Ligand Binding to Four-Atom-Linked Capped Porphyrins

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Received June 16, 1994[⊗]

Abstract: Equilibrium measurements and kinetic behavior from seconds to picoseconds are described for the binding of O_2 or CO to the four-atom-linked capped porphyrin systems Fe(Por)(base), where Por = OC_2OPor (**1**) or OC(CO)NPor (**2**) and base = 1-methylimidazole (1-MeIm) or 1,2-dimethylimidazole (1,2-Me_2Im). Binding of O_2 or CO to the amide-linked system Fe(**2**)(base) would impose nonplanarity on the amide linkages; this does not occur as this system does not bind O_2 or CO even with gas pressures of 100 atm. In contrast, the ether-linked system Fe(**1**)(base), with its sterically crowded pocket, is able to bind CO, but with exceptionally high values of $P_{1/2}^{CO}$ of 100, 40, and 17 Torr at +25, 0, and −20 °C, respectively, for Fe(**1**)(1-MeIm) and ~5 × 10^3 Torr at 25 °C for Fe(**1**)(1,2-Me_2Im). The thermodynamics of binding of CO to Fe(**1**)(1-MeIm) involves a normal entropy term of $\Delta S° = -29(3)$ eu but a less negative than normal enthalpy term of $\Delta H° = -5.8(8)$ kcal/mol. Binding of CO to Fe(**1**)(1-MeIm) is not isosbestic below −30 °C. The value of $P_{1/2}^{O_2}$ of Fe(**1**)(1-MeIm) is 61 Torr at −43 °C; above that temperature the binding is not reversible. Kinetic analysis of Fe(**1**)(1-MeIm)(CO) yields the exceptionally low value of the association constant k_B^{CO} of 18.5(5) M^{-1} s^{-1} and a normal value of the dissociation constant k_B^{-CO} of 0.044(2) s^{-1}. In that system, after subpicosecond photolysis, there is evidence that there may be ~15% geminate recombination occurring with a half-life of 14 ps. This is the first hint of trapping in a model system, but it is still far from the type of trapping seen in proteins, which persists for hundreds of nanoseconds. For the 1-MeIm complex at high pressures of CO, and especially for the 1,2-Me_2Im complex with CO, which can only be detected at high pressures, kinetics observed following photodissociation are complex.

Introduction

The subject of ligand binding to hemoproteins and models, and in particular the origin of CO discrimination in the hemoproteins, remains very active.[1-10] For the oxygen-binding hemoproteins myoglobin (Mb)[11] and hemoglobin (Hb), at least three factors influence ligand binding at the iron center:[12] tension on the iron atom exerted by a proximal ligand, modification of the electronic wave function owing to substituents on the porphyrin ring, and the sterics and electronics of the distal "pocket" in which the ligand sits. Over 20 years ago the first elaborated porphyrin system, an iron porphyrin with a cyclophane strap,[13] was synthesized to explore such distal effects. Since that time large families of elaborated porphyrins have been synthesized, and the binding properties of their metal derivatives for ligands, especially CO and O_2, have been investigated.[3,4,14,15] In addition, a number of Mb mutants have been synthesized and their structural and binding properties elucidated.[1,5,9,10] It is clear that compared with almost all model systems, Mb discriminates against the binding of CO; the usual measure $M = P_{1/2}^{O_2}/P_{1/2}^{CO}$ approaches 4000 in the TPivPP and *five*-atom-linked[16] capped porphyrin systems, but is as low as 6.5 for Mb.[17]

Recently, we described the synthesis of two new *four*-atom-linked capped porphyrins, Por = OC_2OPor (**1**) and OC(CO)NPor (**2**)[18] (Figure 1), that as Fe(Por)(1-MeIm) systems either do not bind CO or O_2 at all (Por = **2**) or bind CO with a $P_{1/2}$

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[⊗] Abstract published in *Advance ACS Abstracts*, November 15, 1994.

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(11) Abbreviations: TPP = tetraphenylporphyrinato dianion; 1,2-Me_2-Im = 1,2-dimethylimidazole; chelated protoheme = protoheme with the chelating arm $NH(CH_2)_3$–imidazole; β-PocPivP = TPP with a 1,3,5-$(CH_2C(O)NH)$-substituted benzene cap attached to three *o*-phenyl positions and a pivaloyl group attached to the fourth *o*-phenyl position, directed opposite the cap; 1-MeIm = 1-methylimidazole; T(*p*-OCH_3)PP = tetrakis(*p*-methoxyphenyl)porphyrinato dianion; Im-pyrroheme = pyrroheme-*N*-[3-(1-imidazolyl)propyl]amide; Anthracene *n,n*-cyclophane = protoheme with $-CH_2NHC(O)(CH_2)_{n-5}$–anthracene–$(CH_2)_{n-5}C(O)NHCH_2-$ strap; TPivPP = tetrakis(*o*-pivaloylphenyl)porphyrinato dianion; C_2-Cap = five-atom-linked capped porphyrin with the linkage being $-C(O)OCH_2CH_2O-$; HbA = adult human hemoglobin; Mb = myoglobin.
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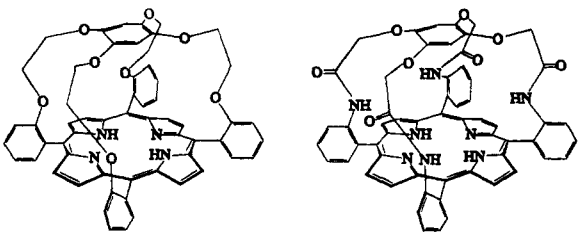

$H_2(OC_2OPor)$ **1** $H_2(OC(CO)NPor)$ **2**

Figure 1. Porphyrins H_2(**1**) and H_2(**2**).

value of about 100 Torr (Por = **1**), 10^3 to 10^4 higher than typical. Here we report the extension of those preliminary equilibrium studies, along with kinetic measurements of the combination reactions, picosecond explorations of any geminate rebinding that might follow photolysis, and nanosecond studies to search for any intermediate processes that might occur. The goals were to confirm and extend the characterization of equilibria, to ascertain whether the major contributor to reduced affinity is less facile association or enhanced dissociation, and, what is most novel, to learn whether the cap, which certainly inhibits approach of the ligand, may actually mimic the protein pocket by trapping a photoinduced geminate pair for some significant time.

