

Metal-Nitroxyl Interactions. 50. EPR Spectra of High-Spin Iron(III) Complexes of Spin-Labeled Tetraphenylporphyrins in Fluid Solution

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EPR spectra were obtained for a series of high-spin iron(III) complexes of spin-labeled tetraphenylporphyrins (P) in fluid solution. Iron EPR signals were observed at room temperature for the spin-labeled complexes and for high-spin iron(III) complexes of tetratolylporphyrin with chloride, fluoride, and dimethyl sulfoxide as the axial ligands. In the complexes with weak iron-nitroxyl interaction the iron relaxation rate was sufficiently fast to collapse the electron-electron spin-spin splitting of the nitroxyl signal. As the strength of the iron-nitroxyl spin-spin interaction increased, the nitroxyl signal broadened and moved to a higher g value. For two of the complexes the iron-nitroxyl interaction was sufficiently strong to cause averaging of the iron $\pm 1/2$ and nitroxyl transitions.

Introduction

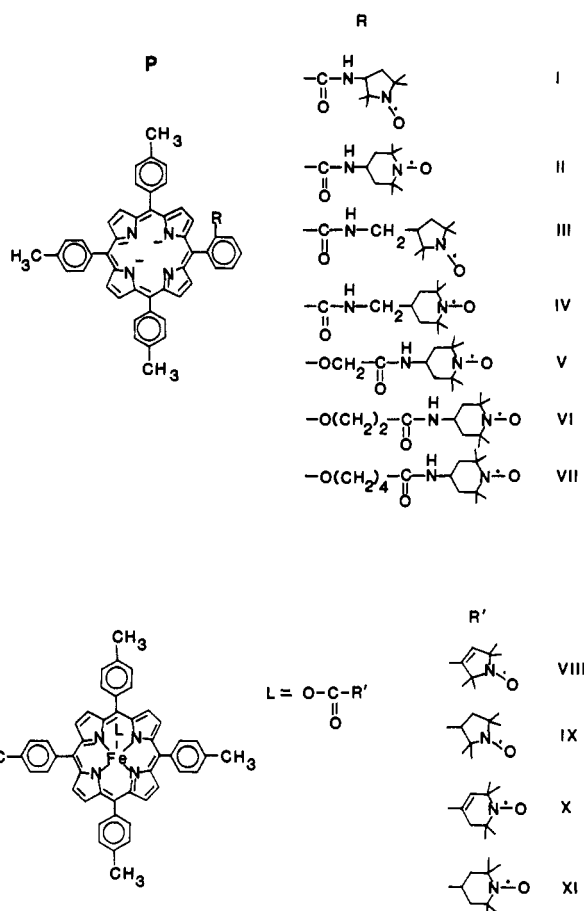
Interaction between a slowly relaxing transition metal with $S = 1/2$ (for example Cu(II), Ag(II), $V^{IV}O$, or low-spin Co(II)) and a nitroxyl radical results in AB patterns in the EPR spectra.¹⁻⁶ Electron-electron spin-spin splitting also has been analyzed recently for spin-labeled complexes of Cr(III) ($S = 3/2$)⁷ and Mn(II) ($S = 5/2$).^{8,9} The exchange contribution to the interaction is determined by the bonding between the two paramagnetic centers. The dipolar contribution depends on the distance between the two centers. In view of the variety of biological systems that contain hemes it was of interest to examine the interaction of nitroxyl spin labels with iron(III) porphyrins.

Previous examples of spin-labeled iron(III) complexes had sufficiently weak iron-nitroxyl interaction that rapid iron relaxation resulted in the observation of "normal" three-line nitroxyl spectra in fluid solution.^{2,10} The complexes studied here were chosen to provide a wide range of magnitudes of the iron-nitroxyl spin-spin interaction. Fluid-solution EPR spectra were obtained for the high-spin iron(III) complexes of ortho-spin-labeled porphyrins I-VII. Iron(III) porphyrins VIII-XI, in which a spin-labeled carboxylate is the axial ligand, were also examined.

