Steric control of CO binding in a totally synthetic heme protein model

(heme models/carbon monoxide-iron complexes/carbon monoxide detoxification/carbonyl myoglobin structure)

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ABSTRACT A family of totally synthetic models for the carbon monoxide adducts of heme proteins has been synthesized and applied to the elucidation of the role of steric effects in the relative detoxification of carbon monoxide. The complexes are designed such that a sheltered void of controllable dimensions encompasses the CO binding site. Systematic variations in the available space for the iron-bound CO produce a wide range of equilibrium binding constants (K_{CO}). An x-ray structure determination of a CO adduct complex having a crowded CO site reveals that the Fe—C≡O linkage is bent, and further, the distortion involves both displacement of the Fe—C vector from the normal to the N_4 plane and bending of the Fe—C—O angle.

Structural analyses of carbonyl hemoglobins and carbonyl myoglobins show a substantial distortion of the FeCO linkage: The oxygen atom is definitely located far from the porphyrin axis, but the indeterminacy of the carbon position makes it unclear whether the FeCO axis is tilted with respect to the N_4 plane or whether the FeCO unit is bent (refs. 1–3 and references cited in ref. 1). The distortion results from crowding by the distal imidazole group, and the subject has been considered in a variety of studies (4–12), including a recently reported cyclophane porphyrin model (13, 14). It has been proposed that the crowding is responsible for the partial detoxification of carbon monoxide in respiring organisms (4, 11). For example, the ratio of equilibrium constants (12–18) for CO and O_2 binding (formation) K_{CO}/K_{O_2} for a protein-free modified heme having no structural counterpart to the distal imidazole (Traylor's chelated protoheme) is 840, whereas the corresponding ratio in myoglobin under comparable conditions is 20 (15). The discrimination against carbon monoxide by myoglobin is evident from the fact that the equilibrium constant for O_2 binding for the chelated protoheme ($2.0 \times 10^6 M^{-1}$) is very close to the value for myoglobin ($1.5 \times 10^6 M^{-1}$). The behavior of a model having an anthracene suspended above a porphyrin has given support to the conclusion that steric effects can weaken CO binding to heme iron (13). Studies on the equilibrium constants for CO and NO binding, both to model compounds and to heme proteins, confirm the role of a steric effect in weakening the binding of CO to myoglobin (19).

In contrast, there is no substantial selective process, steric or otherwise, operating against the binding to iron of CO, as compared to O_2 , in the case of Hb(R) (12). These results generate two related questions, because the FeCO linkage is distorted (1–3) in both crystalline Hb(R)(CO) and crystalline Mb(CO): (i) Do steric effects that push the normally linear FeCO group into some off-axis configuration necessarily decrease the CO affinities of iron(II) complexes? (ii) If yes, are

FIG. 1. Schematic representation of the class of lacunar complexes discussed, emphasizing the presence of a cavity over one axial site of the metal ion.

there structural differences between the carbon monoxide complex of Hb(R)(CO) in aqueous solutions or suspension (20) and in its crystalline state that would account for the apparent contradiction?

The structural origins of modifications in O_2 affinity are well illustrated by the T and R forms of hemoglobin. For O_2 binding, the equilibrium constant is altered by changes in the rate of dissociation and is attributable to constraints placed on the proximal imidazole by protein conformation and the ease with which the iron atom may occupy the N_4 plane of the protoporphyrin (4–11, 21).

We report here a systematic and definitive demonstration that steric effects can indeed control the CO affinity of iron(II). This is shown by direct equilibrium measurements with a family of nonporphyrin heme protein models coupled with key crystal structure analyses. The ligands in our models contain superstructure components that facilitate control of the space available for the binding of CO to an iron(II) atom (22). These so-called *lacunar* ligands are neutral, bicyclic molecules having conformations that incorporate permanent voids in the vicinity of a metal coordination site (see Fig. 1). The efficacy of these synthetic species as biological mimics has been shown through studies on the reversibly formed 1:1 oxygen adducts of the cobalt(II) and iron(II) complexes (23–25). The lacunar structure (Fig. 1) inhibits both dimer formation and proton-promoted electron transfer, the usual modes of irreversible decay of O_2 adducts.

