The Effect of Porphyrin Ruffling on the Intrinsic Binding of Carbon Monoxide in Iron(II) Hybrid Basket-Handle Porphyrins by Multinuclear NMR and FT-IR Spectroscopy

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Carbonyl complexes of single-side hindered hemes, the 'hybrid basket-handle' porphyrins, have been investigated by ^{1}H, ^{13}C, ^{15}N, ^{57}Fe NMR and FT-IR spectroscopy in order to obtain information about the intrinsic properties of the Fe–C bond. The effect upon CO binding of severe distortion of the porphyrin skeleton that occurs in the more tightly packed structures, is discussed. Reduced ability of the iron atom to back-donate d-electrons to the π^*-orbital of the CO ligand as a result of the distortion is suggested to be a mechanism for CO discrimination in these hemes. The ^{57}Fe NMR resonance for the more encumbered structures is shifted by several hundred ppm to lower frequency while the ^{13}C and ^{15}N chemical shifts are very little affected. These data indicate that severe electronic changes occur at the ligand binding-site in the more encumbered structures but they are mainly located at the iron atom. Differences, between the looser and the tighter models, in binding of nitrogen ligands opposite to the carbonyl are reported and provide evidence that the CO affinity is reduced in the tighter complexes. This provides a novel way of probing CO binding and is based on the fact that formation of axial bonds in carbonyl iron(II) porphyrins is an allosteric process.

Intense interest in porphyrin chemistry arose on the suggestion that heme proteins regulate carbon monoxide binding by interfering with the linearity of the Fe–CO bond. 'Capped porphyrins',[1] 'pocket porphyrins',[2] 'basket-handle porphyrins'[3] and many more were designed to achieve variable degrees of constraints on the Fe–CO bond angle. Many of the sterically encumbered heme models showed considerable reductions in ligand binding but without significant off-axis bonding of carbon monoxide. The deviations from the heme normal that have been demonstrated in the few published crystal structures amount to less than 10°,[4] which is too little to account for the observed reductions in CO affinity.[5] No crystal structures have been published of severely hindered carbonyl iron porphyrins. Obviously, other factors determine the observed effects.

Recent structural data on heme proteins[6] reveal that ruffling of hemes is common in nature. An important question, therefore, is whether distortion of porphyrins can affect ligand binding and whether this can in fact be a natural regulatory mechanism.

In the largest of the 'hybrid basket-handle' porphyrins,[3] with 12 carbon atoms in the aliphatic chain, FeBH12 (Fig. 1), CO binds along the heme normal. The increase in steric hindrance which results from making the handle shorter appears not to force the carbonyl ligand into a bent or tilted position, but to cause severe ruffling.[7] However, the ratio of equilibrium constants for binding of carbon monoxide and oxygen ($M = K_{CO}/K_{O_2}$) is strongly reduced in the tighter structures of the series. By making the handle shorter, the desired reduction in M is obtained, but probably not by tilting or bending the CO. The most obvious difference between the looser and tighter structures is the difference in accessibility of the cavity for an approaching ligand, but the degree of ruffling also changes.

One consequence of tightening the binding cavity by diminishing the superstructure is that severe ruffling occurs in the porphyrin plane, the tighter the superstructure, the more ruffling. The effects or ruffling on CO binding has not been investigated in spite of the logical connection between porphyrin structure and iron carbonyl backbonding.

Another consequence of introducing tight steric hindrance on one side of the porphyrin is that CO binding becomes a two-step process.[8] First, the ligand diffuses into the cavity and then the actual bond formation occurs. It is very difficult to obtain accurate data on each step separately. If variations in on- and off-rates with tightness reflect to a large degree the effects on the diffusion step, and an aliphatic chain is a poor model for a protein, it may be that kinetic investigations of these models are of limited use in trying to understand carbon monoxide binding in heme proteins. The rate constants for association and dissociation in sterically hindered heme models of both dioxygen and carbon monoxide show that the discrimination of CO is mainly an effect of differences in dissociation rate.[3] While the rate of O_2 dissociation decreases as the handle gets shorter (which is expected as the diffusion is more obstructed), the off-rate of CO remains fairly constant. Since there is little difference in size for the two molecules their diffusion rates should be similar and one has to conclude that this difference must depend on the intrinsic properties of the iron carbonyl chemical bond.