Experimental Section

Equilibrium Measurements: Materials. Toluene and THF were distilled under N_2 from sodium benzophenone ketyl prior to use. 1-MeIm and 1,2-Me_2Im were dried over KOH, distilled under vacuum, and stored under Ar. Anhydrous $FeBr_2$ was purchased from Strem Chemicals.

Prepurified grade Ar from Gas Tech and CP grade O_2 were passed through molecular sieves to remove residual H_2O. CO mixtures were Matheson-certified standard 0.9960% and 10.23% in N_2. CO was Matheson CP grade. All gases containing CO were passed through coconut charcoal and molecular sieves to remove residual O_2 and H_2O. Porphyrins **1** and **2** were prepared as previously described.[18]

Iron Insertion. Fe^{II}(Por) was prepared by stirring H_2(Por) (100 mg), K_2CO_3, and $FeBr_2$ in 20 mL of 1:1 THF/toluene for 24–48 h in an inert-atmosphere glovebox.[19] After being chromatographed on silica with 10% THF in toluene, the Fe^{II}(Por) product was evaporated to dryness and stored for use in the binding studies.

Base Equilibrium Measurement. K_B (eq 1) was determined from spectrophotometric titration spectra. Aliquots of deoxygenated 1-MeIm,

$$\text{Fe}(\mathbf{1}) + \text{1-MeIm} \leftrightarrows \text{Fe}(\mathbf{1})(\text{1-MeIm}) \qquad (1)$$

either neat or diluted with toluene, were added to a 5 mL toluene solution of Fe(**1**) under Ar in a 1.0 cm optical cell at 25 °C. Absorbance values were corrected for concentration changes, and the data were fit to the Hill equation, as described in earlier work.[20]

O_2 and CO Affinity Measurements. Equilibrium constants for O_2 and CO binding (eq 2, B = base) were determined spectrophotometrically with the use of a 1.0 cm path length, low-temperature optical cell. Slush baths were used to maintain constant temperature. Spectra

$$\text{Fe(Por)(B)} + \text{L} \leftrightarrows \text{Fe(Por)(B)(L)} \quad (\text{L} = \text{O}_2 \text{ or CO}) \qquad (2)$$

were recorded in the 350–500 nm range with a Cary-1E UV–vis spectrophotometer. CO or O_2 was bubbled into a 0.25 or 1 M base/toluene solution of Fe(Por). The partial pressure of the gas was adjusted by mixing 1%, 10%, or 100% CO and Ar, or 100% O_2 and Ar, use being made of flowmeters individually calibrated for each gas. To minimize concentration changes during the experiment, the gas mixture was saturated with toluene at the experimental temperature before being bubbled through the porphyrin solution for 15 min. Reversibility was checked after the last addition by purging again with Ar for 1–2 h. Since only partial ligation of O_2 and CO occurs at 760 Torr, data were fit with the use of the techniques of Collman et al.[21] rather than with the Hill equation.

Kinetic Measurements: Materials. Toluene was distilled over CaH_2. 1-MeIm from Aldrich was dried over KOH and distilled under vacuum. 1,2-Me_2Im, sodium dithionite, and 18-crown-6 were used as purchased from Aldrich. CO from Specialty Products and Equipment, Houston, and O_2 from G. S. Parsons, San Diego, were used as received. Fe(**1**)Cl and Fe(**2**)Cl were prepared as previously described.[18]

Sample Preparation. To 5 mL of degassed methanol were added 18-crown-6 (190 mg, 0.7 mmol) and sodium dithionite (58 mg, 0.33 mmol). The solution was stirred under N_2 for 30 min and then centrifuged to yield a clear solution. Fe(**1**)Cl or Fe(**2**)Cl was dissolved in a minimum of CH_2Cl_2 and then added to a toluene/imidazole mixture that was 1 M in either 1-MeIm or 1,2-Me_2Im until the absorbance at the maximum of the Soret band was about 0.7 in a 1.0 cm path length cell. This corresponds to a concentration of about 5×10^{-6} M. Then 5 mL of the solution was transferred to a tonometer having a total volume of 148.7 mL. Air was removed by several freeze–pump–thaw cycles. The Fe^{III} compound was then reduced to the Fe^{II} complex under Ar by addition of about 2 μL of the sodium dithionite/18-crown-6/methanol solution. For the O_2 solutions, care was taken to use the minimum amount of reducing agent. After sample reduction, the tonometer was again degassed by several freeze–pump–thaw cycles. Finally, CO or O_2 gas was added quantitatively with the use of a gas-tight syringe.

