on the borderline between the set of metals capable of binding to both sites and those which only bind to the C-terminal site.[9,16,25]

A final comment is due on the general utility of thallium(III) as a probe in biochemical problems. From the pulse delay needed to avoid saturation of the signal, T_1 may be estimated of the order of seconds. From the reported rotational correlation time of transferrin of 2×10^{-7} s at 25 °C,[26] the T_1/T_2 ratio at 34.7 MHz is expected to be larger than 10^3. Indeed, the line width at half-peak height is of the order of 100 Hz, which sets T_2 3 orders of magnitude shorter than T_1. It appears therefore that a molecular weight of about 80 000 such as that of transferrin is close to be an upper limit for the practical use of ^{205}Tl as an NMR probe. On the other hand, proteins of lower molecular weight are expected to give rise to much sharper signals and much better signal-to-noise ratios.

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Registry No. Thallium, 7440-28-0; thallium-205, 14280-49-0.

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Mechanism of T_1 Relaxation in ^{13}CO Complexed to an Iron Porphyrin: Implications for CO Bonding in Heme Proteins

T. Perkins,[†,⊥] J. D. Satterlee,*[‡] and J. H. Richards[†]

Contribution No. 6753 from the Departments of Chemistry, California Institute of Technology, Pasadena, California 91125, and the University of New Mexico, Albuquerque, New Mexico 87131. *Received June 10, 1982*

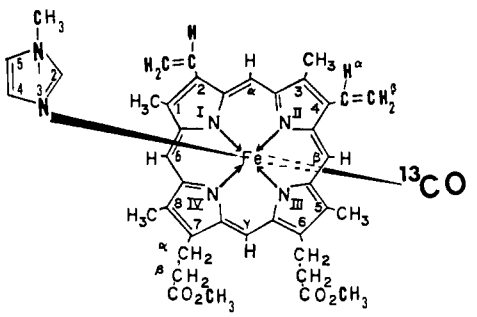
Abstract: Chemical shifts and spin-lattice relaxation parameters for ^{13}CO bonded to the complex 1-methylimidazole-iron protoporphyrin dimethyl ester are reported. The ^{13}CO chemical shifts are much different from those observed in the carbonyl vertebrate hemoglobins, but remarkably similar to that observed for the monomeric hemoglobin component from *Glycera dibranchiata*. The relaxation parameter, T_1, is shown to change dramatically upon ligation to both the hemin model system and to hemoglobin A. The mechanisms of spin-lattice relaxation are dominated by chemical shift anisotropy for ^{13}CO bound to both hemins and proteins, but, in the latter, dipole-dipole forces make an additional contribution to the overall relaxation rate.

Heme prosthetic groups function as the active sites in a number of proteins including ligand-binding enzymes such as catalase and peroxidase, oxygen transport proteins such as hemoglobin, and oxygen storage proteins such as myoglobins. Many of these proteins also bind carbon monoxide, which has provided a basis for infrared and nuclear magnetic resonance spectroscopic studies of the molecular aspects of ligand binding in these heme proteins.[1-11]

In the case of infrared studies, interpretation of results from work on the carbonyl hemoglobins[4,6,8-10] has been greatly aided by observations on various carbonyl heme models that have revealed how cis[2,11] and trans[1,12,13] effects influence the vibrational modes of the CO ligand. These results have enhanced our understanding of interactions in the heme proteins themselves.

In contrast, a less firm basis of model studies exists for understanding the molecular effects which influence the ^{13}C chemical shifts of heme-bound CO, although chemical shifts of carbonyl myoglobins and hemoglobins reconstituted with modified hemes have been shown[14-16] to be sensitive to substitutions around the periphery of the heme. To provide a firmer basis for interpreting NMR spectroscopic properties of ^{13}CO bound to heme proteins, we have studied the NMR properties of the carbonyl hemochrome derived from ferrous (Fe^{2+}) protoporphyrin IX dimethyl ester (I). These data are compared with the NMR properties of adult human hemoglobin (Hb A) and the monomeric hemoglobin fraction from the marine annelid *Glycera dibranchiata*.