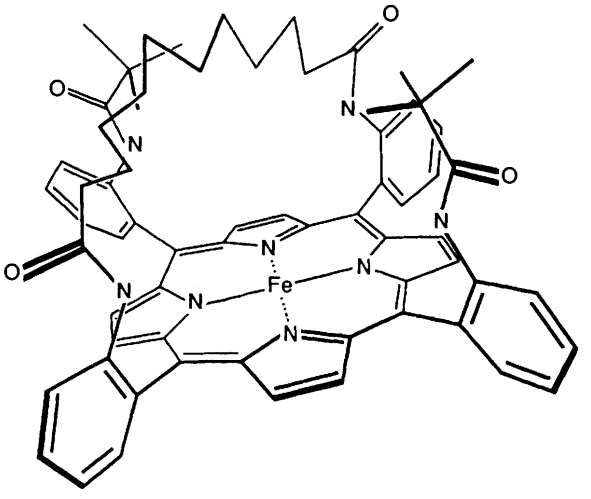

Fig. 1 FeBH12

Ligand binding in the axial positions in carbonyl iron(II)

porphyrins is allosteric.[9] An increase in electron donation from the nitrogen base increases the equilibrium constant for the equilibrium between the iron porphyrin moiety and the base, and also that between the carbon monoxide and the iron porphyrin. Imidazole, the stronger base, binds an order of magnitude stronger than 4-cyanopyridine to carbonyl iron(II) deuteroheme, and CO binds an order of magnitude stronger to the imidazole iron(II) deuteroheme complex than to the corresponding 4-cyanopyridine complex. This provides an opportunity to probe the intrinsic bonding of carbon monoxide by investigating the binding of the nitrogen base. This is easily done by measuring the competitive equilibria with different bases by NMR spectroscopy since the ligand exchange is slow on the NMR timescale.[10]

In this paper we present new vibrational and multinuclear NMR data on the effects of ruffling on the intrinsic CO affinity of the 'hybrid basket-handle' porphyrins where the degree of steric hindrance is systematically increased by shortening the aliphatic chain spanning one side of the porphyrin.

Experimental

All solvents were reagent grade. Dichloromethane was distilled from CaH_2, toluene and THF from sodium and benzophenone prior to use.

Synthesis.—The 'picket fence' porphyrin was synthesized according to the method of Collman *et al.*[11] and the modification by Lindsey.[12] The 'hybrid basket-handle' porphyrins were prepared according to the literature[3] with some minor changes.

The condensations of the α,α,α,α-tetrakis(2-aminophenyl)-porphyrin and two of the diacid chlorides, dodecane-1,10-dicarbonyl dichloride and azelaoyl dichloride, were carried out in dichloromethane medium. In all cases a gas-tight syringe and a syringe pump were used for the addition of the diacid chlorides. This method allowed a slow addition under very dilute conditions.

All substances were purified by flash chromatography on silica gel (particle size 0.040–0.063 mm). A mixture of diethyl ether and acetone (75:25, v/v) was used in the chromatographic separation of α-5,15-[2,2′-(decanediamido)diphenyl]-α,α-10,20-bis(2-aminophenyl)porphyrin. As the material is not very soluble in this medium, it was dissolved in dichloromethane, a small portion of silica gel (12 g to 660 mg substance) was added and the mixture was slowly brought to dryness first under vacuum and finally at normal pressure. The dry silica gel was then loaded on top of the column packed with the eluent.

