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The Effects of Atropisomerism and Porphyrin Deformation on ^{57}Fe Shieldings in Superstructured Hemoprotein Models

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^{57}Fe NMR chemical shifts of superstructured heme model compounds have been found to be extremely sensitive to atropisomerism and deformation (ruffling) of the porphyrin geometry.

Key Words: **^{57}Fe NMR; heme models; atropisomerism; porphyrin deformation.**

There has been increasing interest in the use of ^{57}Fe NMR as a probe of heme protein structure (*1–4*). Iron is a central element of all heme proteins, and the extremely large ^{57}Fe chemical shift range offers, in principle, a very sensitive and direct probe of the functionally interesting diamagnetic, low spin ferrous state. The low natural abundance of ^{57}Fe (2.2%) is readily remedied by isotopic enrichment, and recent studies on heme models suggest that spin–lattice relaxation may be reasonably efficient via the chemical shift anisotropy mechanism (*5*). In this Communication we report, for the first time, that the ^{57}Fe NMR chemical shifts from heme model compounds are extremely sensitive to atropisomerism and provide further evidence in support of Baltzer's suggestion (*6*) that deformation (ruffling) of the porphyrin geometry is a dominant factor in determining ^{57}Fe shieldings.

Both ^{56}Fe(III)–PocPiv and ^{57}Fe(III)–PocPiv (94.5% enriched in ^{57}Fe) superstructured complexes (Fig. 1A) were synthesized and characterized by the methods described previously (*7*). After deoxygenation by flushing a dichloromethane solution with pure argon, the samples were reduced to the iron(II) form using aqueous sodium dithionite solution. After separation of the two phases, the organic layer was transferred under argon into a second vessel containing an excess of 1,2-dimethylimidazole. Separation of the aqueous phase and subsequent evaporation of the organic solvent resulted in a powder which was then loaded into a glass ampule, connected to a vacuum pump, and evacuated at room temperature for 3 h at a pressure of 10^{-4} Torr. The samples were dissolved in deuterated dichloromethane and transferred under argon into the NMR tube via a stainless steel tube. ^{13}CO (99.7% enriched in ^{13}C and 11.9% in ^{18}O) under atmospheric pressure was then introduced to the ^{56}Fe(III) complex while natural abundance CO was introduced to the ^{57}Fe(III) complex to form the carbonylated derivative, and the NMR tubes were sealed under pressure of ~1 atm.

^{13}C NMR spectra 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). ^{57}Fe NMR spectra were recorded at 19.58 MHz with a Bruker AMX-600 equipped with a 10-mm low-frequency multinuclear probe. The 90° pulse width, ~35 μs, was determined on a saturated solution of ferrocene in toluene. Chemical shifts are reported with respect to $Fe(CO)_5$ at 0 ppm, using as a secondary standard a saturated solution of ferrocene in toluene ($\delta \approx 1531$ ppm) (*1, 5*). Chemical shifts are reported with the high frequency positive convention.

Figure 2 shows typical ^{13}C NMR spectra of the ^{13}CO (99.7% enriched in ^{13}C) complex of the Fe(II)PocPiv(1,2-diMeIm) model. The spectrum at 298 K indicates the presence of two strongly overlapped resonances which are better resolved at lower temperature (290 K). The two signals, with relative integrals 3:2, can be attributed to α- and β-atropisomerisation of the fourth untethered picket which occured during the iron(II) insertion into the free base (this entailed the heating of the solution to 80°C). Assignment of the two atropisomers was based on the ring current effect of the benzene ''cap'' on the ^{1}H NMR resonances of the pivalamido picket as well as on the NOE effect between the protons of the methyl groups of the ''β-picket'' and the $-N-CH_3$ protons of the axial imidazole. The ^{13}C-labeled carbon monoxide from commercial sources is normally considerably enriched in ^{18}O, which makes it possible to obtain information on ^{18}O isotope shifts in the same sample. The ^{18}O isotope shift effect on the ^{13}C shielding of the α- and β-atropisomers are different (26.6 and 27.6 ppb, respectively). Jameson and Osten (*8*) suggested that the mass-independent