Conventional Kinetic Measurements. Solutions of Fe(Por)(base) were prepared in the tonometer, as described above. In order to make measurements at a series of increasing CO concentrations, aliquots of the gas were injected through a W-10 septum. After each addition, the sample was allowed to equilibrate while being stirred for 10 min, and then it was placed in a flash photolysis apparatus. A Sunpak Model Auto611 thyristor photographic flashgun placed close to the side of the optical cell provided the photolysis flash. Transient absorbance changes were slow enough to measure with a conventional UVikon-810 spectrophotometer. The absorbance returned to its initial value at long times according to a single exponential decay; the rate constant k_{obs} was determined simply from the $t_{1/2}$ value. Kinetic measurements were also carried out for a series of decreasing gas pressures with the use of analogous methods involving the removal of aliquots of gas, due corrections being made for the volumes required as the pressure diminished. In either case, plots were constructed of k_{obs} against [CO] and the association constant $k_B{}^{CO}$ and dissociation constant $k_B{}^{-CO}$ were obtained from the slope and intercept, according to eq 3.

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

Laser Kinetic Measurements. Kinetic measurements at nanosecond/microsecond or picosecond time scales employed laser apparatus and data analysis that have evolved over the years, but which were described in full recently.[22] Briefly, the nanosecond/microsecond measurements used a dye laser pumped by a pulsed XeCl laser to generate photolysis pulses at 540 nm that were about 2 mJ at the sample over an area of about 0.2 cm^2. The measurement of transient absorbance was performed for one wavelength at a time. Either a continuous tungsten lamp or a long-pulse xenon flash was used, depending on the time scale of interest, and different digitizers were also used for different time scales. A special photomultiplier was used to ensure linearity at high current output. A nonlinear least-squares algorithm was used to fit a kinetic function to the data. The fit could allow for a finite electrical response time, that is, an RC filter.

The picosecond apparatus was very different. A colliding-pulse ring dye laser produced 70 fs pulses at 628 nm that were amplified in four stages pumped by the harmonic of a Nd:YAG laser. Harmonic doubling of the amplifier output produced pulses of about 70 μJ at 314 nm that were used for photolysis. The residual of the amplifier output was

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Table 1. Constants for the Binding of O_2 or CO to Fe(**1**)(base)

base	temp (°C)	$P_{1/2}^{O_2}$ (Torr)	$P_{1/2}^{CO}$ (Torr)
1-MeIm	25		100
	0		40
	−20		17
	−43	61	
1,2-Me_2Im	25	>760	~5000
	−43	>760	

sent around an optical delay line and focused into a continuum generation cell to produce short flashes of white light for the spectroscopic probe. Only one time delay was measured with each laser flash, but a 100 nm wavelength range was detected with the use of an amplified diode array. The delay line was motor driven over a range corresponding to 1 ns, at a step interval that could be as short as 0.167 ps. The laser fired at 10 Hz. Pumped and unpumped spectra were acquired alternately so that absorbance changes could be calculated. Usually a few shots were taken at each delay setting and averaged. Always several complete delay scans were collected, inspected separately to check for sample degradation or other slow drift, and finally averaged to improve signal to noise. Typically, the final transient absorbance matrix had recordings at six hundred wavelengths and a few hundred time delays.

Results and Discussion

Equilibrium Measurements. As expected, the equilibrium constant for base binding $K_B = 2.6 \times 10^5\ M^{-1}$ is in the same range as those reported for other capped porphyrins.[23] Because of earlier observations,[18] preliminary spectral studies of Fe(Por)(base) were carried out in a high-pressure tonometer similar to one described previously,[24] but modified to use a rectangular cell with optical-quality windows. The system Fe(**2**)(base) does not bind O_2 or CO at partial pressures up to 100 atm (10.1 MPa) in mixtures of 1-MeIm/toluene, which should be a favorable solvent. Porphyrin **2** has amide (HN—C=O) linkages; in H_2(**2**) the cap-to-porphyrin distance is 3.90 Å.[18] In Fe(C_2-Cap)(1-MeIm)(CO) this distance is about 5.6 Å[25] so if Fe(**2**)(1-MeIm) were to accommodate a linearly bound CO molecule, the cap would have to move about 1.7 Å further away from the porphyrin. To move the cap would require the four amide linkages to become nonplanar. That this does not occur up to pressures of 100 atm, which corresponds to a ΔG of approximately 11 kJ/mol, is not surprising in view of the large energy barriers to rotation of amides.[26,27]

For the system Fe(**1**)(1,2-Me_2Im) no spectral changes were observed up to O_2 pressures of 1 atm. Higher pressures were not investigated. For this system with CO, $P_{1/2}^{CO} \approx 5 \times 10^3$ Torr. The spectra for this system are largely reversible when CO is removed, but they are not as sharp as usual. Perhaps CO is competing with the 1,2-Me_2Im for binding at the proximal site and there are multiple species at equilibrium. Owing to the high pressures involved, no further equilibrium measurements were carried out for base = 1,2-Me_2Im. Consequently, equilibrium measurements of O_2 or CO binding were restricted to the system Fe(**1**)(1-MeIm). Binding results are summarized in Table 1, and sample spectra are presented in Figure 2. With O_2 binding to Fe(**1**)(1-MeIm) at temperatures above −43 °C, irreversible oxidation occurs and isosbestic behavior disappears; at −43 °C $P_{1/2}^{O_2} = 61$ Torr. With CO binding to Fe(**1**)(1-MeIm), there is a complex equilibrium process below −30 °C and isosbestic behavior is not observed. At these low temperatures a species with a Soret band at 412 nm first appears. Not until a pressure of 78 Torr does the Soret peak for Fe(**1**)(1-MeIm)(CO) begin to appear (Figure 3a). At a CO partial pressure of 760 Torr, formation of this third species is temperature dependent and reversible (Figure 3b). CO binding at higher temperatures is isosbestic, and thermodynamic values have been obtained. The energy of binding is clearly encompassed in the less negative value of $\Delta H°$, as the entropy term is normal (Table 2). While $P_{1/2}^{O_2}$ in this sterically hindered system is perhaps on the high side, $P_{1/2}^{CO}$ is remarkably higher than those reported for hemoproteins and for other model systems (Table 3).

