Paramagnetic ^{19}F N.M.R. Spectra of Iron(III) Porphyrins Substituted with CF_3 Groups and Reconstituted Myoglobin

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Paramagnetic ^{19}F n.m.r. spectra of iron(III) porphyrins substituted with CF_3 groups and metmyoglobin reconstituted with the iron(III) complex of 3-trifluoromethylmesoporphyrin-IX were measured and the ^{19}F shifts compared with the respective chemical shifts of the free ligands and zinc(II) complexes.

Enzymes and substrates chemically modified with fluorine or perfluoroalkyl groups are useful probes for clarifying the structure of enzymes and the interaction between substrates and enzymes by use of ^{19}F n.m.r. spectroscopy. In particular, ^{19}F n.m.r. investigations of paramagnetic metalloenzymes and related metal complexes have given a new insight into their structure elucidation and reactivity. We have prepared the highly symmetrical porphyrin 1,3,5,7-tetrakis(trifluoromethyl)-2,4,6,8-tetraethylporphyrin (**1**)[1] and the dimethyl ester of 3-trifluoromethylmesoporphyrin-IX (**5**)[2] replaceable with naturally occurring heme in hemeproteins and compared the paramagnetic ^{19}F n.m.r. spectra of the iron(III) complexes with those of the corresponding free base and zinc complexes. In addition, the ^{19}F n.m.r. spectrum of the metmyoglobin cyanide reconstituted with the iron complex of the dicarboxylic acid of (**5**) was obtained in D_2O–H_2O.

Table 1 summarizes the ^{19}F n.m.r. signals due to the CF_3 groups of the free bases and their metal complexes. The ^{19}F resonances of the low-spin iron(III) complex (**3**) (S = 1/2) and the high-spin iron(III) complex (**4**) (S = 5/2) showed 5 and 60 p.p.m. downfield shifts respectively, compared with the spectrum of the zinc(II) complex (**2**). Diagnostic ^{1}H n.m.r. spectra of the iron(III) complex of the parent etioporphyrin showed the CH_3 signals at δ 13 and 48 for the low- and high-spin states, respectively.[3] The large differences in the fluorine chemical shifts between the low- and high-spin states are comparable with the differences in the CH_3 chemical

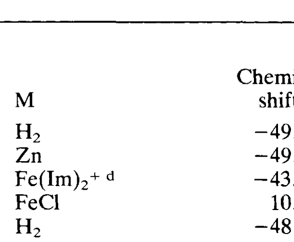

Table 1. ^{19}F n.m.r. chemical shifts for the CF_3 group in compounds (**1**)—(**9**).

Compound	R¹	R²	R³	R⁴	R⁵	M	Chemical shift[a]
(**1**)	CF_3	CF_3	Et	CF_3	Et	H_2	−49.5
(**2**)	CF_3	CF_3	Et	CF_3	Et	Zn	−49.1
(**3**)	CF_3	CF_3	Et	CF_3	Et	$Fe(Im)_2^+$ [d]	−43.5
(**4**)	CF_3	CF_3	Et	CF_3	Et	FeCl	10.8
(**5**)	Me	Me	P^{Me} [b]	P^{Me}	Me	H_2	−48.7
(**6**)	Me	Me	P^{Me}	P^{Me}	Me	Zn	−48.1
(**7**)	Me	Me	P^{Me}	P^{Me}	Me	$Fe(Im)_2^+$	−49.7
(**8**)	Me	Me	P^{Me}	P^{Me}	Me	FeCl	12.8
(**9**)	Me	Me	P[c]	P	Me	$Fe(CN)_2^-$	−50.4[e]

[a] In p.p.m. from $CFCl_3$ in $CDCl_3$. [b] P^{Me} = $CH_2CH_2CO_2Me$. [c] P = $CH_2CH_2CO_2H$. [d] Im = imidazol-2-yl. [e] In D_2O.

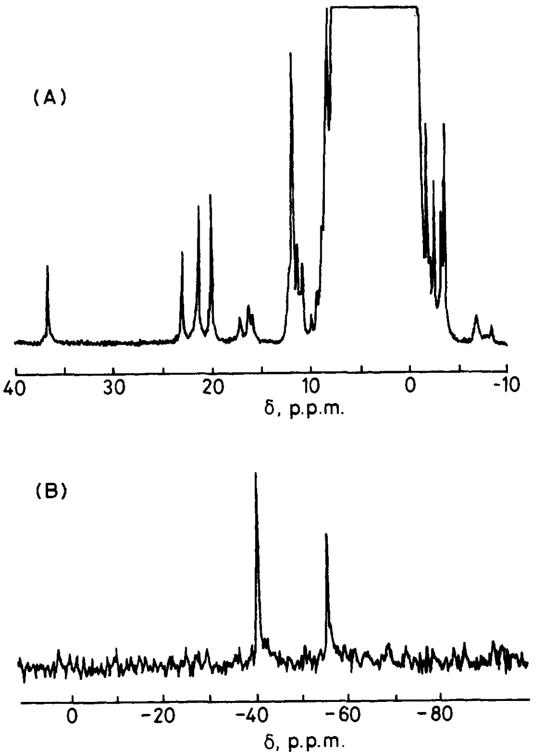

Figure 1. ^1H and ^{19}F n.m.r. spectra of the reconstituted myoglobin cyanide in D_2O. (A) ^1H, DSS (sodium 4,4-dimethyl-4-silapentanesulphonate) reference. (B) ^{19}F, $CFCl_3$ reference.

shifts. Paramagnetic proton chemical shifts for iron(III) porphyrins have been described by two terms: the Fermi contact and dipole–dipole interactions.[4] The present ^{19}F results may be analysed in a similar way to that for proton isotropic shifts. In contrast with the high-spin complex, fluorine chemical shifts of the low-spin complexes (**7**) and (**9**) showed small isotropic shifts relative to that of the zinc(II) complex (**6**), whereas the CH_3 proton resonances showed a 10—15 p.p.m. downfield shift. In the cases of the low-spin complexes (**7**) and (**9**), solvent and axial ligand effects and intermolecular interactions must be taken into account; these may have opposite signs which would explain the very small change in isotropic shift. The fluorine contact shifts for transition metal complexes with fluorine-containing ligands have been estimated to be 2—3 times larger than the proton contact interaction and have the opposite sign if the π-electron spin distribution is constant.[5] The magnitude and sign of fluorine chemical shifts are probably determined by a balance of contact and dipole interactions. It is questionable to consider that a different spin-transfer mechanism is necessary to explain the fluorine chemical shifts of the low-spin iron(III) porphyrins.

The dicarboxylic acid of (**8**) was reconstituted with sperm whale apomyoglobin. Figure 1 illustrates the ^1H and ^{19}F n.m.r. spectra of the metmyoglobin cyanide for the low-spin state. The naturally occurring prosthetic heme and its derivatives can be accommodated in two different ways, normal and reverse, at the initial stage upon reconstitution.[6] The reverse, orientation is obtained by 180° rotation around the *meso* α–γ axis. Both spectra in Figure 1 show the co-existence of normal and reverse orientated hemes in the protein. Two ^{19}F signals at −40.4 and −55.5 p.p.m. for reconstituted myoglobin seem to be indicative of two orientations. Interaction between the amino acid residues and heme may have an effect on the chemical shifts of the CF_3 group. The present ^{19}F n.m.r. studies of paramagnetic iron porphyrins and metmyoglobin provide a diagnostic means for identifying the spin states of the prosthetic group.

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