The linkage between the metal and the nitroxyl in these ortho-substituted porphyrins can adopt a variety of conformations. EPR studies of low-spin iron(III) complexes of porphyrins I-VII demonstrated that the linkage between the porphyrin and the spin label adopted at least two conformations. The electron-electron spin-spin interaction between the iron and the nitroxyl was strongly dependent on the conformation of the linkage.¹¹ Multiple conformations that differed in the strength of the electron-electron spin-spin interaction have also been observed in the frozen-solution EPR spectra of Fe(P)X, (P = V, X = F, Cl, Br).¹² A dependence of the spin-spin interaction on the conformation of the linkage between the porphyrin and the nitroxyl had previously been observed for the analogous Cu(II) and Ag(II) complexes.⁶ Thus, multiple conformations of the metal-nitroxyl linkage are likely to play a role in the fluid-solution behavior of the Fe(III) complexes. Since spin-spin interaction is orders of magnitude smaller for analogous meta- and para-spin-labeled tetraphenylporphyrins than for the ortho-spin-labeled porphyrins, it has been proposed that orbital overlap between the ortho substituent and the porphyrin π system or the metal is a major contribution to the electron-electron coupling pathway.⁶

Experimental Section

Physical Measurements. Visible spectra were obtained on a Cary 14 instrument with the OLIS modification.¹³ Wavelengths of peak maxima are reported in nm. X-Band EPR spectra were obtained on an E9 spectrometer interfaced to Varian 620/L103 or IBM CS9000 laboratory computers or on an IBM ER200 spectrometer interfaced to an IBM CS9000 computer. The lines in most of the EPR spectra were sufficiently broad that the spectra were unchanged by degassing. Therefore, the



spectra discussed below were obtained on air-saturated samples unless otherwise noted. All spectra were obtained at microwave powers that did

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not cause saturation of the signal and with 100-kHz modulation at amplitudes that did not distort the line shapes. Quantitation of the nitroxyl EPR signals was done by comparison of the double integrals of the spectra with double integrals for solutions of known concentrations of 2,2,6,6-tetramethylpiperidin-1-yl or 4-oxo-2,2,6,6-tetramethylpiperidin-1-yl in the same solvent and sample tube. Throughout the ensuing text integration and integrals refer to double integrals of the first-derivative spectra.

Preparation of Compounds. Spin-labeled iron porphyrins Fe(P)Cl, (P = I–VII),¹¹ Fe(TTP)Cl,¹⁴ and (Fe(TTP))₂O^{15,16} were prepared as previously reported. Trinitrobenzene was recrystallized from ethanol. Di-*o*-xylylethane was passed through silica gel and distilled from CaH₂.

Preparation of Solutions for EPR Studies. Fe(P)X. A 1.0-mL aliquot of a 1–5 mM solution of Fe(P)Cl in toluene was shaken with 0.5–1.0 mL of 0.05 M NaOH for 2 min to form Fe(P)OH.^{17,18} The green solution of Fe(P)OH was shaken with 0.5–1.0 mL of 2% HX to form Fe(P)X. For X = Cl, Br, and I the product was red-brown. For X = F the product was green. The visible spectra confirmed that the product was Fe(P)F and not Fe(P)F₂.¹⁹

Fe(P)(Me₂SO)₂⁺. NMR studies have demonstrated the formation of Fe(TPP)(Me₂SO)₂⁺ when Fe(TPP)Cl is dissolved in Me₂SO.²⁰ The visible spectra of Fe(P)Cl dissolved in Me₂SO showed bands at about 325, 415, 497, 531, and 693 nm. Bands at 400 sh, 422, 498, 530, 574, 625, and 677 nm were reported for the bis-Me₂SO complex of an iron(III) picket-fence porphyrin.²¹ The visible spectrum of Fe(TPP)(Me₂SO)₂⁺ had bands at 400, 527, and 660 nm.²² Care was taken to ensure that (Fe(P))₂O was not present in the initial solution of Fe(P)Cl since (Fe(P))₂O is not converted to Fe(P)(Me₂SO)₂⁺ when dissolved in Me₂SO.