The chemical shift studies are aimed at understanding to what degree the protein alters, in a spectroscopically detectable manner,



1-MeIm-I-CO

the environment of heme-bound CO. Several views[3,11,17-19] have been expressed with regard to the functional specificity of heme

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* Author to whom correspondence should be addressed.
⊥ Present address: JEOL USA, Inc., Cranford, NJ 07016.
† California Institute of Technology.
‡ University of New Mexico.

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ligand–globin interactions, and in this work we show additional evidence in support of these arguments.

Another question which bears directly upon the nature of the CO environment concerns the mechanism by which heme-coordinated ^{13}CO undergoes spin–lattice (longitudinal, T_1) relaxation. Elucidation of this mechanism in a model compound such as I would provide an important indication of the mode of such relaxation which is likely to be encountered in both hemoglobins and myoglobins. More importantly, such results may provide a standard with which to compare future results from carbonyl heme proteins. For these reasons we have undertaken a series of T_1 measurements on 1-methylimidazole–I–^{13}CO at magnetic field strengths of 2.35, 4.22, and 8.44 T. The results indicate that the environment of ^{13}CO complexed to the porphyrin complex is dramatically different than that for ^{13}CO bound to heme proteins. The implications of these measurements in determining specific structural modulation of the heme site reactivity in these heme proteins is discussed.

Experimental Section

Ferrous protoporphyrin IX dimethyl ester was prepared from protohemin IX (Sigma) by use of the HCl–methanol procedure.[20] Iron was reintroduced into the product by the method of Adler[21] and the compound was column chromatographed on silica gel. Two successive recrystallizations were subsequently carried out. A uniform, single species was confirmed by proton NMR. Esterification was indicated by the fact that the purified product was insoluble in even basic aqueous solutions but was highly soluble in organic solvents such as chloroform, dimethylformamide, and dimethyl sulfoxide.

NMR Sample Preparation. A heme sample was weighed out and then degassed in a 10-mL syringe. Approximately 5 mL of a 4:1 mixture of previously degassed dimethyl sulfoxide–dimethyl-d_6 sulfoxide (Me_2SO) was then added to the syringe. After the heme had dissolved, the solution was injected into a 10- or 12-mm NMR tube equipped with a 2-mm access port which had been previously sealed with a rubber sleeve and degassed. Approximately 3 mL of 90%-enriched ^{13}CO (Merck) was then injected into the tube; the iron ion was reduced by injection of approximately 15 μL of 0.4 M phosphate buffer solution (pH 7) which had been saturated with dithionite ion. For studies of ferrous protoporphyrin IX dimethyl ester derivatives, aliquots of 1-methylimidazole were injected into the sample, until up to a 3-fold molar excess had been added. For the solvent dependence study, the volume of the sample was 3 mL. An internal capillary was used for the deuterium lock signal.

Instrumentation. Carbon-13 NMR spectra were obtained with three different spectrometers. Low-field measurements were made on a Varian XL-100-15 spectrometer operating at 25.14 MHz in the pulse Fourier transform (FT) mode. Spectra at 45.28 MHz were obtained with a Bruker WH-180 spectrometer. High-field FT spectra were obtained with a Bruker HXS-360 spectrometer with a ^{13}C resonant frequency of 90.5 MHz. For each spectrum the line broadening used was equal to the digital resolution of the transformed spectrum.

T_1 Measurements. Spin–lattice (T_1) measurements were made by using the techniques of inversion–recovery[22] and progressive saturation.[23] In all cases the requirement for use of progressive saturation that $T_2^* << T_1$ was satisfied as determined from a line-width analysis. Delay times were randomly varied in order to eliminate systematic errors. T_1 values were calculated with a nonlinear least-squares program.[24]

Viscosities and Densities. As indicated in the Theory section, the viscosities of the porphyrin solutions affect the relaxation equations due to the variation of rotational correlation time with viscosity. Viscosities were determined by the method of Shoemaker et al.[25] Densities were measured in a 1-mL Weld pycnometer.

