^{57}Fe N.M.R. Spectroscopy of Carbonyl Iron Porphyrins. A New Probe for Heme–Ligand Interactions

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The ^{57}Fe n.m.r. chemical shifts and complex formation constants of some carbonyl iron porphyrin complexes have been measured; the ^{57}Fe n.m.r. chemical shift shows a large upfield shift with increased binding strength between the axial nitrogen ligand and the iron atom.

N.m.r. studies of paramagnetic porphyrin complexes as well as paramagnetic heme proteins have provided a wealth of information about heme–ligand interactions.[1] A sensitive probe for diamagnetic heme models as well as heme proteins, that can provide detailed structural information, seems, however, to be lacking. ^{1}H n.m.r. chemical shifts are affected by ring currents but do not lend themselves to detailed structural interpretations of the heme environment. The ^{13}C n.m.r. shifts of ^{13}C labelled carbonyl groups in model hemes and heme proteins cover roughly 10 p.p.m. There is, however, no simple correlation between chemical shift and chemical structure. The ^{13}C n.m.r. chemical shifts of the carbonyl ligand in the Fe(protoporphyrin-IX)(CO)(pyridine), Fe(PP-IX)(CO)(py), complexes do not differ measurably when the 4-substituted pyridine derivative is varied. Very few ^{15}N n.m.r. studies of iron porphyrins have been published. The potentially most useful nucleus is ^{57}Fe, with spin of 1/2, but it has not been widely used because of its low sensitivity and low natural abundance. Also, long relaxation times have been quoted as being an experimental difficulty. Recently,

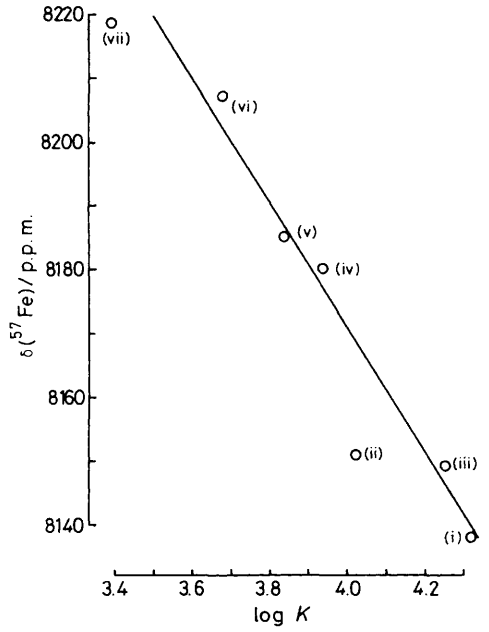

Figure 1. Plot of the ^{57}Fe n.m.r. chemical shift *vs.* log *K* for the equilibrium Fe(PPIX)(CO) + B $\rightleftharpoons$ Fe(PPIX)(CO)(B) in DMF–H_2O (90 : 10) at 25 °C. Bases are (i) = 4-*N*,*N*-dimethylaminopyridine, (ii) = 2-methylimidazole, (iii) = imidazole, (iv) = 4-methylpyridine, (v) = pyridine, (vi) = 4-acetylpyridine, (vii) = 4-cyanopyridine.

however, we showed that the chemical shift scale covers more than 11 000 p.p.m. and that the dominant relaxation mechanism is chemical shift anisotropy (c.s.a.). The use of isotopically enriched materials and high fields, then, partially offsets the low sensitivity and shortens relaxation times. Recently, we and others,[3–5] demonstrated the usefulness of ^{57}Fe n.m.r. spectroscopy in studies of a heme protein, myoglobin–CO (MbCO). In the present communication the significance and some limitations of the ^{57}Fe chemical shift as a structural probe of iron ligand interactions are demonstrated.

The ^{57}Fe n.m.r. experiments were run at 13.055 MHz on a Varian XL-400 n.m.r. system operating at 9.4 T. A solenoid coil probe from Cryomagnet System Inc. with deuterium lock and temperature control was used with 15 mm non-spinning sample tubes, total sample volume 3.1 ml. The 90° pulse width was 65 μs. Typically, 10 000—50 000 transients were recorded using 90° pulses, 0.25 s pulse repetition time, and 5 kHz sweep width. The chemical shifts were referenced relative to external $Fe(CO)_5$ using n-butyl ferrocene (80% in [$^2H_6$]acetone) as a secondary standard, and are reproducible to well within 1 p.p.m. Iron porphyrins, enriched in ^{57}Fe, were prepared in the standard way. Reagents were distilled or recrystallized according to standard procedures. N.m.r. solvents were used as supplied. The sample concentration was 0.005 M in dimethylformamide (DMF)–D_2O 90 : 10 (v/v). ^{1}H n.m.r. spectra were recorded at 400 MHz on a Varian XL 400 operating at 9.4 T. A pulse repetition time of 60 s and an acquisition time of 2 s was used to ensure complete relaxation. Equilibrium constants were measured competitively with 4-*N*,*N*-dimethylaminopyridine (DMAP) and another base competing for the sixth, axial position of the iron atom. Measurement of the integral of the N-methyl resonance of bound DMAP (slow exchange limit) relative to that of the vinyl protons of the iron protoporphyrin provided a measure of the ratio of Fe(PPIX)(CO)(DMAP) to the total amount of porphyrin in solution. From the carefully weighed amounts of reactants the equilibrium constant for the exchange reaction [equation (1)] could be calculated and hence the equilibrium constant for equilibrium (2), if the separately determined equilibrium constant for DMAP was used. Here, B is the axial nitrogen base.