These modifications gave somewhat higher yields. Total yields for the two reaction steps from α,α,α,α-tetrakis-(2-aminophenyl)porphyrin were after recrystallization from dichloromethane–hexane: α-5,15-[2,2′-(dodecanediamido)diphenyl]-α,α-10,20-bis-(*o*-pivalamidophenyl)porphyrin, (BH12), 21%; α-5,15-[2-2′-(decanediamido)diphenyl]-α,α-10,20-bis(*o*-pivalamidophenyl)porphyrin, (BH10), 22%; α-5,15-[2,2′-(nonanediamido)diphenyl]-α,α-10,20-bis(*o*-pivalamidophenyl)porphyrin, (BH9), 25%. The ^{15}N enriched 'picket fence' and BH9 porphyrins were made by the same procedure from isotopically enriched pyrrole.

Identification and purity control of all substances were obtained by TLC and ^1H NMR spectroscopy. The spectra were in good agreement with those reported in the literature.

Fe_2O_3 (both ^{57}Fe enriched and natural abundance material) was then used for the iron insertion according to the procedure described elsewhere.[13]

Preparation of the Carbonyl Complexes.—The chloroiron(III) porphyrin (10–40 mg) was dissolved in [$^2H_8$]toluene (1–4 ml) and the nitrogen ligand (5–20 equiv.) was added. Two or three drops of H_2O were added and the two-phase system was purged with carbon monoxide for 3–4 min. An excess of sodium dithionite (20–25 mg) was added and the flask was sealed and placed in a sonicator. The reaction was complete after 15–60 min. The two phases were separated and the organic layer was dried over Na_2SO_4. Measurement of the spin-lattice relaxation times (T_1) by ^1H NMR spectroscopy was used to monitor the reduction. Any trace of paramagnetic material lowers the T_1 values.

IR Spectroscopy.—Samples with complex concentration of 4–10 mmol dm^{-3} were measured in a CaF cell with a 0.3 mm Teflon spacer on a Perkin-Elmer Model 1800 FT-IR instrument.

NMR Spectroscopy.—The NMR spectra were recorded on a Varian VXR 400 NMR instrument operating at 9.4 T or a Varian Unity 500 at 11.74 T.

^1H and ^{13}C NMR spectra were obtained on the 9.4 T instrument. For the ^{13}C NMR spectra, sample concentrations of 10–15 mmol dm^{-3} in 5 mm NMR tubes gave a sufficiently good carbon monoxide signal after 5000 transients with spectral width of 30 000 Hz and an aquisition time of 1.2 s.

^{15}N *NMR spectroscopy.* The ^{15}N-enriched material was directly observed at 9.4 T. Sufficiently good spectra were obtained with a sample concentration of 10–15 mmol dm^{-3} in 5 mm tubes and 1000 transients. The spectral width was 5000 Hz and the aquisition time 0.5 s. The samples with ^{15}N at natural abundance was studied with a two-dimensional NMR experiment, heteronuclear multiple quantum coherence (HMQC) at 11.74 T. The two-bond spin coupling $^2J_{H-N} = 11$ Hz can be used in an inverse detection correlation experiment where the ^{15}N chemical shift is obtained through observation of the coupling proton. In this way the chemical shift at 25 °C of 10 mmol dm^{-3} solutions of complex, could be obtained in less than six hours of experimental time. Pulse widths of 8.4 μs for ^1H, 18.2 μs for ^{15}N and sweep widths of 7000 Hz in F2 (^1H) and 2000 Hz in F1 (^{15}N) were used together with a relaxation delay of 3 s. The spectra were recorded in the phase-sensitive mode with 32 transients in each of 2 × 128 free induction decays. For comparison we measured the shift of one of the pyridine complexes with the HMQC method as well, using a slightly enriched pyridine (5% ^{15}N). The shifts were in excellent agreement, as expected. Nitromethane (neat) was used as an external reference in all cases.

^{57}Fe NMR spectra were recorded at 13.05 MHz (9.4 T) with sample concentrations of 10–15 mmol dm^{-3} in 15 mm non-spinning tubes with a total volume of 3.1 cm^3. The instrument was equipped with a solenoid coil probe with temperature control. 90 -pulses of 100 μs, 5000 Hz spectral width and an aquisition time of 0.1 s were used. Typically, 300 000 transients were collected. The chemical shifts are referred to $Fe(CO)_5$ and butylferrocene (80% in [$^2H_6$]-acetone) was used as a secondary external reference.