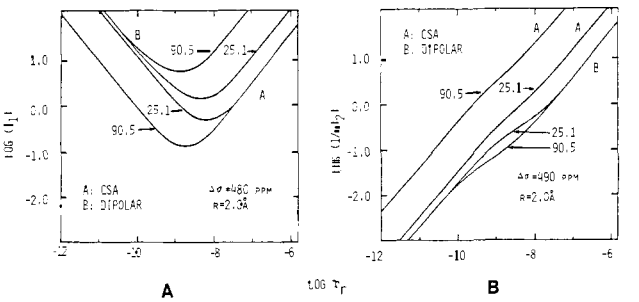

Figure 1. Calculated log–log plots of (A) spin–lattice and (B) spin–spin relaxation for ^{13}CO at 25.14 and 90.5 MHz, using eq 1 and 2, with $\Delta\sigma$ = 480 ppm. Dipole–dipole relaxation times, assuming a carbon–proton distance of 2.0 Å, have been included for comparison.

Theory

Rotational Brownian motion in a magnetic field by molecules containing an NMR-active nucleus ($I > 1/2$) will cause fluctuations in the local magnetic field of that nucleus *if* it possesses an anisotropic chemical shift tensor ($\sigma_{xx} \neq \sigma_{yy} \neq \sigma_{zz}$). These local fluctuations provide a mechanism (chemical shift anisotropy, CSA) for spin–lattice and spin–spin relaxation. If one assumes axial symmetry ($\sigma_{xx} = \sigma_{yy}$) the rate for chemical shift anisotropy relaxation can be described by eq 1 and 2[26] where ω = Larmor

$$1/T_{1_{CSA}} = (2/15)\omega^2(\Delta\sigma)^2(\tau_r/(1 + \omega^2\tau_r^2)) \quad (1)$$

$$1/T_{2_{CSA}} = (1/45)\omega^2(\Delta\sigma)^2[3/(1 + \omega^2\tau_r^2) + 4]\tau_r \quad (2)$$

frequency, $\Delta\sigma = |\sigma_\parallel - \sigma_\perp|$, $\sigma_\parallel = \sigma_{zz}$, $\sigma_\perp = \sigma_{xx} = \sigma_{yy}$, and τ_r = rotational correlation time in solution. Both of these equations assume isotropic rotation in solution. Figure 1 shows log–log plots of T_1 and T_2 relaxation via chemical shift anisotropy at two different frequencies for a case with $\Delta\sigma$ = 480 ppm. In the case of an asymmetric top type ellipsoid, with principal axes $a \neq b \neq c$, undergoing anisotropic reorientation allows eq 1 to become eq 3,[27,28]

$$1/T_{1_{CSA}} = A/T_{1_A} + B/T_{1_B} + C/T_{1_C} \quad (3)$$

where

$$1/T_{1_{A,B,C}} = (2/15)\omega^2(\Delta\sigma)^2(\tau_{A,B,C}/(1 + \omega^2\tau_{A,B,C}^2)) \quad (4)$$

with

$$A = (1/4)(3\cos^2\theta - 1)^2 \quad (5)$$

$$B = 3\sin^2\theta\cos^2\theta \quad (6)$$

$$C = (3/4)\sin^4\theta \quad (7)$$

$$\tau_A = \tau_\perp \quad (8)$$

$$\tau_B = 6\tau_\perp\tau_\parallel(\tau_\perp + 5\tau_\parallel)^{-1} \quad (9)$$

$$\tau_C = 3\tau_\perp\tau_\parallel(2\tau_\perp + \tau_\parallel)^{-1} \quad (10)$$

in which $\tau_\perp$ and $\tau_\parallel$ are the rotational correlation times perpindicular and parallel to the principle symmetry axis, respectively. For bis ligated ferrous heme, which is the subject of this work, we make the reasonable approximation of C_4 symmetry and treat

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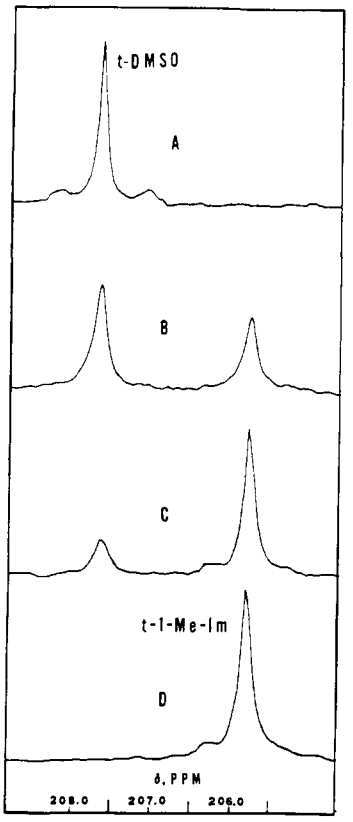

