NOTES *Bull. Chem. Soc. Jpn.*, ***64***, *3199—3201 (1991)*

^{1}H and ^{13}C NMR Relaxation Study of Iron(III) Tetraphenylporphyrin–Imidazole Complexes

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(Received June 10, 1991)

Synopsis. ^{1}H and ^{13}C nuclear relaxation of paramagnetic iron(III) tetraphenylporphyrin–imidazole complexes has been investigated in order to gain insight into the relationship between the paramagnetic relaxation rate and the electron-nucleus distance. The analysis of the nuclear spin–lattice relaxation time (T_1) in terms of the distance indicated that, for the iron porphyrin complexes used, the contribution of the electron–nucleus dipolar coupling no longer contributes significantly to the relaxation process of a proton located at more than 0.75 nm away from the iron. For the complexes studied, the electron spin–lattice relaxation time (T_{1e}) of 6.4 ps was calculated from the T_1 values measured at 26 °C.

Nuclear relaxation has been used to characterize structure and dynamics of molecules. Particularly, in the case of paramagnetic molecules, geometries of complexes in solution, determinations of coordination numbers, and calculations of correlation times have been infered from the investigation of the paramagnetic relaxation.[1—3] Nuclear spin-lattice relaxation time, T_1, in paramagnetic molecules is composed of two terms,

$$T_1^{-1}=T_{1d}^{-1}+T_{1p}^{-1}, \tag{1}$$

where T_{1d} and T_{1p} are the diamagnetic and paramagnetic spin–lattice relaxation times, respectively, and, for the nucleus in the close proximity of the unpaired electron, $T_{1d}^{-1} \ll T_{1p}^{-1}$. T_{1p} is generally expressed in terms of the dipolar and contact interactions between nucleus and unpaired electron as follows,[4—6]

$$T_{1p}^{-1}=(2/15)\gamma_I^2 g^2\beta^2 S(S+1)r^{-6}[3\tau_c/(1+\omega_I^2\tau_c^2) + 7\tau_e/(1+\omega_S^2\tau_e^2)]+(2/3)S(S+1)(A/\hbar)^2\tau_e/(1+\omega_S^2\tau_e^2) \tag{2}$$

and

$$\tau_c^{-1}=T_{1e}^{-1}+\tau_r^{-1}+\tau_{ex}^{-1},\ \tau_e^{-1}=T_{1e}^{-1}+\tau_{ex}^{-1}, \tag{3}$$

where γ_I is the gyromagnetic ratio of the nucleus under consideration and T_{1e}, τ_r, and τ_{ex} are the electron spin–lattice relaxation, the molecular tumbling, and the electron exchange times, respectively. A is the hyperfine coupling constant which is related to the unpaired spin density on the resonating nucleus and the other parameters have been defined previously.[2] The Curie spin relaxation does not contribute significantly to T_{1e} for the present complex.[7—9] In large molecules with highly resolved NMR spectra, $T_{1e} \ll \tau_r$, τ_{ex}, and at high magnetic field, $\omega_I^2 T_{1e}^2 \ll 1$ and $\omega_S^2 T_{1e}^2 > 1$, the nuclear spin–lattice relaxation rate due to the electron-nucleus dipolar coupling is given as,[9,10]

$$T_1^{-1}=(2/5)\gamma_I^2 g^2\beta^2 S(S+1)r^{-6}T_{1e} \tag{4}.$$

Therefore the relaxation rate of a nucleus depends simply on the distance from the unpaired electron and γ_I.

We have measured and analyzed the T_1 values of both ^{1}H and ^{13}C of iron(III) tetraphenylporphyrin–imidazole complexes (structures in Fig. 1). The T_{1e} value of the complex was determined from the analysis of the observed T_1 values. The relationship between the paramagnetic relaxation rate and the electron–nucleus distance is discussed.

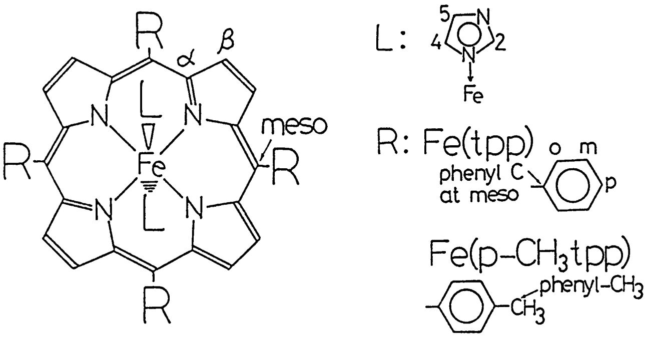

Fig. 1. Structures and numbering system of [Fe(tpp)-$(Im)_2$]Cl and [Fe(p-CH_3 tpp)$(Im)_2$]Cl.