(Fe(P))₂O. A 2-mL aliquot of a 1–3 mM solution of Fe(P)Cl in toluene or chloroform was shaken with 1 mL of 0.1 M NaOH for 5–10 min. The phases were separated, and the solution was kept in the dark at room temperature for 1–2 days for P = I–V, 3–4 days for P = VI, and 7–10 days for P = VII to ensure complete conversion of Fe(P)OH to (Fe(P))₂O.^{17,18}

Iron(III) 5,10,15,20-Tetra-*p*-tolylporphyrin 2,2,5,5-Tetramethyl-1-oxypyrrrole-3-carboxylate (VIII). A 17-mg sample of (Fe(TTP))₂O was added to 0.5 mL of a 20–30 mM solution of 2,2,5,5-tetramethyl-1-oxypyrrrole-3-carboxylic acid in chloroform. The solution was stirred for 2–3 days. The band at 570 nm in the visible spectra due to (Fe(TTP))₂O was replaced by a band at 681 nm due to the five-coordinate monomer. Excess (Fe(TTP))₂O was removed by filtration, and the solution was diluted to 0.2–2.0 mM to obtain solutions for the EPR spectra.

Solutions of spin-labeled porphyrins IX–XI were prepared by the same procedure as for VIII from 2,2,5,5-tetramethyl-1-oxypyrrrole-3-carboxylic acid, 2,2,6,6-tetramethyl-1-oxo-1,2,5,6-tetrahydropyridine-4-carboxylic acid,²³ or 2,2,6,6-tetramethyl-1-oxypiperidine-4-carboxylic acid²³ and (Fe(TTP))₂O.

Computer Simulations. The broad nitroxyl lines in the fluid-solution EPR spectra of Fe(P)X overlapped sharp nitroxyl lines due to small concentrations of nitroxyl impurities in the samples. It was therefore difficult in some cases to estimate the peak-to-peak line width or the *g* value of the broad nitroxyl signal directly from the spectrum. The line widths of the broad signals were determined by computer simulation with a program that used Lorentzian line shapes and a first-order analysis of the nitroxyl nitrogen hyperfine splitting.

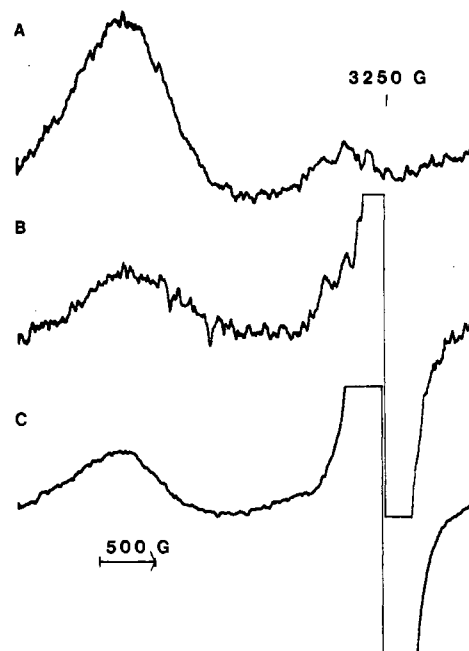


Figure 1. X-Band (9.10-GHz) EPR spectra (4000 G) of (A) 15 mM Fe(TTP)(Me₂SO)₂⁺, (B) 4 mM Fe(P)(Me₂SO)₂⁺ (P = V), and (C) 1 mM Fe(P)F (P = VI) after digital subtraction of the cavity background signal. The spectra were obtained with 100-mW microwave power and 40-G modulation amplitude. The nitroxyl signal is off scale in (B) and (C).

The splittings and shifts of the nitroxyl signals due to spin-spin interaction with slowly relaxing high-spin Fe(III) in the rigid-lattice limit were calculated with a computer program (METNO)^{7,10} that utilized Belford's fourth-order frequency shift perturbation method.²⁴ The Hamiltonian included the Zeeman interactions for both iron and nitroxyl, the iron zero-field splitting, and dipolar and isotropic exchange contributions to the spin-spin interaction.

