^{13}C Chemical Shifts and ^{13}C–^{15}N and ^{13}C–^{57}Fe Spin Coupling Constants as Structural Probes for Haem Environments. ^{13}C N.M.R. Study of the Binding of ^{15}N and ^{13}C Labelled Alkyl Isocyanides to Myoglobin and Synthetic Porphyrins

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Summary A ^{13}C n.m.r. study of ^{13}C and ^{15}N labelled alkyl isocyanides ($R^{15}N^{13}C$) bound to the haem iron(II) atom labelled with ^{57}Fe in natural myoglobin and synthetic tetraphenyl- and octaethyl-porphyrin iron(II) shows that the iron-bound isocyanide ^{13}C chemical shifts and ^{15}N–^{13}C coupling constants are sensitive to variation of the R group and the presence of globin, while the one-bond ^{13}C–^{57}Fe coupling constant is not susceptible to these structural factors.

THE use of isotopically enriched ligand molecules bound to the haem-iron in haemoproteins can be a convenient and effective tool in the n.m.r. spectroscopic study of haemoproteins to probe the structure of the haem environment as well as ligand–haem interactions. ^{13}C Enriched carbon monoxide[1] and isocyanide[2] and ^{15}N labelled cyanide[3] have been utilized for the structural characterization of haemoproteins in Fe^{II} and Fe^{III} low-spin states. We report here new aspects of ^{13}C n.m.r. spectral studies of ^{13}C and ^{15}N labelled alkyl isocyanides ($R^{15}N^{13}C$) bound to the haem Fe^{II} atom of myoglobin and its model porphyrin complex in which the haem iron is enriched with ^{57}Fe. We have studied the ^{13}C–^{15}N spin coupling for the iron-bound isocyanide and ^{13}C–^{57}Fe ligand–iron spin coupling[4] as well as the ^{13}C chemical shifts in relation to the effect of isocyanide–apoprotein interactions on the mode of the iron–ligand binding.

Figure 1 shows representative ^{13}C spectra of iron-bound $RN^{13}C$ and $R^{15}N^{13}C$ of native and ^{57}Fe-enriched myoglobin complexes.† Using ^{57}Fe-labelled sperm whale myoglobin,

† The ^{13}C n.m.r. spectra were recorded at 25·0 MHz and 24 °C in the pulse Fourier transform mode on a Jeol FX-100 spectrometer with deuterium lock. A pulse repetition time of 1·1 s was employed with 14 μs pulse. Chemical shifts are reported in p.p.m. from external Me_4Si. Reduction of native myoglobin (Sigma type III) from Fe^{III} to Fe^{II} states was effected by the addition of $Na_2S_2O_4$. The sample was placed in a 10 mm tube under argon.