The same features that were used to control the O_2 affinities of cobalt(II) and iron(II) complexes have now been used to produce iron(II) complexes with various CO affinities. Further, we herein report an x-ray crystallographic structure determination of the CO adduct of a complex whose CO binding constant shows the effects of the crowding by the bridge component. We

find that the FeCO linkage is distorted in two ways. The Fe—C vector is displaced from the normal to the N₄ plane and the Fe—C—O angle is bent by almost 10° from linear.

MATERIALS AND METHODS

All reagents used were of reagent grade and were used without further purification unless noted. Solvents were invariably distilled prior to use, using standard procedures. Syntheses of ligands as their nickel complexes with subsequent removal of the metal ion have been reported (22). All iron complexes were prepared under an atmosphere of dry nitrogen, using a Vacuum Atmospheres (Hawthorne, CA) glove box (see below).

Conductance measurements were obtained by using an Industrial Instruments (Andover, MA) model RC 16B conductivity bridge with cell constant 0.11 cm⁻¹ at 1000 Hz. Magnetic susceptibilities were measured at room temperature by the Faraday method.

Carbon monoxide binding constants were obtained by using the method of Stevens (24); this involves monitoring electronic spectral changes as a function of carbon monoxide partial pressure with a Cary 17D spectrophotometer. The binding constant K_{CO} was determined by using a nonlinear least-squares fit to the equation

$$A = A_0 + \frac{(\epsilon_{FeLBCO} - \epsilon_{FeLB}) K_{CO}[FeLB]_0(P_{CO})}{1 + K_{CO}(P_{CO})},$$

in which FeLB and FeLBCO represent the five-coordinate precursor iron complex (L, ligand; B, an axial base) and its carbonyl adduct, respectively. A and A₀ represent the observed absorbance at a given partial pressure of CO (P_{CO}) and at $P_{CO} = 0$, respectively.

Crystallographic measurements were carried out with a Syntex P1 automated four-circle diffractometer; details are supplied in text.

Preparation of Iron(II) Lacunar Complexes. Complexes of the composition [Fe(L)Cl]PF₆ (Fig. 1, B = Cl) have been synthesized for the following lacunar ligands (Fig. 1, identified by successive listing of R² and R¹): H, (CH₂)_n, n = 4–6; CH₃,

CH₃, *m*-xylyl. The complexes contain high-spin, five-coordinate iron(II) with magnetic moments in the range between 4.9 and 5.3 Bohr magnetons. Their molar conductances, 1 mM in acetonitrile, range between 125 and 150 Ω⁻¹ mol⁻¹ cm² and are typical of uni-univalent electrolytes. In general, the ligand salt (e.g., H₃[(CH₂)₆(NCH₃Ethi)₂(CH₃)₂[16]tetraene] (PF₆)₃) [Fig. 1, R¹ = (CH₂)₆, R² = CH₃, Ethi = ethylidene] is neutralized in acetonitrile, with an equivalent amount of triethylamine and combined with iron(II), provided either as FeCl₂·2CH₃CN (26) or as FeCl₂·2pyridine (27). Some of the complexes separated from solution as chloride salts, but uniformly good behavior was obtained by metathesis to the PF₆ salts.

* Structure solution details for [Fe{(m-xylyl)(NH₂Ethi)₂(CH₃)₂[16]tetraene-N₄}Cl]Cl·2CH₃OH: 2874 independent autodiffractometer data points collected at 20(1)°C using monochromatized Mo K_α radiation (ω – 2θ scans); space group P2₁/a; a = 10.607(2) Å, b = 24.656(3) Å, c = 10.132(2) Å, β = 79.79(2)°; ρ_{obs} = 1.34 g cm⁻³, ρ_{calc} (Z = 4) = 1.34 g cm⁻³; refinement based on F² using all 2874 data points [1509 > 3σ(I)] yielded R(F) = 0.103 (0.057), R_w(F²) = 0.092 (0.079), and goodness of fit = 1.33 (1.58).