Experimental

Iron(III) tetraphenylporphyrin, Fe(tpp), was purchased from Sigma Chemcal Co. and used without further purification. Iron(III) 5, 10, 15, 20-tetrakis (p-tolyl) porphyrin, Fe(p-CH_3 tpp), was prepared by the literature method[11] and purified using silica-gel column. Since it has been reported that the NMR spectral features of these porphyrin complexes are essentially independent of concentration (5—50 mM)[12], 10 mM iron(III) porphyrin was prepared in $CDCl_3$ with 0.2 M imidazole (Sigma) for the measurements (1 M=1 mol dm^{-3}). ^{1}H and ^{13}C NMR spectra were recorded using a JEOL GSX-270 FT-NMR spectrometer operating at ^{1}H and ^{13}C frequencies of 270 and 67.8 MHz, respectively. T_1 was determined by the inversion recovery method[13] and the experimental error is <20%. Chemical shifts are given in parts per million, ppm, downfield from the signal of tetramethylsilane, with the solvent signal as internal reference.

Results and Discussion

NMR Spectra. ^{13}C NMR spectra of 10 mM iron(III) porphyrin derivatives with 0.2 M imidazole in $CDCl_3$ at 26 °C are shown in Fig. 2. The signal assignments were reported previously[12] and have been confirmed by the T_1 measurements and two-dimensional NMR spectroscopies. The resonances for free imidazole are observed at 122 and 136 ppm as indicated by asterisk in the spectra. Relatively small signal intensity for the free imidazole resonances is due to short pulsing interval used for the measurement which selectively enhances the intensity of more rapidly relaxing paramagnetic resonances. Since it has been reported that the equilibrium

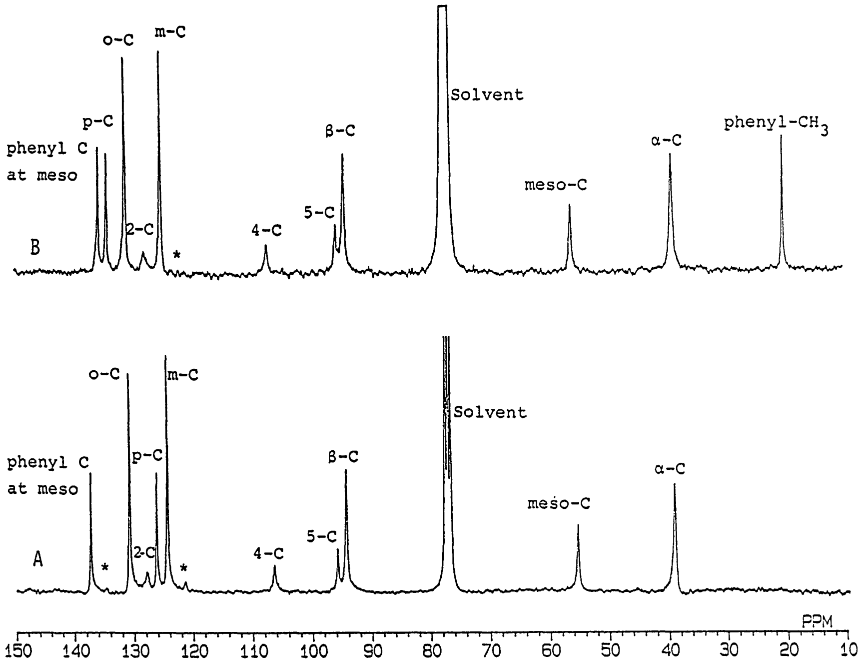

Fig. 2. 67.8 MHz ^{13}C NMR spectra of [Fe(tpp)(Im)$_2$]Cl (A) and [Fe(*p*-CH$_3$ tpp)(Im)$_2$]Cl (B) in CDCl$_3$ at 26 °C. The peak labeling corresponds to the numbering system shown in Fig. 1. The peaks indicated by * are the resonances from free imidazole.

