^{15}N N.M.R. Study of Iron(III) Porphyrin–Imidazole Complex. Observation of the Bound Imidazole Resonances

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^{15}N N.m.r. resonances of the imidazole bound to iron(III) porphyrin have been located and those resonances are expected to serve as potentially powerful probes in characterizing the nature of the iron–imidazole interaction in haemoproteins.

The reactivity of the centre iron in various haemoproteins has been proposed to be primarily influenced by the iron–proximal histidyl imidazole bonding interaction.[1] The iron–histidyl imidazole bond in the haemoprotein can be modulated by both the steric interaction and the hydrogen bonding interaction of N_1H (see the inset of Figure 1) with a protein acceptor.[2,3] The former interaction simply compresses or extends the Fe–N_3 bond and the latter modulates the Fe–N_3 bond through the alteration of the imidazole basicity.

Two spectroscopic probes have been used to specifically monitor the Fe–N_3 bond in the haemoproteins. Resonance Raman spectroscopy[4] provides the Fe–N_3 stretching frequency which can be directly interpreted in terms of the bond energy. On the other hand, ^1H n.m.r. gives the hyperfine shift of N_1H which provides useful information about the unpaired spin delocalization and the strength of the hydrogen bond between N_1H and the acceptor.[5] Combined analysis of both these data allows the separation of the steric and hydrogen bonding contributions to the Fe–N_3 bond.[6] An alternative method for characterizing the electronic structure of the proximal histidyl imidazole is the observation of the ^{15}N resonances because both the steric and hydrogen bonding interactions can be monitored simultaneously by the N_1 and N_3 resonances. In addition, the advantage of observing ^{15}N resonance in a paramagnetic system arises from its narrow linewidth. If the distances from the iron are the same for ^{15}N

Table 1. Separation of hyperfine shifts (δ_{hf}) into pseudo-contact (δ_{pc}) and contact (δ_c) contributions for ^{15}N n.m.r. resonances of the bound imidazole in iron(III) tetraphenyl porphyrin–imidazole complex.

Resonance	δ_{obs}[a]	δ_{dia}[b]	δ_{hf}	g.f.[c]	δ_{pc}	δ_c
N_1	124.8	150.5	−25.7	27.58	23.7	−49.4
N_3	−2115.2	231.5	−2346.7	260.01	223.1	−2569.8

[a] Shifts in p.p.m., in $CDCl_3$ at 30 °C. [b] The value at $T^{-1} \rightarrow 0$ obtained from the Curie plot of Figure 2. [c] Geometric factor, $(3\cos^2\theta - 1)/r^3$, in units of $\times 10^{-3}$ Å^3.

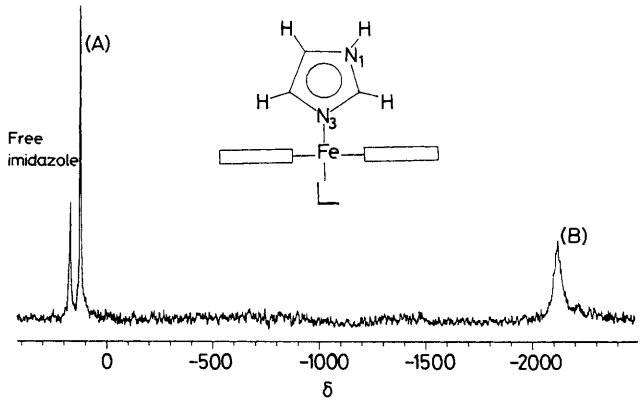

Figure 1. The 27 MHz ^{15}N n.m.r. spectrum of iron(III) tetraphenyl porphyrin–imidazole (enriched to 95% ^{15}N at both N_1 and N_3) complex, 50 mM, in $CDCl_3$ at 30 °C. 3000 Scans with 0.3 s repetition time were used. The chemical shifts are referenced to neat nitromethane. The numbering system of the imidazole ring is indicated in the inset. The resonances arising from the bound imidazole ^{15}N resonances are indicated by (A) and (B).

