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Carbon-13 Nuclear Shieldings as a Novel Method in Estimating Porphyrin Ruffling in Hexacoordinated Superstructured Heme Model Compounds in Solution

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The reversible binding of carbon monoxide and dioxygen has played a central role in studies of heme protein structure and function.[1−4] Initial attention focused on the bent vs linear Fe−C−O unit and on the possibility that the CO binding is inhibited by steric interactions that impede a linear geometry.[5,6] More recently emphasis has been given to polar interactions in the binding pocket,[7−9] ruffling distortion of the porphyrin ring, and expansion of the distal cavity.[10−13] A porphyrin is ruffled when opposite pyrrole rings are counter-rotated so that the meso carbon atoms (C_{meso}) of each pyrrole ring are alternately displaced above and below the mean porphyrin plane.[14] Structural data on heme proteins reveal that ruffling of hemes is common in nature; myoglobin−CO exhibits a tilted CO and an almost flat porphyrin,[15] while in human carbonyl hemoglobin, the tilt is very small and the porphyrin is clearly ruffled.[16] The influence of various porphyrin conformations on the cofactor properties has been widely studied with a variety of physical, theoretical, and spectroscopic techniques,[17] but to date, no reliable method exists to unambiguously determine the type and degree of macrocycle distortion in solution. It is, therefore, of great importance to develop new spectroscopic probes to aid in evaluating the importance of ruffled porphyrin structures in both proteins and model compounds.

In this communication we report, for the first time, that porphyrin ruffling is the dominant factor in determining ^{13}C shieldings of the meso carbons in hexacoordinated superstructured heme model compounds in solution. We have studied by ^{13}C NMR a series of carbonylated heme model complexes exhibiting varying degree of porphyrin ruffling (Figure 1), for which single-crystal X-ray structure determinations have been reported. The ^{13}C NMR resonances of the meso carbons of tetraphenylporphyrin (TPP) derivatives appear in the region around 114 ppm which is relatively free from overlap with other signals.[18] This efficient shielding of the meso carbons can be explained in terms of cyclic conjugation in the 16-membered porphyrin ring.[19]

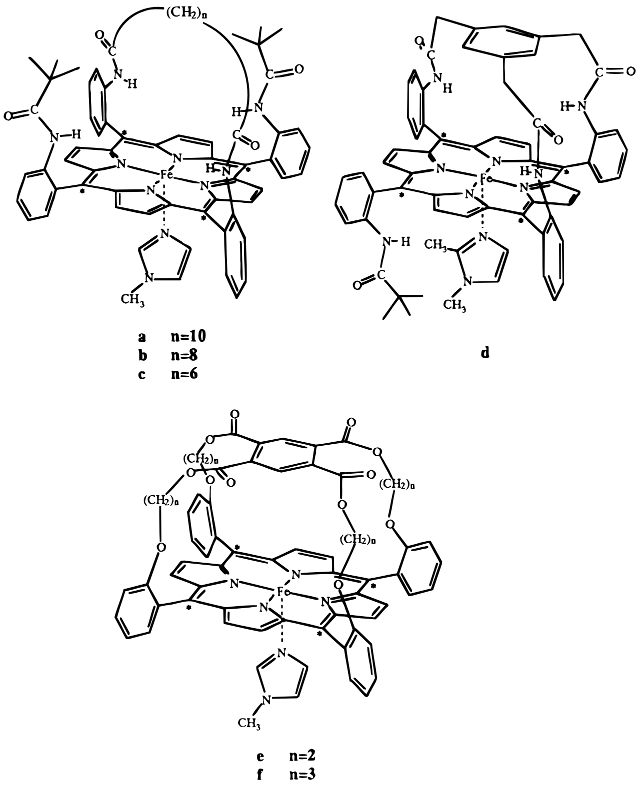

Figure 1. Schematic structures of the heme model compounds studied in this work. The asterisks denote the meso carbons (C_{meso}).