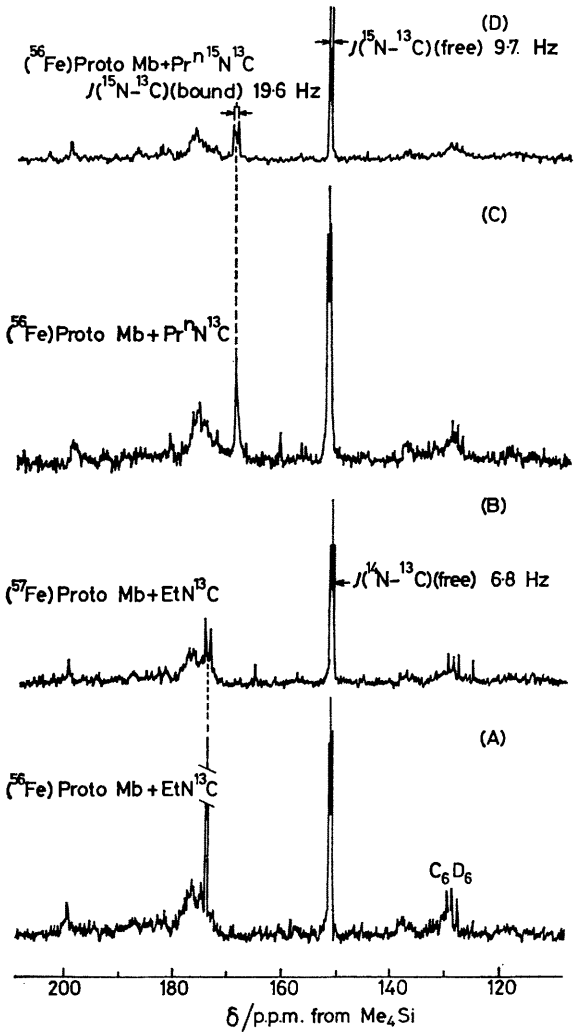

FIGURE 1. ^{13}C N.m.r. spectra of isocyanide complexes of Fe^{II} sperm whale myoglobin. (A) Fe^{II} native myoglobin (5 mM in phosphate buffer) with ^{13}C labelled ethyl isocyanide. 10 K transients were accumulated. (B) EtN^{13}C + Fe^{II} myoglobin reconstituted with the ^{57}Fe enriched protoporphyrin (5 mM). 30 K transients were accumulated. (C) Pr^{n}N^{13}C + myoglobin (5 mM), 10 K transients. (D) Pr^{n15}N^{13}C + myoglobin (5 mM), 10 K transients. All spectra were obtained at 24 °C.

the iron-bound EtN^{13}C exhibits a doublet with J 25·4 Hz, while the native myoglobin complex shows a singlet at the same position. This doublet can be unequivocally attributed to the ^{13}C–^{57}Fe one-bond spin coupling. Figure 1 also shows the one-bond ^{13}C–^{15}N couplings for free and iron-bound Pr^{n15}N^{13}C. A small shoulder to lower field of the doublet for the myoglobin–Pr^{15}N^{13}C complex is due to a small amount of the concomitant mono-enriched species (Pr^{n}N^{13}C). The results for the myoglobin complexes are in the Table. It is worth noting that the ^{13}C chemical shift of the iron-bound RN^{13}C for myoglobin complexes is very sensitive to variation of the alkyl group (R = Et, Pr^{n}, or Bu^{n}), while the ^{13}C–^{57}Fe coupling constant is unchanged. The ^{13}C–^{15}N spin coupling constant is greatly increased on going from the free to the iron-bound isocyanide.

We have also examined the ^{13}C n.m.r. spectra of isocyanide complexes of Fe^{II} tetraphenylporphyrin (TPP) labelled with ^{57}Fe. The TPPFe^{III} was reduced to TPPFe^{II} by adding an appropriate amount of $SnCl_2$ in pyridine solution under argon. Figure 2 shows results for bis-isocyanide complexes of TPP Fe^{II} in pyridine solution. The ^{13}C–^{57}Fe coupling constant is the same for the myoglobin and the model porphyrin complexes. However, the ^{13}C–^{15}N coupling constant for the TPP complex is substantially larger than that for the myoglobin complex. An important feature of Figure 2 is that the ^{14}N–^{13}C coupling observed for the free RN^{13}C is absent for the iron-bound RN^{13}C as is the case for the myoglobin complex (Figure 1). This appears to be due to a change of hybridization of ^{14}N upon co-ordination, rather than to a change of the correlation time associated with molecular motion or to a change in the ^{13}C–^{14}N coupling constant. The one-bond N–C coupling tends to be increased upon co-ordination, as shown by the ^{13}C–^{15}N coupling constants for the free and iron-bound R^{15}N^{13}C. Variation of porphyrin from TPP to OEP (octaethylporphyrin) did not cause any significant difference in the ^{13}C shift and the one-bond spin coupling constants.