Table 1. ^{1}H and ^{13}C Chemical Shifts and T_1s of [Fe(tpp)(Im)$_2$]Cl and [Fe(*p*-CH$_3$ tpp)(Im)$_2$]Cl in CDCl$_3$ at 26 °C

Resonance	[FeTPP(Im)$_2$]Cl		[FeTPP(*p*-CH$_3$)(Im)$_2$]Cl	
	δ/ppm	T_1/ms	δ/ppm	T_1/ms
^{1}H NMR				
β-H	−16.84	26.5	−16.72	23.2
o-H	5.17	52.1	5.10	40.5
m-H	6.28	225.2	6.03	160.3
p-H	6.22	278.6	—	—
Phenyl-CH$_3$	—	—	1.60	257.1
imidazole				
1-H	14.05	16.9	14.05	15.5
2-H	−8.45	<10	−8.35	<10
4-H	10.00	<10	10.06	<10
5-H	8.26	23.0	8.57	21.2
^{13}C NNR				
α-C	38.9	40.6	38.9	29.2
β-C	94.3	54.1	94.2	35.7
meso-C	55.2	40.7	55.9	31.7
Phenyl C at *meso*	137.3	442.5	135.6	155.7
o-C	130.7	200.8	131.0	145.3
m-C	124.3	233.6	125.0	206.2
p-C	126.1	206.6	134.2	909.1
Phenyl-CH$_3$	—	—	20.1	625.0
imidazole				
2-C	127.6	28.7	127.8	16.9
4-C	106.3	23.4	107.1	17.4
5-C	95.7	78.7	95.5	47.6

constant is of the order of 10^6 M^{-2} for imidazole binding to [Fe(tpp)]Cl in CHCl$_3$ at 25 °C,[14] the iron porphyrin should be almost completely in the bis-ligated form. In addition, the fact that both the free and bound imidazole resonances are separately observed is consistent with its slow exchange rate (ca. 10 s^{-1}).[15] ^{1}H NMR spectra of the complexes (not shown) were essentially identical to those reported previously.[16,17] The chemical shifts of ^{1}H and ^{13}C resonances are summarized in Table 1. The comparison of the shift between the two

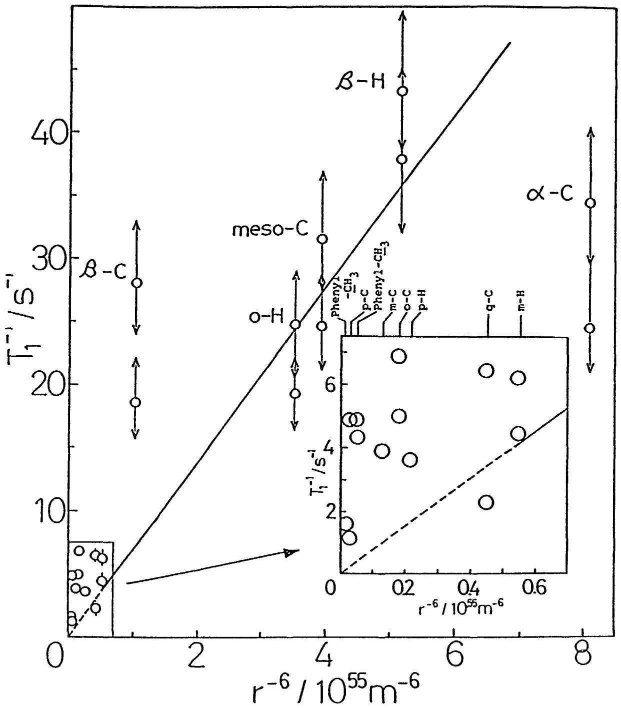

Fig. 3. The plot of T_1^{-1} against r^{-6} for ^1H of the iron porphyrin complexes studied. A straight line passing the origin was drawn with the plots for *o*- and *m*-H. For the ^{13}C data, T_1^{-1} were plotted against r^{-6} $(\gamma_C/\gamma_H)^2$. The plots near the origin was expanded in the inset.

complexes indicates that the influence of the methyl substitution on the resonance frequency is localized at the phenyl resonances.

T_1 Analysis. The observed T_1 values are included in Table 1. The T_1 values of the corresponding nuclei, except the phenyl C at *meso* and the *p*-C, between the two complexes agree within the experimental error of 20%. The difference in the T_1 value of the phenyl C at *meso* between the two complexes may be attributed to the change in the contact contribution, i.e. the second term in Eq. 2, and the presence of the unpaired spin density at this carbon has been suggested earlier.[12] The fact that the T_1 value for the *p*-C in [Fe(tpp)(Im)$_2$]Cl is considerably smaller than that in [Fe(*p*-CH$_3$ tpp)(Im)$_2$]Cl indicates the significance of the attached ^1H for the relaxation of this carbon. Relatively large difference in the T_1 value of the bound imidazole carbons between the two iron porphyrin complexes may be attributed to the difference in the contact contribution.