Measurement of Relative Equilibrium Constants.—A complex of the iron porphyrin, carbon monoxide and one nitrogen base was prepared as above and its proton NMR spectrum was recorded. Since the chemical exchange of nitrogen bases is slow on the NMR time scale, an addition of a second nitrogen ligand in a suitable amount results in sharp signals for the two different complexes with integrals of comparable size. The integrals of the four species (the two different complexes and the two free nitrogen ligands) were measured to give the relative equilibrium constants for the two competing equilibria. The K_{rel} values are reproducible to within 10% and checked crosswise.

Table 1 Vibrational frequencies of the C–O bond stretch for carbon monoxide bound to iron(II) porphyrins in [$^2H_8$]toluene

	ν_{CO}/cm^{-1}	
Nitrogen ligand	FeBH9	FeBH12
4-Acetylpyridine[a]	1955	1966
Pyridine[a]	1953	1963
1-Butylimidazole[a]	1948	1959
1-Methylimidazole[b]	1948	1958
DMAP[a]	1947	1950

[a] This work. [b] Ref. 16. DMAP = *N*,*N*-dimethyl-4-aminopyridine.

Table 2 ^{15}N NMR chemical shift of the pyrrolic nitrogens in carbonyl complexes of heme models

Complex	δ/ppm[a,b]
FeBH9(CO)(py)	−226.8
FeBH9(CO)(buim)	−227.2
FePF(CO)(py)	−229.7
FePF(CO)(buim)	−229.7

[a] Relative to neat nitromethane. A positive chemical shift is a shift to higher frequency. [b] In [$^2H_8$]toluene at 25 °C. PF = 'picket fence' porphyrin, py = pyridine and buim = 1-butylimidazole.

Table 3 ^{15}N NMR chemical shift of the co-ordinating nitrogen in the ligand

Complex	δ/ppm[a,b]
FeBH9(CO)(py)	−114.3
FeBH9(CO)(bium)	−166.8
FeBH12(CO)(py)	−115.2
FeBH12(CO)(buim)	−167.6

[a] Relative to neat nitromethane. A positive chemical shift is a shift to higher frequency. [b] In [$^2H_8$]toluene at 25 °C. py = pyridine and buim = 1-butylimidazole.

Table 4 ^{13}C NMR chemical shifts of the carbon monoxide in carbonyl complexes of 'hybrid basket-handle' iron complexes

Complex	δ/ppm[a]
FeBH9(CO)(py)	207.28
FeBH9(CO)(buim)	206.26
FeBH9(CO)(*s*-buam)	205.19
FeBH10(CO)(py)	206.53
FeBH12(CO)(py)	206.05
FeBH12(CO)(buim)	205.31

[a] In [$^2H_8$]toluene. py = pyridine. buim = 1-butylimidazole and *s*-buam = *sec*-butylamine.

Results

Vibrational Frequencies.—The C–O stretching frequencies for some carbonyl complexes of FeBH12 and FeBH9 with different nitrogen ligands are listed in Table 1 together with some values from the literature. The signal is easily determined as it occurs around 1950 cm^{-1} where no other signals are found. For both porphyrins the stretching frequency of the C–O bond decreases when a more electron donating ligand is used. The series of FeBH12 complexes shows generally higher stretching frequencies and a larger range from the largest to the smallest value than does the FeBH9 series. The larger range of the FeBH12 series is mainly due to the low stretching frequency of the DMAP complex.

NMR Chemical Shifts.—The multinuclear chemical shifts of the pyrrolic nitrogens, the co-ordinating nitrogen in the ligand, the carbon of the bound carbon monoxide and the iron atom itself were recorded.

The ^{15}N chemical shifts of the pyrrolic nitrogens were measured using ^{15}N-labelled material in the pyrrolic positions.

