Dioxygen and Carbon Monoxide Binding to Apolar Cyclophane Hemes: Durene-Capped Hemes

Shantha David,[†] Brian R. James,*[,†] David Dolphin,[†] Teddy G. Traylor,*[,‡,§] and Marco A. Lopez[§]

Contribution from the Department of Chemistry, University of British Columbia, Vancouver, British Columbia, V6T 1Z1, Canada, and Department of Chemistry, University of California, San Diego, La Jolla, California 92093-0506

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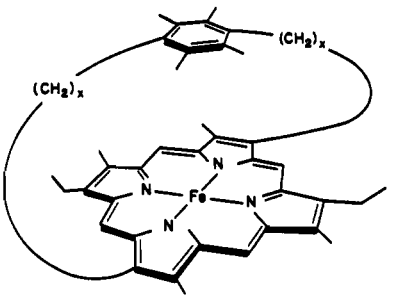
Abstract: Detailed solution kinetic and equilibria data (mainly in toluene) are presented for the reversible binding of CO and O_2 to the five-coordinate hemes Fe(por)B, where B is 1,5-dicyclohexylimidazole or 1,2-dimethylimidazole (chosen to mimic the R- and T-states, respectively) and por = the dianion of some durene-capped porphyrins with variable length linking methylene straps on either side of the durene moiety (4/4, 5/5, or 7/7 methylenes). Use of spectrophotometric equilibrium titrations from 30 to −50 °C, stopped-flow data, and laser flash photolysis under either CO or CO/O_2 mixtures, has allowed for determination of on and off rates, equilibrium constants, and, in the case of the 4/4-system, thermodynamic constants for the binding. Increasing steric hindrance provided by the durene cap, in the order 7/7 < 5/5 < 4/4, is generally less than expected from studies with other heme derivatives; in combination with the complete absence of polarity effects, as within nonpolar distal sites, the durene hemes exhibit poor differentiation between CO and O_2. However, the distorted 4/4-derivative discriminates between CO and O_2 in a novel way through a "proximal effect" associated with deformation of the porphyrin skeleton from planarity, the effect being largely reflected by an increased CO dissociation rate.

Reversible dioxygen carrying hemoproteins show considerable differentiation in the coordination of small ligands such as CO and O_2 to the iron center. In efforts to elucidate the extent to which this differentiation is governed by steric versus electronic or polar factors associated with the *distal* environment of the heme (surrounding the vacant sixth site), numerous sterically-encumbered iron(II) porphyrin model systems, which attempt to mimic the protein active sites, have been synthesized and studied.[1-7] Consistency within studied systems strongly suggests that while steric effects can alter affinity for both O_2 and CO, electronic factors largely enhance O_2 affinity without appreciably affecting CO affinity.[7]

Available crystallographic data, although limited to a few model systems,[1b,8] indicate an essentially planar porphyrin macrocycle; however, substantial doming of the porphyrin skeleton toward the coordinated fifth (*proximal*) ligand is revealed in the crystal structures of some hemoproteins (e.g., deoxy-Mb[9] and MbO_2[10]) and may be expected to influence ligand affinity. In two isolated cases of model hemes,[11,12] some degree of porphyrin buckling is revealed in the structural determination, although the influence on heme reactivity is not clear. In one instance, porphyrin doming was invoked to explain the low O_2 affinity of a TPP-derived C_2-cap-pyridine strapped heme which had an axial pyridine base attached to the porphyrin periphery at two opposite benzene rings.[13] The lack of structural information and kinetic data to date, however, limits conclusive analysis.

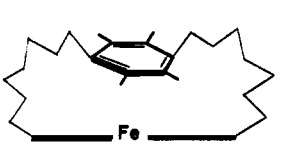


I

x = 4 ; Fe(durene-4,4)

x = 5 ; Fe(durene-5,5)

x = 7 ; Fe(durene-7,7)



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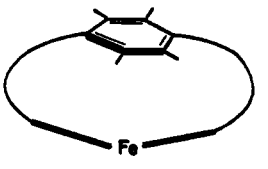

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† University of British Columbia.

‡ Deceased June 14, 1993.

§ University of California.

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For the purpose of systematically investigating the effect of porphyrin deformation on ligation to the sixth site of the heme without changes in the polarity of the environment, the durene-capped hemes (I)[14]—having varying degrees of enforced skeletal distortion—seemed ideally suited. The reported UV–visible spectral trends of the free-base porphyrins and six-coordinate heme complexes of isocyanides, CO, and O_2 (bound on the capped side of the heme, opposite the proximal imidazole) indicate distortion of the porphyrin macrocycle increasing in the order 7/7 < 5/5 < 4/4, with the 7/7-system of the series remaining essentially planar.[15] Crystallographic data reveal a severely distorted porphyrin skeleton in the smallest capped durene-4/4 hemin chloride derivative. It has an angle of 43° between the two pyrrole rings linking the cap to the porphyrin periphery, while the corresponding angle between the other two pyrrole rings is 17.2° (based on a coplanar angle of 0°).[16]

In the present study, we investigate the detailed binding behavior, mainly in toluene, of the five-coordinate durene-capped hemes (containing axial base B = 1,5-dicyclohexyl-(DcIm) or 1,2-dimethylimidazole(1,2-Me_2Im) toward CO and O_2. Increasing steric hindrance provided by the durene cap, in the order 7/7 < 5/5 < 4/4 is clearly exemplified by the affinities of the hemes toward bulky isocyanides.[17] In the case of CO and O_2, however, steric hindrance to binding is less than that expected from studies with other heme derivatives, even with the tightest capped 4/4-heme; in combination with the complete absence of polarity effects, as within nonpolar distal sites, these durene hemes exhibit poor differentiation between CO and O_2. One exception in the series is found with the distorted 4/4-derivative, a novel system which appears to discriminate between CO and O_2 binding through "proximal effects" associated with the severe deformation of the porphyrin skeleton from planarity.

Experimental Section

Preparation of five-coordinate complexes in toluene, Fe^{II} (durene-por)(B) where B = an imidazole base, from the four-coordinate hemes was carried out as described previously.[15] Toluene was distilled over CaH_2 immediately prior to use. *o*-Dichlorobenzene was washed with concentrated H_2SO_4, aqueous Na_2CO_3, and water, dried over Na_2SO_4, and distilled from CaH_2 prior to use. *N*-Methylimidazole, *N*-MeIm (Aldrich), was distilled under reduced pressure from KOH, 1,2-Me_2Im was recrystallized from heptane, and DcIm was prepared as previously described.[1a] *tert*-Butylisocyanide and tosylmethylisocyanide (TMIC) (Aldrich) were used as received. N_2 was passed through Ridox and molecular sieve columns to remove trace O_2 and H_2O. CO and O_2 were passed through molecular sieve columns. The solubilities of CO and O_2 used were 1.0×10^{-5} M Torr^{-1} and 1.2×10^{-5} M Torr^{-1} in toluene and 6.8×10^{-6} M Torr^{-1} and 8.0×10^{-6} M Torr^{-1} in CH_2Cl_2, respectively, at 20 °C.[18]

Equilibrium Titrations. [ν(CO) bands for the six-coordinate Fe(durene-por)B(CO) species in toluene were found in the range 1972–1990 cm^{-1}; attempts to measure $\nu(O_2)$ were unsuccessful [David, S. Ph.D. Dissertation, University of British Columbia, Vancouver, BC, 1985]. Titration with CO or O_2 was accomplished by admitting prediluted CO/N_2 or O_2/N_2 mixtures into a tonometer while being measured on a manometer. The heme solution was shaken well and allowed to equilibrate to the temperature in the spectrophotometer. A large tonometer, with a solution/volume ratio of ~100:1, minimized any changes in partial pressure of the gas over the solution during mixing. Spectra were recorded on a Cary 17 spectrophotometer in the 700–250-nm region. For temperatures down to −10 °C, a Haake (model FK) circulating thermostatting bath connected to a cell-holding Dewar was used to maintain a constant temperature. A 6-cm path length quartz cell mounted inside a small Dewar was used for lower temperatures, down to −50 °C; the Dewar was filled with a slush bath of appropriate temperature to equilibrate the solution contained within the quartz cell.

