^{15}N NMR of Sterically Distorted Cyanomet Haems

Gladys Avilés and Chi K. Chang*

Department of Chemistry, Michigan State University, East Lansing, Michigan 48824, USA

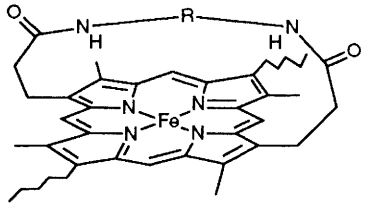
Sterically distorted Fe–CN bonds created by 'strapped' iron(III) porphyrins exhibit a large upfield shift in the cyanide ^{15}N NMR signal, making the steric effect on the distal side an important consideration in interpreting the ^{15}N NMR spectra of cyanomet haemoproteins.

Changes in the chemical environment of the ^{15}N nucleus cause an unusually large response in its NMR chemical shift. Variations in protonation state, hydrogen bonding and metal ligation typically produce large shifts.[1] ^{15}N NMR spectra of the cyanomet form of various haemoproteins have been reported[2–7] and show a range between δ 1055 and 412 (from nitrate reference). There appear to be at least two factors that influence the C^{15}N chemical shift. In the study of dicyano-Fe^{III} protoporphyrin, Goff[5] demonstrated that hydrogen bonding can cause an 82 ppm upfield shift. Goff also studied the mixed imidazole cyano-Fe^{III} protoporphyrin, which showed an upfield shift of 277 ppm associated with the deprotonation of the *trans*-imidazole ligand. These results demonstrate that *trans* ligand and proton donor clearly play major roles in dictating the C^{15}N signal. A third factor in cyanide binding that may influence ^{15}N NMR spectra is the geometric distortion of the cyanomet complex. Up to now the steric effect on the C^{15}N chemical shift was unknown. The steric hindrance by distal residues in the haem pocket has a major effect on the ability of haemoproteins to bind ligands.[8,9] It is, therefore, of importance to examine such effects using synthetic models. Previously, we have used 'strapped' haems equipped with a covalently linked 13-, 14-, or 15-atom hydrocarbon chain across one face of the haem to illustrate the distal steric effect on CO and O_2 bindings as well as on the stretching vibrations of the Fe–C–O, Fe–C–N and Mn–N–O complexes.[9–12] In this communication, we demonstrate the significant shift of the C^{15}N signal produced by these sterically hindered haems, making this study the first documented case of such an effect.

The ^{15}N NMR spectrum of dicyano-Fe^{III} porphyrins† was measured at 22 °C in dimethyl sulfoxide (DMSO) (3–5 mmol dm^{-3} in haem with a tenfold excess of KC^{15}N). The NMR spectrum of (C^{15}N$^-$)$_2$Fe^{III} **NSP** exhibits two peaks, one at δ −100 (free C^{15}N$^-$) and the other at δ 743 corresponding to the bis-cyano complex. The NMR of the strapped porphyrin series typically has three peaks. One corresponds to the free cyanide (δ − 100) and the other two, to the two bound cyanide ligands. For the Fe**SP15** the two peaks appear at δ 686 and 669 (Table 1). By decreasing the strap length there is a consistent upfield shift for both cyanide peaks. The (C^{15}N$^-$)$_2$Fe**SP12** shows peaks at δ 503 and 386, an upfield shift of 239 and 357 ppm with respect to the no strap Fe**NSP**. In addition, the spacing between the two peaks of a given strapped porphyrin becomes larger as the strap becomes shorter (Fig. 1).

