} = k_B^{O_2}(O_2) + k_B^{-O_2} \qquad (9)$$

$[O_2]$, k^{-O_2} also becomes negligible. In our experience, other photolysis processes which sometimes occur in the absence of O_2 can contribute to this fast process at low O_2 concentration, often making the intercept of eq 9 unreliable.

The rate constant for the second, slower process is given by the rate constant for $BHmO_2$ dissociation times the fraction of Hm going to BHmCO, this fraction being $k_B^{CO}(CO)$ divided by all the rate processes in eq 8.

$$k_{slow} = \frac{k_B^{-O_2}k_B^{CO}(CO)}{k_B^{O_2}(O_2) + k_B^{-O_2} + k_B^{CO}(CO) + k_B^{-CO}} \qquad (10)$$

We usually keep CO constant and measure the fast and slow processes at zero or varying concentrations of O_2. Depending upon the binding constant for O_2 and realizing that k_B^{-CO} is very small, two extreme situations are almost invariably achievable.[1b,35]

Case I. $k_B^{CO}(CO) \gg k_B^{-O_2}$. In this case the Gibson equation[35] holds and eq 10 becomes, in reciprocal form,

$$\frac{1}{k_{slow}} = \frac{1}{k_B^{-O_2}} + \frac{k_B^{O_2}(O_2)}{k_B^{CO}(CO)k_B^{-O_2}} \qquad (11)$$

Case II. $k_B^{-O_2} \gg k_B^{CO}(CO)$. Equation 2 becomes

$$k_{slow} = \frac{k_B^{-O_2}k_B^{CO}(CO)}{k_B^{O_2}(O_2) + k_B^{-O_2}} = \frac{k_B^{CO}(CO)}{1 + K_B^{O_2}(O_2)} \qquad (12)$$

or

$$\frac{1}{k_{slow}} = \frac{1}{k_B^{CO}(CO)} + \frac{K_B^{O_2}(O_2)}{k_B^{CO}(CO)} \qquad (13)$$

In both of these cases and under all other conditions except those where other species are formed, a plot of $1/k_{slow}$ vs. O_2/CO has a slope equal to the O_2 equilibrium constant divided by the known quantity $k_B^{CO}(CO)$.

The slope of a plot of $1/k_{slow}$ vs. O_2/CO gives $K_B^{O_2}$, but the intercept cannot be readily interpreted from such plots unless one establishes whether one of these limiting cases applies. Examples of cases I[25] and II[1b] have been demonstrated. We recommend the use of plots of $k_B^{CO}(CO)/k_{slow}$ vs. O_2 at fixed [CO] using eq 14 as the best method for assessing the slow phase. The required

$$\frac{k_B^{CO}(CO)}{k_{slow}} = \frac{k_B^{CO}(CO)}{k_B^{-O_2}} + 1 + K_B^{O_2}(O_2) \qquad (14)$$

$k_B^{CO}(CO)$ is simply obtained from photolysis in the absence of O_2. The slope of such plots immediately gives $K_B^{O_2}$, and it is accurately determined regardless of which approximation holds. A single measurement of $k_B^{CO}(CO)$ followed by a single measurement of k_{slow} after O_2 is added reveals whether case II applies. Since $K_B^{O_2}(O_2) = HmO_2/Hm$, a small change in k_{obsd} upon addition of dioxygen means that oxygen is incompletely bound and $k_B^{-O_2}$ is larger than $k_B^{CO}(CO)$.[1b,33b] For example, at 5.6×10^{-4} M CO and 2.7×10^{-3} M O_2 in benzene, the values of $k_B^{CO}(CO)/k_{slow}$ are 160 for chelated protoheme (case I) and 1.4 for the Fe(6,6-cyclophane) **1** (case II) showing that **1**–B is only partially oxygenated at this concentration. However, case I is not so simple and requires a plot of eq 14 for its identification. When k_B^{-CO} is slow and can be ignored, the intercept (int) of eq 14 is rigorously defined as

