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^{57}Fe NMR OF IRON(II) LOW SPIN HEMES

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^{57}Fe NMR signals were obtained by using a steady-state free precession method for a bis(pyridine) complex of [$^{15}N_4$,^{57}Fe]-meso-tetraphenylporphine and a bis(pyrrolidine) complex of [$^{15}N_4$,^{57}Fe]-meso-p-tolylporphine at 7260 and 7340 ppm low field from that of pentacarbonyliron(0) ($Fe(CO)_5$), respectively.

Many physicochemical techniques have been applied to hemoproteins to ellucidate electronic structures of heme iron. However, experimental determination of the electronic structures of oxygenated hemes awaits further examinations.$^{1)}$ We have started ^{57}Fe NMR studies of heme complexes to determine the electronic structures of hemes directly from experiments. The observation of ^{57}Fe NMR is hampered by the extremely poor sensitivity (0.2% of the ^{13}C), the low natural abundance (2.2%) and the possibly long relaxation time. The ^{57}Fe NMR resonance of $Fe(CO)_5$ and ferrocene (bis(η-cyclopentadienyl)iron(II)) could be detected in the pure liquid or a concentrated solution using a steady-state free precession technique and the quadriga Fourier transform technique.$^{2-5)}$ Chemical shifts in the NMR spectra of ^{57}Fe NMR for various iron complexes have been reported recently by a direct detection.$^{6)}$ These include data of iron complexes coordinated only with nuclei with rare magnetic moments. We have succeeded in determining chemical shifts in the ^{57}Fe NMR for heme complexes in which ^{57}Fe nuclei were coordinated by nuclei with magnetic moments (^{15}N, and ^{14}N). This is the first detection of ^{57}Fe NMR for the iron complexes having ligand nuclei with non-zero magnetic moments.

The ^{57}Fe NMR was measured with a Bruker CXP-300 NMR spectrometer incorporated with a Bruker ^{57}Fe probe which can be tuned between 10.2 and 9.2 MHz. The static field B_0 = 7.05 T (corresponding to a 300 MHz proton Larmor frequency) gave ≃9.7 MHz resonance frequencies for the ^{57}Fe nuclei. The 90° pulse width was 180 μs which affords 10.1×10^{-4} T H_1 field, when the transmitter voltage was 200 V (peak to peak value). The observations were carried out under internal ^{2}H lock using deuterated solvents except for $Fe(CO)_5$ which was used as neat liquid. A Bruker temperature controller (B-VT-1000) was employed and calibrated by measuring the frequency differences between the resonances of ethyl alcohol.$^{7)}$ The ^{57}Fe NMR spectra for hemes in neat pyridine-d_5 or pyrrolidine-C^2HCl_3 (50 : 50, v/v) solutions were measured at the spinning rate of 18 Hz with sample tubes of 15 mm diameters. ^{15}N-enriched α,β,γ,δ-meso-tetraphenylporphine (TPP) and α,β,γ,δ-meso-tetra-p-tolylporphine (TTP) were prepared from ^{15}N-labelled pyrrole (Commisariat A L'energie Atomique, Saclay, France, 95% enrichment), and benzaldehyde or p-tolylaldehyde by the usual method.8) Incorporation of ^{57}Fe (Harwell, Oxon, Great Britain, 95.45% enrichment) was accomplished by Rothemund's method.9) Bis(pyridine) and bis(pyrrolidine) Fe^{II} low spin complexes of [$^{15}N_4$,^{57}Fe]-TPP or [$^{15}N_4$,^{57}Fe]-TTP were prepared by the reported methods.10-12) $Fe(CO)_5$ was purchased from Strem Chemicals, Inc., (Massachusetts, USA) and ferrocene and potassium hexacyanoferrate(II) ($K_4[Fe(CN)_6]$) were obtained from Wako Chemicals, Inc. as their guaranteed grade reagents. Benzene-d_6, and pyridine-d_5 were purchased from E. Merk Japan Ltd. and pyrrolidine was obtained from Wako Chemicals, Inc.

Since the chemical shifts of ^{57}Fe NMR of iron complexes were anticipated to be fairly large, the sweep-width should be taken as wide as possible. However, because sweep-width larger than 10×10^3 Hz gave artificial signals, we had to measure the spectra with rather small sweep-width of 8×10^3 Hz. We covered the chemical shift of 10000 ppm lower field than that of $Fe(CO)_5$ by variation of carrier wave frequency. The ^{57}Fe NMR observation using the usual one pulse FT method could not afford any significant resonances with enough signal to noise ratios (S/N's). This may be partly due to the very short apparent T_2^* value which describes the decay of the NMR signal due to inhomogeneity of the field B_0, chemical exchanges and others. As discussed by Schwenk et al. if T_2^* is much shorter than T_1 and T_2, a large loss of NMR signal will result.2-6) A steady-state free precession of the spins is achieved by applying periodic and coherent pulses to the probe sample. After a time, which is long compared with the relaxation time T_1, a stationary motion of the magnetization has built up. At the end of the free precession, the resulting transverse component of the magnetization induces the NMR signal. The pulse period may be short compared with T_1 and T_2. Apart from the time for RF pulses, no waiting time for the relaxation is necessary, and any loss of signal due to the short T_2^* time can be avoided, independent of the relaxation times T_1 and T_2. As a references of S/N and observation methods, the ^{57}Fe NMR of ferrocene was measured by both Schwenk's method and the conventional one pulse FT method. Schwenk's method yielded the ^{57}Fe NMR signal at the frequency (9.73056 MHz) with S/N of about 3 after two hours of observation (Fig. 1). Compared with this, the one pulse method failed to give any meaningful ^{57}Fe NMR signals more than a noise level. From the comparison of ^{57}Fe nucleus concentrations which were 4.5×10^{-2} and 2.2×10^{-2} mol dm^{-3} for bispyridine-[$^{15}N_4$,^{57}Fe]-TPP and ferrocene, respectively, the ^{57}Fe NMR signal for the former should be observed within one hour if the half linewidths were similar among them.