Kinetic Measurements. Carbon monoxide dissociation rates were obtained using a Durrum stopped flow (Model 110) apparatus. Equal volumes of toluene solutions of TMIC and Fe^{II}(por)(B)(CO) (B = DcIm or 1,2-Me_2Im) were preequilibrated to 20 °C and rapidly mixed in a 2-cm path length cell compartment. The change in absorbance versus time for the formation of Fe^{II}(por)(B)(TMIC), under pseudo-first-order conditions, was monitored spectrophotometrically in the 460–250-nm region.

Laser flash photolysis under either CO or CO/O_2 mixtures was used to determine CO kinetic constants and kinetic and some equilibrium constants for O_2 binding. Aliquots of CO and/or O_2 were syringed through a septum plug into a degassed tonometer containing the heme solution. The entire tonometer was then clamped horizontally in a water-bath thermostatted to 20 °C, and the heme solution was stirred magnetically for 15 min. Photolysis was accomplished using a Phase-R DL 2100 D tunable flash lamp pumped-dye laser with a rating of 0.5 J/pulse; the decay rate constant of the laser was $\sim 1.7 \times 10^6$ s^{-1}, with a pulse width of 400 ns. A detailed description of the apparatus and methods used has been provided elsewhere.[19]

Results

Within the durene-capped heme series, *N*-MeIm was prevented from coordinating at the distal side of the heme only in the case of the smallest capped durene-4/4 derivative; in neat *N*-MeIm the visible spectrum for this heme showed no trace of the characteristic α and β bands indicative of six-coordination.[15] For the purpose of making useful comparisons between the binding behavior of the five-coordinate systems for all three durene hemes, the sterically hindered bases DcIm and 1,2-Me_2Im were chosen to mimic the R- and T-state systems, respectively. For steric reasons, these bases do not coordinate on the capped side of the heme to give a six-coordinate complex, Fe(por)$(B)_2$. Visible spectral changes in the 700–460-nm region indicated that less than 10% of the bis-base species is formed, even for the largest capped 7/7-derivative, in heme solutions containing base concentrations of up to 1 M in either base.[15]

CO Binding. With the exception of the Fe(por)(DcIm) systems, where por = durene-7/7 or durene-5/5, for which CO affinities were too high to be followed by direct titration, all $P_{1/2}^{CO}$ values were obtained via the simple addition of CO/N_2 mixtures to the five-coordinate hemes. Typical spectral changes observed in the 380–600-nm range are shown in Figure 1. In each case, values of $P_{1/2}^{CO}$ were the same within ±20% over a 10-fold range in added [base], 0.1 < [B] < 1.0 M (Tables 1 and 2) as determined by standard type Hill plots.

The K_B^{CO} for the two systems Fe(durene-7/7)(DcIm) and Fe(durene-5/5)(DcIm) were necessarily determined by competitive binding methods analogous to those used previously.[1a] The titrations were carried out by following the CO displacement of either *N*-MeIm or *t*-BuNC from under the durene cap according to eq 1

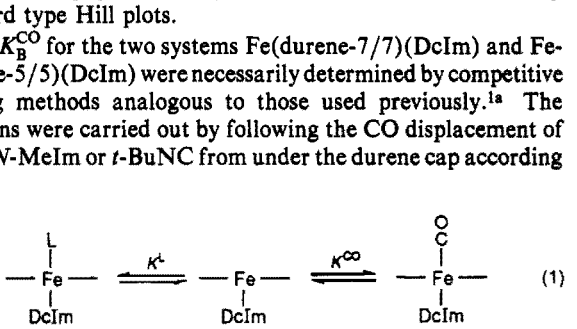

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Table 1. Constants for CO and O_2 Binding to R-State Model Hemes[a]

no.	compound	k_B^{CO} (M^{-1} s^{-1})	k_B^{-CO} (s^{-1})	$P_{1/2}^{CO}$ (Torr)	$k_B^{O_2}$ (M^{-1} s^{-1})	$k_B^{-O_2}$ (s^{-1})	$P_{1/2}^{O_2}$ (Torr)	$k_B^{O_2}/k_B^{CO}$	$M = P_{1/2}^{O_2}/P_{1/2}^{CO}$	ref
				Pyrrole-Substituted Hemes[b]						
1	chelated protoheme	1.1×10^7	0.025	0.00023	6.2×10^7	4000	5.6	6	2.4×10^4	1b
2	chelated mesoheme	1.1×10^7	~0.05	0.0005	9.0×10^7	5000	4.9	8	1.0×10^4	1b
3	Fe(anthracene-7/7)(DcIm)	6.0×10^6	0.05	0.0009	6.5×10^7	1000	1.4	11	1.5×10^3	1a
4	Fe(anthracene-6/6)(*N*-MeIm)	3.0×10^4	0.05	0.17	1×10^5	800	700	3	4.1×10^3	1a
5	Fe(adamantane)(*N*-MeIm)	9.2×10^3	0.05	0.57	1.5×10^5	690	300	16	5.3×10^2	1b
6	Fe(pyridine-5/5)(*N*-MeIm)	6.3×10^2	0.24	37	1.1×10^4	68	540	17	14	1c
7	Fe(durene-7/7)(DcIm)	9.5×10^5	0.014	0.0015	9.8×10^6 [f]	10^4 [h]	152 [j]	10	9×10^4	tw
		1.1×10^6 [d]	(0.02)[e]		(1.2×10^7)	(4×10^4)[i]	(~64)[k]	11		
8	Fe(durene-5/5)(DcIm)	1.1×10^7	0.13	0.0012	1×10^8 [g]	10^5 [h]	83 [j]	9	7×10^4	tw
			(0.29)[e]				(~64)[k]			
9	Fe(durene-4/4)(DcIm)	3.0×10^6	0.7	0.023	8.2×10^7 [f]	10^5 [h]	139 [j]	20	~6×10^3	tw
						(9×10^4)[i]	(160)[k]			
10	Fe(durene-4/4)(*N*-MeIm)	4.9×10^6	0.7	0.014			69 [j]			tw
							(82.6)[k]			
				TPP-Derived Hemes[c]						
11	chelated picket-fence	3.6×10^7	0.0078	2.2×10^{-5}	4.3×10^8	2900	0.58	12	2.7×10^4	2
12	Fe(MedPoc)(*N*-MeIm)	1.5×10^6	0.0094	6.5×10^{-4}	1.7×10^7	71	0.36	11	5.5×10^2	2
13	Fe(PocPiv)(*N*-MeIm)	5.8×10^5	0.0086	1.5×10^{-3}	2.2×10^6	9	0.36	4	2.7×10^2	2
14	Fe(C_2-cap)(*N*-MeIm)	9.5×10^5	0.05	5.4×10^{-3}			23		4.3×10^3	3
15	Fe(C_3-cap)(DcIm)	4.1×10^6	0.17	4.1×10^{-3}						3
16	chelated TPP	4.2×10^6	0.04	1×10^{-3}	2.9×10^7	30,000	81	7	10^5	2 and 22
17	Fe(6,6,6,6 ether cyc)(*N*-MeIm)			100						23
18	Fe(6,6,6,6 amide cyc)(*N*-MeIm)			>1 atm						23

[a] In benzene or toluene unless stated otherwise; the abbreviations used for the various porphyrins are generally the commonly accepted ones—the complete structures are given in the relevant references; tw = this work. [b] At 20 °C. [c] At 25 °C. [d] CH_2Cl_2 at 20 °C. [e] Measured directly (k_B^{-CO} by displacement of CO by TMIC). [f] From plots of k_{obs} vs O_2 concentration (eq 5). [g] From a single rate determination. [h] $k_B^{O_2}/K_B^{O_2}$ value. [i] Intercepts of plots as in *f*. [j] Kinetic determination. [k] Equilibrium determination.