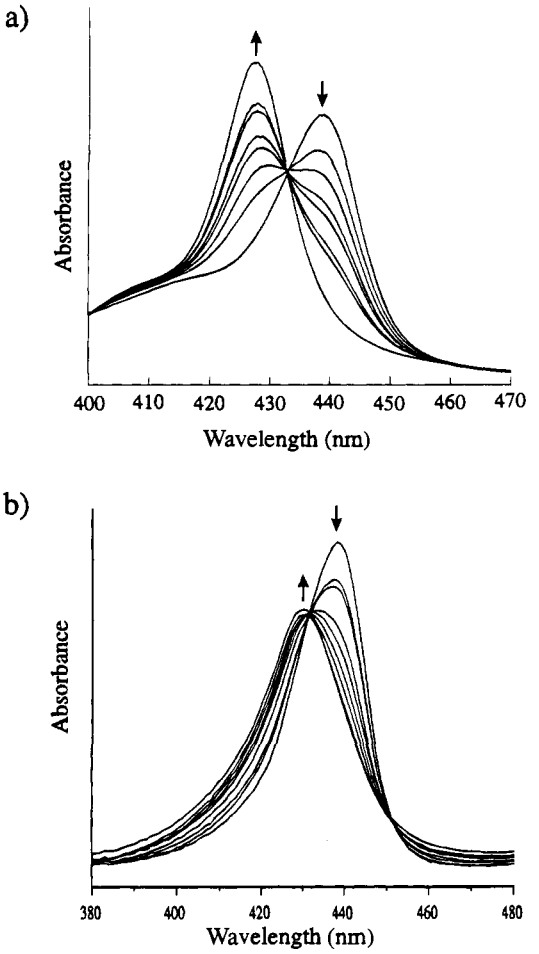

Figure 2. Binding to Fe(**1**)(1-MeIm): (a) CO binding at 0 °C, P^{CO} = 0, 8, 20, 30, 37, 59, 78, 760 Torr; (b) O_2 binding at −43 °C, $P^{O_2} = 0$, 15, 30, 58, 87, 150, 224, 507, 760 Torr.

Kinetics of Overall Association/Dissociation. The kinetics of binding to Fe(**1**)(base) could only be measured for CO and base = 1-MeIm. When conventional flash photolysis kinetic studies were attempted on Fe(**1**)(1,2-Me_2Im)(CO) in 1,2-Me_2-Im/toluene, the equilibrium fraction of carbonylated species at 760 Torr partial pressure of CO was too small to give absorbance changes large enough to analyze, although there were slight spectral changes indicative of dissociation of a small amount of ligated species. The high-pressure cell could not be used with the conventional flash apparatus. The kinetics of oxygen binding to Fe(**1**)(base) could not be measured; absorbance changes were either too small or too fast to measure with the UVikon spectrophotometer, which is consistent with the observation that reversible oxygen binding does not occur at 25 °C.

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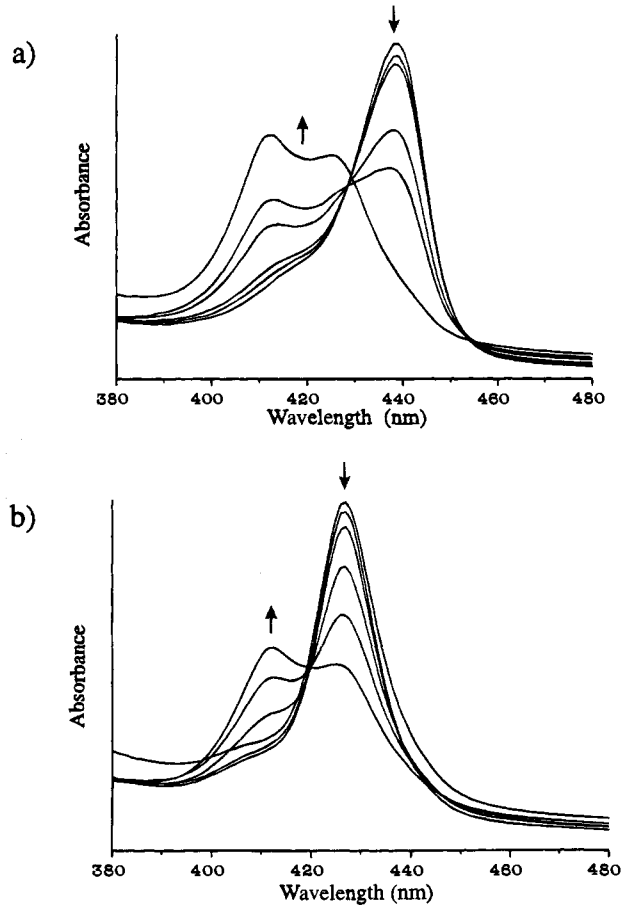

Figure 3. Binding of CO to Fe(**1**)(1-MeIm): (a) −52 °C, $P^{CO} = 0.05$, 3.1, 7.6, 26, 78, 760 Torr; (b) $P^{CO} = 760$ Torr, $t = 17$, −7, −22, −37, −45, −52 °C.

Table 2. Thermodynamic Values for CO Binding to Iron(II) Porphyrin Complexes and Hemoproteins

complex	$\Delta H°$ (kcal/mol)	$\Delta S°$ (eu)	ref
HbA, pH 7.4	−17.7[a]		42
HbA, isolated chains, pH 7.4	−21.8(4)		43
HbA, isolated chains, pH 7.4	−24.4(4)		43
Fe(TPP)(1,2-Me_2Im)	−13(1)	−26(2)	44
chelated protoheme	−17.5	−34	45
Fe(β-PocPivP)(1,2-Me_2Im)	−14(1)	−28(3)	44
Fe(OC_2OPor)(1-MeIm)	−5.8(8)	−29(3)	this work

[a] This value is the average for the four monomers in Hb.