FIG. 2. ORTEP diagrams showing the side and front views of lacunar complexes. (Upper) [Fe{(m-xylyl)(NH₂Ethi)₂Me₂[16]tetraene-N₄}Cl]⁺ (Fig. 1, M = Fe^{II}, B = Cl, R¹ = *m*-xylyl, R² = H), an example of a lid-on isomer. (Lower) [Fe{(m-xylyl)(NMe₂Ethi)₂Me₂[16]tetraene-N₄}Cl]⁺ (Fig. 1, M = Fe^{II}, B = Cl, R¹ = *m*-xylyl, R² = CH₃), an example of a lid-off isomer.

RESULTS AND DISCUSSION

Dimensions of the Void—X-Ray Structures. X-ray crystal structures have been completed on two of the chloro complexes (detailed reports will appear elsewhere): [Fe{(m-xylyl)(NH₂Ethi)₂(CH₃)₂[16]tetraene-N₄}Cl]Cl·2CH₃OH* (Fig. 1, B = Cl, R¹ = *m*-xylyl, R² = H) and [Fe{(m-xylyl)(NCH₃Ethi)₂(CH₃)₂[16]tetraene-N₄}Cl]PF₆[†] (Fig. 1, B = Cl, R¹ = *m*-xylyl, R² = CH₃) [referred to as (NH)₂ and (N—CH₃)₂, respectively, throughout the following discussion]. The ligands differ only in that the hydrogen atoms on the bridge nitrogens in the first are replaced by methyl groups in the second. The effect of this seemingly small structural change is great; it produces isomerically different structures and very different cavity sizes.

ORTEP drawings of the structures are given in Fig. 2. In both structures the iron atom is five-coordinate, square-pyramidal and located below the N₄ plane, away from the cavity, by 0.65 Å in the (NH)₂ derivative and by 0.54 Å in the (N—CH₃)₂ case. These displacements are reminiscent of those in native heme proteins (17, 28). The average Fe—N distances of 2.112 Å (NH)₂ and 2.117 Å (N—CH₃)₂ are slightly greater than those for comparable porphyrin structures, but consistent with the high-spin configuration. The Fe—Cl distances are 2.307 Å (NH)₂ and 2.326

† Structure solution details for [Fe{(m-xylyl)(CH₃N₂Ethi)₂(CH₃)₂[16]tetraene-N₄}Cl]PF₆: 9230 independent autodiffractometer data points collected at -73(3)°C using monochromatized Mo K_α radiation (ω – 2θ scans); space group P1; a = 8.575(1) Å, b = 13.788(2) Å, c = 14.383(2) Å, α = 84.15(1)°, β = 76.32(1)°, γ = 104.80(1)°; ρ_{obs} = 1.46 g cm⁻³, ρ_{calc} (Z = 2) = 1.47 g cm⁻³; refinement based on F² using all 9230 data points [3042 > 3σ(I)] yielded R(F) = 0.154 (0.066), R_w(F²) = 0.112 (0.085) and goodness of fit = 1.21 (1.60).

Å (N—CH₃)₂ and relate to the displacement of the iron from the N₄ plane.

The (NH)₂ derivative has a tall, narrow cavity (Fig. 2 *Upper*), whereas the (N—CH₃)₂ species has a short, wide cavity (Fig. 2 *Lower*). The bridge is attached to the parent macrocycle through its two trigonal planar nitrogen atoms, and this leads to isomerism as shown diagrammatically in structures I and II. Both represent side views of the complex.

I (lid-on isomer) is the arrangement in the (NH)₂ species, and II (lid-off isomer) is the arrangement found in the (N—CH₃)₂ complexes. The lid-on (NH)₂ cavity is 7.57 Å high in front and 5.46 Å high at the rear, while in the lid-off (N—CH₃)₂ substituted species the corresponding dimensions are 5.02 Å and 3.94 Å. The slopes of the cavity roofs are 53° (dihedral angle relative to the N₄ plane) in the (NH)₂ derivative and 30° in the (N—CH₃)₂ case. The difference in width is comparable: 5.20 Å for (NH)₂ and 7.37 Å for the (N—CH₃)₂ derivative (the distance between the ethylidene carbons).