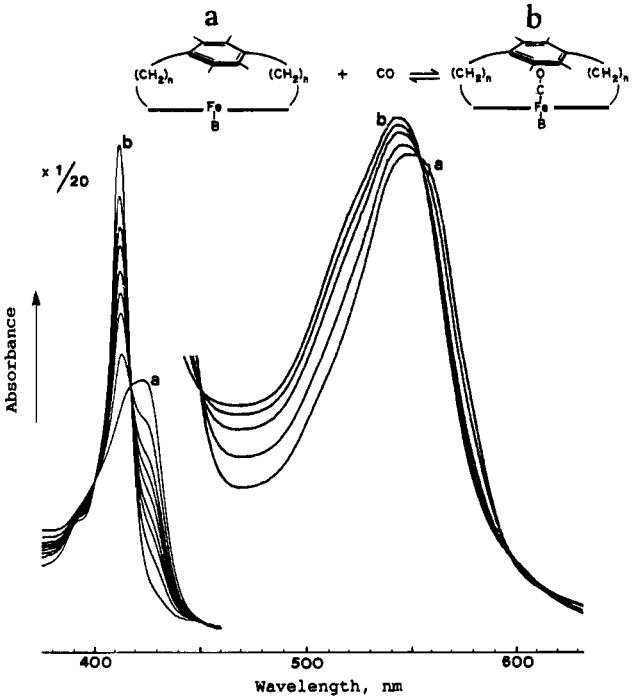

Figure 1. Isosbestic spectra changes in the 380–600-nm region for the equilibrium: Fe(durene-5/5)(1,2-Me_2Im) + CO ⇌ Fe(durene-5/5)-(1,2-Me_2Im)(CO) at 20 °C in toluene; added P_{CO} = 0.0119, 0.0242, 0.0363, 0.0529, 0.0816, 0.117, and 0.240 Torr and final pressure of 1 atm.

where

$$K^{CO} = \frac{K^L[L]}{[CO]} \qquad (2)$$

and L = *N*-MeIm and *t*-BuNC for the 7/7- and 5/5-systems, respectively. In each case, K^L was independently determined by direct titration with the respective five-coordinate heme, Fe(por)-(DcIm), as already described.[17] A high concentration of DcIm (1 M) was maintained in order to prevent base displacement by either L or CO. Clean isosbestic points were obtained in spectral changes for both systems; K^{CO} values obtained from standard Hill plots are converted into $P_{1/2}^{CO}$ values for comparison with other systems (Table 1).

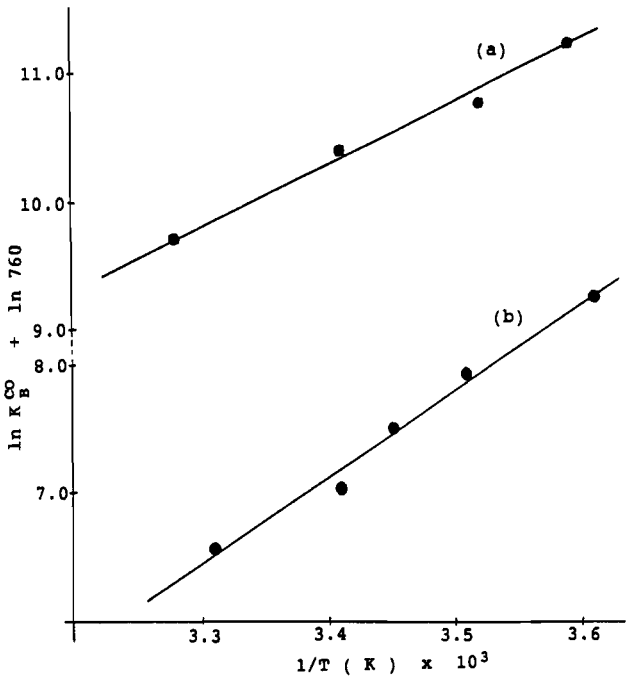

Figure 2. van't Hoff plots for the equilibria: Fe(durene-4/4)(B) + CO ⇌ Fe(durene-4/4)(B)(CO) measured in toluene from 0 to 30 °C. (a) B = DcIm, gives $\Delta H°$ = –9.4 kcal/mol and $\Delta S°$ = –11.6 eu. (b) B = 1,2-Me_2Im, gives $\Delta H°$ = –13.7 kcal/mol and $\Delta S°$ = –32.4 eu.

For the Fe(durene-4/4)(B) systems, B = DcIm and 1,2-Me_2-Im, $P_{1/2}^{CO}$ values were measured at different temperatures within the 0–30 °C range. Plots of ln K_B^{CO} versus $1/T$ (K) provided $\Delta H°_{CO}$ and $\Delta S°_{CO}$ thermodynamic constants for these systems (Figure 2 and Table 3).

Table 2. Constants for CO and O_2 Binding to T-State Model Hemes[a]

no.	compound	k_B^{CO} (M^{-1} s^{-1})	k_B^{-CO} (s^{-1})	$P_{1/2}^{CO}$ (Torr)	$k_B^{O_2}$ (M^{-1} s^{-1})	$k_B^{O_2}$ (s^{-1})	$P_{1/2}^{O_2}$ (Torr)	$k_B^{O_2}/k_B^{CO}$	$M = P_{1/2}^{O_2}/P_{1/2}^{CO}$	ref
				Pyrrole-Substituted Hemes[b]						
19	deuteroheme (2-MeIm)	1.0×10^6	0.45	0.045						19a
20	Fe(OEP)(1,2-Me_2Im)		0.56[d]	0.045						tw
21	Fe(durene-7/7)(1,2-Me_2Im)	1.3×10^5	0.04 (0.05)[d]	0.030			2.19×10^3		9×10^4	tw
22	Fe(durene-5/5)(1,2-Me_2Im)	1.1×10^6	0.52 (1.1)[d]	0.048			2.31×10^3		6×10^4	tw
23	Fe(durene-4/4)(1,2-Me_2Im)	6.3×10^5	4.2 (4.8)[d]	0.66			2.45×10^3 (8.78×10^2)[f]		$3–5 \times 10^3$	tw
				TPP-Derived Hemes[c]						
24	Fe(TpivPP)(1,2-Me_2Im)	1.4×10^6	0.14	8.9×10^{-3}	1.06×10^8	4.6×10^4	38	76	4.3×10^3	2
25	Fe(MedPoc)(1,2-Me_2Im)	2.1×10^5	0.053	0.026	5.2×10^6	800	12.4	25	4.8×10^2	2
26	Fe(PocPiv)(1,2-Me_2Im)	9.8×10^4	0.055	0.067	1.9×10^6	280	12.6	19	2.16×10^2	2
27	Fe(TTPPP)(1,2-Me_2Im)			0.27			508		5.6×10^4	4
28	Fe(TPP)(1,2-Me_2Im)			0.14						26
29	Fe(C_2-cap)(1,2-Me_2Im)			0.20[e]						26

[a] In toluene; the abbreviations used for the porphyrins are the commonly accepted ones—the complete structures are given in the relevant references; tw = this work. [b] At 20 °C. [c] At 25 °C. [d] Measured experimentally either from a plot of k_{obs} vs concentration or directly by displacement of CO by TMIC. [e] Corresponding values for the C_3- and C_4-cap systems are 0.14 and 4.1 Torr, respectively (ref 26). [f] Extrapolation from equilibrium determination at low temperature.

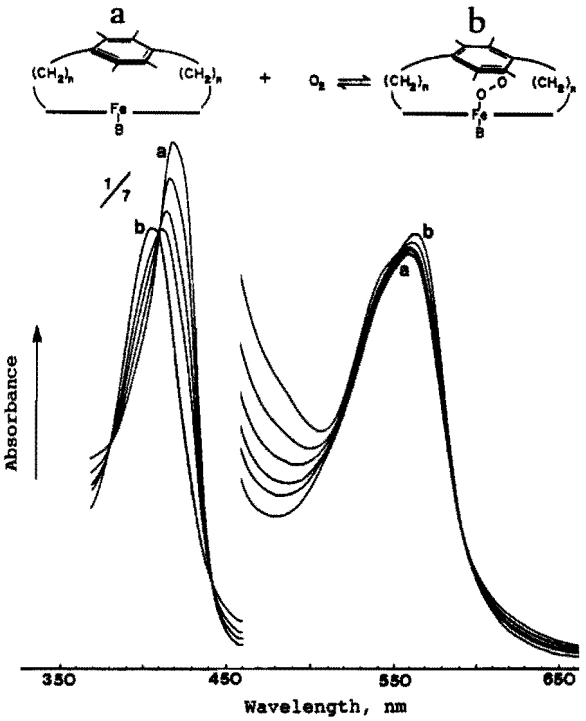

Figure 3. Isosbestic spectral changes in the 360–650-nm region for the equilibrium: Fe(durene-4/4)(*N*-MeIm) + O_2 ⇌ Fe(durene-4/4)(*N*-MeIm)(O_2) at 20 °C in toluene; added P^{O_2} = 24, 64, 124 Torr and final pressure of 1 atm.