Bimolecular association of Fe(**1**)(1-MeIm) with CO after conventional photolysis at 25 °C was characterized for increments of CO at 20 mL intervals, corresponding to concentrations from 0.90 to 6.45 mM. In a separate run, the association kinetics were characterized as CO was removed. Figure 4 shows data from both series. Over the range of concentrations of CO employed, the fraction of Fe having a bound CO varied from less than 30% to more than 70%. As expected, the amplitude of the absorbance change induced was smaller when less Fe was ligated. From the slope and intercept we obtain $k_B{}^{CO} = 18.5(5)\ M^{-1}\ s^{-1}$ and $k_B{}^{-CO} = 0.044(2)\ s^{-1}$. Consequently, we have $K_{eq} = k_B{}^{CO}/k_B{}^{-CO} = 420\ M^{-1}$, which, given the solubility of CO in toluene at 25 °C of 7.6×10^{-3} mol L^{-1} atm^{-1},[28] is equivalent to $P_{1/2}{}^{CO} = 180$ Torr, in fair agreement with the value of 100 Torr derived from the equilibrium data. The association rate constant is very low, but the dissociation rate constant is much closer to normal (Table 4). The cap must be effective in blocking access of CO; it must move 1.7 Å away from the porphyrin in order to accommodate a linear Fe−C−O linkage. The presence of the cap increases the energy of both the bound state and the transition state. But the cap does not induce strain that is reflected in bond breaking.

Nanosecond Kinetic Measurements. The primary goal of the nanosecond measurements was to search for concentration-independent recombination over tens or hundreds of nanoseconds that might be attributed to trapping a ligand within the distal cavity created by the cap, similar to the now well-known trapping in the protein pocket.[22,29,30] We could not detect any such nanosecond geminate rebinding for Fe(**1**) for any of the four combinations of ligand (O_2, CO) and base (1-MeIm, 1,2-Me_2Im). Nevertheless, the nanosecond experiments complemented the conventional measurements, as changes as a function of wavelength were measured more conveniently. The nanosecond measurements reproduced the rate constant listed above for bimolecular recombination of Fe(**1**)(1-MeIm)(CO) for CO partial pressures below 760 Torr and added the following information: (1) The absorbance change varies with wavelength as expected from the static difference spectrum, with a large negative value (bleach) at 427 nm, an isosbestic point near 433 nm, and a transient absorption maximum at 438 nm. At shorter wavelengths, there is another isosbestic point at about 398 nm and a broad transient absorption below that. (2) Faster processes, if any, occurring at any times between 50 ns and 1 s contribute less than a few percent to absorbance changes for CO partial pressures up to 760 Torr.

Nanosecond laser experiments were also carried out for ligand partial pressures above 760 Torr. For Fe(**1**)(1-MeIm)(CO) recombination kinetics are more complex at higher pressures, as indicated both by multiexponential decay curves at any particular wavelength and by time-resolved difference spectra that change over time. Figure 5 shows results at 13.8 atm of CO. The difference spectrum measured at early times, displayed for 20 μs, is that expected for an Fe(**1**)(1-MeIm)−Fe(**1**)(1-MeIm)(CO) system. It is similar to the transient observed at low CO pressures, as discussed above. At higher CO concentrations, however, this spectrum evolves with a rate near 10^4 s^{-1} (only weakly dependent on CO pressure) into a new spectrum. As displayed in Figure 5 for 300 μs, this second transient difference spectrum has a maximum near 410 nm, which is reminiscent of the observations for low-temperature equilibria. The new spectrum ultimately decays and the original equilibrium returns in a process that is approximately exponential with a second-order rate constant that matches that of the CO bimolecular combination measured in conventional kinetics. As the CO pressure is increased further, up to 100 or 200 atm, the slow phase becomes multiexponential, so that at least three exponentials are required to model all of the absorbance change. The most reasonable explanation is that a variety of forms of the dissociated species exist. Scheme 1 displays the minimum number of intermediates and the kinetic relations among them needed for a complete analysis of the capped porphyrin systems discussed here. This kinetic scheme is already simplified by the assumptions that there are no other potential ligands present in solution and that no imidazole will ever bind under the cap. After photolytic dissociation of CO, loss of B is expected to be very fast.[31] However, addition of B

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Table 3. O_2 and CO Binding to Iron(II) Porphyrin Complexes and Hemoproteins[a]

	$P_{1/2}^{O_2}$ (Torr)	$P_{1/2}^{CO}$ (Torr)	$M = P_{1/2}^{O_2}/P_{1/2}^{CO}$	ref
Fe(TPP)(1,2-Me_2Im)[b]				46
Fe(T(*p*-OCH_3)PP)(1,2-Me_2Im)[b]	5.3[f]	0.08		46
chelated protoheme[c]	5.6	2.5×10^{-4}	2.2×10^4	47
Im-pyrroheme[d]	0.2[f]	0.088		48
anthracene 7,7-cyclophane[c]	1.4	9×10^{-4}	1.5×10^3	49
anthracene 6,6-cyclophane[c]	7×10^2	0.17	4×10^3	49
Fe(TpivPP)(1,2-Me_2Im)[b]	38	8.9×10^{-3}	4.3×10^3	21, 50
Fe(*β*-PocPivP)(1-MeIm)[b]	0.36	1.5×10^{-3}	2.7×10^2	44
Fe(*β*-PocPivP)(1,2-Me_2Im)[b]	12.6	6.7×10^{-2}	2.2×10^2	44
Fe(C_2-cap)(1-MeIm)[b]	23, 0.10[f]	5.4×10^{-3}	4300	20, 46
Fe(C_2-cap)(1,2-Me_2Im)[b]	4000, 27[f]	0.2	2.0×10^4	20, 46
Fe(OC_2OPor)(1-MeIm)[b]	61[g]	100, 40,[h] 17[i]		18, this work
Fe(OC(CO)NPor)(1-MeIm)[b]	$>7.7 \times 10^4$	$>7.7 \times 10^4$		this work
Mb (elephant)[e]	0.62	9.5×10^{-2}	6.5	17
Mb (horse)[e]	0.70	1.8×10^{-2}	39	51
HbA(R)[e]	0.22(α),[j] 0.36(*β*)[j]	1.4×10^{-3} [j]	160(α), 260(*β*)	44, 52–55
HbA(T)[e]	40(α),[j] 140(*β*)[j]	0.3[j]	130(α), 470(*β*)	44, 54–56