Two complexes of each porphyrin were investigated. Pyridine and 1-butylimidazole were chosen as the nitrogen bases for solubility reasons. The chemical shifts are presented in Table 2. For the 'picket fence' porphyrin complexes, the pyrrolic nitrogen had the same shift, regardless of which of the two bases was used. In the 'hybrid basket-handle' porphyrin a small shift to lower frequency was observed with the more electron donating ligand 1-butylimidazole.

Only one signal for the pyrrolic nitrogens was found in all the investigated complexes. As the samples were enriched in ^{57}Fe, the signal was a doublet with $^1J_{Fe-N}$ = 8.0–8.3 Hz. The coupling constants can be measured very accurately and seem to vary irregularly within this narrow range.

The shift of the co-ordinating nitrogen of the ligand was measured for pyridine and 1-butylimidazole in complexes of FeBH12 and FeBH9 (Table 3). The pyridine signals were obtained by direct observation of complexes with ^{15}N-enriched pyridine.

The shifts of the butylimidazole complexes were measured at natural abundance of ^{15}N by heteronuclear multiple quantum coherence techniques (HMQC).

The ^{13}C NMR resonance for heme-bound carbon monoxide usually appears in the high frequency region around 205 ppm. We measured this shift for some complexes in the 'hybrid basket-handle' series. The results are listed in Table 4. Comparison of complexes with the same nitrogen ligand *e.g.* pyridine, shows that the chemical shift increases slightly when the handle becomes shorter. Going from 12 to 10 carbon atoms in the chain, shifts the signal 0.48 ppm to higher frequency. Taking away one more carbon atom from the handle shifts the signal another 0.75 ppm. Varying the nitrogen ligand causes a shift to lower frequency as the ligand becomes more electron releasing. The coupling to the ^{57}Fe nucleus remains constant in the series ($^1J_{Fe\ C}$ = 27.2 Hz).

We recently reported the ^{57}Fe chemical shifts for some carbonyl complexes of the 'hybrid basket-handle' porphyrins.[14] The nitrogen ligands were imidazoles or pyridines. Here we present the shift for a primary amine complex of the tightest structure. The amine is *sec*-butylamine and the shift value falls into the same region as the shifts of the pyridine and butylimidazole complexes. The ^{57}Fe chemical shifts are summarized in Table 5.

Table 5 ^{57}Fe NMR chemical shifts of some carbonyl iron(II) porphyrin complexes in [$^2H_8$]toluene at 25 °C.

Complex	δ/ppm[a]
FePF(CO)(py)	8124[b]
FePF(CO)(buim)	8110[b]
FeBH12(CO)(py)	8055[b]
FeBH12(CO)(buim)	8036[b]
FeBH10(CO)(py)	7717[b]
FeBH10(CO)(buim)	7728[b]
FeBH9(CO)(py)	7488[b]
FeBH9(CO)(buim)	7500[b]
FeBH9(CO)(*s*-buam)	7502[c]

[a] Relative $Fe(CO)_5$. [b] Ref. 14. [c] This work. PF = 'picket fence' porphyrin, py = pyridine, buim = 1-butylimidazole and *s*-buam = *sec*-butylamine.

Equilibrium Measurements.—Competition between two nitrogen bases for the sixth binding position of a carbonylated iron(II) porphyrin can be described as in Scheme 1. Since the

Table 6 Relative equilibrium constants for binding of nitrogen bases to carbonyl complexes of FeBH9 and FeBH12

Nitrogen ligand	K_{rel}[a] FeBH9	FeBH12
Pyridine	1	1
1-Methylimidazole	25	78
1-Butylimidazole	36	195
DMAP	58	150

[a] Definition in Scheme 1. DMAP = *N*,*N*-dimethyl-4-aminopyridine.

