# $^{57}$Fe N.M.R. Spectroscopy of Heme Proteins: Chemical Shift Anisotropy and Relaxation Parameters of Carbonylmyoglobin

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The first observation of $^{57}$Fe n.m.r. signals in a protein, carbonylmyoglobin, is reported, together with a determination of the $^{57}$Fe relaxation times and chemical shift anisotropy.

Extensive n.m.r. studies of heme proteins and model compounds, such as porphyrins, have been undertaken with $^{1}$H and $^{13}$C, and to some extent $^{15}$N.[1] $^{57}$Fe, with a spin of 1/2 and a large range of chemical shifts that are sensitive to substituent effects,[2,3] has been little studied because of its extremely low sensitivity ($7.4 \times 10^{-7}$ that of $^{1}$H at natural abundance, 2.2%). Recently, however, $^{57}$Fe n.m.r. studies of two porphyrins, enriched to about 90%, have been reported.[3,4] We present here the first $^{57}$Fe n.m.r. data on a heme protein, carbonylmyoglobin (MbCO).

The $^{57}$Fe n.m.r. experiments were run at 16.326 MHz on a Nicolet NT-500 n.m.r. system operating at 11.74 T. A home-built solenoidal coil probe, with deuterium lock and temperature control, was used with 10 mm diameter non-spinning sample tubes. Observations were made with 30 μs pulsewidths (90° pulse ≡35 μs), a spectral width of 10 kHz, and a pulse repetition time of 51 ms. Myoglobin (Sigma) was reconstituted with $^{57}$Fe protoporphyrin-IX (PP-IX) according to standard procedures. The sample used was 1.1 ml of 10 mM MbCO, enriched to 90% in $^{57}$Fe, at pH 7 with 25% $D_2O$ in $H_2O$ as solvent. Temperature was maintained at 15 °C (± ~0.5 °C).

Figure 1 shows the $^{57}$Fe n.m.r.spectrum of MbCO, acquired in a period of 10 h under the conditions described. A line-broadening of 50 Hz was applied. The chemical shift of 8234 p.p.m., relative to $Fe(CO)_5$ as a reference, compares with values of 8211 p.p.m. for Fe(PP-IX)(CO)(py) in pyridine (py)[3] and about 7300 p.p.m. for two other porphyrins.[4] After correction for the line-broadening that was deliberately introduced and for broadening due to change in chemical shift with temperature fluctuation, the true linewidth is about 100 (±10) Hz, corresponding to a $T_2$ of about 3.2 ms. From a progressive saturation experiment (repetition times of 17, 51, and 102 ms), we can estimate a very rough value of $T_1$ of 30 ms.

For $^{57}$Fe in t-butylferrocene we showed previously that relaxation at 16.3 MHz is due almost solely to chemical shift anisotropy (C.S.A.).[3] It is reasonable to assume the C.S.A. mechanism to be dominant here too, since little else could relax the $^{57}$Fe effectively in a diamagnetic protein.[5] From the standard equations for $T_1$ and $T_2$ determined by C.S.A,[5] we can calculate the rotational correlation time $\tau_c = 35$ ns and the C.S.A. = 3050 p.p.m., at 15 °C. (A value of $\tau_c = 19$ ns for MbCO at 36 °C from $^{13}$C n.m.r. studies has been reported.[6]) For comparison, the model compound $^{57}$Fe(PP-IX)(CO)(py) has a $^{57}$Fe $T_1$ of 0.17 s at 23 °C,[3] and a $T_1$ of a *meso* $^{13}$C of 0.18 s. From the usual relation for dipolar relaxation of the $^{13}$C nucleus,[5] a value of $\tau_c = 2.4 \times 10^{-10}$ s can be obtained, and from that a C.S.A. of 4200 p.p.m. calculated. Within the several experimental errors involved, these values of C.S.A. are thus of comparable magnitude.

$^{57}$Fe n.m.r. chemical shifts are highly temperature dependent. From measurements in the 10—30 °C range, we estimate a temperature coefficient of chemical shift of −1.6 p.p.m./°C for MbCO, as compared with +2.3 for $Li_4Fe(CN)_6$, +0.7 for

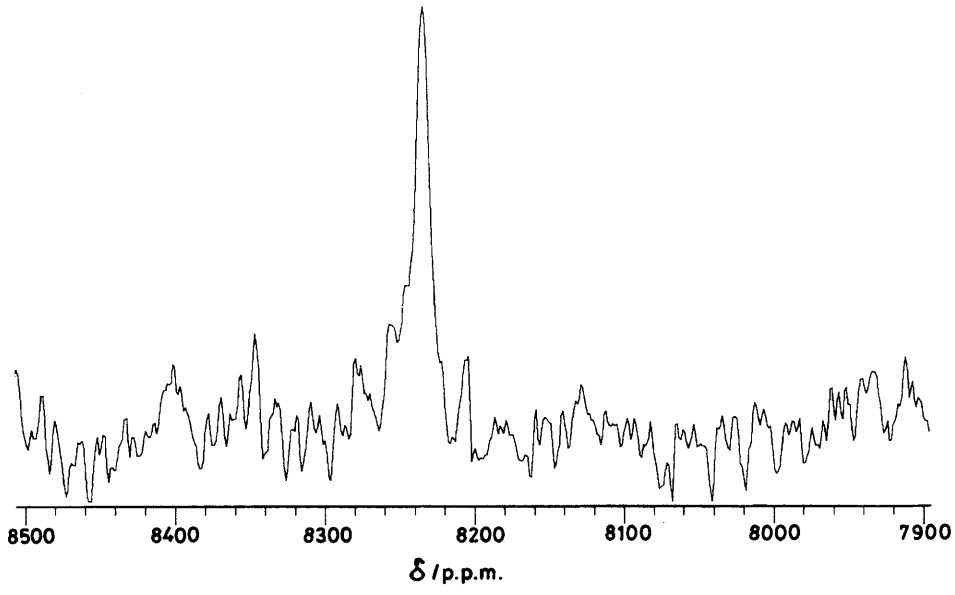


**Figure 1.** $^{57}$Fe N.m.r. spectrum of 1.1 ml of 10 mM MbCO in $H_2O$–$D_2O$ (3 : 1), pH 7, 15 °C. Total experimental time 10 h. Line-broadening 50 Hz.

t-butylferrocene, and +2.1 for Fe(bipyridyl)$_3$Cl$_2$. The negative coefficient for MbCO is interesting but may result from a combination of factors.

These results demonstrate the feasibility of $^{57}$Fe n.m.r. studies of heme proteins with enriched materials. The use of a 500 MHz spectrometer is helpful in some respects but may not be optimum. For small molecules, under extreme narrowing conditions, sensitivity for $^{57}$Fe n.m.r. improves with increasing magnetic field, since signal/noise increases approximately as $B_0^{3/2}$; $T_1$ decreases as $B_0^2$, thus permitting more rapid pulse repetition; and the C.S.A. contribution to linewidth is usually not very great. With large molecules, such as proteins, the frequency dependence of $T_1$ is more complex, and the shortening with increased magnetic field may become smaller or absent. Nevertheless, the $B_0^{3/2}$ dependence remains, and higher field still provides better integrated signal/noise. The increased C.S.A. may, however, lead to substantially broader lines, and hence reduce peak signal/noise approximately as $B_0^2$, making sensitivity only slightly field dependent.

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