Results and Discussion

Iron(III) complexes of porphyrins I–VII, which have nitroxyl spin labels attached to the ortho position of one phenyl ring, were examined. The iron porphyrins were studied as the five-coordinate axial halides (Fe(P)X), as the Me₂SO bisadducts (Fe(P)(Me₂SO)₂⁺), and as the μ -oxo dimers ((Fe(P))₂O). Four iron porphyrins, VIII–XI, with nitroxyl carboxylates as the axial ligand were also studied. The axial ligands were selected to give only high-spin iron complexes.

Aggregation of metalloporphyrins in fluid solution can perturb the physical properties of the monomers. Although aggregation is less extensive for high-spin iron(III) complexes of tetraarylporphyrins than for natural porphyrins,²⁵ NMR studies of Fe(TTP)X have shown that aggregation is significant at 5–30 mM concentration at –30 °C.²⁶ Several methods have been proposed to minimize the effects of aggregation. Aggregation is greater at higher concentrations, so dilution can decrease aggregation. When excess diamagnetic porphyrin is added to a solution of a high-spin iron porphyrin, the tendency to aggregate may remain constant, but more of the dimers will contain a paramagnetic iron porphyrin and a diamagnetic porphyrin rather than two paramagnetic iron porphyrins and so the impact of aggregation on the magnetic properties of the iron porphyrin is decreased.^{27,28} Trinitrobenzene forms a molecular complex with a pyrrole ring

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of Fe(TTP)Cl that decreases the tendency of the porphyrin to aggregate.^{26,29} The EPR spectra of the spin-labeled iron porphyrins in fluid solution were independent of concentration over the range 0.1–5.0 mM. Addition of 10 mM trinitrobenzene or 5–10 mM diamagnetic porphyrin to 1 mM solutions of several of the spin-labeled iron porphyrins had no effect on the EPR line shape. It was therefore concluded that the effects of aggregation on the fluid-solution continuous-wave EPR spectra were not significant in this study.

Iron EPR Signal. The EPR spectra of Fe(P)X (P = I–VII, X = F, Cl) in toluene solution and Fe(P)(Me₂SO)₂⁺ (P = I–VII) in Me₂SO solution at room temperature had broad iron signals at low field with maxima at about $g = 6$ (Figure 1). These low-field signals became broader and more difficult to detect when the axial ligand was Br or I. Similar broad iron signals were observed in the room-temperature EPR spectra of Fe(TTP)F, Fe(TTP)Cl, and Fe(TTP)(Me₂SO)₂⁺. These iron signals were observed at high microwave powers and spectrometer amplification after the background signal from the cavity and quartz Dewar insert was subtracted from the spectra.

The line width of the iron EPR signal for Fe(P)F (P = II) was smaller (about 500 G) in di-*o*-xylylene solution than in toluene solution (about 900 G). The room-temperature viscosities of di-*o*-xylylene and toluene are about 30³⁰ and 0.5 cS,³¹ respectively. The observation that a change in viscosity by about a factor of 60 caused less than a factor of 2 change in line width indicates that the spectra are close to the slow-tumbling limit with respect to the $\pm 1/2$ iron transitions. Further indication that these spectra are near the slow-tumbling limit can be obtained by examination of the relationship between the tumbling correlation time and the magnitude of the anisotropy in the spectrum. It seems reasonable to assume that the tumbling correlation times for the iron tetraphenylporphyrins are similar to tumbling correlation times for copper(II) tetraphenylporphyrins. In the EPR spectrum of copper(II) tetraphenylporphyrin in toluene solution the dependence of the line widths on nuclear spin (m_I) is given by line width (G) = 22 + 13.4 m_I + 2.7 m_I^2 . The use of these coefficients in Chasteen's equations³² for Kivelson's model³³ of molecular motion indicates a tumbling correlation time, τ_R , of (0.9–1.0) $\times 10^{-10}$ s/rad in toluene at room temperature. The anisotropy in the observed iron EPR spectrum ($g = 6$ to $g = 2$) is $\Delta\omega = 7.6 \times 10^{10}$ rad/s. Since τ_R^{-1} is about one-sixth of $\Delta\omega$, some motional broadening of the spectrum is expected although the tumbling is much too slow to fully average the anisotropy of the spectrum.