$$\text{FeP(CO)} + \text{base 1} \overset{K_1}{\rightleftharpoons} \text{FeP(CO)(base 1)}$$

$$\text{FeP(CO)} + \text{base 2} \overset{K_2}{\rightleftharpoons} \text{FeP(CO)(base 2)}$$

$$K_{rel} = \frac{K_2}{K_1} = \frac{[\text{FeP(CO)(base 2)}]}{[\text{FeP(CO)(base 1)}]} \times \frac{[\text{base 1}]}{[\text{base 2}]}$$

Scheme 1

exchange of bases is slow on the NMR time scale the resonances of both complexes and the free bases appear in the ^{1}H NMR spectrum and the integrals can be measured and compared. Using this method, relative equilibrium constants for some bases were obtained. The results are presented in Table 6.

The two iron porphyrins differ in overall range and order of the relative equilibrium constants. Pyridine has the smallest binding constant among the chosen bases for both porphyrins, while 1-butylimidazole has the largest for FeBH12 and *N*,*N*-dimethyl-4-aminopyridine for FeBH9. The largest K_{rel} value for FeBH9 is 60 while FeBH12 shows a maximum K_{rel} value of nearly 200.

Discussion

Vibrational Frequencies.—The stretching frequencies of the C–O and Fe–C bonds for a large number of natural as well as synthetic heme–CO complexes can be found in the literature.[15] The former stretch is easily observed by IR spectroscopy and the Fe–C stretch by resonance Raman. An approximately linear inverse correlation between the two stretching frequencies is observed.[15c–e] Vibrational data are important because they say something about the intrinsic properties of the bonds.

We assume that the inverse relation between the two stretching frequencies is also valid for the 'hybrid basket-handle' complexes. The observed decrease in the C–O bond stretching frequency, which is also reported for unprotected iron(II) tetraphenylporphyrin,[15b] when a more electron releasing ligand in the sixth binding position is used, is therefore believed to correspond to an increase of the Fe–C bond frequency. This is the expected behaviour if the stretching frequencies are interpreted in terms of bond strength. Through the back-bonding mechanism which is well established for carbonyl complexes of flat, unprotected hemes,[9] the iron atom donates d-electrons back to the empty π^*-orbital of the CO unit. The more electron density the iron atom receives from the nitrogen ligand, the more it can back-donate to the carbonyl and hence strengthen the Fe–C bond. The two hemes FeBH9 and FeBH12 show the same behaviour and seem to be equally sensitive to the exchange of nitrogen ligands except for co-ordination of the most electron releasing base, *N*,*N*-dimethyl-4-aminopyridine. The co-ordination of DMAP to the larger structure of FeBH12 results in a substantially larger change in the C–O stretching frequency than for the corresponding complex of FeBH9. In comparison with the pyridine complex one possibility is that the back-donation ability becomes slightly reduced in the tighter structure of FeBH9 and is therefore not equally sensitive to the stronger electron donating ability of the nitrogen ligand. The perturbance of the porphyrin plane in these hemes, known as ruffling, is expected to increase when the hydrocarbon chain is shortened.[4b,8] This ruffling may cause a change in the orbital symmetry of the iron atom, which could explain the reduction of electron back-donation ability.

The 'hybrid basket-handle' porphyrin series shows an increase of the Fe–C stretching frequency from 488 to 506 cm^{-1} for the CO/1-methylimidazole adducts of the molecules upon shortening the chain from 12 carbon atoms to 9 and a decrease of the C–O stretch from 1958 to 1948 cm^{-1} for the same complexes.[16] The fact that complexes of FeBH12 generally show a higher C–O stretching frequency than the corresponding complexes of FeBH9, suggests that the tighter structures bind CO more strongly than the looser ones. This does not agree with the thermodynamic and kinetic data of CO binding. It has been suggested that changes in local polarity may be the reason for this behaviour.[16] The series of 'pocket' porphyrins exhibit no such trend in the C–O stretching frequencies when the structure becomes more tightly packed.[2] Indeed those values vary quite irregularly, which shows how difficult it is to interpret differences in C–O stretching frequencies between different hemes. On the other hand, changes in the C–O stretching frequency upon substitution of the nitrogen base opposite to the carbonyl seem to lend themselves to an interpretation.

