Very Long-range Isotope Shifts in the Proton NMR Spectra of Deuteriated Haemins

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Very long-range isotope shifts have been observed in the 500 MHz proton NMR spectra of several deuteriated haemins; these shifts are consistent with a redistribution of delocalised π spin density upon fractional resolution, owing to deuteriation, of the ground state orbital degeneracy of the low-spin iron(III) ion.

The proton NMR spectra of paramagnetic haems and haem proteins have proven to be a remarkably sensitive probe of the environment and electronic structure of the haem unit.[1] The low-spin biscyano complexes of iron(III) protoporphyrin IX (Fig. 1) have been particularly amenable to study because the proton resonances are well dispersed but narrow because of short electron relaxation times.[2] These complexes also exhibit an asymmetric distribution of π-electron density and unpaired electron spin which is very sensitive to substituent effects.[3] We now report a previously unrecognized consequence of this sensitivity, namely the presence of unusually long-range (>10 bond) isotope shifts in the proton NMR spectra of several deuteriated haemins. These long-range shifts are quite unlike those measured for diamagnetic molecules[4,5] because they represent a rare example of the effect of isotopic substitution on the hyperfine shift.

Isotope shifts were observed for several deuteriated haemins[6,7] **1–5** (Table 1), but are seen most clearly in the methyl region of the 500 MHz proton NMR spectra of porphyrins **2** and **4** (Fig. 2). The three peaks seen for many of the methyl signals are the result of partial deuteriation at the β-vinyl position. Addition of non-deuteriated material enhanced either the most upfield or most downfield signal. Of the two peaks remaining for each methyl group, the middle one was assigned to mono-deuteriated porphyrin and the third peak to di-deuteriated porphyrin. Rather surprisingly, the middle signal for the 8-methyl group of **2** was resolved into two peaks of approximately equal height, corresponding to *cis* or *trans* mono-deuteriation. This gives some indication of how sensitive the hyperfine shifts in haemins are to isotopic substitution. The 8-methyl group is no less than 10 bonds away from the site of deuteriation, but still experiences a different hyperfine shift when the $2\beta_{cis}$ or $2\beta_{trans}$ position is selectively deuteriated. To the best of our knowledge, such long-range effects are unique and set new limits for the distance over which deuterium isotope effects can be readily measured by proton NMR spectroscopy.

The unusual nature of these isotope shifts prompted us to investigate further how they are transmitted over such long distances and what information the shifts provide about the electronic effects of deuteriation. There are several mechanisms by which deuteriation could change the hyperfine shifts. However, the observed pattern of isotope shifts can be most readily explained by a change in the contact contribution, with increasing contact shifts for the deuteriated and opposite pyrrole rings and decreasing contact shifts for the two pyrrole

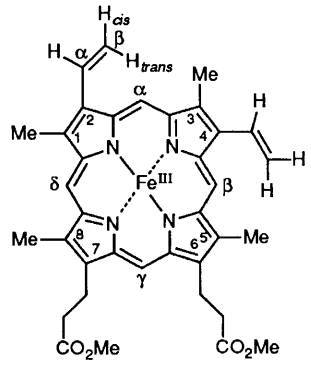

Fig. 1 Iron(III) protoporphyrin IX dimethyl ester

Table 1 Observed deuterium isotope shifts ($\delta_D - \delta_H$; ppb) for iron(III) biscyano protoporphyrin IX dimethyl ester complexes[a]