O_2 Binding. Reversible oxygenations at room temperature were achieved from all the Fe(durene-por)(B)(O_2) complexes with B = DcIm; a high base concentration 1 M was necessary in order to prevent irreversible oxidation to the μ-oxo dimer. Within the series, the most stable oxy complexes (with respect to formation of μ-oxo species) were formed with the most hindered 4/4-durene capped systems (B = DcIm or *N*-MeIm). Direct O_2 titration of these durene-4/4 complexes at room temperature gave absorbance changes with sharp isosbestic points, indicating clean conversion of the oxygen species (Figure 3) and no trace of oxidation over a 1-h period. Reasonable estimates (±25%) of $K_B^{O_2}$ for durene-7/7 and -5/5 hemes, where B = DcIm, could also be obtained at room temperature; deoxygenation after full formation of Fe(por)(B)(O_2) indicated <20% heme oxidation during a period of ~20 min under O_2. The affinity constants for O_2 binding to the durene systems with B = 1,2-Me_2Im were too low ($P_{1/2}^{O_2}$ too high)

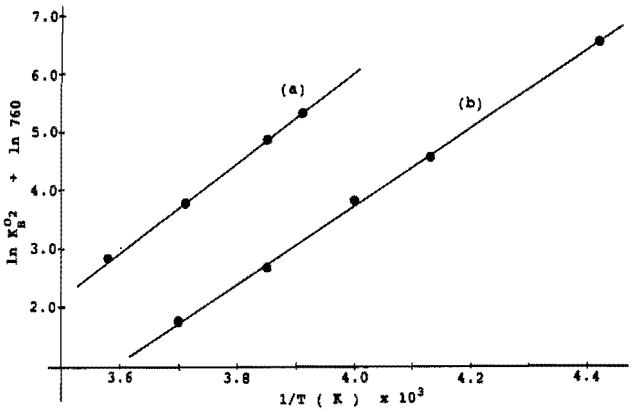

Figure 4. van't Hoff plots for the equilibria: Fe(durene-4/4)(B) + O_2 ⇌ Fe(durene-4/4)(B)(O_2) measured in toluene from 0 to -50 °C. (a) B = DcIm, gives ΔH° = -15.3 kcal/mol and ΔS° = -49.5 eu. (b) B = 1,2-Me_2Im, gives ΔH° = -13.1 kcal/mol and ΔS° = -45.5 eu.

Table 3. Thermodynamic Constants for CO and O_2 Binding[a]

compound	ΔH°_{CO} (kcal/mol)	ΔS°_{CO} (eu)	$\Delta H^\circ_{O_2}$ (kcal/mol)	$\Delta S^\circ_{O_2}$ (eu)
Hb	-17.4		-13.6 to -15.5	-27.7 to -31.7
Mb			-15.3 to -21.0	-38.1 to -56.1
chelated protoheme	-17.5	-34	-14.0	-35
chelated picket-fence			-16.3	-40
Fe(TpivPP)(1,2-Me_2Im)			-14.3	-42
Fe(PocPiv)(1,2-Me_2Im)	-13.9	-28		
Fe(TPP)(1,2-Me_2Im)	-12.8	-26.1		
Fe(C_2-cap)(*N*-MeIm)			-10.5	-27.9
Fe(durene-4/4)(DcIm)	-9.4	-11.6	-15.3	-49.5
Fe(durene-4/4)-(1,2-Me_2Im)	-13.7	-32.4	-13.1	-45.5

[a] Standard state 1 atm. Error estimates from linear regression analysis for the durene hemes are within ±1 kcal for ΔH° and ±20% for ΔS° values. Except for this work on the durenes, the data are taken from ref 2.

to allow measurement at room temperature. These values were determined kinetically. For Fe(durene-4/4)(B) complexes, where B = DcIm and 1,2-Me_2Im, measurement of $P_{1/2}^{O_2}$ values at various temperatures down to -50 °C provided thermodynamic constants, $\Delta H^\circ_{O_2}$ and $\Delta S^\circ_{O_2}$, from van't Hoff plots for both systems (Figure 4, Table 3).

CO Association. Direct recombination of CO to the Fe(por)(B) complex, following photolysis of the CO adduct, Fe(por)-

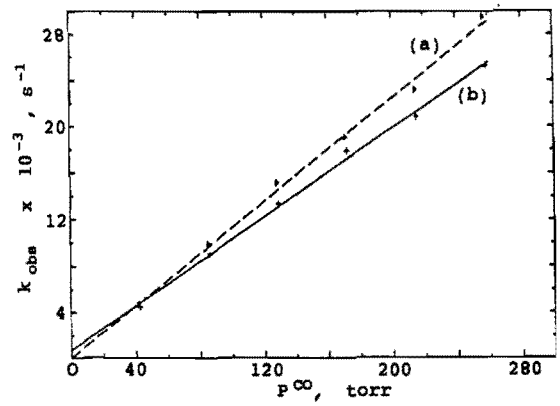

Figure 5. Determination of k^{CO} for the equilibrium: Fe(durene-5/5)(DcIm) + CO ⇌ Fe(durene-5/5)(DcIm)(CO) at 20 °C in toluene. Data measured at (a) 425 nm, gives k^{CO} = 111.8 $Torr^{-1}$ s^{-1} and at (b) 415 nm, gives k^{CO} = 95.7 $Torr^{-1}$ s^{-1}. Average k^{CO}_{DcIm} = 1.1 × 10^7 M^{-1} s^{-1}.

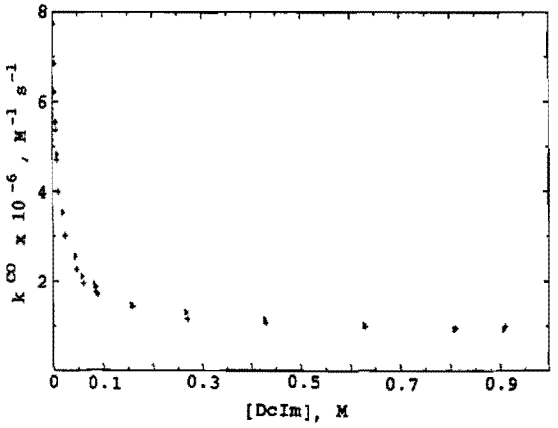

Figure 6. Determination of k^{CO} at varying [DcIm] for the equilibrium: Fe(durene-7/7)(DcIm) + CO ⇌ Fe(durene-7/7)(DcIm)(CO) at 20 °C in toluene at two wavelengths, 412 (+) and 425 nm (▲); at [DcIm] = 0.9 M, k^{CO}_{DcIm} = 9.5 × 10^5 M^{-1} s^{-1}.