The changes in the ^{15}N isotropic shift of cyano-haem complexes arise from interactions between the Fe^{III} and the C^{15}N$^-$, taking place through bond (contact contributions) and through space (dipolar contributions). Cyanide is a strong-field ligand that ensures a low spin state for the iron(III) haem. In this configuration, d_{xz} and d_{yz} orbitals are above d_{xy}, separated by an energy gap μ. The bending of one cyano ligand brought about by shortening of the strap should reduce μ by lowering d_{xz} and d_{yz} relative to d_{xy}. Loew[3] showed that a decrease in μ causes a decrease in the magnetic anistropy, which results in an upfield shift for both cyano nitrogens bound to the iron. While such dipolar contribution is a part of the overall shift, the principal effect is spin delocalization from the iron to the ^{15}N atom. For the bent or tilt cyano group, because of poorer σ-orbital overlapping with the Fe^{III}, the lower effectiveness in unpaired spin transfer should cause a major upfield shift. Morishima and coworkers[7] suggested the possibility that a bent or tilt Fe–C–N bond may enhance the overlap between the metal dπ and cyanide pπ orbital and predicted a downfield shift. Our observation that both C^{15}N$^-$ ligands exhibit significant upfield shifts when one ligand is distorted, clearly demonstrates that such π-interactions cannot be an important mechanism to increase the level of spin transfer to the nitrogen atom.

In mixed ligand complexes, prepared by addition of 1.5 equiv. each of 1,5-dicyclohexylimidazole (DCIm) and KC^{15}N to Fe^{III} porphyrins in DMSO, the spectra exhibit only two peaks: the free cyanide and the one corresponding to the monocyano complex. Since the bulky 1,5-dicyclohexylimidazole is unable to form a hexacoordinate complex with any of the strapped Fe^{III} porphyrins,[9] it is virtually certain that the cyanide is bound on the strapped side in these mixed complexes. The C^{15}N signal for the mixed ligand complexes appears further downfield than for the dicyano complexes. The *trans* effect may be understood by the better π-accepting ability of the imidazole, which would remove electron density from the π-antibonding C–N orbital with subsequent strengthening of the C–N bond and more efficient spin transfer to the nitrogen.[5] With the strapped model compounds, a 150 ppm upfield shift was observed on going from the Fe**NSP** to Fe**SP12**. While the magnitude of the shift here is less drastic



SP12 R = $-(CH_2)_4-$

SP13 R = $-(CH_2)_5-$

SP14 R = $-(CH_2)_6-$

SP15 R = $-(CH_2)_7-$

NSP R = $-(CH_2)_3CH_3$, $CH_3(CH_2)_3-$

Table 1 ^{15}N NMR chemical shifts for strapped porphyrins

Porphyrin complex	^{15}N chemical shift (δ in/ppm)
Fe**NSP**(CN$^-$)$_2$	743.0
Fe**SP15**(CN$^-$)$_2$	686.0; 669.5
Fe**SP14**(CN$^-$)$_2$	636.9; 582.9
Fe**SP13**(CN$^-$)$_2$	607.2; 522.6
Fe**SP12**(CN$^-$)$_2$	503.6; 386.3
Fe**NSP**(CN$^-$)(DCIm)	984.5
Fe**SP15**(CN$^-$)(DCIm)	945.0
Fe**SP12**(CN$^-$)(DCIm)	834.4

† The hitherto unreported **SP12** was synthesized by coupling putrescine and the porphyrin diacid chloride. All new compounds have been adequately characterized by spectroscopic analyses.