[a] At 25 °C, unless otherwise noted. [b] Toluene. [c] Benzene. [d] CH_2Cl_2. [e] H_2O, pH ~7. [f] −43 °C. [g] −45 °C. [h] 0 °C. [i] −20 °C. [j] 20 °C.

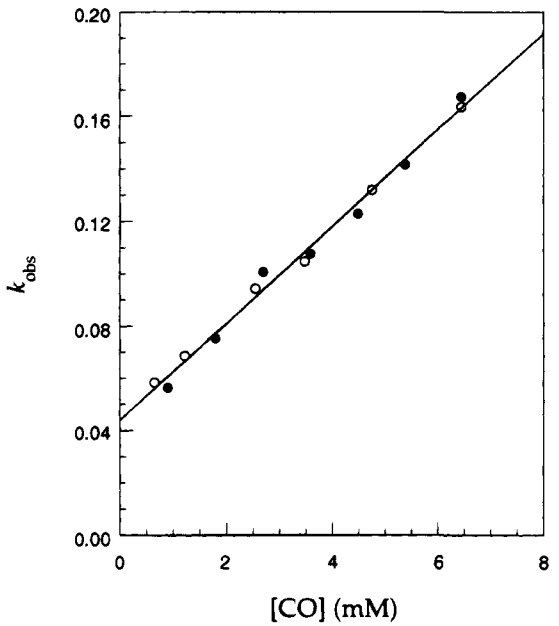

Figure 4. k_{obs} for bimolecular association of CO to Fe(**1**)(1-MeIm) as a function of CO concentration: ●, measured as CO was added; ○, measured as CO was removed. The slope yields $k_B^{CO} = 18.5(5)$ M^{-1} s^{-1}, and the intercept gives $k_B^{-CO} = 0.044(2)$ s^{-1}.

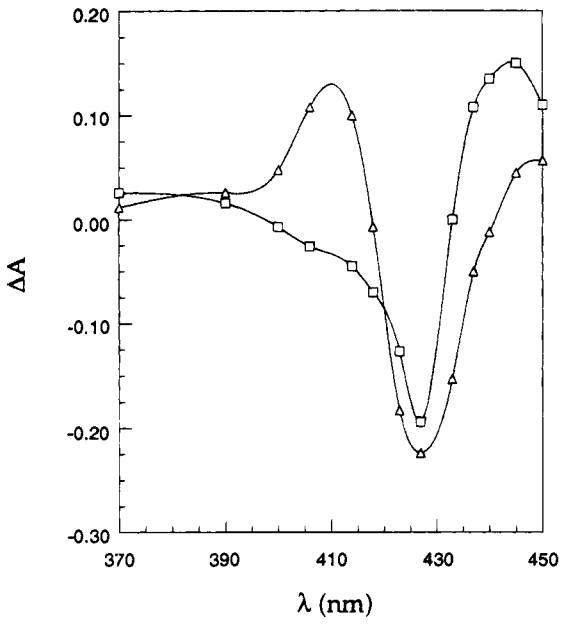

Figure 5. Transient difference spectra measured following nanosecond flash photolysis of Fe(**1**)(1-MeIm) under 13.8 atm pressure of CO: □, data observed at 20 μs; △, data observed at 300 μs after photolysis.

Table 4. Kinetic Data for CO Binding to Iron(II) Porphyrin Systems and Hemoproteins

compound	k_B^{CO} (M^{-1} s^{-1})	k_B^{-CO} (s^{-1})	ref
Mb (elephant)[a]	52.9	0.0068	17
Mb (horse)	5.0×10^5	0.017	51
HbA(R), α chain[a]	4.6×10^6	0.009	54, 55
chelated protoheme[b]	1.1×10^7	0.025	47
anthracene 6,6-cyclophane[b]	3×10^4	0.05	49
anthracene 7,7-cyclophane[b]	6×10^6	0.05	49
Fe(C_2-cap)(1-MeIm)[c]	9.5×10^5	0.05	46, 57
Fe(OC_2OPor)(1-MeIm)[c]	18.5	0.044	this work

[a] Aqueous, pH 7–7.4, 20 °C. [b] Benzene. [c] Toluene.

to four-coordinate iron porphyrins is also fast,[32] and Scheme 1 makes it clear that at high B and low CO concentrations, the species a and b dominate. Our kinetic studies, as well as the spectra recorded at various equilibria, show that these conditions can be met at room temperature, as long as CO partial pressures remain below 760 Torr. At higher CO pressures and lower temperatures, we cannot avoid significant populations of mono-