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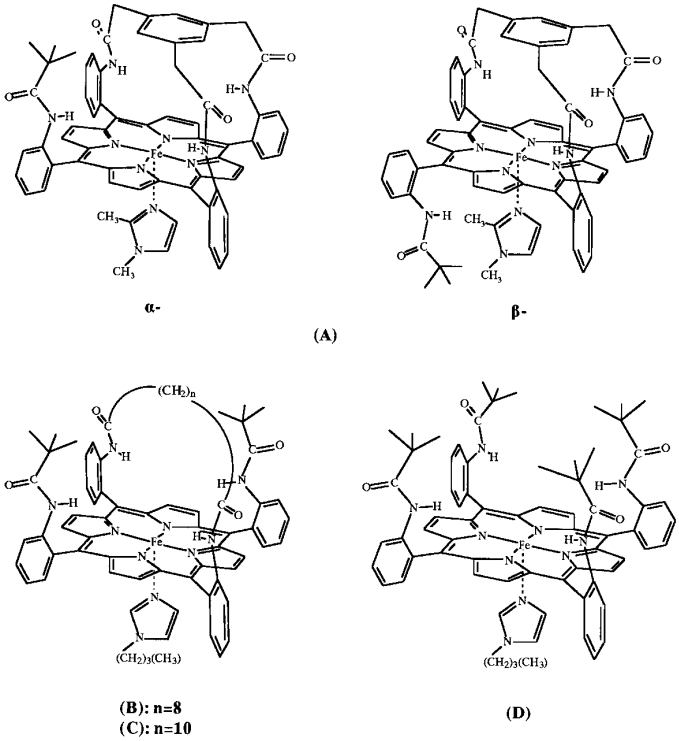

FIG. 1. Schematic structures of: (A) Fe(II)PocPiv(1,2-diMeIm); (B) Fe(II)Piv_2C_{12}(1-BuIm); (C) Fe(II)Piv_2C_{10}(1-BuIm); (D) Fe(II)picket fence (1-BuIm) superstructured heme model compounds.

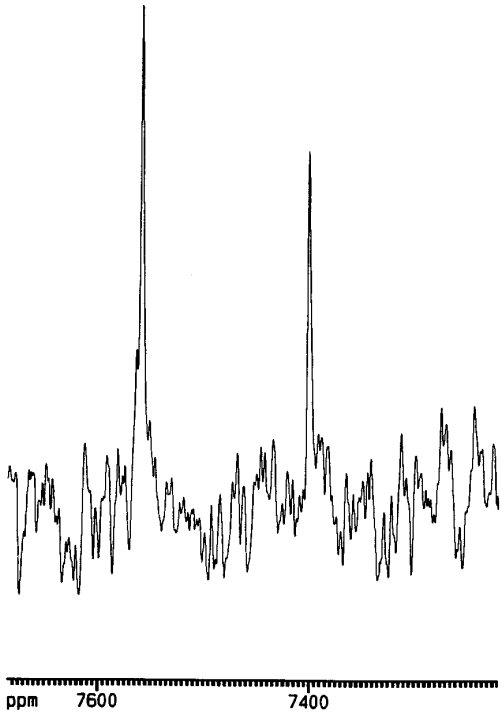

FIG. 3. ^{57}Fe NMR spectrum of the ^{57}Fe(II)PocPiv(1,2-diMeIm) (CO) (94.5% enriched in ^{57}Fe) adduct in CD_2Cl_2, saturated solution ($\leq$20 mM) at 298 K using a Brüker AMX-600 instrument, with $T_{acq} \sim 0.21$ s, number of scans = 327,680, 20 h total acquisition time, 130 μs preacquisition delay to avoid acoustic ringing phenomena (*15*), sensitivity enhancement by a line broadening function of 50 Hz, and relaxation delay = 20 ms.

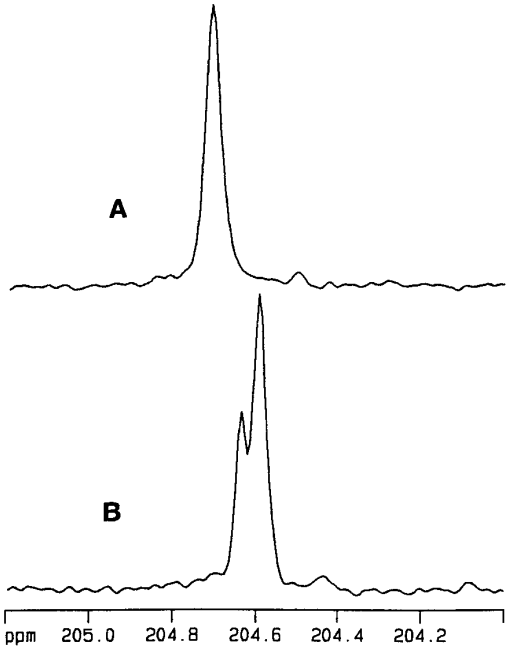

FIG. 2. ^{13}C NMR spectra of the ^{13}CO complex (99.7% enriched in ^{13}C and 11.9% in ^{18}O) with model A, saturated solution in CD_2Cl_2 at: (A) 298 K and (B) 290 K using a Bruker AMX-400 MHz instrument, with $T_{acq} \sim$ 0.8 s, number of scans = 5000, after resolution enhancement by a Gaussian exponential function.

part of the dynamical factors of the one-bond isotope shifts are largely determined by the bond length. In general the magnitude is greater for the stronger bond which implies that the CO bond lengths of the two atropisomers may be different.