In the series of "hybrid" porphyrins[20] (**a**−**c**) the central distal steric hindrance is achieved by an aliphatic chain constrained in a plane perpendicular to the macrocycle by two lateral pivalamido

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(18) ^{13}C NMR spectra of the ^{13}CO complexes (99.7% enriched in ^{13}C) of the heme model compounds of Figure 1 were obtained at 100.62 MHz with a Bruker AMX-400 instrument equipped with a high-resolution probe (5-mm sample tubes). The chemical shifts were determined relative to the resonance position of the solvent (CD_2Cl_2 ~53.8 ppm). Saturated solution (≤10 mM) in CD_2Cl_2, at 298 K, number of scans 2000−4000 depending on concentration.

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(20) Abbreviations: 1-MeIm, 1-methylimidazole; 1,2-diMeIm, 1,2-dimethylimidazole; **a**, α-5,15-[2,2′-(dodecanediamido)diphenyl]-α,α-10,20-bis-[*o*-(pivaloylamido)phenyl]porphyrin(1-MeIm); **b**, α-5,15-[2,2′-(decanediamido)diphenyl]-α,α-10,20-bis[*o*-(pivaloylamido)phenyl]porphyrin(1-MeIm); **c**, α-5,15-[2,2′-(octanediamido)diphenyl]-α,α-10,20-bis[*o*-(pivaloylamido)phenyl]porphyrin(1-MeIm); **d**, 5,10,15-(1,3,5-benzenetriacetyl)tris(α,α,α-*o*-aminophenyl)-20-[α-*o*-(pivaloylamido)phenyl]porphyrin(1,2-diMeIm); **e**, 5,10,-15,20-[pyrromellitoyltetrakis[*o*-(oxyethoxy)phenyl]]porphyrin(1-MeIm); **f**, 5,10,15,20-[pyrromellitoyltetrakis[*o*-(oxypropoxy)phenyl]]porphyrin(1-MeIm).

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Table 1. Shieldings of Meso Carbons, $\delta(^{13}C_m)$, Average C_{meso} Shieldings, $\delta(^{13}C_{m,av})$, ^{13}C Shieldings of the Fe−^{13}CO Unit, $\delta(^{13}CO)$, Crystallographic C_{meso} Average Displacement, $|C_m|$, and Fe−C−O Angle of the Heme Model Compounds of Figure 1

compd	$\delta(^{13}C_m)$ (ppm)	$\delta(^{13}C_{m,av})$ (ppm)	$\delta(^{13}CO)$ (ppm)	$\|C_m\|$ (Å)	Fe−C−O (angle/deg)
a	114.7 114.1	114.4	205.0	0.155[b]	180.0[b]
b	114.5 113.5	114.0	205.3	0.29[c]	178.9[c]
c	114.5 112.5	113.5	206.0	0.44[c]	178.3[c]
d	113.8 113.2 112.4	113.0	204.7	0.53[d]	172.5[d]
e[a]	114.9	114.9	202.1	0.04[e] 0.03[e]	172.9[e] 175.9[e]
f	115.3	115.3	203.0	0.01[f]	178.0[f]

[a] There are two independent Fe(C_2-Cap)(1-MeIm)(CO) molecules within the asymmetric part of the unit cell. [b] Reference 21. [c] Reference 10. [d] Reference 22. [e] Reference 12. [f] Reference 13.

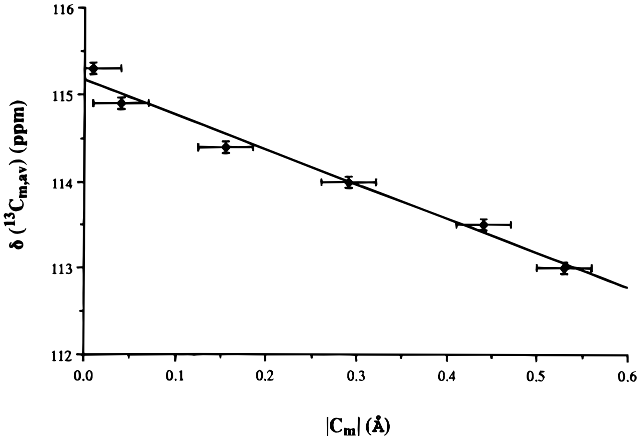

Figure 2. Plot of $\delta(^{13}C_{m,av}$, ppm) vs $|C_m|$ (Å) of the heme model compounds of Figure 1. The vertical bars denote the accuracy of the NMR measurements (determined from at least two independent measurements), while the horizontal denote the accuracy of the X-ray structures.