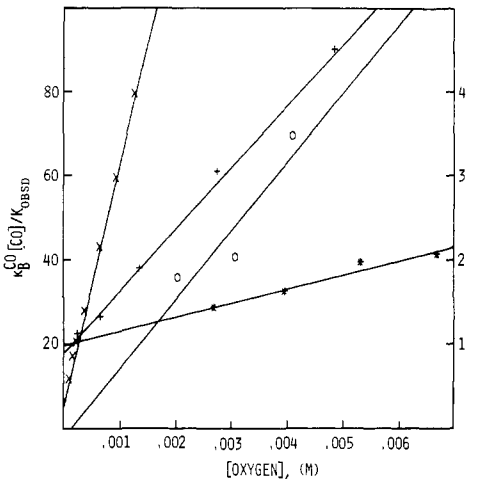


Figure 7. Plots of $k_B^{CO}(CO)/(k_{obsd})$ for the second, slow phase of return to heme–CO after photolysis of heme solutions in benzene at 20 °C in the presence of constant CO and variable O_2 concentrations. (+) Chelated protoheme, 7 μM, CO = 6.8×10^{-3} M, data recorded at 420 nm, $k_B^{CO}[CO] = 74800\ s^{-1}$. (○) Chelated protoheme, 7 μM, CO = 5.5×10^{-4} M, data recorded at 420 nm, $k_B^{CO}[CO] = 6100\ s^{-1}$. (×) 7,7-Cyclophane **2**, 10 μM, CO = 1.2×10^{-3} M, DCIm = 0.033 M, data recorded at 415.5 nm, $k_B^{CO}[CO] = 6050\ s^{-1}$. (*) 6,6-Cyclophane **1**, 7 μM, CO = 5.6×10^{-4} M, DCIm = 0.75 M, data recorded at 417 nm, $k_B^{CO}[CO] = 23.0\ s^{-1}$; right-side ordinate.

$$\mathrm{int} = \frac{k_B^{CO}(CO)}{k_B^{-O_2}} + 1 \qquad (15)$$

which becomes $k_B^{CO}(CO)/k_B^{-O_2}$ for limiting case I and identically 1.0 for case II. In any case, the intercept and its error (d_{int}) is all the information that is required to evaluate $k_B^{-O_2}$ and its error $dk_B^{-O_2}$ (assuming no error in $k_B^{CO}(CO)$).

$$k_B^{-O_2} = \frac{k_B^{CO}(CO)}{\mathrm{int} - 1} \qquad (16)$$

$$dk_B^{-O_2} = \left(\frac{k_B^{CO}(CO)}{(\mathrm{int} - 1)^2}\right) d_{int} \qquad (17)$$

As the intercept becomes close to 1, greater precision in the intercept is required to obtain $k_B^{-O_2}$ from eq 16 as in eq 17. (For $k_B^{CO}(CO) = 8000$, an error in the intercept of 1 at int 3 becomes an error in $k_B^{-O_2}$ of 2000.)

Plots of data for chelated protoheme according to eq 14 are shown in Figure 7 for two different CO concentrations (lines 1 and 2). The plot (+) corresponds to a case where the Gibson analysis applies (case I) and the intercept = 17.8 = $k_B^{CO}[CO]/k_B^{-O_2}$. At lower carbon monoxide concentrations (plot (○)) neither limiting case applies. In the plot of eq 14 the intercept for this rather poor data set is negative, and this error in $k_B^{-O_2}$ reveals that $k_B^{-O_2}$ is not well defined. However, the slope of this plot, $1.6 \pm 0.7 \times 10^4$, is within experimental error of that in plot (+), 1.5×10^4. Thus, $K_B^{O_2}$ is determined in both cases. The data for the 7,7-cyclophane **1** are plotted as (×) in Figure 7. In this case the intercept = 4.4 allows an estimate of $k_B^{-O_2}$ but not as accurately as in the case of chelated protoheme in which the plot was at higher CO concentration and thus gave a larger intercept. An additional data set for the 6,6-cyclophane heme is shown in the lower part of the figure (*). In this case the intercept is within experimental error of 1.0, and $k_B^{-O_2}$ cannot be obtained (case II). The slope of the plot still determines $K_B^{O_2}$. However, the rate changes are small, and $K_B^{O_2}$ is less accurately determined. Two separate experiments gave values of $K_B^{O_2}$ = 120 and 170 M^{-1}.

Data (Table III) for the chelated protoheme and heme cyclophanes **1** and **2** were obtained by this modified Gibson method. For the cyclophanes and chelated protoheme, the fast phase was

also studied. The slope of a plot of k_{fast} vs. $[O_2]$ was used to obtain $k_B^{O_2}$. This eliminates errors which might occur if other processes (not dependent on O_2) are also present in the fast relaxation. The $k_B^{-O_2}$ independently calculated from $k_B^{O_2}$ and $K_B^{O_2}$ (both obtained from slopes) is in good agreement with that found from the intercept of eq 14 in those cases where $k_B^{CO}(CO) \gg k_B^{-O_2}$. We believe that the slopes of eq 9 and 14 constitute the most reliable kinetic measures of $K_B^{O_2}$, $k_B^{O_2}$, and thus $k_B^{-O_2}$ because neither depend upon achievement of proper conditions. A similar approach was taken by Momenteau.[37]

When these methods are used, with precautions to ensure independence of kinetic behavior from base concentration, it is possible to obtain both dioxygen and carbon monoxide equilibrium constants with the same confidence, if not quite the same absolute accuracy as by direct titration.

Kinetic constants for isocyanide binding are listed in Table IV and those for carbon monoxide and dioxygen binding in Table III. Other published data are included for comparison.