Figure 2. ^{13}CO chemical shifts of ferrous protoporphyrin IX DME-^{13}CO in Me_2SO: (A) 0.015 M FePPIXDME-^{13}CO, (B) sample (A) plus 2.3 μL of 1-MeIm, (C) sample (A) plus 4.6 μL of 1-MeIm (0.8:1 mole ratio), (D) sample of (A) plus 13 μL of 1-MeIm (2:1 mole ratio). All spectra were taken at room temperature.

the molecule as axially symmetric ($a \neq b = c$). With this approximation θ equals zero for ^{13}CO bound to the heme ferrous ion and eq 3 reduces to eq 1.

Results and Discussion

Chemical Shifts. The ^{13}C NMR spectra of the ^{13}CO-hemochrome complex show a single peak in the region near 200 ppm downfield from Me_4Si. Equilibration of ^{13}CO with ferrous protoporphyrin IX dimethyl ester (FePPIXDME) dissolved in 4:1 Me_2SO:Me_2SO-d_6 leads to a single peak 207.7 ppm downfield from Me_4Si (Figure 2A). Addition of aliquots of 1-methylimidazole (1-MeIm) causes a second peak to appear at 205.8 ppm; addition of further 1-MeIm causes the relative intensity of the second peak to increase until, at a 1-MeIm:heme ratio of 2:1, only a peak at 205.8 ppm is observed. Accordingly, we assign the peak at 207.7 ppm to the complex Me_2SO-FePPIXDME-^{13}CO, in which Me_2SO occupies the axial ligand position trans to the CO ligand. In fact, previous observations[29,30] have shown that Me_2SO coordinates to iron porphyrins, presumably through oxygen. Displacement of the axial Me_2SO by 1-MeIm yields the complex 1-MeIm-FePPIXDME-^{13}CO (I) which is responsible for the resonance at 205.8 ppm.

Figure 3 shows the results of titrating 1-MeIm-I-^{13}CO with excess amounts of 1-MeIm. The 1-ppm change in the chemical shift parallels a decrease in the C-O stretching frequency which Maxwell and Caughey observed for carbonylhemochromes when 1-MeIm was used as a solvent.[19] Although Caughey and Maxwell had performed infrared experiments, while we are concerned with NMR results, the correlation between these two methods is striking. It is worth noting that the shifts in parameters found here correlate in the same manner as those found in carbonyl-ligated heme proteins.[14,15]

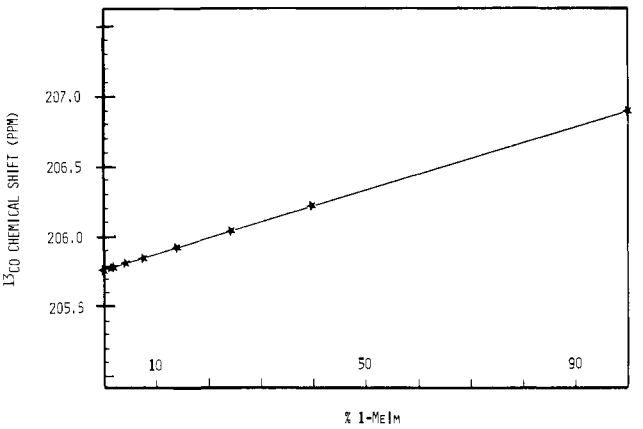

Figure 3. Solvent dependence of the carbon-13 resonance position of ^{13}CO coordinated to compound I. The chemical shifts were measured relative to internal Me_4Si. The line drawn through the points is the least-squares fit of the data. Spectra were taken at room temperature.