^{15}N *NMR Chemical Shift of the Pyrrolic Nitrogens.*—The data in Table 2 show that the chemical shift of the pyrrolic nitrogens in the 'picket fence' porphyrin with the flat, unperturbed skeleton does not change at all upon changing the electron donating ability of the nitrogen ligand. In the 'hybrid basket-handle' porphyrin with nine carbon atoms in the chain, a small shift to higher field is observed. Although this shift is indeed very small, it indicates that in the 'hybrid basket-handle' case, the electron structure around these nitrogens changes somewhat. In the flat porphyrin case, the increase in donated electrons apparently does not affect the electronic structure of the pyrrolic nitrogens.

The difference in chemical shift between the two hemes is only *ca.* 3 ppm. This difference is surprisingly small as the geometry of the porphyrin plane differs between the two hemes. The distortion of the porphyrin skeleton that occurs in the encumbered molecules, does not apparently affect the electronic structure of the pyrrolic nitrogens very much.

The fact that just one signal was found for the pyrrolic nitrogens, shows that at room temperature all four nitrogens are magnetically equivalent in both porphyrins. The distortion of the porphyrin skeleton affects the four nitrogens equally.

^{15}*N NMR Chemical Shift of the Coordinating Nitrogen in the Nitrogen Ligand.*—As can be seen in Table 3, the shift of the coordinating nitrogen differs substantially between the two different ligands. However, for the same base, the shift difference between the two complexes is less than 1 ppm. The small shift observed goes in the same direction for both bases. A factor that may influence this shift is the distance between the iron and the nitrogen ligand. The ligand could possibly come closer in the tighter structure of FeBH9 as the phenyl rings are pulled away from the side of the porphyrin, reducing the steric hindrance.

The very subtle differences among the ^{15}N NMR shifts for nitrogens of the same type indicate that the paramagnetic term in Ramsey's equation is not dominant for these complexes. The shift scale for ^{15}N is indeed very large but the surrounding nitrogens appear to be insensitive to this kind of structural and electronic change.

^{13}C NMR Chemical Shift of the Carbon Monoxide Ligand.—The only carbon atom directly bound to the iron is that of the carbon monoxide ligand. For both FeBH9 and FeBH12 the shift of that carbon goes to lower frequency when a more electron releasing nitrogen ligand is coordinated. This is also reported for carbonyl complexes of iron(II) tetraphenylporphyrin.[15b] In the light of the back-bonding theory it is reasonable to interpret this upfield shift as being due to an increased electron density around the carbon atom. The more electron density the iron atom gains from the nitrogen ligand, the more it can donate back to the empty π*-orbital of the CO ligand.

A shift to lower frequency is also achieved by increasing the length of the handle. This is clearly seen in a comparison of the shifts between the different porphyrins in the 'hybrid basket-handle' series complexed with the same ligand *e.g.* pyridine. Apparently the carbon atom of the carbon monoxide has less electron density when the handle is shortened. Again an explanation of this could be a decreased back-donation ability in the tighter structures due to a change in the orbital symmetry caused by the distortion of the porphyrin plane.

The effect on the ^{13}C NMR chemical shift reported for the 'pocket' porphyrin series of making the pocket smaller is opposite to those reported here.[17] This may be due to a different kind of porphyrin plane distortion and ^{13}C NMR spectroscopy may be a tool in the investigation of skeleton perturbance.

The coupling constant to the ^{57}Fe nucleus did not give any further information as it does not vary between the different complexes. Also the reported coupling constant for the CO complex of the 'pocket' porphyrin, $^{1}J_{Fe-C} = 27.4$,[17] is very similar to those of the 'hybrid basket-handle' porphyrin series. The crystal structure of a 'pocket' porphyrin complex[4a] has shown that the carbonyl ligand is somewhat displaced from the heme normal but this apparently does not affect the coupling constant.