Complex	Same ring	Opposite ring	Adjacent ring	Deuteriated group
1 2α[$^2H_1$]			−9 (8Me)	−60 (H2β_{cis}), −37 (H2β_{trans})
2 2β[$^2H_2$]	+30 (1Me)	+ve sh (5Me)	−19 (3Me)	H2β_{cis}[$^2H_1$] −12 (H2α), −22 (H2β_{trans})[d]
			−23 (8Me)	H2β_{trans}[$^2H_1$] −45 (H2α), −40 (H2β_{cis})[d]
			−ve sh (H4α)	
3 4α[$^2H_1$]				−59 (H2β_{cis}), −39 (H2β_{trans})
4 4β[$^2H_2$]	+32 (3Me)	+ve sh (8Me)	−20 (5Me)	H2β_{cis}[$^2H_1$] −12 (H2α), obscured (H2β_{trans}),[d] H2β_{trans}[$^2H_1$] −49 (H2α), −38 (H2β_{cis})[d]
5 5Me[$^2H_3$]	+30 (6CH_2)[b]	+14 (1Me)	−28 (8Me)	[c]
			−21 (H4α)	
			+8 (H4β_{cis})	
			−ve sh (7CH_2)[b]	

[a] The 500 MHz proton NMR spectra were recorded at 25 °C on a Nicolet NM500 spectrometer, using 5 mmol dm^{-3} solutions of chloroiron(III) porphyrin in [$^2H_4$]methanol with an excess of KCN in D_2O. The methanol signal was used as an internal reference (δ 3.31). ppb = 1 in 10^9. +ve and −ve sh indicate positive or negative shoulders upon addition of non-deuteriated material. [b] The methylene signals were not specifically assigned, but the methylene group with a positive isotope shift is presumably on the deuteriated ring. [c] Isotope shifts (δD − δH) of +110 ppb per deuterium have been reported for partially deuteriated methyl groups.[12] In diamagnetic porphyrins this effect is only −16 ppb.[13] [d] Isotope shifts for mono-deuteriated porphyrin complex.