Table I. ^{13}CO Chemical Shifts of Various Heme Complexes

complex	chemical shift,[a] ppm	ref
Me_2SO-I-^{13}CO	207.7	this work
1-MeIm-I-^{13}CO (Me_2SO solvent)	205.8	this work
1-MeIm-I-^{13}CO (1-MeIm solvent)	206.9	this work
human hemoglobin, α chains	207.5	15, 16, 31
β chains	207.1	15, 16, 31
sperm whale myoglobin	208.7	15, 16, 31
G. dibranchiata	206.2	17

[a] Shifts are downfield from Me_4Si, error limits are ±0.1 ppm.

Compared to the resonances of heme-coordinated ^{13}CO in mammalian hemoglobins and myoglobins,[14] the resonances from this model system appear shifted upfield. The data in Table I show that the ^{13}C NMR spectrum of 1-MeIm-I-^{13}CO in Me_2SO more closely resembles the spectrum obtained from the monomeric hemoglobin (Hb-II) of the marine annelid *Glycera dibranchiata*,[15] whereas in 1-MeIm solution the ^{13}CO chemical shift is similar to that of HbA. The chemical shifts of ^{13}CO in heme proteins which are reported in Table I differ slightly from those reported previously[14,15] due to more accurate calibration of the operating frequency of the spectrometer.[31]

Previously in heme proteins we have correlated higher field ^{13}CO chemical shifts with the absence of a histidine at position E-7 in the primary amino acid sequence of the globin polypeptide.[6] We have hypothesized that these shifts were the spectroscopic manifestation of an interruption in the nucleophilic interaction which could take place between the histidine at position E-7 and the heme-coordinated CO in mammalian hemoglobins.[6,17] In all species examined to date in which the E-7 residue was histidine, the ^{13}CO resonances exhibited chemical shifts further downfield than 207 ppm, relative to Me_4Si. However, in *Glycera* Hb-II, where the E-7 residue is a leucine, which renders a nucleophilic interaction impossible, the ^{13}CO resonance occurs at 206.2 ppm, a position very close to the ^{13}CO resonances found in the model system under study.[15] In support of this argument is the fact that the ^{13}CO resonance of the model complex in 1-MeIm solvent shifts *downfield*, toward the chemical shifts observed for normal vertebrate hemoglobins which have a histidine at position E-7.

Although we clearly understand that analogies which may be drawn between a hemochrome complex in Me_2SO or 1-MeIm solution and the heme pocket of a native protein are necessarily limited, we do feel that under the conditions of our experiments this model system contains legitimate properties possessed by all hemoglobin heme pockets: (1) a hydrophobic environment, and (2) the absence (or presence) of a specific distal residue capable

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Table II. Spin-Lattice Relaxation Times of ^{13}CO Bound to 0.015 M 1-Methylimidazole-Protoheme IX Dimethyl Ester in Me_2SO-Me_2SO-d_6 (4:1)

T (±1.5, °C)	ν, MHz	T_1,[a] s	τ_r,[b] $\times 10^{10}$ s	$\lvert\Delta\sigma\rvert$,[c] ppm
21.0	25.14	2.3 ± 0.2		
	45.28	0.63 ± 0.23	4.1 ± 0.8	574 ± 122
	90.5	0.19 ± 0.02		
29.0	25.14	2.6 ± 0.2		
	90.5	0.20 ± 0.02	3.2 ± 0.6	614 ± 63

[a] Errors are standard deviation obtained from a nonlinear curve fit. [b] τ_r calculated by using a solvent radius of 2.1 ± 0.1 Å and a heme radius of 7.2 ± 0.2 Å as estimated from the crystal structure of α-chlorohemin. The error limits reflect the uncertainty in the viscosity measurements and radius estimates. [c] Obtained with eq 14. Error limits obtained by using a propagation of errors analysis.

of interacting with CO. Indeed, the chemical shift data presented here lend further support to the view that the spectroscopic properties of heme-coordinated CO reflect specific, interpretable interactions within the heme pocket.[6,17]

