that R-state hemoglobin shows the same kinetics, equilibrium, and binding enthalpies for O_2 and CO as does chelated protoheme,[13] indicate that such differentiation is not a significant factor in hemoglobins and related dioxygen-transporting heme proteins.

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Nonbonding Steric Effect on CO and O_2 Binding to Hemes. Kinetics of Ligand Binding in Iron-Copper Cofacial Diporphyrins and Strapped Hemes

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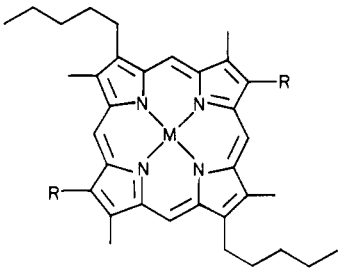
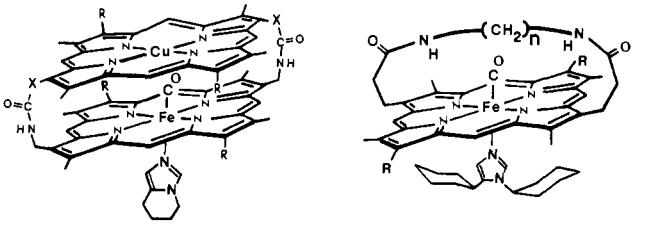
X-ray crystallography showed that the structures of carbon monoxide liganded hemoglobins (Hb) and myoglobins (Mb) exhibit a bent or tilted FeCO linkage with respect to the porphyrin ring,[1-5] whereas in heme model compounds the FeCO bond is linear and perpendicular to the heme plane.[6,7] The origin of the distorted configuration in the proteins is attributed primarily to nonbonding steric interactions of the axial ligand with residues at the distal side. An assumption is made that ligands such as O_2 and NO, which preferentially form bent complexes, should encounter less steric hindrance when bound in the heme pocket.[8,9] It has been proposed that in Hb and Mb, the distal steric effect would decrease the affinity ratio of CO vs. O_2 and is responsible for the detoxification of CO poisoning in respiratory systems.[10-13] A comparison of ligand binding constants of proteins and model compounds often shows that many heme models have a larger CO vs. O_2 affinity ratio (M value) than the proteins. However, such a comparison does not necessarily constitute a correlation between the distal steric effect and affinity as the ligand binding constants of heme models can be drastically altered by medium effects.[14,15] Indeed, Traylor and co-workers have shown that a 5-coordinate protoheme-imidazole model binds both O_2 and CO in aqueous suspension with equilibrium and kinetic parameters almost identical with R-state isolated hemoglobin chains.[14-17] In other cases, for example, T-state hemoglobin and notably myoglobins have very small M values which cannot be duplicated with simple heme compounds.

It is therefore of importance to examine the steric effects on ligand affinity using synthetic models equipped with varying degrees of steric hindrance at the distal side. Several porphyrin models of this kind have been prepared[18-20] and recently an iron complex with a bent CO has been shown,[21] but kinetic rates of ligand binding to the hindered hemes are not available. We wish to report the equilibria and kinetic rates of CO and O_2 binding to two hindered heme systems. One is mixed metal cofacial diporphyrins in which an inert copper porphyrin[22] is tightly linked to the heme, thereby providing a compression from above to the coordinating ligand. The second system is iron cyclophane porphyrins where a hydrocarbon chain is strapped across one face of the heme. Depending on the chain length, the strap would mostly exert a side-way shearing strain to the gaseous ligand.



1, R = CH_2CH_2COCl; M = Cu
2, R = CH_2CH_2COCl; M = 2H
3, R = CH_2COCl; M = Cu
4, R = CH_2NH_2; M = 2H
5, R = CH_2NHAc; M = Fe

Cofacial diporphyrins have been synthesized by coupling porphyrin diamines with diacid chlorides under high dilution conditions.[23] Thus reactions of **1** and **4** and **3** and **4** in CH_2Cl_2-pyridine afforded the copper-free base dimer 5 and dimer 4, respectively. Insertion of iron was accomplished by using the ferrous sulfate method.[24] The strapped hemes 13, 14, and 15 were synthesized by reacting **2** with 1,5-diaminopentane, 1,6-hexanediamine, and 1,7-diaminoheptane, respectively, followed by iron insertion. All porphyrins were characterized by visible,