Discussion

In order to study the effect of increasing steric encumbrance upon the reaction of hemes with ligands, we must first show that the structural alterations made for this purpose do not introduce other changes which affect ligation. It is known that increasing proximal basicity of the fifth ligand,[13a,21c,33] increasing the side-chain electron donation to the heme,[21c,38] or increasing solvent polarity[21c,39-41] causes the oxygen binding to increase, principally through decreased dissociation rates.[13d,25b,38] These changes have little effect on CO binding.[38] Introduction of "proximal pull" by changing the fifth ligand from 1-methylimidazole to 2-methylimidazole,[11a,b,21c,25b,29] or otherwise introducing strain into the Fe–Im bond,[13d] thus tending to deform the six-coordinated state, decreases the affinities of both dioxygen and carbon monoxide. It has also been suggested[39a] and recently documented[33b,c,42,43] that polar groups such as amides, when positioned near the iron center, increase oxygen binding by a "local polarity" effect.

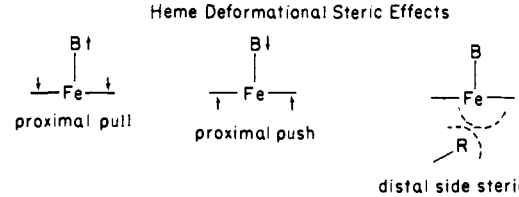
We have designed the two cyclophanes **1** and **2** to minimize these effects. First there are two lines of evidence that indicate that heme deformation does not result from attaching the cyclophane strap to form **1** and **2**. The affinity constant of the smallest Fe(6,6-cyclophane) (**1**) for 1-methylimidazole is 3×10^3 M^{-1} (Table I) compared to 4.5×10^3 M^{-1} for imidazole binding to the electronically similar deuteroheme dimethyl ester.[29b] The 7,7,7,7-cyclophane (FeC_2cap) **5**, which is known to be distorted,[44] binds 1-methylimidazole 10 times poorer than does the uncapped iron tetraphenylporphyrin[21c] (see Table I). Secondly, the porphyrin group in adamantane 1,3-porphyrin Fe(6,6-cyclophane), **9–P**, has been shown by X-ray crystallography to be flat.[20c] This cyclophane has 15 atoms connecting to the 1,13 positions in the porphyrin compared to 16 for the anthracene Fe(6,6-cyclophane), **1**, and is otherwise identical. We therefore conclude that neither the 6,6- nor 7,7-cyclophane model compound is either distorted or strained toward adding a fifth ligand.

Secondly, we have chosen an octaalkylporphyrin without substitution at the meso positions in order to maintain electronic effects like those in mesoheme or protoheme and their derivatives. Similar imidazoles are used as fifth ligands in the cyclophanes and reference compounds.

The "local polarity" effects are minimized by using anthracene as the cap and benzene as the solvent. However, the two amide groups in the side chains (Figure 1) could impart some local polarity and thus affect dioxygen (but not CO) binding. Since polarity effects are displayed in dioxygen dissociation rates, we can set some limit on this effect by observing dissociation rates.[13d] The $k_B^{-O_2}$ for chelated mesoheme, Fe(7,7-cyclophane)–DCIm (**1**–DCIm), and Fe(6,6-cyclophane)–DCIm (**2**–DCIm) are 4500, 1000, and 800 s^{-1}, respectively, in benzene (Table III). By this criterion there is a local polar effect in the cyclophanes, but it is small.

By contrast the pocket hemes **10** and **11** (*n,n,n*-cyclophanes), having three amide groups which are brought closer to the iron site as *n* decreases, show large decreases in $k_B^{-O_2}$ as *n* decreases (Table III). Recent studies of Momenteau,[33] Suslick,[42] and Chang[43] make it quite clear that such polar groups must reduce $k_B^{-O_2}$ and make it very reasonable to assume that this large decrease (from $k_B^{-O_2} = 2900$ to 9 s^{-1}) is entirely due to a polar effect. This interpretation brings the results with the "pocket hemes" into concordance with all the other steric effect studies in which it was concluded that steric effects on dioxygen and carbon monoxide are similar. It is important to notice that, within experimental error, $k_B^{-O_2}$ for our two cyclophanes is the same. We can conclude that the introduction of the anthracene 7,7- or 6,6-cyclophane attachment has not significantly altered electronic or solvent environment or changed the heme planarity. This is further documented by the rather small difference with regard to diatomic molecule ligation between chelated mesoheme and the 7,7-cyclophane, seen in Table III. Therefore, we can confidently attribute changes in ligation inside the cyclophane to steric effects, although comparison with non-cyclophane model compounds requires a small correction for local polar effects. Because the 7,7-cyclophane behaves like non-cyclophane mesoheme compounds, our estimation of steric effects does not depend upon the choice of reference compounds. In the tetraphenylporphyrin series, this is not the case. The 5,5,5-cyclophanes (pocket hemes) **10** and **11** display affinity reductions compared to picket fence heme but not compared to large ring capped hemes such as **5** or **6** or tetraphenylheme itself (Table III).