The values of the iron relaxation time, T_2 , calculated from the line widths of the EPR signals are as follows: Fe(TTP)F, 2.4×10^{-11} s (900-G line width); Fe(TTP)(Me₂SO)₂⁺, 2.3×10^{-11} s (950-G line width); Fe(TTP)Cl, 1.9×10^{-11} s (1150-G line width). The comparison of the line widths in toluene and di-*o*-xylylene solution suggests that about half of the line width may be due to motional broadening. Under these conditions T_1 is probably within a factor of 2 of T_2 . NMR studies of iron porphyrins have obtained values of T_1 for Fe(TPP)Cl or Fe(TTP)Cl: 1×10^{-11} ,³⁴ 3×10^{-11} ,³⁵ and 4×10^{-11} s.²⁹ Thus, similar estimates of T_1 are obtained from NMR and EPR. Some difference between the values of the relaxation times obtained by EPR and NMR might be expected due to the fact that the NMR spectra reflect the interaction of the nuclei with all of the electron spin states of the iron, but the EPR signal is for the $-1/2$ to $+1/2$ transition. In view of the paucity of data available for relaxation times for transition metals it is of interest to note that these values of T_1 are about

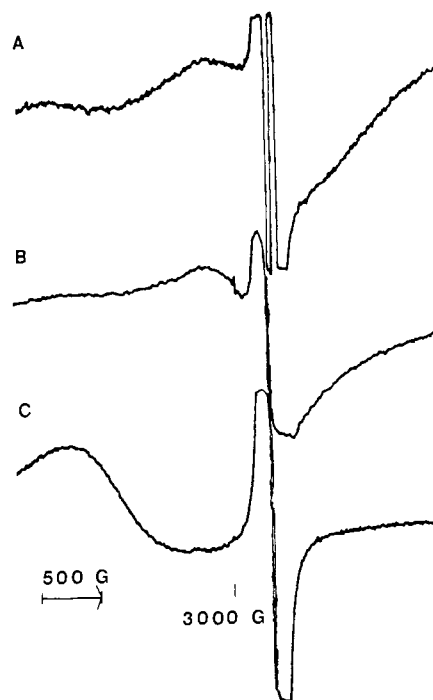


Figure 2. X-Band (9.10-GHz) EPR spectra (4000 G) of iron porphyrins with nitroxyl carboxylates as axial ligands in chloroform/toluene solution at room temperature: (A) 17 mM IX; (B) 9 mM X; (C) 12 mM XI. The spectra were obtained with 20-G modulation amplitude and 150-mW microwave power.

1 order of magnitude shorter than observed for Fe(P)Cl (P = V) at 90 K.¹²

T_1 is expected to decrease with increasing zero-field splitting (ZFS).³⁵ Literature values for the ZFS parameter D are as follows: Fe(TPP)Cl, 6.0,³⁶ 6.5,³⁷ 7.0 cm⁻¹;³⁸ Fe(TPP)Br, 12.5 cm⁻¹;^{39,40} Fe(TPP)I, 13.5 cm⁻¹;⁴⁰ Fe(TPP)(Me₂SO)₂⁺, 10 cm⁻¹.⁴¹ Since the value of D for Fe(protoporphyrin IX)F is 5.6 cm⁻¹,⁴² and ZFS values for iron tetraphenylporphyrins tend to be smaller than for natural porphyrins,^{36,38} the value of D for Fe(P)F is probably between 3.0 and 5.0 cm⁻¹. The shorter value of T_1 obtained for Fe(TPP)Cl than for Fe(TTP)F is consistent with a larger ZFS for Fe(TPP)Cl than for Fe(TTP)F. D for Fe(P)X with X = Br, I is substantially larger than for X = Cl. The line widths for the iron EPR signals in Fe(P)Br and Fe(P)I are therefore expected to be substantially greater than for Fe(P)Cl, which is consistent with the difficulty in observing the iron signals for complexes with these axial ligands. The estimated value of T_1 for Fe(TTP)(Me₂SO)₂⁺ was about the same as for Fe(TTP)Cl, despite the larger ZFS for Fe(TTP)(Me₂SO)₂⁺ than for Fe(TTP)Cl. The higher viscosity of Me₂SO than of toluene may permit less motional averaging of the ZFS in Me₂SO solution than in toluene. Iron signals were not observed in the fluid-solution EPR spectra of (Fe(P))₂O, which is consistent with the expectation that T_1 is shorter in the oxo-bridged dimers than in the monomers.