R = n-pentyl

Fe-Cu-4 X=(CH_2)
Fe-Cu-5 X=$(CH_2)_2$

FeSP-13 n=5
FeSP-14 n=6
FeSP-15 n=7

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Table I. Kinetic and Equilibrium Constants for Binding of CO and O_2 (20–22 °C)[a]

compd	solvent	l', M^{-1} s^{-1}	l, sec^{-1}	$P_{1/2}^{CO}$, torr	k', M^{-1} s^{-1}	k, sec^{-1}	$P_{1/2}^{O_2}$, torr	M	ref
Mb	H_2O	$3–5 \times 10^5$	0.02–0.04	0.014 ~0.025	$1 \sim 2 \times 10^7$	10 ~ 30	0.5 ~ 1	20 ~ 40	27
Hb, isolated α^{SH}	H_2O	4×10^6	0.013	0.0024	5.0×10^7	28	0.3	130	8
chelated protoheme	H_2O[b]	3.6×10^6	0.009	0.0018	2.6×10^7	47	1.0	560	14
chelated mesoheme	H_2O[b]	11×10^6	0.019	0.0013	2.2×10^7	23	0.58	450	15a, 14
chelated mesoheme	toluene	8×10^6	0.03	0.0004	5.3×10^7	1700	3.2	8×10^3	15a, 14
deuteroheme + *N*-MeIm	benzene	12×10^6	0.028	0.0002					
Fe-Cu-4 + 0.2 M *N*-MeIm	benzene	2.0×10^4	0.02	0.1	5.2×10^5	160	31[e]	310	this work
Fe-Cu-4 + 1.0 M THPIm[c]	benzene	2.2×10^4	0.03	0.13	5.0×10^5				this work
Fe-Cu-5 + 0.2 M THPIm[c]	benzene	9.0×10^4	0.02	0.02	1.8×10^6	91	5[e]	250	this work
FeSP-13 + 0.2 M *N*-MeIm	benzene	6×10^2	0.07	12					this work
FeSP-14 + 1.0 M DCHIm[d]	benzene	8×10^3	0.04	0.5	3×10^5				this work
FeSP-15 + 0.2 M DCHIm[d]	benzene	9.1×10^4	0.04	0.05	1.7×10^6	250	15	300	this work

[a] Rates were calculated using the following solubilities: 1 torr of CO = 1×10^{-5} M (benzene, toluene), 1.35×10^{-6} M (H_2O); 1 torr of O_2 = 1×10^{-5} M (benzene, toluene), 1.8×10^{-6} M (H_2O). [b] Suspended in 2% CTAB or MTAB. [c] THPIm: 5,6,7,8-tetrahydroimidazo[1,5-*a*]-pyridine. [d] DCHIm: 1,5-dicyclohexylimidazole. [e] $P_{1/2}^{O_2}$ obtained from direct titrations and from kinetic measurements agreed within 20%.

NMR, and mass spectra and elemental analyses. With the Cu–H_2 diporphyrins, a second copper could be inserted and the resultant $(Cu)_2$ diporphyrins were characterized by EPR spectra.[23b,d] The zero-field splitting parameter[25] of the diporphyrin 4 indicated that the two rings are spaced less than 3.8 Å apart which undoubtedly would impose steric strain to a linear axial CO ligand.

CO and O_2 binding to the ferrous hemes were studied in benzene solution containing excess *N*-alkylimidazoles. The nitrogen base was chosen such that it can only form 5-coordinate heme. *N*-Methylimidazole was bulky enough to meet this criterium only with the very tightly gapped Cu-Fe-4 and FeSP-13 but was not satisfactory for Cu-Fe-5 nor FeSP-15 as considerable competition of CO binding at the hindered site by a second imidazole can be observed. We therefore used a "tall" bicyclic imidazole for the dimers and a "fat" dicyclohexylimidazole[26] for the strapped hemes; using these bases no competition was observed.[33] Kinetic rates were determined by a flash photolysis method[15] which permits direct measurements of CO and O_2 association rates as well as the O_2 dissociation rates. Typical relaxation curves along with the corresponding absorption spectra of different complexes were shown in Figure 1. The CO and O_2 association rates were obtained under pseudo-first-order conditions, and the CO dissociation rates were calculated by $L = l'/l$. The oxyheme complex formed in the Cu–Fe dimers was so stable (no oxidation detectable even after 12 h at room temperature) that the $P_{1/2}^{O_2}$ values can be measured directly by gas titration, and as such, they provided an independent check on the O_2 off rates derived from the kinetic equations.[15,27] All rates and equilibrium constants for models and relevant heme proteins are tabulated in Table I.