Relaxation. In order to interpret the magnetic relaxation data, it is necessary to note that the overall rate of spin–lattice relaxation can be described by an equation like eq 11. This summarizes the fact that the general rate of relaxation is due to contributions from several mechanisms, potentially. For eq 11 the definition of each term is DD = nuclear dipole–dipole relaxation, SC = scalar relaxation, CSA = chemical shift anisotropy relaxation, P = paramagnetic relaxation, Q = quadrupolar relaxation, and SR = spin-rotation relaxation. For the heme model system discussed in this paper the scaler, quadrupolar, paramagnetic, and dipole–dipole relaxation rates do not contribute to the overall rate of relaxation and may be neglected.[22] If one observes the relaxation rates of the ^{13}C nucleus at two different field strengths, eq 12 will describe the difference. That is, two potential mechanisms contribute to the observed relaxation rate. We reason that for a molecule as large as the model complex, I, the spin-rotation contribution to the overail rate should be negligible, or at least much less than the chemical shift anisotropy contribution.[32,33] Therefore, eq 12 reduces to eq 13, and it is predicted that the observed relaxation behavior of ^{13}CO in I should be dominated by the chemical shift anisotropy term.

$$1/T_{1_{tot}} = 1/T_{1_{DD}} + 1/T_{1_{SC}} + 1/T_{1_{CSA}} + 1/T_{1_P} + 1/T_{1_Q} + 1/T_{1_{SR}} \quad (11)$$

$$\Delta(1/T_{1_{tot}}) = \Delta(1/T_{1_{CSA}}) + \Delta(1/T_{1_{SR}}) \quad (12)$$

$$\Delta(1/T_{1_{tot}}) = \Delta(1/T_{1_{CSA}}) \quad (13)$$

The chemical shift anisotropy term, $\Delta\sigma$, can be explicitly derived by combining eq 1 and 13:

$$|\Delta\sigma| = [(\Delta(1/T_{1_{tot}}))/((2/15) \times |\omega_A^2/(1 + \omega_A^2\tau_r^2) - \omega_B^2/(1 + \omega_B^2\tau_r^2)|\tau_r)]^{1/2} \quad (14)$$

where $\omega_A > \omega_B$. From eq 14 one can determine $|\Delta\sigma|$ directly from relaxation measurements obtained at two different frequencies (ω_A and ω_B) and the measured viscosity, which allows independent determination of τ_r (see Experimental Section). It should be noted that if there is any contribution to the total relaxation rate from mechanisms other than chemical shift anisotropy[34] then

$$\Delta(1/T_{1_{tot}}) < \Delta(1/T_{1_{CSA}})$$

Thus, the value for $|\Delta\sigma|$ obtained from eq 14 will be a lower bound. Figure 4 shows a set of typical progressive saturation stack plots obtained at various frequencies. Table II summarizes the relaxation data obtained at three frequencies (field strengths). Table III summarizes the chemical shift information for solid ^{13}CO and for three carbonyl complexes. One observes a significant decrease in $\sigma_\perp$ (from −123 ppm for solid ^{13}CO to −207 ppm for 1-MeIm–I–^{13}CO) which reflects the altered electronic environment of the carbon nucleus.[35,36] The increase in $\sigma_\parallel$ for 1-MeIm–I–CO is probably a consequence of the ring-current shift, due to the π network of the heme.[37] Such an effect of the heme ring current can also explain the significant difference between $\Delta\sigma$ for 1-MeIm–I–^{13}CO and $Fe(CO)_5$.

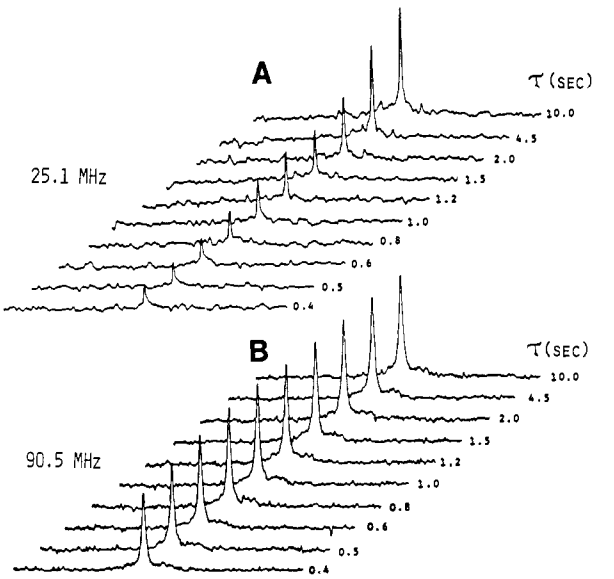

Figure 4. ^{13}CO progressive saturation T_1 stack plot measurements of 0.015 M 1-MeIm–I–^{13}CO taken at 29.0 ± 1.5 °C: (A) 25.14 MHz, (B) 90.5 MHz. Note the increase in line width in (B) compared to (A) which is characteristic of relaxation which is dominated by CSA.