Kinetic and Mechanistic Consequences of Steric Effects. Two kinds of steric effects have been postulated for hemes and hemoproteins: a heme deformation effect moving the iron either toward (proximal push)[11e,20d] or away from (proximal pull)[7,8,10,11a,25b,45] the incoming ligand and a steric effect in which some nonbonding group interferes with the approaching or bound ligand. The proximal deformation could preferentially stabilize



either the five- or six-coordinated state, resulting in decreased or increased ligand affinity relative to a simple, flexible heme. The proximal pull effect, which is thought to be responsible for the R-to-T-state change in hemoglobin,[5,7,45] has been demonstrated in model compounds by introducing repulsive groups or bending strain in the proximal base (e.g., 2-methylimidazole).[11a,29b] This effect was shown to increase the CO dissociation and decrease the association rates approximately equally and increase O_2 dissociation without greatly altering the O_2 association rates.[13d,25]

The effect on CO binding kinetics resembles that which is common in transition-metal carbonyl compounds[46] such as

(37) Momenteau and Lavalette[33b] have substituted eq 8 into eq 10 to obtain $k^{-O_2} = (k_{slow})(k_{fast})/k_B^{CO}(CO)$, making three measurements at constant CO with and without O_2. This method avoids assumptions and gives $k_B^{-O_2}$ as a function of three measured rates on the same system. However, it is still necessary to determine either $K_B^{O_2}$ or $k_B^{O_2}$ from slopes rather than single determinations.

(38) Traylor, T. G.; White, D. K.; Campbell, D. H.; Berzinis, A. P. *J. Am. Chem. Soc.* **1981**, *103*, 4932.

(39) (a) Brinigar, W. S.; Chang, C. K.; Geibel, J.; Traylor, T. G. *J. Am. Chem. Soc.* **1974**, *96*, 5597. (b) Traylor, T. G.; Berzinis, A.; Campbell, D.; Cannon, J.; Lee, W.; McKinnon, D.; Mincey, T.; White, D. K. In "Biochemical and Clinical Aspects of Oxygen"; Caughey, W. S., Ed.; Academic Press: New York, 1979; pp 455–476.

(40) Stynes, D. V. *Can. J. Spectrosc.* **1981**, *26*, 109.

(41) Jones, R. D.; Summerville, D. A.; Basolo, F. *Chem. Rev.* **1979**, *79*, 139.

(42) Suslick, K. S.; Fox, M. M. *J. Am. Chem. Soc.* **1983**, *105*, 3507.

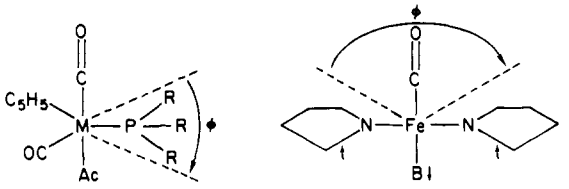
(43) Chang, C. K. *J. Am. Chem. Soc.* submitted.

(44) (a) Jameson, G. B.; Ibers, J. A. *J. Am. Chem. Soc.* **1980**, *102*, 2823. (b) Sabat, M.; Ibers, J. A. *Ibid.* **1982**, *104*, 3715.

(45) (a) Takano, T. *J. Mol. Biol.* **1977**, *110*, 537. (b) *Ibid.* **1977**, *110*, 569.

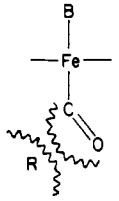
(46) Darensbourg, D. J.; Kudaroski, R.; Schenk, W. *Inorg. Chem.* **1982**, *21*, 2448.

$C_5H_5MoAc(CO)_2PPh_3$, studied by Barnett and Pollman.[47,48] The CO dissociation rates were plotted against the calculated steric "cone angle ϕ" to reveal an R size vs. k^{diss} effect. In the case



of binding of CO to five-coordinated hemes, we suggest that steric hindrance in B would likewise decrease the N–Fe–N angle, increasing repulsion between CO and the pyrrole nitrogens.

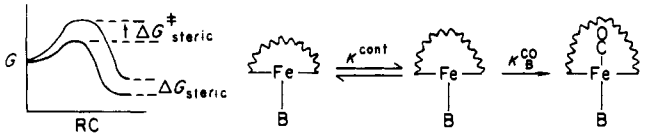
In its earlier formulations, distal side steric effect was considered similar to the example give above, in that the CO was found to be bent off the heme normal,[10] which it adopts in model compounds,[9] and the CO stretching frequency was considered abnormally low in heme protein carbon monoxide complexes.[10] Such



deformation was considered, like that caused by the large phosphines in organometallic carbonyl compounds discussed above, to destabilize the heme–CO complex, thus resulting in lower affinities for carbon monoxide in heme proteins than in model compounds.[10-12]

Our first reservation concerning this appealing theory was based upon the discovery that chelated protoheme, in MTAB suspension, binds carbon monoxide with the same affinity as does R-state hemoglobin.[13] But this conclusion rests upon the assumption that heme solvation energies in the two systems are similar. This reservation is now strongly supported by the finding that distal steric effects in CO binding are entirely in the association rates in model compounds (see Table III). All available data are consistent with the notion that steric effects are seen almost exclusively in the association rates for the ligands carbon monoxide, dioxygen, and isonitriles. We propose that this is a general property of distal side steric effects.