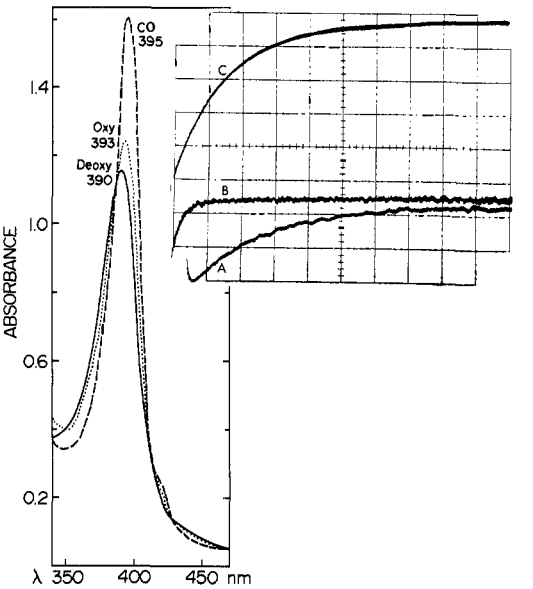

Figure 1. Absorption spectra of various forms of Fe-Cu-4 and the corresponding oscilloscope traces for the regeneration of Fe–O_2 and Fe–CO after flash photolysis. The sample in 0.2 M *N*-methylimidazole/benzene solution was monitored at 398 nm. Time scales for the relaxation curves were 0.5 s (C), 2 ms (B), and 0.2 ms/division (A). The signals from photomultiplier were processed through an absorbance converter[32] before recorded on scope and fed into a digital computer.

The most striking results shown by Table I is that, indeed, distal steric hindrance can affect ligand binding, but this effect is manifested only in the ligand association rate constants and has almost no effect on the off rates, in agreement with the predictions made earlier by Moffat et al.[8] and consistent with the isocyanide

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(33) However, we did observe a positive shift in l' as a function of *N*-methylimidazole concentration for FeSP-13 and FeSP-14. At *N*-MeIm concentration ranging from 0.1–3.0 M, FeSP-13 had an l' increase of about 200%. On the other hand, CuFe-4 had a corresponding shift of less than 20% (0.1–1.0 M). This indicates that with l' less than about 10^4, equilibria on the proximal side (base-off[37]) present a somewhat serious problem. For this reason, any O_2 vs. CO comparisons for FeSP-13 and FeSP-14 are probably not reliable and, therefore, are not discussed. With l' greater than 2×10^4, proximal base equilibria should present little difficulty. Indeed, l' of FeCu-5 or FeSP-15 is unaffected by base concentration.

Table II. ν_{CO} of Sterically Hindered and Unhindered CO Hemes[a]

compd	medium	$\nu^{12}CO$	$\nu^{13}CO$
Fe-Cu-4	neat *N*-MeIm	1960	1915
	0.1 M *N*-Ph_3CIm in CH_2Br_2	1967	1924
FeSP-13	neat *N*-MeIm	1962	
	0.1 M *N*-Ph_3CIm in CH_2Br_2	1967	
heme 5	neat *N*-MeIm	1955	1910
	0.1 M *N*-Ph_3CIm in CH_2Br_2	1966	1922

[a] Spectra were obtained by using a Perkin-Elmer 283B Spectrometer interfaced with computer.