(B)(CO), was monitored separately at λ_{max} for both Fe(por)(B)(CO) and Fe(por)(B) species (B = 1,2-Me_2Im and DcIm) in the 460–350-nm region. Identical k^{CO} rate constants (±15%, Figure 5) were obtained from data at both wavelengths according to eq 3.

$$k_{obs} = k^{CO}[CO] + k^{-CO} \quad (3)$$

To confirm that CO binding was indeed occurring at the sixth coordination site without base displacement, experiments were carried out where [B] was varied at constant P_{CO}. For all three systems (P_{CO} ~ 200 Torr), k^{CO} (±15%) was found to be independent of [B] down to 0.3 M (Figure 6). The k^{CO}_B values obtained within this range of [B] indeed correspond to CO coordination on the capped side of the five-coordinate heme complex. As [B] is decreased below 0.1 M, however, the kinetics are complicated by CO association via the base-elemination mechanism.[19a]

CO Dissociation. The high rate of CO dissociation from the Fe(durene-4/4)(1,2-Me_2Im)(CO) system allowed k^{-CO} determination directly from the k_{obs} versus [CO] plot according to eq 3. The extrapolation to [CO] = 0 afforded a value of k^{-CO} ~ 4.8 s^{-1}, within an acceptable range of the calculated value of 4.2 s^{-1} obtained from the K^{CO} and k^{CO} constants (Table 2). For all other systems whose CO dissociation rates were too low to be measured accurately by the above method, k^{-CO} was determined by isocyanide displacement of CO, analogous to the method used previously.[1a] Initially, sufficient CO was added to fully form the six-coordinate Fe(por)(B)(CO) complex. This was then rapidly treated with a deaerated solution of TMIC, and the rate of formation of the six-coordinate Fe(por)(B)(TMIC) complex followed spectrophotometrically. Relative concentrations of CO and TMIC were chosen such that K^{TMIC}[TMIC] > K^{CO}[CO] (by >10 fold), when TMIC effectively displaced CO from the sixth coordination site. Further, a high [B] (~1.0 M) was maintained in all cases so that significant base displacement by either CO or TMIC did not occur. Because k^{RNC}_B[RNC] ≫ k^{-CO}, the rate of Fe(por)(B)(TMIC) formation, under these conditions, is determined exclusively by the rate at which CO dissociates from the heme. The first-order rate constants (k^{-CO}) obtained were consistent over a 10-fold variation in [CO]/[TMIC], and identical rates (±25%) were obtained when monitoring at λ_{max} corresponding to either Fe(por)(B)(CO) or Fe(por)(B)(TMIC) species (Tables 1 and 2). In all cases, values of k^{-CO}_B, determined by experiment and by calculation from K^{CO} and k^{CO}, are considered to be in reasonable agreement (within 2-fold) for the different methods used. This further confirms the validity of the results.

Unfortunately, because of the poor affinity of the five-coordinate durene-4/4 hemes toward all isonitriles (including *n*-BuNC), the CO dissociation rates for these systems with B = DcIm or *N*-MeIm could not be obtained in this way, and the values quoted (Table 1) are calculated from K^{CO} and k^{CO} values.

O_2 Kinetics. Photolysis of a B(Hm)CO adduct, where Hm = Fe(por), in the presence of CO/O_2 mixtures provides kinetic and equilibrium constants for O_2 binding. These are derivable from the Gibson method,[20] which is used extensively for hemoprotein systems[21] and model hemes.[1,2,5,6] The technique utilizes the difference in O_2 and CO association rate constants ($k^{O_2}_B$ > k^{CO}_B, typically 10-fold), so that for appropriately chosen [O_2]/[CO] mixtures, photolysis of the B(Hm)CO complex initially leads to fast O_2 association, which is then followed by slow O_2/CO equilibration back to the heme–CO complex, eq 4

$$O_2 + B(Hm)CO \underset{k^{CO}_B}{\overset{h\nu}{\rightleftharpoons}} B(Hm) + CO + O_2 \underset{k^{-O_2}_B}{\overset{k^{O_2}_B}{\rightleftharpoons}} B(Hm)O_2 \quad (4)$$

The high overall affinity for CO (K^{CO}_B[CO] > $K^{O_2}_B$[O_2]) ensures that the equilibrium eventually lies toward that of the fully formed CO species. The two rates, "fast" and "slow", are sufficiently separated so that independent first-order traces may be obtained and analyzed. The fast rate directly affords $k^{O_2}_B$ and $k^{-O_2}_B$ from a plot of eq 5

$$k_{fast} = k^{O_2}_B[O_2] + k^{-O_2}_B \quad (5)$$

The slow rate in general is given by eq 6

$$k_{obs} = k^{-O_2}_B\left(\frac{k^{CO}_B[CO]}{k^{CO}_B[CO] + k^{O_2}_B[O_2] + k^{-O_2}_B}\right) \quad (6)$$

For all the durene systems, $k^{-O_2}_B \gg k^{CO}_B$[CO], and thus $K^{O_2}_B$ is determined using eq 7 via a plot of k^{CO}_B[CO]/(k_{obs}) versus [O_2].

$$\frac{k^{CO}_B[CO]}{(k_{obs})} = K^{O_2}_B[O_2] + 1 \quad (7)$$

Typically, a solution of B(Hm)CO was prepared under a known [CO], and then aliquots of O_2 were syringed into the tonometer. For $k^{-O_2}_B$ determination (eq 6), the heme solution was flashed while being monitored at the λ_{max} for B(Hm)O_2. The relatively low O_2 affinity of the durene heme systems necessitated the addition of higher O_2 pressures to achieve any noticeable change in absorbance during formation of B(Hm)O_2. At these higher [O_2], however, the observed rate constants for the 5/5 system

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(21) Antonini, F.; Brunori, M. *Hemoglobin and Myoglobin in Their Reactions with Ligands*; North-Holland Publishing Co.: Amsterdam, 1971; p 212.

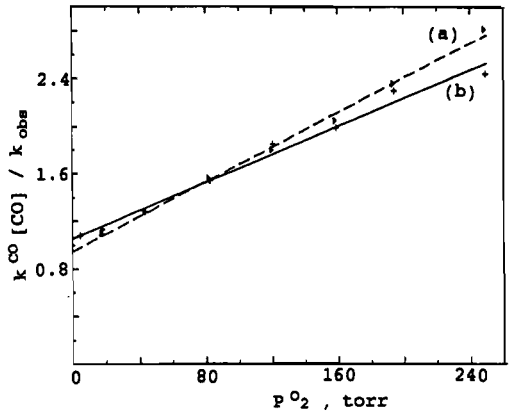

Figure 7. Kinetic determination of K^{O_2} for the equilibrium: Fe(durene-7/7)(DcIm) + O_2 ⇌ Fe(durene-7/7)(DcIm)(O_2) at 20 °C in toluene; data plotted according to eq 7. Data at 425 nm (a) give $K^{O_2} = 7.3 \times 10^{-3}$ Torr^{-1}; data at 412 nm (b) give $K^{O_2} = 5.9 \times 10^{-3}$ Torr^{-1}. Average $P^{O_2}_{1/2} = 152$ Torr.

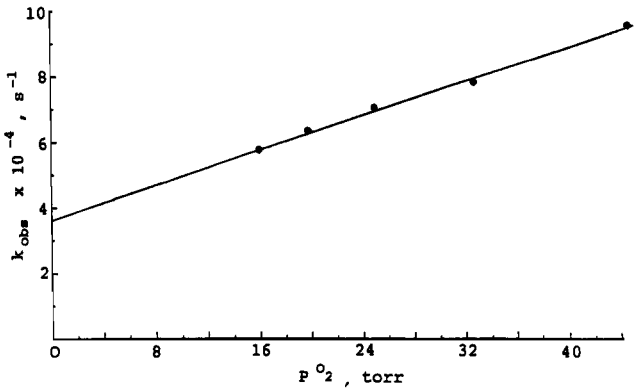

Figure 8. Determination of k^{O_2} for the equilibrium: Fe(durene-7/7)-(DcIm) + O_2 ⇌ Fe(durene-7/7)(DcIm)(O_2) at 20 °C in toluene. Data at 405 nm; slope = 118 Torr^{-1} s^{-1} gives $k^{O_2}_{DcIm} = 9.8 \times 10^6$ M^{-1} s^{-1}; intercept gives $k^{-O_2}_{DcIm} \approx 4 \times 10^4$ s^{-1}.

were close to the decay rate constant of the laser beam. In this case a single O_2 pressure was used to estimate $k^{-O_2}_B$.

The kinetic determination of $K^{O_2}_B$ from the "slow rate" gave consistent results (±25%) at λ_{max} values corresponding to both B(Hm) and B(Hm)CO species (Figure 7); these $K^{O_2}_B$ values are in reasonable agreement (within about 2-fold) with those measured by equilibrium titration, once again confirming the validity of results obtained from both methods (Table 1). Values of $k^{-O_2}_B$ could be estimated from the intercepts of eq 5 for the 4/4- and 7/7-systems (Figure 8).