Finding that distal steric effects on ligand binding are displayed primarily in the association rates suggests that distal side steric effects are not directly related to repulsion in the bound state, as previously suggested, but are governed by the limited access to the heme face. Kinetically, we observe that the transition state and bound state are equally affected by steric encumbrance. A suggested[1b,c] mechanistic interpretation consistent with these observations, and those in hemoproteins, comprises a rapid conformational equilibrium among almost equal energy states, some of which deny access to the heme. After ligand binding, the number of such states is reduced. In this case $k^{steric} \approx K^{conf}$, analogous to conformational effects upon reactivity discussed by Winstein et al.[49]



We can now conclude that the several effects are characterized by different kinetic changes as shown in Table V.

Independence of Proximal and Distal Side Steric Effects. Since the proximal side steric effect (proximal pull or T-state effect) involves conformational destabilization of the bound state toward

(47) Barnett, K. W.; Pollman, T. G. *J. Organomet. Chem.* **1974**, *69*, 413.
(48) Brown, H. C. *J. Chem. Soc.* **1956**, 1248.
(49) Winstein, S.; Holness, N. J. *J. Am. Chem. Soc.* **1955**, *77*, 5562.

Table V. Kinetic Characteristics of Steric, Electronic and Solvent Effects[a]

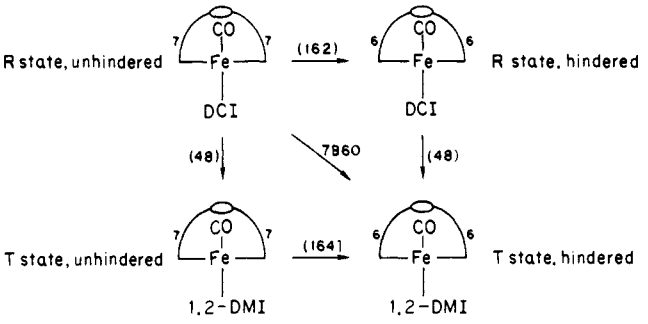
	association rates			dissociation rates		
	CO	RNC	O_2	CO	RNC	O_2
proximal pull	dec.		NC	inc.		inc.
distal steric hindrance	dec.	dec.	dec.	NC	NC	NC[b]
increased solvent polarity or electron donation	NC[c,d]		NC[d]	NC		dec.

[a] dec., decrease; NC, little or no change; inc., increased; blank, not determined. References appear in the previous discussion. [b] The decreased dissociation rates in some cyclophanes are discussed, and this conclusion is justified in the following section. [c] Small decreases have been observed. [d] Small changes have been observed. However, the effects of ligand solubilities complicate these studies.

Table VI. Carbonyl Stretching Frequencies of Heme–CO Complexes

	solvent	ν_{CO}, cm^{-1}	ref
Fe(6,6-cyclophane)(MeIm)(CO), **1**–CO	$CHCl_3$	1975	this work
Fe(7,7-cyclophane)(MeIm)(CO), **2**–CO	$CHCl_3$	1966	this work
Fe(adamantane 6,6-cyclophane)(MeIm)(CO), **9**–CO	$CHCl_3$	1959	20c
Fe(C_2cap)(MeIm)(CO), **5**–CO	toluene	2002	21d
Fe(C_2cap)(1,2-Me_2Im)(CO), **5**–CO	toluene	1999	21d
Fe(C_3cap)(1,2-Me_2Im)(CO), **6**–CO	toluene	1984	21d
Fe(PocPiv)(MeIm)(CO), **11**–CO	C_6D_6	1964	21d
Fe(TPivPP)(MeIm)(CO)	C_6D_6	1969	11d
Fe(TPP)(1,2-Me_2Im)(CO)	toluene	1972	21d
MbCO	aqueous	1931, 1945, 1970	10b
HbCO	aqueous	1951	10a
chelated protoheme	Me_2SO	1951	27

dissociation and distal side steric effect does not (involving groups away from the heme), it would be expected that these two effects might act independently. The diagram shown below for 6,6- and 7,7-cyclophanes **1** and **2**, taken from Table III and the Results section, shows that this is the case.



The numbers in parentheses show the observed reduction in CO affinity ΔK_B^{CO} in making the change in the direction of the arrow. Substitution of simple hemes for the 7,7-cyclophane does not substantially change this diagram. Clearly, steric effects on CO binding are the same for the R-state as for the T-state models. These results suggest that proximal and distal side steric effects can independently control ligand affinities in hemoproteins.