"pickets". Upon increasing the steric hindrance by shortening the chain, CO affinities are decreased, indicating a steric discrimination against CO. However, X-ray structure determinations of these complexes reveal that the Fe−C−O unit is both linear and normal to the mean porphyrin plane.[10,21] All contacts between the terminal oxygen atom and the aliphatic bridging chain are longer than 4 Å. The steric constraints are mainly released by a pronounced ruffling of the porphyrin macrocycle; the tighter the superstructure, the more ruffling. For complex **a** the average C_{meso} shielding, $\delta(^{13}C_{m,av})$, is 114.4 ppm. This model exhibits the smaller absolute crystallographic C_{meso} average perpendicular displacement, $|C_m|$, from the 24-atom mean porphyrin core in this series. Upon shortening the chain, the degree of ruffling increases (C_{meso} average displacement is 0.29 and 0.44 Å for complexes **b** and **c**, respectively (Table 1)), resulting in a significant shielding of the C_{meso} resonance (114.0 and 113.5 ppm for complexes **b** and **c**, respectively).

The superstructure of the "pocket" model (Figure 1, **d**) consists of a benzene cap attached to three out of four of the porphyrin phenyl substituents via *o*-amide linking groups and a fourth free pivalamido picket. The X-ray crystal structure of the β-atropisomer of **d** (in which the fourth picket is in the "down" position) shows the Fe−C−O angle to be 172.5°.[22] The modest distortion of the Fe−C−O unit is accompanied by considerable ruffling of the porphyrin periphery. Model **d** exhibits the most ruffled porphyrin plane among the compounds of Figure 1, and this is reflected in the C_{meso} resonance which is the most shielded (113.0 ppm).

The series of "cap" models consists of a 1,2,4,5-substituted benzene cap connected by four linkages of the type $-(C{=}O)O(CH_2)_nO-$ to the ortho positions of the phenyl rings of the TPP. In the case of "C_2-cap" ($n = 2$, Figure 1, **e**) the refined X-ray structure shows the presence of two crystallographically independent porphyrin molecules with the Fe−C−O groups distorted from linearity (172.9 and 175.9°, Table 1).[12] Interestingly, the porphyrin ruffling is very small, presumably because of the constraint of the cap. Only a single ^{13}C resonance is observed for the Fe−^{13}CO moiety, which shows that the interconversion rate of the two conformers is, presumably, fast on the NMR time scale. In the "C_3-cap" ($n = 3$, Figure 1, **f**) the Fe−C−O group is close to linearity; the degree of porphyrin ruffling is the smallest among the six models studied in this work,[13] and this results in the most deshielded C_{meso} resonance (115.3 ppm).

When the average shieldings of the meso carbons, $\delta(^{13}C_{m,av}$, ppm), are plotted against the absolute crystallographic average displacement of the C_{meso} relative to the porphyrin core mean plane, $|C_m|$ (Å), then, an excellent linear correlation is observed (Figure 2), which can be expressed as

$$\delta(^{13}C_{m,av}, \text{ppm}) = 115.16 - 4.0049|C_m| (\text{Å})$$

with a correlation coefficient of 0.985. From the above correlation it is evident that even small variations of porphyrin ruffling result in a significant change in ^{13}C shieldings of the meso carbons which vary by ca. 2.3 ppm between the two extremes and in a regular fashion, giving a shielding with increased ruffling.[23] This is consistent with some sp^3 character[24] of the meso carbons relative to that expected in flat porphyrins. Furthermore, contrary to earlier claims,[11] ^{13}C shieldings of the Fe−^{13}CO unit do not correlate either with the degree to which the CO unit is distorted from its linear configuration or with porphyrin ruffling (Table 1).

In summary, we have shown the great potential of the shieldings of meso carbons in structural studies of porphyrin distortion in superstructured heme model compounds in solution. Further investigations of synthetic models of hemoglobin and cytochrome P-450 are currently in progress.

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