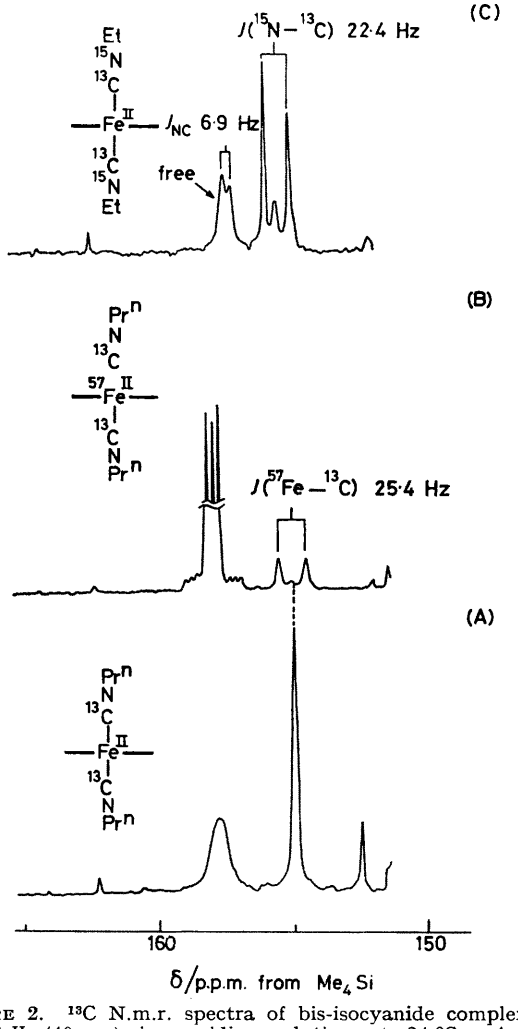

FIGURE 2. ^{13}C N.m.r. spectra of bis-isocyanide complexes of TPPFe^{II} (40 mM) in pyridine solution at 24 °C. A pulse repetition time of 1·5 s was employed to collect 10 K transients. (A) (Pr^{n}N^{13}C)$_2$–TPPFe^{II} in pyridine. The ^{13}C–^{14}N coupling for free isocyanide is not well resolved. (B) (PrN^{13}C)$_2$–TPP^{57}Fe^{II} in pyridine. (C) (Et^{15}N^{13}C)$_2$–TPPFe^{II} in pyridine.

TABLE. ^{13}C N.m.r. data for free and iron-bound isocyanide of myoglobin and TPP complexes

	Free isocyanide		Myoglobin–isocyanide complex			Bis-isocyanide complex of TPP		
Isocyanide	$\delta(^{13}C)$ /p.p.m.	$J(^{13}C–^{15}N)$ /Hz	δ (^{13}C) /p.p.m.	J ($^{13}C–^{15}N$) /Hz	J ($^{13}C–^{57}Fe$) /Hz	$\delta(^{13}C)$ /p.p.m.	$J(^{13}C–^{15}N)$ /Hz	$J(^{13}C–^{57}Fe)$ /Hz
EtNC	151·5	9·7	174·4	18·5	25·4	155·2	22·4	—
Pr^nNC	152·2	9·7	169·2	19·6	25·4	154·8	—	25·4
Bu^nNC	152·1	—	167·7	—	25·4	—	—	—

Figures 1 and 2 show that the isocyanide ^{13}C chemical shift tends to move downfield upon co-ordination for myoglobin, in contrast to the upfield shift for the TPP complex. This substantial difference in the ^{13}C shifts of the iron-bound isocyanide between the myoglobin and the TPP or OEP complexes may be attributable to the globin–ligand interaction and/or the *trans*-effect caused by the change of the fifth ligand of the haem iron. Analysis of the origins of the effect of variation of the R group in the alkyl isocyanides and the effect of globin on the iron-bound $RN^{13}C$ shifts is complex. One possible factor is the influence of a change in the R group or in the fifth ligand on the strength of the sixth ligand–iron bonding interaction *via* a steric or electronic effect. As the $^{13}C–^{57}Fe$ coupling constant is invariant (25·4 Hz), while the $^{13}C–^{15}N$ coupling varies substantially in going from the myoglobin to the porphyrin complexes, the ^{13}C chemical shift of the iron-bound $RN^{13}C$ may be influenced by the structure of the bound isocyanide, possibly by a change in CNR bond angle due to the globin–ligand steric interaction. The iron-bound isocyanide of the myoglobin complex may be forced to take a bent structure in the haem crevice, while the model porphyrin complexes are free from this deformation. Considerable chemical shift changes on going from ethyl to butylisocyanide for the myoglobin complex appear to be caused by this effect.

This work was supported by grants from the Ministry of Education, Japan.

(*Received, 7th February* 1979; *Com.* 122.)

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