Structural Differentiation of CO and O_2. The wide variation in the CO to O_2 affinities in hemoproteins[20e] requires some explanation. Based upon X-ray crystal structure studies and upon variations in carbon monoxide stretching frequencies, the distal steric differentiation discussed above was proposed. The idea that bound carbon monoxide, which assumes a natural orientation perpendicular to the heme plane, would suffer greater steric hindrance than does dioxygen which assumes a bent configuration has been accepted and widely discussed. First, the proposed correlations of carbon monoxide stretching frequencies with carbon

monoxide affinities and with steric effects are shown by the data summarized in Table VI to be incorrect. Carbon monoxide stretching frequencies correlate with solvent polarity and electronic effects rather than steric hindrance.[13c,50]

Second, our results presented here and elsewhere, particularly with **1**, **2**, and **9**, are inconsistent with more than about 3-fold steric differentiation of CO and O_2. Finally, other studies,[22b] which appear to show steric differentiation, have not been corrected for local polar effects. This correction can be made by using the kinetic criteria for steric effects previously suggested. One can take values of $\Delta P_{1/2}^{CO}$ (from Table III) as an indication of the magnitude of expected steric effects and the value of $k_B^{O_2}/k_B^{CO}$ as a kinetic measure of steric differentiation. Thus, we see that in benzene or toluene, this ratio for unhindered hemes ranges from 7 to 10 and with the hindered compounds from 3.3 to 17. However, it is interesting that both the anthracene 6,6-cyclophane **1** and the 5,5,5-cyclophane ("FePocP") **10** have lowest values of $k_B^{O_2}/k_B^{CO}$, indicating, if anything, that both differentiate *against* dioxygen by steric effects.

Adamantane 6,6-cyclophane, **9**, and the cofacial 5,5-cyclophane ("Cu 5") **8** show somewhat higher $k_B^{O_2}/k_B^{CO}$ ratios, 17 and 16, respectively. These compounds, therefore, seem to show a slight steric differentiation favoring O_2 over CO.

Having concluded that steric hindrance does not significantly differentiate between CO and O_2, we must explain the large variations in the affinity ratios, $K_B^{CO}/K_B^{O_2}$, among the cyclophanes and hemoproteins. These vary among model systems from about 300 to about 10^5. The first clue to these variations is the large change in M value ($K_B^{CO}/K_B^{O_2}$ ratio) from about 8000 for chelated mesoheme in benzene to 270 in the cetyltrimethylammonium bromide suspension in water (calculated from Table III). The polarity of these micelles has been estimated to be higher than that of dimethylformamide.[14] Similar results have been obtained by Suslick with tetra(2,4,6-triphenyl)phenylheme.[42] When the solvent effect is combined with previously established effects of proximal base, side-chain electronic effects,[21c,38] and hydrogen bonding,[33d,43] all of which have dominant effects on dioxygen affinity, very large changes in $K_B^{CO}/K_B^{O_2}$ can be obtained.

Moffatt et al.[8b] have used these kinetic criteria (k^{O_2}/k^{CO}) to examine steric effects in hemoproteins. Although the ratio of affinities among hemoproteins changes from K^{CO}/K^{O_2} of about 6000 to less than 4, the value of k^{O_2}/k^{CO} changes little, ranging from 3 to 30, just as in the case of the models discussed above. This latter value of 30 represents the largest possible steric differentiation yet seen in hemoproteins as judged by kinetic criteria. Therefore, the proteins seem to behave like the simple cyclophane model systems in showing little steric differentiation between CO and O_2. In proteins as well as model compounds, steric effects seem to be displayed only in the association rates.

Additional evidence for small differentiation between bent and linear ligands was provided by Romberg and Kassner.[50] The ratios of nitric oxide to carbon monoxide binding, K_B^{NO}/K_B^{CO}, with protoheme 1-methylimidazole, hemoglobin, and myoglobin were found to be 2000, 2400, and 15 000, respectively. This confirms our previous conclusions that hemoglobin binds linear and bent ligands without steric differentiation but myoglobin shows a small differentiation which might be due to steric effects. However, $k_B^{O_2}$ is about 3 times smaller in myoglobin than in hemoglobin α chains, suggesting contributions from other effects which could also affect NO binding.

We conclude that differentiation of CO and O_2 can be effectively brought about with changes in solvent polarity, electronic effects, and hydrogen bonding *but not with distal side steric effects alone*.

Comparison with Proteins. Comparisons between model hemes and proteins can be made in terms of ligation-free energy differences. A ligation-free energy difference, $\delta\Delta G$, for the cyclophanes and hemoproteins can be calculated by subtracting their respective ligation-free energies from those of the unhindered but structurally similar model chelated protoheme.

$$\delta\Delta G = -RT \ln(K_H^1/K_D^1) \qquad (18)$$

In eq 18, K_D^1 is the equilibrium constant for binding the ligand, l, to chelated protoheme and K_H^1 is the corresponding constant for the hindered cyclophane hemes or hemoproteins. $\delta\Delta G$ may be expressed in terms of a sum of "effects" related to differences between the reference compound (chelated protoheme) and the heme or protein in question.

$$\delta\Delta G = \delta\Delta G_{steric} + \delta\Delta G_{solvent} + \delta\Delta G_{electronic} + \delta\Delta G_{other} \qquad (19)$$