Table III. ^{13}CO Chemical Shifts and Shielding Tensors[a]

compd	$\sigma_\parallel$[b]	$\sigma_\perp$[b]	σ_{AV}[c] (±10)	$\Delta\sigma$[d]	ref
^{13}CO	283	−123	12	406 ± 30	36
$Ni(^{13}CO)_4$	294	−146	1	440 ± 44	32
$Fe(^{13}CO)_5$	253	−155	−19	408 ± 41	32
1-MeIm–(I)–^{13}CO	377	−207	−12	584 ± 132	this work

[a] Units are in ppm. [b] Calculated given the two equations $\Delta\sigma = |\sigma_\parallel - \sigma_\perp|$ and $\sigma_{AV} = (\sigma_\parallel + 2\sigma_\perp)/3$. [c] Equation for σ_{AV} given in footnote *b*. Chemical shifts are based on an absolute scale, with $\sigma_{AV} = 12 \pm 10$ for ^{13}CO. Positive σ_{AV} denotes upfield shift. [d] Sign of $\Delta\sigma$ is positive.[32]

Because we have established that CSA is the dominant mechanism of relaxation for heme-bound ^{13}CO in isolated hemin complexes, it is instructive to consider whether CSA also contributes significantly to the relaxation of ^{13}CO bound to heme proteins. T_1 data obtained[38] on ^{13}CO–HbA, previously analyzed by assuming only a dipolar mechanism for relaxation, have been reinterpreted by using the following expresion:

$$1/T_{1_{tot}} = 1/T_{1_{CSA}} + 1/T_{1_{DD}} \quad (15)$$

where $1/T_{1_{CSA}}$ is given by eq 1 and $1/T_{1_{DD}}$ by eq 16, where $\gamma_x =$

$$1/T_{1_{DD}} = \gamma_x^2\gamma_s^2\hbar^2S(S+1)\sum_{i=1}^{n}[(1/12)J_0(\omega_x - \omega_s) + (3/2)J_1(\omega_x) + (3/4)J_2(\omega_x + \omega_s)] \quad (16)$$

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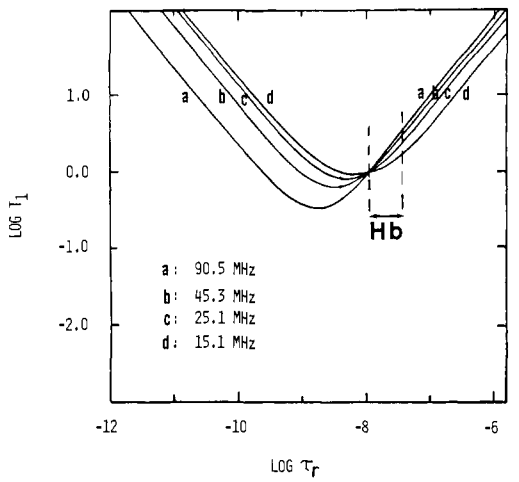

Figure 5. Calculated log-log plots of spin-lattice relaxation for ^{13}CO, using eq 1, with $\Delta\sigma$ = 276 ppm and r_{eff} = 2.0 Å. The region where correlation times for HbA generally occur is shown.

magnetogyric ratio of the observed nucleus (^{13}C), γ_s = magnetogyric ratio of the relaxing nucleus (^1H), $\hbar$ = modified Planck constant, S = spin of the relaxing nucleus ($S = {}^1/_2$ for ^1H), and

$$J_0(\omega_x - \omega_s) = (24/15)(r_i^{-6})(\tau_r/(1 + (\omega_x - \omega_s)^2\tau_r^2))$$

$$J_1(\omega_x) = (4/15)(r_i^{-6})(\tau_r/(1 + \omega_x^2\tau_r^2))$$

$$J_2(\omega_x + \omega_s) = (16/15)(r_i^{-6})(\tau_r/(1 + (\omega_x + \omega_s)^2\tau_r^2))$$

with r_i = distance between nucleus x (^{13}C) and nucleus s_i (^1H), ω_x = Larmor frequency of the observed nucleus (^{13}C), ω_s = Larmor frequency of the relaxing nucleus (^1H), and τ_r = rotational correlation time.