Discussion

The results obtained on the binding of CO to the durene-capped hemes exemplify the necessity for obtaining and analyzing both kinetic and equilibrium constants (Tables 1 and 2). Although the $P^{O_2}_{1/2}$ values for the durene-7/7 and -5/5 systems are almost identical, the magnitudes are in fact equally offset by significant differences in association and dissociation rate constants for these systems. (For the sake of consistent comparison between all three durene heme systems, k^{-CO}_B values calculated from K^{CO}_B and k^{CO}_B will be used in discussion below.) Compared with the "open" chelated meso- and protoheme systems, the durene-7/7 heme exhibits a 10-fold reduction in CO association rate, whereas the durene-5/5 and -4/4 systems both unexpectedly show little reduction in k^{CO}_B values relative to that of the open hemes, despite their reduced overall affinity toward bulky isocyanides.[17] To ascertain whether this reduction in k^{CO}_B was characteristic of the 7/7-system and not caused by a preferred orientation of toluene solvent molecules obstructing coordination at the sixth site, a k^{CO}_B determination was also carried out in CH_2Cl_2. Under otherwise identical conditions, however, no significant change in k^{CO}_B was observed between the two solvents, thus ruling out the possibility of solvent participation interfering with CO association to the 7/7-system (Table 1). This observed reduction in CO association rate with little change in dissociation rate for the 7/7-heme, relative to the open heme systems, is therefore interpreted in terms of distal steric hindrance toward the binding of CO, imposed by the presence of the durene cap. That a distal steric effect shown toward an incoming ligand is largely manifested in a reduced rate of association, with little change in dissociation rate, is widely accepted from studies involving the ligation of bulky isocyanides to heme proteins[24] and that of CO and O_2 to numerous sterically hindered model hemes.[1,2,7] A central steric effect of the durene-7/7 cap is clearly illustrated by a reduction in the overall binding constant for a bulky ligand such as *tert*-butylisocyanide (680-fold),[17] relative to an open heme, but seems unlikely for the binding of a less sterically demanding diatomic to a "large-capped" system. A plausible explanation is that the 7/7-cap, with 14 methylene carbon linkages, may prefer a "pendulous" conformation in which the rotating durene moiety is suspended close to the center of the porphyrin plane, obstructing ligand coordination to the Fe atom (II); an upfield shift of the durene methyl signal in the ^{1}H NMR spectrum for the free base 7/7-porphyrin, relative to those of the 5/5- and 4/4-porphyrins, provides some evidence for this suggestion.[14] A crystal structure of a large cyclophane reported by Cruse *et al.*[25] indicates such a "collapse" of a large cyclophane. A similar explanation has been proposed to account for the low affinity of a Fe(C_4-cap)(B) system toward the binding of CO relative to the smaller C_3- and C_2-capped analogues (Table 2; footnote *e*).[26] For the 7/7-system, this suggestion is substantiated further by other relevant kinetic data. An analogous comparison for the binding of dioxygen to the durene-7/7 system once again reveals about a 6- to 8-fold reduction in $k^{O_2}_B$ relative to open chelated hemes, not significantly different from that for CO (Table 1). Steric restriction imposed by the durene cap, therefore, diminishes ligand access to the Fe for both CO and O_2 alike, with no significant discrimination shown toward either diatomic molecule.

The most striking feature of the CO kinetics within the durene series is the high rates of association and especially for the 5/5- and 4/4-hemes (Tables 1 and 2). For instance, the durene-4/4 heme with six methylene carbon linkages less than the 7/7 exhibits a higher k^{CO}_B than the larger analogue, despite the complete failure of the hindered 4/4-system to coordinate bulky isocyanide ligands to the distal side of the heme.[17] Further, the durene-5/5 heme, with the same number of adjoining atoms linking the cap to the porphyrin periphery as the pyridine-5/5, has an ~10^4-fold higher association rate for CO than the latter system. Particularly in the case of the 4/4-durene complexes, these k^{CO}_B values are accompanied by high rates of CO dissociation from the heme. An explanation for this "deviant" behavior most likely derives from the porphyrin plane distortion inherent in these two smaller capped durene systems as evidenced in the solution visible spectra,[15] structural analysis,[16] and affinity toward bulky isocyanides;[17] the structural data[16] refer to the durene-4/4 hemin chloride (see Introduction), while the solution spectral data[15] refer to the trends in the visible spectra of the hemin chlorides, the free-base porphyrins, and six-coordinate heme species. For example, for the free bases, the 7/7-species shows band intensities in the order

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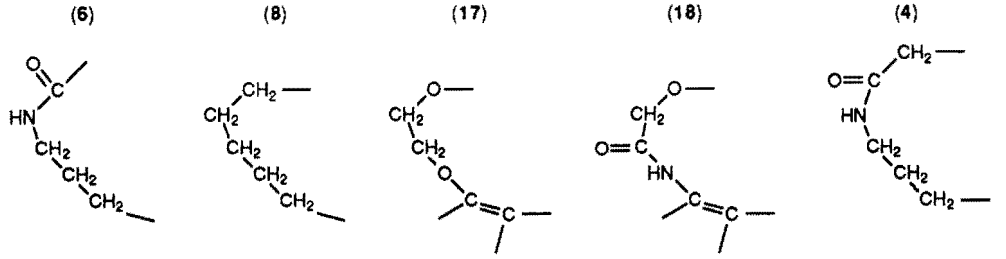
(23) Johnson, M. R.; Seok, W. K.; Ibers, J. A. *J. Am. Chem. Soc.* **1991**, *113*, 3998–4000.
(24) Mims, M. P.; Porras, A. G.; Olson, J. S.; Noble, R. W.; Peterson, J. A. *J. Biol. Chem.* **1983**, *258*, 14219–14232.
(25) Cruse, W. B.; Kennard, O.; Sheldrick, G. M.; Hamilton, A. D.; Hartley, S. G.; Battersby, A. R. *J. Chem. Soc., Chem. Commun.* **1980**, 700–701.
(26) Shimizu, M.; Basolo, F.; Vallejo, M. N.; Baldwin, J. E. *Inorg. Chim. Acta* **1984**, *91*, 247–250.

Chart 1

Compound (see Tables 1 and 2)



	(6)	(8)	(17)	(18)	(4)
No. of Connectors	2	2	4	4	2
$P_{1/2}^{CO}$ (torr)	37	10^{-3}	100	>760	0.17

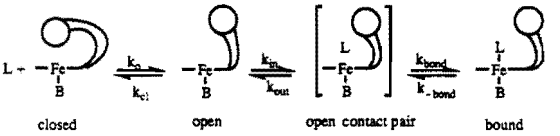
IV > III > II > I, typical of a planar system, while the 5,5- and 4/4-species show increased intensity of III relative to IV such that the 4/4-species shows the relative intensity pattern III > IV > II > I (a rhodo-type spectrum), and this arises from severe porphyrin plane distortion.[27] To consider the 5/5-complex first, placement of a more rigid diagonal strap, relative to that in the 7/7-case, presumably induces doming of the porphyrin skeleton in such a manner as to give a "basket-shaped" conformation (**III**) that provides the small incoming CO ligand free access to the iron; subsequent to Fe–CO bond formation, however, porphyrin plane distortion apparently causes destabilization of the bound carbonyl and results in an increased CO dissociation rate by 5–10-fold. The doming effect would become even more pronounced for the tighter capped durene-4/4 system and could give rise to the approximately 5-fold increase in CO off rate relative to the 5/5; the lower association rate for CO (4-fold) might be due to the reduced accessibility to the sixth coordination site because of the smaller cap.