For CO and isocyanides, solvent and heme electronic differences are considered unimportant for the comparisons given in Table VII and the $\delta\Delta G$'s may be equated to ΔG_{steric}. Olson has interpreted isocyanide binding to hemoproteins in terms of both steric hindrance and a component associated with proposed hydrophobic interactions between the protein and more hydrophobic isocyanides.[31a,c] Hydrophobic effects in the protein would favor isocyanide binding compared with the model studies in benzene. Therefore, if anything, the calculated $\delta\Delta G$'s in Table VII for isocyanide binding may underestimate the steric effects in the proteins. We have not attempted to separate the hydrophobic effect as Olson has done. For the isocyanides used in our work, the hydrophobic factors should not be large enough to change our conclusions.

Because of the greater dependence of O_2 binding on solvent and electronic factors, the interpretation of $\delta\Delta G$ is more difficult for this ligand. For this reason $\delta\Delta G$'s for the cyclophanes are calculated using the more electronically similar chelated mesoheme as the reference heme. This comparison shows (assuming $\Delta G_{solvent} + \Delta G_{other} = 0$) no steric effect in 7,7-cyclophane **2** and significant steric effects in anthracene 6,6-cyclophane (**1**) and adamantane 6,6-cyclophane (**9**) which are rather close to those for CO.

For Mb and Hb the chelated protoheme is a valid electronic reference heme, but solvation differences are important. The very large $\delta\Delta G$ based on chelated protoheme in benzene is largely attributable to the poor binding of O_2 to chelated protoheme in benzene. If one uses the O_2 affinity of chelated protoheme in aqueous CTAB or MTAB as the reference, much smaller $\delta\Delta G$'s are obtained. One still cannot decide whether the ~1 kcal/mol difference is steric or due to solvation differences between the heme in the micelle and that in the protein. Given that O_2 affinity in model hemes is sensitive to the environment, it is clearly impossible to unambiguously resolve the question of whether O_2 suffers a steric effect in the proteins by comparison of O_2 binding constants with any model, since no model can adequately duplicate the environment found in the protein. For this reason, M values ($K_B^{CO}/K_B^{O_2}$) should not be used to characterize steric effects since $K_B^{O_2}$ is much too sensitive to other factors.

With these uncertainties in mind, examination of Table VII reveals that steric effects on binding of ligands to the sterically hindered cyclophanes **1** and **9** are generally greater than those in hemoglobin or myoglobin and steric effects in the 7,7-cyclophane **2** are smaller than those of these heme proteins. These effects have been discussed in detail by Olson.[31c]

Table VII. Ligation-Free Energy Differences[a] $\delta\Delta G$ (kcal/mol) at 298 K for Cyclophane Hemes or Hemoproteins

ligand	**1**[e]	**9**[f]	**2**[g]	Mb	Hb(R)α
n-BuNC	5.3		2.7	5.3	5.1
t-BuNC	8.2	5.1	4.5	7.2	8.3
TMIC	5.4	6.2	1.0	7.4	
CO	3.8	4.2	0.8	1.6	−0.4
O_2	3.0	2.5	−0.8	2.6	−2.3
	2.9[b]	2.4[b]	−0.7[b]	0.5[c]	1.3[c]
CH_3Im[d]	5.7		1.2		

[a] Relative to chelated protoheme in benzene unless stated otherwise (see eq 18). [b] Relative to chelated mesoheme in toluene. [c] Relative to chelated protoheme in aqueous CTAB suspension. [d] Relative to $K^B_B = 6 \times 10^4$ M^{-1} for deuteroheme DME. [e] Anthracene 6,6-cyclophane. [f] Adamantane 6,6-cyclophane. [g] Anthracene 7,7-cyclophane.

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Conclusion. Distal steric effects in anthracene heme cyclophanes and other cyclophanes can be large. They reside almost exclusively in the ligand association rates, suggesting a control by a conformational preequilibrium. Distal steric effects differentiate ligands by gross size and shape but do not significantly differentiate linear from bent binding diatomic molecules. Variations in K^{CO}/K^{O_2} are found to be the result of changes in polar rather than steric effects.