The relaxation rates of the iron porphyrin ^1H resonances are plotted against r^{-6} in Fig. 3. The plots for the ^{13}C resonances are also included in the figure in the form of T_1^{-1} vs. r^{-6} $(\gamma_C/\gamma_H)^2$, because Eq. 4 predicts that the ratio of the T_1^{-1} values for the proton and carbon located equidistantly from the metal is $(\gamma_C/\gamma_H)^2$. Since it has been already shown that the unpaired electron density on the *o*-, *m*-, and *p*-C in these complexes is negligible,[12] a straight line passing through the origin was drawn with the plots for the phenyl ^1H resonances. The T_{1e} value can then be calculated from the slope of this line using Eq, 4. The T_{1e} value of 6.4 ps was obtained and is well within the typical value for iron(III) low spin complexes (2—8 ps).[18] The interpretation of the relaxation rates for the porphyrin ring nuclei requires the knowledge about the spin density of the porphyrin skeleton. The plots in Fig. 3 do not converge to the origin as $r^{-6}\rightarrow 0$, but the relaxation rates are larger than those expected from Eq. 4 when $r^{-6}<0.5\times 10^{55} m^{-6}$. These results reflect that the paramagnetic dipolar relaxation mechanism is not a predominant relaxation process for a proton located more than 0.75 nm away from the iron in the present complexes and the distance is expected to be reduced with increasing the molecular weight. It has been shown that the interpretation of the nuclear relaxation in paramagnetic hemoproteins, based on Eq. 4, tends to underestimate the iron–nucleus distance and the error becomes larger for nucleus oriented further away from the paramagnetic center.[19]

References

1) T. J. Swift,"NMR of Paramagnetic Molecules; Principles and Applications," ed by G. N. La Mar, W. D. Horrocks, Jr., and R. H. Holm, Academic Press, New York (1973), Chap. 2.

2) I. Bertini and C. Luchinat,"NMR of Paramagnetic Molecules in Biological Systems," The Benjamin/Cummings Publishing Company, Inc., (1982), Chap. 2.

3) I. Bertini, C. Luchinat, and L. Messori, *Metal Ions Biol. Syst.*, **21**, 47 (1986).

4) I. Solomon, *Phys. Rev.*, **99**, 559 (1955).

5) N. Bloembergen and L. D. Morgan, *J. Chem. Phys.*, **34**, 842 (1961).

6) N. Bloembergen, *J. Chem. Phys.*, **27**, 572 (1957).

7) M. Gueron, *J. Magn. Reson.*, **19**, 58 (1975).

8) K. Wüthrich, J. Hochmann, R. M. Keller, G. Wagner, M. Brunori, and C. Giacometti, *J. Magn. Reson.*, **19**, 111 (1975).

9) A. J. Vega and D. Fiat, *Mol. Phys.*, **31**, 347 (1976).

10) S. W. Unger, T. Jue, and G. N. La Mar, *J. Magn. Reson.*, **61**, 448 (1985).

11) R. G. Little, J. A. Anton, P. A. Loach, and J. A. Ibers, *J. Heterocyl. Chem.*, **12**, 343 (1975).

12) H. M. Goff, *J. Am. Chem. Soc.*, **103**, 3714 (1981).

13) R. L. Vold, J. S. Waugh, M. P. Klein, and D. E. Phelps, *J. Chem. Phys.*, **48**, 3831 (1968).

14) F. A. Walker, M. -W. Lo, and M. T. Ree, *J. Am. Chem. Soc.*, **98**, 5552 (1976).

15) J. D. Satterlee, G. N. La Mar, and T. J. Bold, *J. Am. Chem. Soc.*, **99**, 1088 (1977).

16) G. N. La Mar and F. A. Walker, *J. Am. Chem. Soc.*, **95**, 1782 (1973).

17) G. N. La Mar and F. A. Walker, "The Porphyrins," ed by D. Dolphin, Academic Press, New York (1979), Vol. IV, Chap. 2.

18) G. N. La Mar, "Biological Applications of Magnetic Resonance," ed. by R. G. Shulman, Academic Press, New York (1979), Chap.7.

19) Y. Yamamoto, K. Iwafune, N. Nanai, A. Ōsawa, R. Chûjô, and T. Suzuki, *Eur. J. Biochem.*, **198**, 299 (1991).