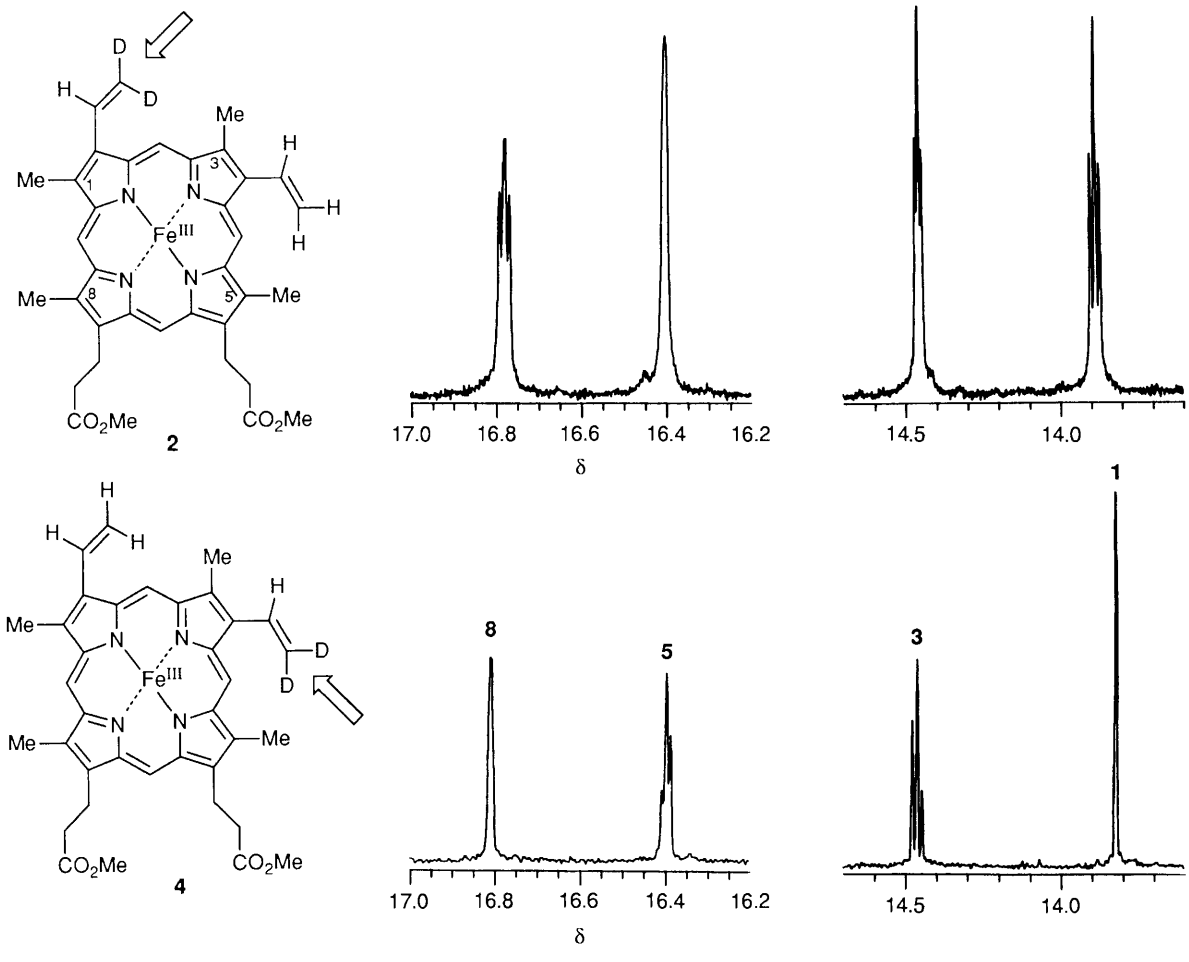

Fig. 2 Methyl regions of the resolution enhanced 500 MHz proton NMR spectra of low-spin iron(III) biscyano protoporphyrin IX dimethyl ester complexes **2** (2-β-[$^2H_2$] 50% overall deuteriation, composed of 30%-[$^2H_2$], 45%-[$^2H_1$] and 25%-[$^2H_0$]) and **4** (4-β-[$^2H_2$] 60% overall deuteriation, composed of 35%-[$^2H_2$], 48%-[$^2H_1$] and 17%-[$^2H_0$]). The 8, 5, 3 and 1 above the peaks in the lower spectrum are the methyl assignments.[2]

rings adjacent to the deuteriation site. This redistribution of π spin density between pairs of opposite pyrrole subunits has previously been shown to result from raising the orbital degeneracy of a four-fold symmetric iron(III) haem,[8,9] and is the basis for the large π spin asymmetry found in the majority of low-spin iron(III) haem proteins where the degeneracy is completely resolved by the π bonding with the axial histidyl imidazole.[1,8,9] The very modest redistribution of spin density between the two pairs of opposite pyrroles for the deuteriated haemins must be due to weak perturbation of the symmetry by deuteriation. Similar resolution of an orbitally degenerate ground state upon mono-deuteriation that leads to a major redistribution of π spin density has been observed by ESR for the benzene anion radical.[10]

The isotope shifts for the deuteriated pyrrole ring also provide information about the relative electron-donating ability of deuteriated and non-deuteriated substituents. The haem methyl contact shifts arise from porphyrin-to-iron π charge-transfer which introduces π spin density into the 3e π orbital.[1] Hence, the lone unpaired electron spin goes to the less strongly antibonding component of the 3e π orbitals, which will be the one with the weakest interaction. From the fact that deuteriation of a methyl weakens this interaction for the perturbed pyrrole subunit, we can infer that the C^2H_3 group must be a weaker electron-donating group than is CH_3. This conclusion is completely consistent with the results obtained from isotope effects in diamagnetic molecules.[11] Moreover, since the vinyl group is generally electron-withdrawing, the increase in the contact shift of the pyrrole upon deuteriation of that group argues that $-C^2H=C^2H_2$ is more efficient in withdrawing electron density than is $-CH=CH_2$. Hence, the net effect of deuteriation for both the vinyl and methyl groups is a decrease in electron-donating ability.

In summary, we have measured isotope shifts in deuteriated haemins that operate over much greater distances than isotope shifts in diamagnetic systems. These isotope shifts arise from a change in the contact contribution to the hyperfine shift and provide valuable information about the electron-donating or -withdrawing ability of the deuteriated substituent.

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