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Registry No. **1**, 93985-01-4; **1**-(MeIm), 93985-21-8; **1**-(MeIm)$_2$, 93985-23-0; **1**-(MeIm)(*n*-BuNC), 93985-05-8; **1**-(MeIm)(*t*-BuNC), 93985-06-9; **1**-(MeIm)(TMIC), 93985-07-0; **1**-(MeIm)($C_6H_{11}NC$), 93985-08-1; **1**-(*n*-BuNC), 93985-36-5; **1**-(*t*-BuNC), 93985-24-1; **1**-(TMIC), 93985-25-2; **1**-($C_6H_{11}NC$), 93985-26-3; **1**-(*n*-BuNC)$_2$, 93985-30-9; **1**-(*t*-BuNC)$_2$, 94089-29-9; **1**-(TMIC)$_2$, 93985-31-0; **1**-($C_6H_{11}NC$)$_2$, 93985-32-1; **1**-(DCIM)(CO), 93985-04-7; **1**-(MeIm)(CO), 93985-18-3; **1**-(1,2-Me$_2$Im)(CO), 93985-16-1; **1**-(DCIM)(O_2), 94024-66-5; **2**, 93985-02-5; **2**-(DCIM), 93985-22-9; **2**-(MeIm)$_2$, 93985-20-7; **2**-(DCIM)(MeIm), 93985-19-4; **2**-(DCIM)(*n*-BuNC), 93985-09-2; **2**-(DCIM)(*t*-BuNC), 93985-10-5; **2**-(DCIM)(TMIC), 93985-11-6; **2**-(*n*-BuNC), 93985-27-4; **2**-(*t*-BuNC), 93985-28-5; **2**-(TMIC), 93985-29-6; **2**-(*n*-BuNC)$_2$, 93985-33-2; **2**-(*t*-BuNC)$_2$, 93985-34-3; **2**-(TMIC)$_2$, 93985-35-4; **2**-(DCIM)(CO), 93985-03-6; **2**-(MeIm)(CO), 93985-17-2; **2**-(1,2-Me$_2$Im)(CO), 93985-15-0; **2**-(DCIM)(O_2), 94024-65-4; TMIC, 36635-61-7; MeIm, 616-47-7; 1,2-Me$_2$Im, 1739-84-0; DCIm, 80964-44-9; CO, 630-08-0; O_2, 7782-44-7; *n*-BuNC, 2769-64-4; *t*-BuNC, 7188-38-7; $C_6H_{11}NC$, 931-53-3; $C_6H_{11}NH_2$, 108-91-8; $C_6H_{11}CHO$, 2043-61-0; chelated protoheme, 76747-88-1; chelated protoheme-(*n*-BuNC), 93985-12-7; chelated protoheme-(*t*-BuNC), 93985-13-8; chelated protoheme-(TMIC), 93985-14-9.

Model Studies of Iron-Tyrosinate Proteins

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Abstract: The phenolate-to-iron(III) charge-transfer transition in a series of iron(III) phenolate complexes has been investigated. NMR contact shifts for the phenolate protons are well correlated with the visible absorption maxima of the complexes and the Fe^{III}/Fe^{II} redox potentials. The results indicate that the energy of the phenolate-to-iron(III) charge-transfer band is sensitive to the crystal field strength of the other ligands coordinated to the ferric center. The stronger the other ligands are, the higher the energy of the phenolate charge-transfer band. The blue shift of the charge-transfer band is also reflected in smaller contact shifts for the phenolate protons and a more negative Fe^{III}/Fe^{II} redox potential. On the basis of these studies, the probable identities of ligating species in transient dioxygenase intermediates are deduced. These studies also demonstrate that a square-pyramidal complex with an apical and a basal phenolate can give rise to phenolate charge-transfer bands of quite different energies. A dioxygenase active site approaching such a structure is proposed. Lastly, the axial ligand in Fe(salen)OC_6H_4-4-CH_3 is shown to be an excellent model for tyrosine in resonance Raman studies of iron-tyrosinate proteins. Isotopic substitution studies on the model complex show that the ca. 570-cm^{-1} feature found in these proteins cannot be assigned solely to an Fe-O stretching vibration.

The coordination of tyrosine to metal centers in proteins is a structural feature recently found for a number of metalloproteins.[1] Resonance Raman spectroscopy has played a crucial role in elucidating this coordination feature in the transferrins,[2-5] the catechol dioxygenases,[6-10] and the purple acid phosphatases.[11-13] These comprise the new subclass of iron-tyrosinate proteins.[7] Resonance-enhanced phenolate vibrations observed at ca. 1170, 1270, 1500, and 1600 cm^{-1} result from excitation into the tyrosinate-to-iron(III) charge-transfer bands and are the characteristic Raman spectral signature for these proteins. In several cases, a prominent low-frequency feature near 570 cm^{-1} is also observed[10,12,14] and is proposed to arise from Fe-O modes. To assign this vibration, we have studied the Raman spectrum of a ferric *p*-cresolate complex employing ^{54}Fe, ^{18}O, and ^{2}H isotopic substitutions.

The factors affecting the energy of the phenolate-to-iron(III) charge transfer transition have also been investigated. The tyrosinate-to-iron(III) charge-transfer bands in the proteins are clearly sensitive to the ligand environment, as evidenced by the broad range of colors exhibited by iron-tyrosinate proteins. The charge-transfer transition in Fe(III) transferrins can be shifted simply by changing the Lewis base function on the synergistic anion,[15] while the binding of a variety of substrates and inhibitors to the catechol dioxygenases gives rise to complexes with absorption maxima spanning the 400-600-nm region.[16-20]

Although previous studies have investigated spectral shifts in iron phenolate complexes,[21,22] recent observations on the catechol

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