The one-bond ^{57}Fe–^{13}C couplings, observed via ^{13}C NMR, were found to be 25 and 26 Hz for the α- and β-atropisomer, respectively, and are not accurate monitors of the various steric and electronic interactions in superstructured heme model compounds (*9*). On the contrary, the ^{57}Fe NMR spectrum of complex A (Fig. 3) indicates the presence of two distinct resonances with a chemical shift difference of 157.9 ppm and relative integrals 3:2. This provides direct evidence that severe electronic changes which occur at the ligand binding site of the two atropisomers are mainly expressed at the iron atom, and not at nearby ^{13}C or ^{15}N.

Baltzer and Landergren (*6*) suggested that the magnitudes of the shielding changes due to medium effects, difference in coordination of the axial ligand residue, and the effect of forcing the carbonyl off-axis are substantially smaller than those occuring as a result of perturbations of the porphyrin core. This was suggested to be the case of the so-called ''hybrid'' models B and C which have two pivalamido pickets (as in the unconstrained ''picket fence'' porphyrin D)

TABLE 1

^{57}Fe Shieldings and Most Significant Structural Features for CO Binding in the Superstructured Heme Model Compounds of Fig. 1

Compound	$\delta(^{57}Fe)$ [ppm]	C_{meso} mean displacement [Å]	Fe–C–O [°]	Fe–C [Å]	Fe ··· M[a] [Å]
A α-atropisomer	7402.0[b]	—	—	—	—
β-atropisomer	7559.9[b]	0.53[d]	172.5(6)[d]	1.768(7)[d]	5.36[d]
B	7728.0[c]	0.29[e]	178.9(5)[e]	1.752(4)[e]	6.81[e]
C	8036.0[c]	0.155[e]	180.0(0)[e]	1.728(6)[e]	8.43[e]
D	8110.0[c]				

[a] Fe ··· M is the distance between the centroid of distal cap or strap and the Fe atom; it defines the distal pocket size.
[b] Data from the present work.
[c] Data from Ref. (*6*).
[d] Data from Ref. (*12*).
[e] Data from Ref. (*11*); 1-MeIm complex.

on each side of an amide handle of variable length linked in a cross-trans configuration. The X-ray structures of the ''hybrid'' complexes show that the Fe–C–O unit is both linear and normal to the mean porhyrin plane (*10, 11*). All contacts between the terminal oxygen atom and the aliphatic bridging chain are longer than 4 Å. The porhyrin plane in all models is clearly ruffled; the shorter the aliphatic chain, the more it is ruffled. Kim *et al.* (*12*) have reported the X-ray crystal structure of the β-atropisomer of complex A in which the fourth picket group is in the ''down'' position. The Fe–C–O bond angle was found to be 172.5°, and the off-axis displacements of the C and O atoms are 0.18 and 0.38 Å, respectively. The Fe atom is only 0.001 Å out of the 24-atom least-squares plane toward the CO ligand, and the Fe–C bond length (1.768(7) Å) appears to be longer than that with the 1-MeIm adducts. The modest distortion of the Fe–C–O unit is accompanied by a significant shifting of the benzene ''cap'' away from the bound CO ligand and a considerable ruffling of the porphyrin periphery (Table 1).

From Table 1 it is evident that the ^{57}Fe chemical shifts vary by more than 500 ppm between the various models and in a seemingly regular fashion, giving an increased shielding with increased ruffling. It can therefore be concluded, in agreement with Baltzer's work (*6*), that the effect of perturbation of the iron *d* orbital energies on ^{57}Fe shieldings is much larger than that observed upon changing the ligands in a strictly octahedral complex.

Interestingly, ruffled porhyrins may be of biological significance since myoglobin–CO exhibits an almost flat porphyrin (*13*) while in human carbonyl hemoglobin (*14*) the plane is clearly ruffled. Further research in this direction is in progress in our laboratories.

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