The tendency of the bridges in this family of ligands to produce bending of metal-donor linkages that are normally linear is shown dramatically by the bis(isothiocyanato)-cobalt(III) complex with the (N—CH₃)₂ (CH₂)₆ ligand, which has an N-bonded NCS ligand in the cavity. Although the Co^{III}-NCS group is almost always linear, the Co—N—C linkage inside the cavity is bent from linearity by 32° in that structure (23). In view of this, it is reasonable to suggest that the lid-on (NH)₂ complex should be better able to accommodate CO than the lid-off (N—CH₃)₂ isomer; this is because smaller changes in the shape of the cavity are required in the lid-on case.

Control of CO Affinity by Bridging Groups. Two sets of equilibrium constants are presented in Table 1. For the chloride-free systems, the order of stabilities of CO adducts is (NH)₂ *p*-xylyl > (N—CH₃)₂ *m*-xylyl ≈ (NH)₂ (CH₂)₅ > (N—CH₃)₂ (CH₂)₄ [named according to Fig. 1 with format (NR²)₂ R¹]. In our earlier studies involving dioxygen binding to the cobalt

complexes of the same ligands we found that the entropy changes associated with adduct formation all had the same values, within experimental uncertainties (unpublished results). The assumption that the same relationship holds for CO adducts provides a convenient method for temperature extrapolation of *K*_{CO} so that a semiquantitative comparison can be made. The temperature dependence of *K*_{CO} for the (N—CH₃)₂ *m*-xylyl complex has been studied with care (seven temperatures, from −30°C to +20°C) and Δ*H* = −9.8 (±0.6) kcal mol^{−1} and Δ*S* = −38 (±2) cal mol^{−1} K^{−1} (standard state of 1 torr) (1 torr = 133 Pa; 1 kcal = 4.184 kJ). On this basis the estimated values of *K*_{CO} at 10°C for the various complexes, in the sequence listed above, are (torr^{−1}): 3.7, 0.16, 0.14, 0.03. Thus the binding constants differ by a factor of well over 100, with the variations paralleling expectation on the basis of the cavity size and shape.

We have completed a detailed study of the multiple equilibria occurring in the system containing [Fe{(*p*-xylyl)(NH*Et*)₂(CH₃)₂[16]-tetraeneN₄)Cl}]⁺ (Fig. 1, R¹ = *p*-xylyl, R² = H) with carbon monoxide in acetonitrile solution at 0°C. From the chloride dependence of the observed CO binding constant, *K*_{obs}, we deduce that the following equilibria occur simultaneously:

The apparent CO binding constant is related to the defined equilibrium constants by Eq. 4.

$$K_{\text{obs}} = K_1 + \frac{K_3(K_2 - K_1)[\text{Cl}^-]}{1 + K_3[\text{Cl}^-]}. \quad [4]$$

From the magnitudes of *K*₃ [2.0 × 10⁵ M^{−1} for the (NH)₂ *p*-xylyl case] it becomes clear that useful comparisons can be made at specific, fixed values of chloride concentration of 1 mM, and a temperature of 0°C, in acetonitrile solution. It is an impressive fact that the CO adduct of the (N—CH₃)₂ (CH₂)₆ bridged species is so stable that we can confidently conclude *K*_{CO} > 100 torr^{−1}. The complex having a bridge shorter by only one member (NH)₂ (CH₂)₅ is so much weaker that the constant is too small to measure under these conditions (*K*_{CO} < 10^{−3} torr^{−1}). We consequently conclude that the change in *K*_{CO} is by at least five orders of magnitude. In our discussion of the crystal structures of the chloro complexes we predicted that the lid-on (NH)₂ *m*-xylyl derivative would form more stable complexes than the lid-off (N—CH₃)₂ *m*-xylyl species. The two values of *K*_{CO} differ by a factor of almost ten and in the direction expected.

Synthesis and Characterization of Solid CO Adducts. The iron(II)-chloro complexes undergo reaction with CO and nitrogen bases to produce carbonyl adducts that are readily crystallized and characterized (Eq. 5).