Recent picosecond kinetic studies[28] on the adamantane cyclophane heme reactions with isocyanides have revealed two conformations of the cyclophane. One of these has the adamantane practically in van der Waals contact with the iron (as in the crystal structure), and the other is open for diffusion controlled ligation. The ratio of the closed to open forms is about 10^3. Similar studies indicate multiple conformations of heme proteins as well.[29]

In the present case, the larger (7,7) cap can rotate to bring the durene close to the iron atom, as indicated in the crystal structure of a cyclophane porphyrin reported by Cruse *et al.*[25] On the other hand, the crystal structure of the 4,4-durene cyclophane indicates severe porphyrin distortion and a preferred conformation of the durene cap which leaves more space between the cap (5.6 Å) and the iron[16] than does the adamantane cyclophane (2.7 Å) in its preferred, closed conformation.[1b,28] Thus a conformational change prior to ligation is required for the adamantane heme (**5**) but not the 4,4-durene system (**9**).

The 5,5-durene cyclophane (**8**) behavior might be explained similarly by having a deformed porphyrin similar to that of the 4,4-durene system (**9**). However, comparison with the 5,5-pyridine cyclophane system (**6**) and other cyclophane hemes of similar sizes suggests additional effects. Both cyclophanes (**6**) and (**8**) have k_B^{CO} values around 0.2 s^{-1}, indicating similar deformation of the porphyrin. But the 5,5-durene system adds CO (k_B^{CO}) with the same rate constant as does the unhindered chelated protoheme (10^7 M^{-1} s^{-1}), whereas the 5,5-pyridine complex reacts 16 000 times slower ($k_B^{CO} = 6 \times 10^2$ s^{-1}). This clearly indicates that the number of connecting atoms in cyclophanes does not necessarily determine the steric hindrance toward ligation. This is made even more pointedly with the two 6,6,6,6-cyclophanes (including two atoms of the phenyl ring in the connections) reported by Johnson *et al.*[23] in which one shows a $P_{1/2}^{CO}$ of 100 Torr and the other does not bind CO at atm pressure. A series of connecting groups along with the $P_{1/2}^{CO}$ values for the (5,5 or 6,6,6,6) cyclophanes is shown in Chart 1. The connections are those between the porphyrin carbon and the arene carbons. It is noteworthy that those cyclophanes which display a large steric effect (*vs* chelated protoheme) have amide linkages or double bonds (of arenes) as part of the cyclophane linkage. The 5,5-durene cap is the only cyclophane having only $-CH_2-$ groups. This not only reduces the pocket polarity but also allows greater conformational flexibility in the cyclophane. One explanation of the rapid binding of 5,5- (and perhaps the 4,4-) durene capped hemes is that, unlike adamantane heme cyclophanes, where the closed to open conformational ratio favors the closed by about 10^3, the cyclophanes with $-CH_2-$ connections might have similar populations of open and closed forms.

It is now rather well-established from picosecond kinetic studies of both cyclophane systems and heme proteins that the binding process includes conformational changes as part of the "diffusional process" which assembles the contact pair.[30] This is illustrated with a cyclophane heme below. Similar, but more complex mechanisms can be written for heme proteins. In this view, the



distal steric effect is determined by the term ($k_o k_{in}/k_{cl}$), which includes the preequilibrium. This preequilibrium is in turn governed by the conformational freedom of the system and not by the steric interaction of the bound state. This opening of the cyclophane cap is seen directly in the crystal structures of the C_2-cap porphyrin and the C_2-cap CO complex (**14**).[31] The aromatic centroid separations are 4 and 5.6 Å, respectively, allowing room for CO to bind with a small reduction in affinity. Again, the open and closed conformations might not be very different in energy. That the rate of dissociation of this CO complex is not appreciably faster than that of the chelated tetraphenylporphyrin CO complex (**16**) suggests that this con-

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(31) Kim, K.; Ibers, J. A. *J. Am. Chem. Soc.* **1991**, *113*, 6077–6081.

Table 4. Comparison of M Values for Model Hemes in Toluene and o-Dichlorobenzene

compound	toluene	o-dichlorobenzene	ref
Fe(durene-7/7)(DcIm)[a]	9×10^4	2×10^4	tw
Fe(durene-5/5)(DcIm)[a]	7×10^4	1×10^4	tw
Fe(durene-4/4)(DcIm)[a]	$\sim 6 \times 10^3$	1×10^3	tw
Fe(pyridine-5/5)(DcIm)[a]	14	5	1c
Fe(TTPPP)(1,2-Me_2Im)[b]	5.6×10^4	1.4×10^4	4

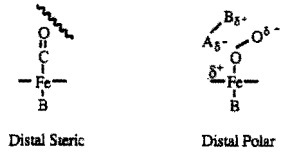
[a] Data at 20 °C; tw = this work. [b] Data at 25 °C.

formational opening does not "destabilize" the bound state or the transition state for bond formation.

Some molecular modeling studies on these systems have been initiated;[32] data on the Fe(durene-7/7)(DcIm) species reproduce the relative CO/O_2 binding preference and suggest that the Fe-(CO or O_2) groups are "pushed up" toward the distal side, with more motion for the CO ligand. Of note also, the motion of the 7/7-durene strap is predicted to be at least twice that of the 5,5-pyridine strap.

R- to T-State Change. Replacement of the proximally coordinated N-alkylimidazole (e.g., N-MeIm or DcIm) with a sterically hindered imidazole substituted in the 2-position (e.g., 1,2-Me_2Im) results directly in a substantial reduction in O_2 and CO affinity (compare Tables 1 and 2).[2-4] Steric interaction between the 2-methyl groups and the porphyrin plane, as the Fe attempts to take up a more central position on coordination of a sixth ligand, is believed responsible for the lowered affinity of the latter system relative to the unhindered case.[33] This type of nonbonded interaction, which is considered to contribute to the very low affinity of T-state relative to R-state hemoglobin,[34] has led to the hindered and unhindered models being labeled T- and R-state systems, respectively. For both the intrinsically distorted 5/5- and 4/4-hemes, the "T-state effect" of introducing a 2-substituted axial base simply causes a further decrease in association rate as well as an increase in dissociation rate relative to that for the corresponding R-state complex. An unusual feature about the Fe(durene-7/7)(1,2-Me_2Im) system is that compared to the simple, open T-state systems, deuteroheme(2-MeIm) and Fe(OEP)(1,2 Me_2Im), the 7/7 heme displays a 10-fold reduction in both association and *dissociation* rates. This unusual drop in k_B^{CO} may be accounted for by a required conformational change prior to dissociation, which in essence multiplies the intrinsic dissociation rate constant by a preequilibrium constant (*vide supra*). In other words, the special arrangement of the strap, which initially hinders coordination of CO to the five-coordinate heme, may adopt a different orientation in order to accommodate the bound CO in the six-coordinate complex; conversely, the energy now required to disrupt the conformation of this state will tend to retard CO dissociation from the heme. The T-state durene-7/7 system is apparently more sensitive to the conformational demands of the cap than is the R-state complex where ligand dissociation is, in general, much slower. The extremely low rates of CO association and dissociation for the hemoprotein horseradish peroxidase,[35] where a crowded distal environment is implied, are probably similarly related to conformational changes.

Differentiation of Carbon Monoxide and Oxygen Binding. Simple five-coordinated hemes in hydrocarbon solutions bind carbon monoxide much more strongly (>10 000 times) than dioxygen. This is expressed as the M value ($M = K^{CO}/K^{O_2}$) for the system.[36] This value varies from a high of about 90 000 to a low of 3, with heme proteins having values in the ~100 range. There has been much discussion of the mechanistic basis of the variation in the M values. The discussions focused mainly on distal steric effects, i.e., steric interference with the binding of the ligand and distal polar effects, especially dipolar or ionic groups near the bound ligand (including solvation effects). The former proposed a larger steric effect on CO and the latter suggested a larger polar effect on dioxygen. The third variation



which affects binding—the effect of enforced doming—seems to affect both CO and O_2 binding (*vide supra*) and is usually not considered significant in determining M values.[36]

In the durene series, a hydrophobic cap provides a nonpolar distal environment similar to that of the solvent medium, toluene, in which (lack of) polarity effects experienced by a bound dioxygen should be the same for all three systems. Indeed, the $P_{1/2}^{O_2}$ values (Tables 1 and 2) are essentially the same within each of the R- and T-state systems, entirely consistent with the absence of distal polar interactions. Differentiation in the binding of CO and O_2 must then result from steric and/or solvation factors. Considering the latter effect first, on changing the solvent from toluene (ϵ = 2.4) to o-dichlorobenzene (ϵ = 9.9) there is about a 4–7-fold drop in M value for all three durene hemes (Table 4). This is in line with data from studies on other heme systems,[3,4] where O_2 affinity in the more polar solvent has been attributed to greater stabilization of the dipolar FeO_2 moiety relative to that of Fe–CO (see below). Although data for direct comparison with an "open" system are not available, the reduction in M value indicates that solvent orientation is not restricted within the distal cavity of any durene heme and that solvation effects are essentially the same for all three systems.