^{57}Fe NMR Chemical Shift.—We recently reported the ^{57}Fe NMR chemical shifts for some carbonyl complexes of the 'hybrid basket-handle' porphyrins.[14] The shift differences between the loose structures of FeBH12 and the more crowded complexes of FeBH9 are very large, in contrast to the ^{15}N and ^{13}C chemical shifts. The CO–pyridine complex of FeBH9 is shifted 567 ppm to lower frequency relative to the corresponding complex of FeBH12. The shift difference between the latter complex and that of the unconstrained 'picket fence' porphyrin is in turn 69 ppm.

The ^{57}Fe NMR shift scale is very large and the shift is sensitive to such parameters as solvent and temperature. As discussed previously,[14] all these effects are small compared to those occurring in the complexes of the tightest 'hybrid basket-handle' porphyrins. Obviously, the distortion of the porphyrin plane causes serious electronic changes in these carbonyl complexes. According to the minor alternation in the chemical shift of the surrounding atoms, these electronic changes are principally located on the iron atom. Such effects on the orbital symmetry could most likely give rise to a decrease in the back-bonding ability.

In order to check that these extreme shifts of the FeBH9 complexes were not dependent on the π-character of the nitrogen base, we measured the ^{57}Fe chemical shift of the CO–*sec*-butylamine complex of this porphyrin. The value fitted the pattern well, and also confirmed the previously reported shift behaviour of FeBH9 upon binding of a more electron donating base *trans* to the carbonyl ligand. In contrast to other iron porphyrins such as protoporphyrin IX, 'picket fence' and the largest 'hybrid basket-handle' porphyrin, the signal for FeBH9 and FeBH10 carbonyl complexes, is shifted to higher frequency when a more electron donating base is used. The origin of this behaviour has been discussed elsewhere.[14]

Relative Nitrogen Ligand Binding Constants.—The difficulties in probing the intrinsic properties of the Fe–C bond in carbonylated hemes are obvious. The thermodynamic and kinetic data of CO binding reflect association or dissociation processes that are the sum of at least two steps.[8] An indirect method for investigating the Fe–C bond is to study the binding of the nitrogen base in the unhindered position opposite the carbonyl ligand, as this is a single step process that is intimately related to the CO binding through the back-bonding mechanism. From Table 6, it can be seen that back-bonding also exists in these encumbered heme models.

The results in Table 6 show that the difference in the range of K_{rel} values between the two 'hybrid basket-handle' porphyrins FeBH9 and FeBH12 is notable. The increase in electron releasing capacity in the series of nitrogen bases, makes the binding to the iron porphyrin more favourable. This is much more pronounced for the larger of the two hemes, and decreased back-bonding ability in carbonyl complexes of FeBH9 is the most likely explanation. When the iron atom donates less electron density to the carbon monoxide, it does not attract electrons from the nitrogen ligand to the same extent.

Conclusions

These spectroscopic data show that significant electronic changes occur around the iron atom in the more tightly packed complexes of the 'hybrid basket-handle' porphyrins. This is most certainly caused by distortion of the planarity of the porphyrin skeleton. The results also indicate that the electron back-donation ability of the iron atom to the carbonyl ligand is reduced in these complexes. Such decreased back-bonding capacity may be the reason for the large rate constants for dissociation of carbon monoxide in complexes of the tighter structures in the 'hybrid basket-handle' series. It is known from X-ray crystal structures that some natural hemes are ruffled in a similar fashion[6] and therefore such a mechanism of CO discrimination may be used by the natural hemes to regulate binding of gas ligands.

More accurate structural data of heme models should be of great value, but the structures of the most severely distorted complexes have not appeared in the literature. It is also not clear to what extent the molecular structure changes between solution and solid state, so structural information from NMR techniques might be useful. In addition ^{57}Fe NMR can be a helpful tool in further studies of the intrinsic properties of the binding of gas ligands to both heme models and proteins.

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Paper 1/5054K
Received 3*rd October* 1991
Accepted 30*th October* 1991