For several cases the reaction is relatively complete under an atmosphere of CO in the presence of excess nitrogen base. Equal weights (0.250 g) of iron(II) chloro complex and axial base were dissolved in a mixed solvent composed of 5 ml of acetonitrile and 30 ml of alcohol. The solution was exposed to CO in a Schlenk flask. Dropwise addition of a solution of 0.5 g of ammonium hexafluorophosphate dissolved in ethanol yielded a red-orange precipitate. Such complexes were prepared for ligands having a variety of bridges and axial bases. The solids are

Table 1. Equilibrium constants for the formation of the CO adducts in CH₃CN solutions

Bridge	Temp., °C	Axial base	Cl [−] , mM	<i>K</i> , torr ^{−1}
(NH) ₂ <i>p</i> -xylyl	0	CH ₃ CN	0	7.9
(N—CH ₃) ₂ <i>m</i> -xylyl	−10	CH ₃ CN	0	0.84
(N—CH ₃) ₂ <i>m</i> -xylyl	0	CH ₃ CN	0	0.43
(N—CH ₃) ₂ <i>m</i> -xylyl	9.8	CH ₃ CN	0	0.16
(NH) ₂ (CH ₂) ₅	10	CH ₃ CN	0	0.14
(N—CH ₃) ₂ (CH ₂) ₄	−8.6	CH ₃ CN	0	0.10
(N—CH ₃) ₂ (CH ₂) ₆	0	CH ₃ CN, Cl [−]	1	>100*
(NH) ₂ (CH ₂) ₅	0	CH ₃ CN, Cl [−]	1	<10 ^{−3}
(NH) ₂ <i>p</i> -xylyl	0	CH ₃ CN, Cl [−]	1	0.025
(NH) ₂ <i>m</i> -xylyl	0	CH ₃ CN, Cl [−]	1	0.010
(N—CH ₃) ₂ <i>m</i> -xylyl	0	CH ₃ CN, Cl [−]	1	0.0012

* Based on the conservative assumption that a 5% change in level of saturation of the equilibrium could be detected spectrophotometrically.

FIG. 3. Stereoscopic pairs of ORTEP diagrams of a carbon monoxide complex, showing how the bridging group of the lacunar ligand forces bending of the FeCO linkage. (*Upper*) View defining the cavity dimensions. (*Lower*) View emphasizing the bending of the FeCO linkage.

generally stable in air for days but, in solution, reaction with air occurs within minutes, or hours, depending on the particular bridging group and axial ligand. The CO adducts have been characterized by the usual physical measurements as well as elemental analyses, and a complete x-ray structure determination has now been completed for one crucial case.

Carbon-13 NMR spectra show mirror symmetry for cases involving symmetrical axial bases. Unsymmetrical bases such as imidazole double the number of resonances because they destroy the mirror plane (the planar bases are constrained to lie between the two six-membered rings of the macrocycle ligand).

Crystal Structure of a CO Adduct.[‡] The phenomenon of the distortion of the Fe—C—O linkage in natural systems has already been emphasized. There is no precedent for such bending in model systems (29–31), and thus it was highly desirable to structurally characterize a CO adduct of an iron(II) lacunar complex to determine unambiguously the nature of the bending in the Fe—C—O linkage. This has been accomplished by using a particularly appropriate example, $[\text{Fe}((\text{CH}_2)_5(\text{NHethi})_2\text{Me}_2[16]\text{tetraeneN}_4)(\text{CO})(\text{Py})](\text{PF}_6)_2 \cdot \text{CH}_3\text{OH}$ (detailed reports will appear elsewhere). Stereo ORTEP drawings of the molecule are shown in Fig. 3. The structure analysis was carried out

at $-40(1)^\circ\text{C}$ to reduce thermal motion and improve the precision of the results; the equality of chemically equivalent bonds, essentially flat difference map, and the location and refinement of the hydrogen atoms testify to its high quality.[‡] The estimated standard errors in the bond parameters are small, $\pm 0.005 \text{ \AA}$ for C—C and C—N distances, and realistic, and the details of the structure can be interpreted with confidence.