From the spin-spin coupling constant between ^{57}Fe and ^{15}N (≈8 Hz),13) the ^{57}Fe NMR linewidth for the heme can be evaluated around 40 Hz as described below. The ^{57}Fe NMR signal will be split into five components by the spin coupling between ^{57}Fe and four ^{15}N, and the axial two ^{14}N nuclei further separate them into totally fifteen lines. The spin-spin coupling constant between ^{57}Fe and ^{15}N was determined to be ≈8 Hz from ^{15}N NMR observation of bis(pyridine)-[$^{15}N_4$,^{57}Fe]-TPP13) as mentioned above and that between ^{57}Fe and ^{14}N will be estimated to be around 6 Hz.[14] Schwenk's method gave the ^{57}Fe NMR signal after about twenty hours of accumulation as shown in Fig. 2 for bis(pyridine)-[$^{15}N_4$, ^{57}Fe]-TPP. The spin-spin coupling could not be resolved in the spectra. This may be due to the limitation of the measuring method which needs the small memory size and the wide sweep-width to make fast repetition possible. Schwenk's method was applied for the bis(pyrrolidine) complex of [$^{15}N_4$, ^{57}Fe]-TTP, which yielded the ^{57}Fe NMR spectrum similar to that of Fig. 2. Observed ^{57}Fe NMR chemical shift values for the above heme complexes as well as those for several other complexes are summarized in Table 1.[15]

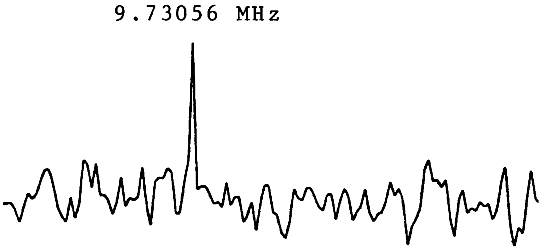

Fig. 1. The ^{57}Fe NMR spectrum of ferrocene obtained by Schwenk's method. The 22.2° pulses were applied periodically (30 Hz) to the ferrocene C_6H_6-d_6 solution (1 mol dm^{-3}) at 302 K.

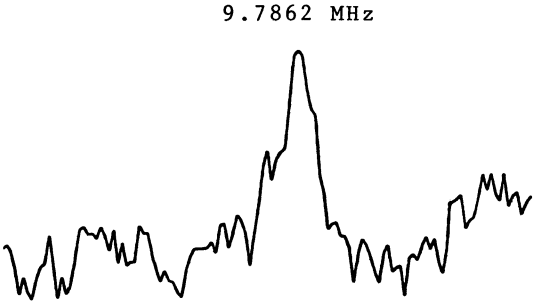

Fig. 2. The ^{57}Fe NMR spectrum of [$^{15}N_4$, ^{57}Fe]-TPP(py)$_2$ obtained by Schwenk's method. The 22.2° pulses were applied periodically (30 Hz) to the heme solution (4.6 x 10^{-2} mol dm^{-3}) at 302 K.

Table 1. ^{57}Fe NMR for Various Complexes

Complex	Solvent	Field Reference $\nu(^{57}Fe)/\nu(^{73}Ge)$	Chemical Shift/ppm
$Fe(II)TTP(Pyrr)_2$ a)	Pyrr-$CDCl_3$	0.93497 c)	7341
$Fe(II)TPP(Py)_2$ b)	Py-d_5	0.93489 c)	7258
$K_4[Fe(CN)_6]$	D_2O	0.93047 c)	2495
Ferrocene	C_6D_6	0.929576 d)	1531
$Fe(CO)_5$	Neat	0.928155 d)	0

a) Pyrr : Pyrrolidine. b) Py : Pyridine. c) Values obtained from Schwenk's method. d) Values obtained from one pulse method with enough accumulation.

Since it takes very long time to get ^{57}Fe NMR signal of $Fe(CO)_5$, it is not advisable to refer the ^{57}Fe frequencies to that of a ^{57}Fe standard. The ^{73}Ge NMR frequency of $GeCl_4$ in a 90% C^2HCl_3 solution (10.46774 MHz), which is observable in few minutes with the same probe, and which is referred to the Larmor frequency of ^2H in ^2H_2O with high accuracy, was used as a standard for the field reference.[6]

Large chemical shift values observed for the ^{57}Fe nuclei are consistent with those for other transition metal nuclei. The theory on ^{59}Co NMR chemical shift in Co^{III} complexes has been described by Griffith and Orgel,[16] Freeman et al.,[17] Betteridge and Goldings,[18] and LaRossa and Brown.[19] The chemical shifts were related to ligand field strengths of the bound ligands and the energies of the electronic d-d transitions. An increasing ligand field strength results in an increase in energy separation between the ground and excited states, and, therefore, leads to decreases in both residual paramagnetism and downfield chemical shifts. The above chemical shift theory should be applied carefully to complexes with different ligand field symmetries. Comparison among the complexes with a similar symmetry indicates somewhat larger ligand field strength for $[^{15}N_4,{}^{57}Fe]$-TPP$(Py)_2$ than that for $[^{15}N_4,{}^{57}Fe]$-TTP$(Pyrr)_2$ from the difference in the downfield chemical shifts in the ^{57}Fe NMR signals.

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