Comparing the durenes shows essentially no change in M from the 7,7- to the 5,5-systems, while the 4,4-durene cyclophane shows a reduction of about 10 in the M value (Table 1). Although this is a modest change within the scale mentioned, it does represent some differentiation of CO and O_2 and, considering the similar O_2 affinities of the three systems, this differentiation results from discrimination against coordination of CO. There is little reduction in the rate of CO binding. The difference between the 4,4-durene and the longer, open chain systems is mainly in the increased off rate (k_B^{CO}) for the former (0.7 s^{-1}) and is attributed to porphyrin deformation (see below). On the other hand, the dioxygen off rate ($k_B^{O_2}$) is not increased. It is the increased k_B^{CO} which accounts for the differentiation. This is not considered to be due to a distal steric effect because, based on current hypotheses,[1,2,7] these are reflected largely in decreased association rates (k_B^{CO}). The increase in k_B^{CO} observed (i.e., to > about 0.2 s^{-1}) is not observed in heme proteins and will probably occur only in highly distorted heme systems such as **8**, **9**, **17**, **18**, and perhaps **6**. It is an indication that the extreme T-state deformation has more effect on k_B^{CO} than on $k_B^{O_2}$ and therefore represents a special case of discrimination against CO. No such discrimination is observed in heme proteins.

A comparison of the binding dynamics and equilibria among different series is very informative. In particular, the polar 5,5-cyclophane (**6**) and the nonpolar 5,5-cyclophane (**8**) show a very large difference in M values, changing from 14 to 70 000, a factor of 5000. This change can be attributed principally to changes in dioxygen off rates ($k_B^{O_2}$), which change from 68 to 10^5 s^{-1}, a factor of about 1500. The same conclusion is reached within the "picket-fence heme" and "pocket heme" series. The $k_B^{O_2}$ value decreases in the series: chelated tetraphenyl heme (**16**: 30 000 s^{-1}), chelated picket-fence heme (**11**: 2900 s^{-1}), pocket heme (**13**:

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Table 5

	K_{CO} (M^{-1})	K_B^{CO} (M^{-1})
durene-4/4	$\sim 4 \times 10^5$ [a]	$\sim 4 \times 10^6$ [b]
planar heme	$\sim 6.6 \times 10^4$ [c]	$\sim 2 \times 10^8$ [d]

[a] Determined by titration of CO to the four-coordinate heme at 20 °C in toluene as described for K_B.[15] [b] DcIm as base (Table 1). [c] Fe(TPP) in toluene at 20 °C (ref 37). [d] Chelated mesoheme (Table 1).

9 s^{-1}), an overall decrease of 3000 times; k_B^{-CO} changes by only a factor of 5. Similar comparisons are seen in the cyclophane series (**1–6**). These comparisons, and the study of Chang *et al.* in another system,[5] demonstrate the dominant effect of dioxygen dissociation rates on the differentiation of CO and O_2. These values of $k_B^{-O_2}$ have, in turn, been attributed to changes in the polar environment around the presumably dipolar $Fe^{\delta+}$–$OO^{\delta-}$ species.

Differentiation in the T-State Systems. Since the magnitude of differentiation (M values) in the durenes is paralleled in the T-state system (with B = 1,2-MeIm), an unfavorable steric interaction between the proximal base and porphyrin is unlikely to be the cause of this discriminatory effect. Instead, this effect must result from the porphyrin plane doming inherent in the 4/4-heme, which is present to a similar extent in both R- and T-state systems. Interestingly, the introduction of skeletal distortion in the 5/5-system appears to affect the CO and O_2 binding to a similar extent, the M value being not much different from that of the 7/7-heme; as the "doming effect" becomes more severe, however, CO binding is further hindered, whereas O_2 binding is not diminished significantly. In contrast to the reduced affinity for CO ligation on the distal side of a five-coordinate durene-4/4 complex is the somewhat enhanced coordination of this ligand to the unhindered side of the four-coordinate durene-4/4 heme, relative to an analogous comparison for a planar system (see Table 5). This difference may be a reflection of the Fe position in relation to the porphyrin plane and/or the extent of σ/π overlap allowed Fe and CO as a result of increased distortion of the Fe–porphyrin system in the durene-4/4 complexes; either or both of these effects could serve to enhance ligation of CO to the unhindered side of the four-coordinate heme and, conversely, diminish affinity for the ligand to the distal side of a five-coordinate system.

Thermodynamic Considerations for CO and O_2 Binding. Studies from one of our laboratories[1a] and from Collman and co-workers[2] have independently suggested that for the binding of CO to hindered model hemes, the transition state and carbonylated complex both suffer steric hindrance to a similar degree. These interpretations were based on the grounds that reduction in CO affinity is predominantly expressed in *decreased association rates*, with little change in the dissociation rate for encumbered systems relative to open hemes. Changes in dioxygen affinity, on the other hand, are reflected by a decrease in association and dissociation rates; this is clearly exemplified in the hindered Fe(PocPiv) and Fe(Med Poc) systems relative to the picket-fence hemes (Table 1). The binding of O_2 is therefore considered to be influenced by factors which affect both the transition state and the ground state of the dioxygen complex.

Severe distortion of the porphyrin plane, as is evidenced in the durene-4/4 complexes, however, gives rise to thermodynamic consequences different from those of other systems for the binding of CO. The decrease in CO affinity for the 4/4-heme relative to the 5/5-system is largely reflected by an increase in the rate of *dissociation* from the former, to the extent of ~60% and ~80% of the difference in the ligation free energy changes in the R- and T-state systems, respectively (from Tables 1 and 2), indicating that destabilization of the *bound*-CO is the determining factor in the lowered affinity for CO. This may be clearly defined as a "proximal" effect on CO ligation unlike a distal steric hindrance, the kinetic consequence of the latter effect being manifested as a reduction in the rate of CO association. That the bound CO complex suffers greater destabilization than the Fe–O_2 complex as a result of porphyrin distortion is a reflection of the different nature of the Fe–CO and Fe–O_2 bonds.

Although the apparently random variation in $\Delta H°$ and $\Delta S°$ values for studied model hemes renders meaningful comparisons between systems difficult, an attempt can be made to interpret thermodynamic constants for CO and O_2 binding within the R- and T-state durene-4/4 complexes (Table 3). Clearly, the binding of CO involves a smaller unfavorable entropy change ($\Delta S°$) for both R- and T-state systems relative to that for O_2; this is consistent with a smaller overall loss of entropy (including more restriction of solvent motion by the polar dioxygen ligand) as a result of destabilization of the CO-bound state for this distorted heme. On these grounds, the more negative entropy change for the T-state system (with even more pronounced destabilization in the six-coordinate state as compared to the R-state) seems contradictory; however, the reduced flexibility inherent in the six-coordinate T-state complex could conceivably demand a greater loss of entropy in order for bond formation to occur on CO binding to the five-coordinate complex, relative to that required for the R-state 4/4-complex.

Conclusion

The durene-capped hemes with a completely hydrophobic distal pocket reversibly coordinate O_2 and CO. The lack of polarity effects, which typically enhance the binding of O_2 relative to CO, results in poor differentiation between these two ligands within the series, and the ligand binding kinetics reveal the true dimensions of steric effects. The discrimination between O_2 and CO is attributed to distortion (doming) of the porphyrin skeleton, as evidenced especially in the 4/4-durene heme and results largely from destabilization of the bound CO as reflected in an increased rate of dissociation from the heme.

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