The ORTEP drawings clearly show the coordination sphere of the iron(II) with the macrocycle bound equatorially, pyridine bound axially, and carbon monoxide bound axially within the cavity. The low-spin iron(II) ion is displaced but $0.05 \text{ \AA}$ out of the N_4 plane of the macrocycle, toward the pyridine. The average iron–macrocycle nitrogen bond distance of $1.992(5) \text{ \AA}$ is typical of low-spin iron(II) porphyrin complexes (17, 29–31). The iron–pyridine bond distance of $2.052(3) \text{ \AA}$ is the shortest reported for iron(II)–pyridine–carbon monoxide adducts. The iron–carbon and carbon–oxygen distances are $1.792(4) \text{ \AA}$ and $1.150(5) \text{ \AA}$, respectively.

By far the most significant feature of the structure is the bent Fe—C—O linkage, which has an angle of $170.6(5)^\circ$. All other structures reported for model systems exhibit essentially linearly bound CO molecules (29–31). Furthermore, the carbon monoxide bends both at the iron center and at the carbon atom (Fig. 4). This bending is caused by steric interactions between the bound CO and the pentamethylene bridging group. The cavity shape is quite restrictive, with a width of $6.28 \text{ \AA}$ between the bridge nitrogen atom centers ($2.88 \text{ \AA}$ when corrected for van der Waals radii). The bridge is also quite low, with measured van der Waals heights of between $3.83 \text{ \AA}$ and $5.31 \text{ \AA}$ to the N_4 plane. Most importantly, the methylene hydrogen atoms of the

[‡] Structure solution details for $[\text{Fe}((\text{CH}_2)_5(\text{NHethi})_2\text{Me}_2[16]\text{tetraeneN}_4)(\text{CO})(\text{Py})](\text{PF}_6)_2 \cdot \text{CH}_3\text{OH}$ (Py, pyridine): 4964 independent autodiffractometer data points collected at $-40(2)^\circ\text{C}$ using monochromatized Mo K_α radiation ($\omega - 2\theta$ scans); space group $P2_1/m$, $Z = 2$; $a = 10.115(3) \text{ \AA}$, $b = 16.468(3) \text{ \AA}$, $c = 11.926(3) \text{ \AA}$, $\beta = 112.78(1)^\circ$; refinement based on F^2 using all 4964 data points [$2332 > 3\sigma(I)$] yielded $R(F) = 0.105(0.054)$, $R_w(F^2) = 0.120(0.104)$ and goodness of fit = 1.17 (1.48). The solvent MeOH and PF_6^- anion were completely ordered.

FIG. 4. Distortion of the Fe—C≡O group from linearity.

bridge point into the cavity at the bound CO. Thus it appears that there is little room within the cavity to accommodate a ligand. As a result, the CO oxygen atom is but 2.62 Å and 2.92 Å from hydrogen atoms on the methylene bridge carbons. The first of these distances is actually less than the van der Waals contact distance of 2.7 Å (32), suggesting an intramolecular non-bonded interaction, which may contribute to the observed multiple bending of the FeCO linkage. The bridge itself is in turn distorted, with the central carbon being pushed as far as possible from the bound CO. Curiously, the carbonyl oxygen atom is 2.77 Å from the *para*-hydrogen atom of the pyridine belonging to the next molecule in the lattice. However, the packing diagram shows that this interaction is in a direction that would, in fact, be expected to oppose the bending of the CO and force it toward a more linear orientation. Thus we expect that the degree of bending of the Fe—C—O linkage is probably greater in solution, where this specific intermolecular packing interaction is unimportant.

Conclusions. The following conclusions are justified by the studies summarized here.

(i) Steric effects have been used to distort the normally linear FeCO linkage in a series of synthetic complexes.

(ii) These steric effects both bend the FeCO linkage and displace the Fe—C bond from the perpendicular to the N_4 plane.

(iii) These steric effects can alter the CO affinities of closely related iron(II) complexes by as much as five orders of magnitude. Earlier studies have documented the fact that electronic effects do *not* accompany the changes in the bridge structure (22, 24).

(iv) CO affinity is more sensitive to the steric effects than is O_2 affinity.

(v) Phenomena that are limited to the solid state may increase

or decrease the extent of distortion of the FeCO linkage so that the results of x-ray structure determination must be applied to the solution state with caution.

(vi) This model system may be useful to those studying steric effects on small molecule binding by heme proteins.

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