$$\text{Fe(PPIX)(CO)(B)} + \text{DMAP} \rightleftharpoons \text{Fe(PPIX)(CO)(DMAP)} + \text{B} \quad (1)$$

$$\text{Fe(PPIX)(CO)} + \text{B} \rightleftharpoons \text{Fe(PPIX)(CO)(B)} \quad (2)$$

Determination of equilibrium (2) for B = DMAP was done spectrophotometrically at 25 °C by titration of a CO-saturated solution of Fe(PPIX) with base. The uncertainty introduced by the possibility of having two equivalents of CO bind to the iron is probably very small, but in any case it does not affect the relative magnitude of the equilibrium constants. The experimental titration curve does not suggest that Fe(PPIX)$(DMAP)_2$ is present.

The dependence of the ^{57}Fe n.m.r. chemical shift of several carbonyl iron porphyrin complexes on log *K* for the corresponding complex formation constants is shown in Figure 1. The correlation is excellent for the structurally similar pyridines and for imidazole (Im), but a small deviation is found for the sterically hindered 2-methylimidazole, (2-MeIm). This compares well with the results of others using other techniques.[6] The chemical shift range covered is close to 80 p.p.m. and if the chemical shifts for the imidazolate anion complex[5] and $MbCO^{3-}$[5] are included, almost 200 p.p.m. are covered. This provides a sizable range for the observation of changes in iron–ligand interactions. Obviously, the ^{57}Fe chemical shift can also be plotted *vs.* the pK_a of the conjugate acids of the bases and for the substituents with similar results. Therefore, electron density donation through σ bonds is important for ^{57}Fe n.m.r. However, it is known that orbital overlap is significant in imidazole binding to the iron atom and the deviation observed for 2-MeIm may be due to changes in overlap as a result of steric hindrance. This introduces some uncertainty in the interpretation of chemical shifts. A change in binding interaction may take place without an effect on the chemical shift if there is a simultaneous change in heme–ligand geometry. The magnitude of this effect is not yet clear.

Another factor that influences chemical shifts is the solvent. Changing the solvent from 4-methylpyridine (4-MePy) to DMF–D_2O causes a 32 p.p.m. upfield shift for Fe(PPIX)(CO)(4-MePy). Interpretation of iron–ligand binding strength in MbCO on the basis of a comparison between MbCO and Fe(PPIX)(CO)(Im) chemical shifts may therefore be difficult considering the drastic change in environment.[5] Also, it has not yet been demonstrated how tilting of the carbonyl group relative to the porphyrin plane will affect the chemical shift. The temperature dependence of the chemical shift must also be accounted for. In spite of these effects it seems logical to interpret the difference in chemical shift between the imidazole complex and MbCO, amounting to almost 80 p.p.m., as being due to stronger binding in the complex than in the protein.

These results demonstrate the feasibility of using ^{57}Fe n.m.r. chemical shifts as a structural probe in heme models and heme porphyrins, provided some precautions are taken in interpreting the results. The chemical shift range is large and chemical shift changes may be interpreted in terms of ligand binding changes. The sensitivity of the chemical shifts to these interactions suggest that ^{57}Fe n.m.r. spectroscopy may be very useful in studying allosteric effects in heme proteins.

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References

1 H. M. Goff, in 'Iron Porphyrins', eds. A. B. P. Lever and H. B. Gray, Addison–Wesley Publishing Co., Reading, Mass., 1983, ch. 4.
2 L. Baltzer, E. D. Becker, B. A. Averill, J. M. Hutchinson, and O. A. Gansow, *J. Am. Chem. Soc.*, 1984, **106**, 2444.
3 L. Baltzer, E. D. Becker, R. G. Tschudin, and O. A. Gansow, *J. Chem. Soc., Chem. Commun.*, 1985, 1040.
4 H. C. Lee, J. K. Gand, T. L. Brown, and E. Oldfield, *J. Am. Chem. Soc.*, 1985, **107**, 4087.
5 G. N. LaMar, C. M. Dellinger, and S. S. Sankar, *Biochem. Biophys. Res. Commun.*, 1985, **128**, 628.
6 J. P. Collman, J. I. Brauman, T. J. Collins, B. I. Iverson, G. Lang, R. B. Pettman, J. L. Sessler, and M. A. Walters, *J. Am. Chem. Soc.*, 1983, **105**, 3038.