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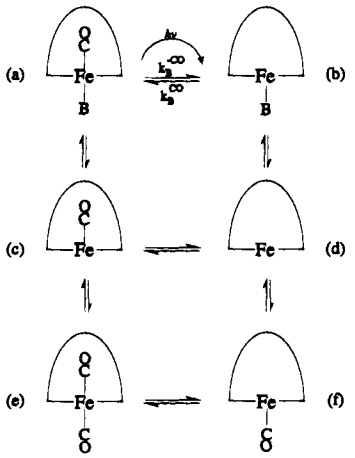
Scheme 1



CO species lacking B but having a bound CO ligand (species c and f). It is likely that the spectra of the two mono-CO species would be similar, but their kinetics would be very different. Such species would absorb near 410 nm,[31] and we observe such

an absorption in binding studies of CO to Fe(**1**)(1-MeIm) below −30 °C. Even for four-coordinate iron porphyrins, where the porphyrins are not capped and hence coordination of CO is more facile, bis-CO species should not be prominent at CO pressures of 760 Torr.[31,32] However, we cannot rule out small concentrations of species e at very high pressures of CO. Although we could fit more of our data by making assumptions about which species dominate, we cannot offer compelling arguments for the uniqueness of a given kinetic fit. However, the species a and b of Scheme 1 are those that are important for comparison with Mb and Hb, where B is always present as the side chain of the proximal histidine group.

With 1,2-Me_2Im as the proximal base, photolysis can only be observed at high CO pressures, where some CO is bound. The situation is even more complex, in that the equilibrium spectra suggest that a second minor component is present already at equilibrium and can be photolyzed to give additional contributions to the observed relaxation processes. The transient difference spectrum observed immediately after photolysis is essentially that expected for simple loss of CO from the six-coordinate complex. At later times, the difference spectrum evolves and includes a feature absorbing near 410 nm. However, a minimum of three exponentials is required to fit the measured decay under all conditions. They occur in approximately the same time regimes as for the analogous 1-MeIm system at high CO pressures. Probably there is no set of conditions for which the rate limiting process is CO addition to five-coordinated Fe(**1**)(1,2-Me_2Im) species. It is more likely that recombination proceeds predominately through loss of 1,2-Me_2Im, addition of CO, and return of 1,2-Me_2Im. However, there are clearly multiple species present, not in rapid equilibrium, presumably including those with CO bound on the proximal side.

There is no feature of the nanosecond transient spectroscopy observed in any of the complexes under any conditions that might be attributed to concentration-independent geminate recombination on that time scale.

We attempted measurements on O_2 complexes. The time resolution should have been sufficient to measure any plausible bimolecular recombination, but no transient signals were ever observed. Thus, if there were any O_2-liganded species present, the photodissociated O_2 was recombining in an efficient, geminate process that was too fast to measure with the nanosecond apparatus. If all the Fe were ligated by O_2, we should have been able to detect transients with dissociation quantum yields as low as 1%. It is likely that there was little, if any, of the desired six-coordinated O_2 species present. Quantitative limits are difficult to assign, because samples degraded by irreversible oxidation over the duration of an experimental run. From the equilibrium studies, such oxidation is known to occur above −43 °C. Although these capped systems appeared somewhat more stable than the less encumbered systems, they did not survive well for the full time required for laser measurements. No differences were noticed between 1-MeIm and 1,2-Me_2Im as the proximal base; neither displayed any transient signal.

Picosecond Kinetic Measurements. Picosecond laser measurements were successful only for Fe(**1**)(1-MeIm)(CO), and they were carried out only for a CO pressure of 760 Torr at 25 °C. The high-pressure cell could not be used with the picosecond apparatus, so 1,2-Me_2Im solutions were not measured. The Fe(**1**)(1-MeIm)(O_2) system was examined, but the conditions of the picosecond experiments resulted in accelerated photodegradation, making any interpretation very dubious.

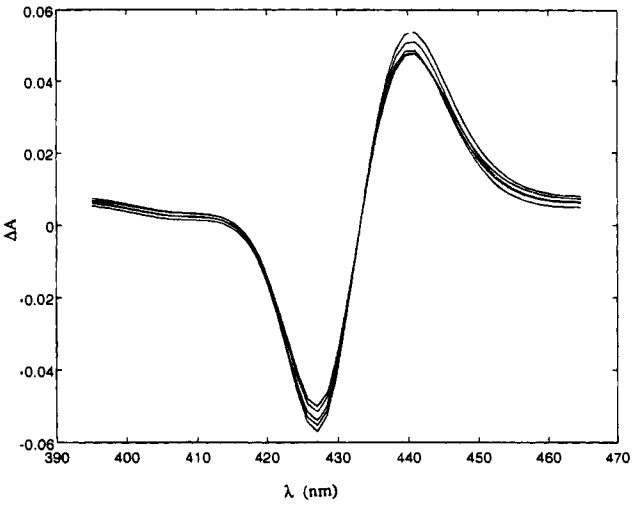


Figure 6. Transient difference spectra recorded following picosecond flash photolysis of Fe(**1**)(1-MeIm) under 760 Torr of CO. Traces are displayed for 10 ps intervals and show a monotonic decrease of both the bleach near 427 nm and the transient absorption near 440 nm. Between the first two curves, there is an extra decay or shift to shorter wavelengths of another absorption near 450 nm.

Measurements on Fe(**2**)(base) were not attempted, since this system does not bind either CO or O_2.