Figure 5 is a plot of eq 15, with $\Delta\sigma$ = 276 ppm (an estimate from our initial analysis of the T_1 data) and r_{eff} = 2.0 Å.[39] From this analysis it is clear that, in the case of HbA, the rotational correlation time is such that, regardless of whether CSA dominates the relaxation rate, a dipolar field dependence will be observed for ^{13}CO bound to HbA ($\tau_r > 10^{-8}$).

Figure 6 shows the weighted least-squares fit of the HbA–^{13}CO T_1 data, using eq 15. The most important result of this analysis is the dramatic decrease in $\Delta\sigma$ for the ^{13}CO when bound to HbA (194 ± 47 ppm) vs. that for the 1-MeIm–I–^{13}CO complex (584 ppm). Such a change in $\Delta\sigma$ is consistent with the concept that the CO experiences greater asymmetry in its electronic environment when it is bound to hemoglobin A. This is consistent with our view that the role of the distal histidine is more than simply to offer steric repulsion to ligand binding in the sixth heme coordination site. Certainly a nucleophilic donor–acceptor interaction between histidine E-7 and heme-bound ^{13}CO would result in a breakdown of the axial symmetry of the ^{13}CO molecule.[35] We further note that the value for the effective proton distance for potential relaxing nuclei within the heme pocket is in good agreement with that estimated from the crystal structure of carbonyl myoglobin.[40]

(39) The initial value of 276 ppm came as the result of a nonweighted least-squares analysis of our initial relaxation data. This was later refined by a weighted nonlinear least-squares method to produce the value of $\Delta\sigma$ (197 ppm) which is reported as the final result. Employing this latter value neither significantly alters the form of Figure 5 nor affects the conclusions which we have presented.

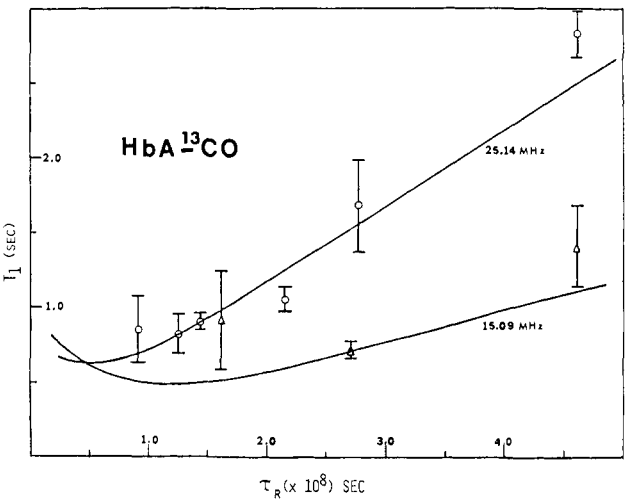

Figure 6. Two variable (ω, τ_r) weighted nonlinear least-squares fits of ^{13}CO–HbA spin-lattice relaxation times, using eq 16. The parameters obtained are $\Delta\sigma$ = 194 ± 37 and r_{eff} = 1.81 ± 0.2 Å. The data are from ref 38.

Summary

Understanding the environmental factors which influence the ^{13}CO chemical shift and spin-lattice relaxation in protohemin models provides a basis for interpreting these NMR parameters in carbonyl hemoglobins and myoglobins. This work illustrates the analysis of such data. For hemin model systems chemical shift anisotropy is determined to dominate heme-coordinated ^{13}CO's relaxation rate. For heme proteins the analysis must also consider relaxation from neighboring protons which line the heme pocket, so that the observed relaxation rate is due to both chemical shift anisotropy and dipole–dipole mechanisms.

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Registry No. Me_2SO-I-^{13}CO, 84303-11-7; 1-MeIm-I-^{13}CO, 84303-12-8; ^{13}CO, 1641-69-6; hemoglobin A, 9034-51-9.

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