binding results of Traylor.[34] Since it has previously been shown that CO and O_2 association rates are nearly independent of medium and heme electronic effects and that the O_2 off rates are very much affected by the local polarity of the ligand binding site,[28,29] it is futile to directly compare the O_2/CO affinity ratio (M) of different model compounds. However, when we compare only the association rate data we find, relative to chelated mesoheme, for FeCu-5 or FeSP-15 a CO reduction of 90-fold while O_2 is reduced by 30 (a reduction ratio of 3) and for FeCu-4 a CO reduction of 400-fold with O_2 being reduced by 100 (a reduction ratio of 4). This unequal reduction of CO and O_2 association rates may be considered as an evidence for the steric differentiation of O_2 and CO. This steric selectivity nonetheless does not explain why we cannot obtain the degree of differentiation observed for Mb, i.e., chelated protoheme or R-Hb vs. Mb has a reduction ratio of at least 5, even though our model compounds have more steric hindrance built into them than does Mb, as reflected by CO on rates. Neither can we reconcile the fact that there is essentially no change in the on rate reduction ratio nor the M value going from FeCu-5 to FeCu-4 while the structural data as well as the CO on rates indicate clearly that the FeCu-4 has a tighter gap than FeCu-5. If the bending of CO is responsible for the differentiation, it would have to show in the 4 to 5 comparison. One possibility is that the differentiation is not proportional to the steric hindrance; it reaches a maximum and then decreases as the steric effect becomes too great. Unfortunately, in the present study we found it is difficult to have a system whose CO on rate is in the neighborhood of 5×10^5 M^{-1} s^{-1}, to compare with Mb. Cofacial diporphyrins with longer linkages, e.g., FeCu-6 and FeCu-7 exhibit kinetic rates similar to FeCu-5 since the two porphyrin rings have a tendency to assume a slipped conformation and maintain a tight gap, as shown by X-ray studies;[35] thus these compounds offered no insight. On the other hand, hemes equipped with longer straps tend to form 6-coordinate hemochromes with the excess base.

Although the present study does not provide a definitive answer as to whether or not steric bulk at the ligand binding site can selectively reduce the *affinity* of CO vs. O_2, surely the kinetic results imply that models which bind CO 2-3 orders of magnitude slower than Mb should decisively indicate whether there is any relation between ligand affinity and ν_{CO}. Table II summarizes the ν_{CO} of some of the synthetic compounds measured in different solvents. It is evident that the influence of medium is far greater than the steric effect. *There is no correlation between ν_{CO} and the ligand affinity.*[30] While it is unclear whether ν_{CO}, which is a function of the bond order between C and O, should be sensitive to slight distortion at the C-Fe bond, the lack of any significant change in ν_{CO} suggests that the bond nature in hindered hemes is not very different from those in a normal octahedral geometry. The unequal reduction of the CO and O_2 association rates by the steric bulk implies that such differentiations must be related to the bond-forming processes. Szabo[31] has suggested that CO-heme transition state resembles product while O_2 heme has a more reactant-like transition state. That is to say since the Fe-CO bond formation requires shorter contact, the CO molecules must be in closer proximity than O_2 to attain transition state. Any steric barricade at the heme binding site therefore would hinder CO coordination more than O_2 coordination.

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The present study also indicates that it would be a unique synthetic challenge to prepare heme models[36] that match Mb's kinetic behavior. So long as we showed that bending of CO cannot be solely responsible for the large differentiation observed in Mb, other factors such as the basicity of the proximal base, preequilibrium of the heme conformation inside the protein pocket, etc., have to be taken into consideration.[37] The synthesis of other sterically hindered, 5-coordinate hemes is under way.

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A New Mechanism for Photosubstitution of Organometallic Complexes. Generation of Substitutionally Labile Oxidation States by Excited-State Electron Transfer in the Presence of Ligands

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Photosubstituion remains one of the most important reactions of inorganic and organometallic complexes.[1,2] Photosubstitution occurs by dissociative and associative pathways[1-4] involving loss of a ligand from the excited state or ligand addition to the excited state as the key step. By either of these mechanisms the quantum yield for substitution can be no greater than 1. We wish to report results that establish a new mechanism for light-induced ligand substitution where quantum yields can, and do, exceed 1. Photoinitiated substitution via the generation of substitution labile, metal-centered radicals by cleavage of metal-metal bonds can also lead to substitution with quantum yields that exceed 1.[5] The basis of our new mechanism is that a unit change in the oxidation state of the metal can have profound consequences on the substitution lability. Classic examples include the pairs of complexes derived from Cr^{3+}/Cr^{2+} and $Co^{3+/2+}$ where the 2+ oxidation states yield labile complexes, and the 3+ states yield inert complexes.[6] Accessing substitution labile oxidation states of metal complexes by ligand-to-metal charge transfer is known (eq 1), but the net

$$Co(NH_3)_5Br^{2+} \xrightarrow[H_2O]{h\nu} Co^{2+} + 5NH_4^+ + Br^- \qquad (1)$$

result is not substitution on the original complex.[2] We now describe results that show that excited-state electron transfer can

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