Photolysis of Fe(**1**)(1-MeIm)(CO) with subpicosecond laser pulses at 314 nm and subsequent time-resolved absorbance measurements over the range 380−510 nm reveal transient spectra that are similar to those recorded at nanosecond times. As these spectra are similar in shape and amplitude to those observed with CO complexes of other protein and model systems,[33] photochemical bond breaking is essentially 100% efficient. Spectral and temporal changes are complex over the first few picoseconds, but then evolve according to a simple exponential decay to a constant plateau (Figure 6). The complex spectral evolution at early times is similar to that seen in all heme−CO complexes[33] and is therefore attributed to decay of hot-band absorption as vibrational relaxation of the photolyzed molecules proceeds, possibly with an admixture of features involving the decay of excited electronic states. This behavior has a characteristic time constant of a few picoseconds and is virtually complete by about 10 ps. In the present instance of Fe(**1**)(1-MeIm)(CO), Figure 6 illustrates what was observed with four separate sample preparations: after the initial fast phase, there is an additional small percentage decay of the transient spectrum that requires several tens of picoseconds to complete and involves little or no spectral evolution. We attribute this to geminate recombination of CO with the Fe center. We propose that the cap is able to trap the CO long enough to allow bond formation to occur in competition with escape of the CO molecule from the cage. We estimate the fractional recombination to be about 15(5)%, the lifetime of the caged pair to be 20(5) ps, and the observed rate constant to be 5×10^{10} s^{-1}. The fractional recovery of spectral changes on the picosecond time scale is larger than we have observed previously for CO bound to either proteins or model systems (except in highly viscous solvents[33]). The 20 ps time scale for these changes is also slower than those observed in any systems studied previously. Consequently, this is the first observation made with toluene as solvent that seems likely to involve geminate

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recombination of CO. More recently, we have detected[34] a very similar feature, also attributed to geminate recombination of CO, in a picosecond study of a TPivPP porphyrin system. At present, emphasis must be placed on the similarity of the putative geminate recombination of CO in the two systems, despite their very different geometries. The precision of our measurements is not sufficient to distinguish possible small differences between the systems. Such differences might be more evident with some other ligand that shows more geminate recombination. Only small diatomics are candidates, since only they might conceivably bind under the cap. In fact, the TPivPP system is the first model system where we were able to measure the geminate recombination of O_2. Unfortunately, analogous measurements on the present capped systems were unsuccessful. On the other hand, the recovery of transient absorption on the picosecond time scale is still smaller for CO in these special cases than is observed for any other ligand of the dozen or more that we have characterized.[22,35-41] We conclude that most of the dissociated CO escapes from under the cap, even when that cap is as confining as in **1**. There is nothing resembling the trapping observed in proteins, which persists for hundreds of nanoseconds. It is surely an oversimplification to assume a constant rate for bond formation. If there is a trapped CO, it is likely that the probability for bond reformation evolves continuously after photolysis, for the short times involved here. Nevertheless, if the bond formation is assumed to occur with constant probability over the trapping time, these numbers imply an elementary rate constant for bond formation of $(5-10) \times 10^9$ s^{-1}, which is somewhat faster than we inferred from previous studies.[33]

Conclusions

The present study confirms that these four-atom-linked capped porphyrin systems Fe(Por)(base), with Por = OC_2OPor (**1**) or OC(CO)NPor (**2**), discriminate against CO binding to an extent unknown in previous models or in the natural systems. The system Fe(**2**)(base) does not bind O_2 or CO at partial pressures up to 100 atm (10.1 MPa) in mixtures of 1-MeIm/toluene, which should be a favorable solvent, probably because the four amide linkages would be forced into nonplanarity for binding to occur. The ether-linked system Fe(**1**)(base), with its sterically crowded pocket, has exceptionally high values of $P_{1/2}^{CO}$ of 100, 40, and 17 Torr at +25, 0, and −20 °C, respectively, for Fe(**1**)(1-MeIm) and $\sim 5 \times 10^3$ Torr at 25 °C for Fe(**1**)(1,2-Me_2Im). Binding of CO to Fe(**1**)(1-MeIm) is not isosbestic below −30 °C. The value of $P_{1/2}^{O_2}$ for Fe(**1**)(1-MeIm) is 61 Torr at −43 °C; above that temperature the binding is not reversible. Kinetic analysis of Fe(**1**)(1-MeIm)(CO) yields the exceptionally low value of $k_B^{CO} = 18.5(5)$ M^{-1} s^{-1} and the normal value of $k_B^{-CO} = 0.044(2)$ s^{-1}. It is reasonable to assign responsibility for this discrimination against CO binding of Fe(**1**)(base) to the long-hypothesized steric effects, especially to those centrally induced by the essentially symmetric 1,2,4,5-attachment of the benzene cap. It is clear that the dominant effect is on association, which is reduced dramatically. In contrast, dissociation is close to normal. Thus, the movement of the cap about 1.7 Å further from the porphyrin for ligation to occur raises the energy of the transition state at the same time that it raises the energy of the bound state.

Laser flash photolysis of Fe(**1**)(1-MeIm)(CO) confirmed that no faster processes intervene between the very slow bimolecular phase and geminate recombination that is complete in less than 100 ps, if it occurs at all. The model apparently accomplishes (and even exceeds) some of the protein functions that might be attributed to distal effects, but it in no way provides anything like the "pocket" proteins have, which can contain either CO and O_2 for hundreds of nanoseconds. Since the picosecond transient absorbance recovery is both larger and slower than we have observed previously in any other model heme−CO complex, but only by about a factor of 2, we have some justification in concluding that the pocket of Fe(**1**)(1-MeIm) can contain the dissociated ligand for a characteristic time of 20 ps and result in a geminate recombination efficiency of about 15%.

Acknowledgment. This work was supported by NIH Grant T32-GM-08326 (T.M.G.), NSF Grant CHE-9114613 (D.M.), and NIH Grant HL-13157 (J.A.I.). We dedicate this paper to the late Professor Teddy G. Traylor who organized this collaboration.

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