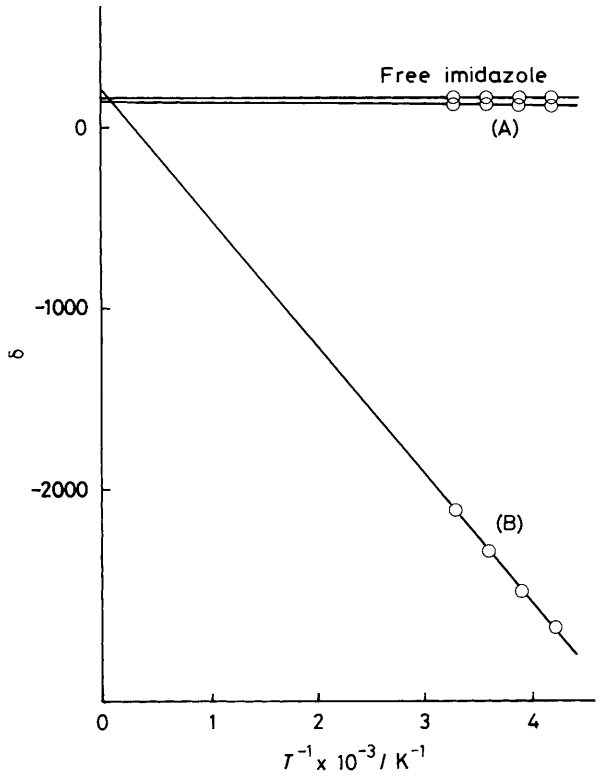

Figure 2. Curie plots, observed shift *vs.* reciprocal of temperature, for the resonances observed in the spectrum of Figure 1.

and ^1H nuclei and metal-centred dipolar relaxation is predominant, the line-width of the ^{15}N resonance should be about 1% of that of the ^1H resonance due to $\gamma_H/\gamma_N \sim 10$.

This Communication presents the first observation of the bound imidazole ^{15}N n.m.r. resonances in iron(III) tetraphenyl porphyrin–imidazole (enriched to 95% ^{15}N at both N_1 and N_3) complex in $CDCl_3$, which are expected to serve as potentially powerful probes in characterizing the nature of the iron–imidazole interaction. The 27 MHz ^{15}N n.m.r. spectrum of the iron(III) tetraphenyl porphyrin–imidazole complex in $CDCl_3$ at 30 °C is illustrated in Figure 1. In the spectrum, two resonances, (A) and (B), are observed at 124.8 and −2115.2 p.p.m., besides the signal from free imidazole which is observed as a single resonance at 172.6 p.p.m. due to the rapid tautomerization reaction at ambient temperature.[7] Considering the exchange rate of imidazole between its free and bound states, $\sim 14\ s^{-1}$,[8] it is reasonable to assume that the resonances A and B arise solely from the bound imidazole.

Resonances (A) and (B) exhibit distinctly different line-widths of 52 and 708 Hz, respectively. Since the paramagnetic relaxation contributions dominate in the present case, the broader resonance, (B), may be assigned to N_3 because of its closer distance to the iron and hence the resonance (A) to N_1. The geometric factors, with respect to the porphyrin plane, predict a large deshielding pseudo-contact contribution for these ^{15}N resonances relative to their diamagnetic shifts. Therefore large upfield shifts of resonance (B) due to direct unpaired electron density transfer *via* the Fe–N_3 bond is consistent with the assignment of this resonance to N_3. From the line-width of 708 Hz for the N_3 resonance, the value of 9 Hz is calculated for the line-width of the N_1 resonance using the known distance of Fe–N_1 (4.10 Å) and Fe–N_3 (1.97 Å)[9] and the assumption of a predominant metal-centred dipolar relaxation mechanism. The larger observed line-width for the N_1 resonance indicates that other paramagnetic relaxation mechanisms contribute to their relaxation process. Additionally, difference in observed shift between the N_1 and N_3 resonances dictates that the unpaired electron density transferred from the iron to the imidazole ring is localized, to a large extent, at N_3.

The N_1 and N_3 signals were observed at different temperatures, −35 to 30 °C, and their Curie plots, observed shift *vs.* reciprocal of temperature, are shown in Figure 2. The chemical shift of the resonance from free imidazole is essentially independent of temperature. The Curie plots for the N_1 and N_3 resonances are fairly straight over the temperature range examined and the values at $T^{-1} \rightarrow 0$ of 150.5 and 231.5 p.p.m. are obtained for the N_1 and N_3 resonances, respectively. The pseudo-contact shifts for these

resonances can be calculated from the equation obtained by Goff[10] with their geometric factors. Using the values at $T^{-1} \rightarrow 0$ as tentative diamagnetic reference shifts, the contributions to their hyperfine shifts are separated and the results are summarized in Table 1. A large difference in the unpaired electron density between N_1 and N_3 nuclei is apparent from their δ_c values.

The utility of the model compound study in facilitating the location and interpretation of the n.m.r. resonances in the haemoproteins has been well documented.[10,11] The present results indicate the potentiality of studying the proximal histidyl imidazole ring in low-spin forms of the haemoproteins by ^{15}N n.m.r. Their resonances should be observed for the haemoprotein with the natural abundance ^{15}N-level, although genetically ^{15}N-labelled protein[12] would be desirable, using effective data acquisition enabled by their fast relaxation rates. The N_1 resonance may overlap with the resonances from the protein but its relatively short relaxation time may allow selective observation of the signal. Work is in progress involving the penta-co-ordinated iron(II) porphyrin–imidazole complex and deoxy form of haemoproteins.

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