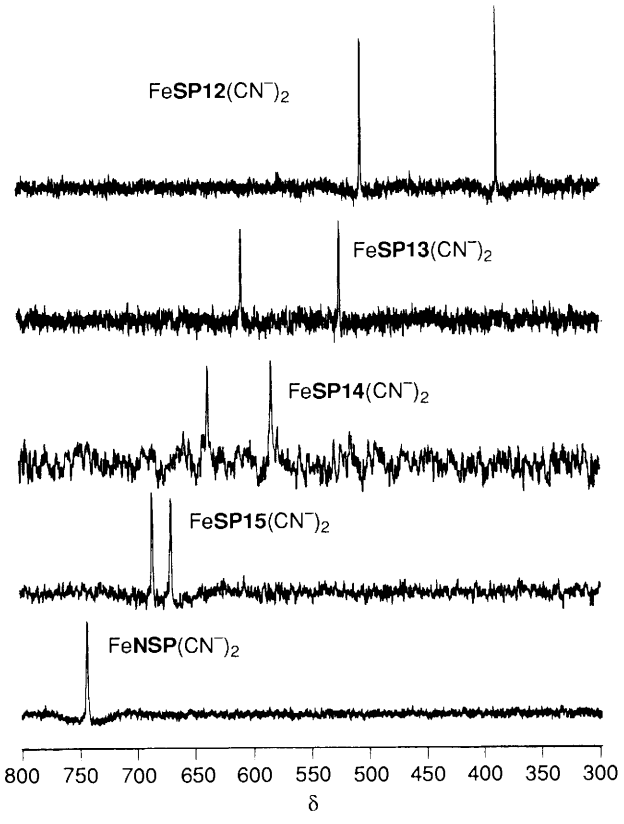


Fig. 1 ^{15}N NMR spectra of the dicyano-Fe^{III} complex of various porphyrins, in DMSO, with chemical shift referenced to KNO_3. Measurements were made on a Varian VXR-500, in 8 mm tubes with typically 30 000 to 60 000 scans.

than for the bis-cyanide series, the trend is undoubtedly the same. The smaller range of shift should not be surprising since the Fe–CN binding is stronger in this series and perhaps the spin transfer from Fe to N is less sensitive to geometric restrictions.

Cyanide is often used as a reporter group to probe the haem pocket environment. Haemoglobin, myoglobin and cytochrome c have the signal range between δ 1055 and 847 while horseradish peroxidase, lactoperoxidase, chloroperoxidase and cytochrome P-450 appear in a much upfield region of δ 412–578.[4–7] It has been shown that a negatively charged *trans* ligand and hydrogen bonding at the distal axial cyanide ligand might induce a large upfield shift.[4,5] These are the common structural features believed to be important for scission of the O–O bond of peroxide in the peroxidases. We now have shown that any steric constraint that renders the Fe–C–N linkage off axis of the haem normal would also produce a significant upfield shift. However, because the shift is in the same direction as those caused by the other two factors, it is not obvious as to what extent the large upfield shift observed in cyanomet peroxidase is due to steric perturbations. Having the well-defined model compounds as benchmarks, future interpretations of the $C^{15}N$ NMR of biological systems will have to include the consideration of steric effects.

This work was supported in part by the National Institutes of Health grant GM36520.

Received, 6th March 1991; Com. 1/01055G.
Received in revised form 1st October 1991

References

1 K. Kanamori and J. D. Roberts, *Acc. Chem. Res.*, 1983, **16**, 35.
2 I. Morishima, T. Inubushi, S. Neya, S. Ogawa and T. Yonezawa, *Biochem. Biophys., Res. Commun.*, 1977, **78**, 739.
3 I. Morishima and T. Inubushi, *J. Chem. Soc., Chem. Commun.*, 1977, 616.
4 I. Morishima and T. Inubushi, *J. Am. Chem. Soc.*, 1978, **100**, 3568.
5 D. V. Behere, E. Gonzalez-Vergara and H. M. Goff, *Biochim. Biophys. Acta*, 1985, **832**, 319.
6 D. V. Behere, D. C. Ales and H. M. Goff, *Biochim. Biophys. Acta*, 1986, **871**, 285.
7 Y. Shiro, T. Iizuka, R. Makino, Y. Ishimura and I. Morishima, *J. Am. Chem. Soc.*, 1989, **111**, 7707.
8 K. Moffat, J. F. Deatherage and D. W. Seybert, *Science*, 1979, **206**, 1035.
9 B. Ward, C.-B. Wang and C. K. Chang, *J. Am. Chem. Soc.*, 1981, **103**, 5236.
10 N.-T. Yu, E. A. Kerr, B. Ward and C. K. Chang, *Biochemistry*, 1983, **22**, 4534.
11 T. Tanaka, N.-T. Yu and C. K. Chang, *Biophys. J.*, 1987, **52**, 801.
12 N.-T. Yu, S.-H. Lin, C. K. Chang and K. Gersonde, *Biophys. J.*, 1989, **55**, 1137.
13 G. M. H. Loew, *Biophys. J.*, 1970, **10**, 196.
14 G. N. LaMar, J. S. De Ropp, V. P. Chacko, J. D. Satterlee and J. E. Erman, *Biochim. Biophys. Acta.*, 1982, **708**, 317.