The $g = 6$ iron EPR signal for a series of heme proteins in fluid solution was reported by Asakura et al.⁴³ The line widths were 500–700 G and decreased to 100–250 G upon addition of NaF. It was noted that the anisotropic splitting of the iron signal was large compared to the rotational correlation time so the fluid-solution and frozen-solution spectra were similar. Hershberg and

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Chance subsequently reported 500–1000-G line widths for the iron EPR signal in ferric myoglobin and ferric catalase at room temperature.⁴⁴ The similarity between the spectra reported in ref 43 and 44 and the spectra obtained in this study suggests that EPR spectra of iron porphyrins in fluid solution are more readily observed than has generally been assumed.

EPR Spectra for Iron Porphyrins with Axial Nitroxyl Ligands.

The EPR spectra of IX–XI in fluid solution at room temperature are shown in Figure 2. In these complexes the nitroxyl is coordinated via a carboxylate group as the axial ligand on the iron porphyrin, which provides a relatively direct pathway for iron–nitroxyl spin–spin interaction. The sharp signals near $g = 2$ are due to about 10% unreacted nitroxyl acid that was present in the equilibrium mixture. The spectra of VIII and XI (Figure 2C) were similar to each other and included a single broad line at a g value intermediate between those expected for the iron $\pm^{1/2}$ and nitroxyl transitions. This spectrum is assigned to an averaging of the iron $\pm^{1/2}$ and nitroxyl transitions as a result of strong exchange interaction. This averaging requires $J > 0.2 \text{ cm}^{-1}$. The spectra do not contain resolved features that could be assigned to nitroxyl that is interacting with iron in the $m_s = \pm^{3/2}$ or $\pm^{5/2}$ spin states. Due to the large ZFS in these complexes the signal for nitroxyl that is strongly interacting with iron in these spin states is likely to be spread over a wide range of magnetic fields and may be too broad to detect. The observation of the signal in Figure 2C indicates that when the iron–nitroxyl interaction is strong, the iron relaxation rate is not fast enough to average the signals for nitroxyl that is interacting with iron $m_s = \pm^{1/2}$ with signals for nitroxyl that is interacting with iron $m_s = \pm^{3/2}, \pm^{5/2}$.

The spectra of IX and X (Figure 2A,B) show broad iron $m_s = \pm^{1/2}$ and nitroxyl signals. The observation of separate iron and nitroxyl signals indicates a weaker exchange interaction than that observed for VIII or XI (Figure 2C). The nitroxyl signals are shifted toward the position of the averaged signal shown in Figure 2C, which indicates substantial iron–nitroxyl interaction. Due to the large fraction of the signal intensity that is present in the wings of a Lorentzian line, wide scans were required to estimate the intensities of the nitroxyl signals. Integration of a spectrum with a sweep width about 14 times the peak-to-peak line width gives 85% of the theoretical area of a signal that is centered in the spectrum.⁴⁵ These wide sweeps introduce uncertainty in the base line. Scans with 4000-G sweep widths were integrated after subtraction of the background signal. Integrals of the nitroxyl signals accounted for about one-fourth to one-third of the expected intensity. Although there is substantial uncertainty in the integrals of these broad signals, the low values suggest that only a portion of the nitroxyl signal is detected. This observation is consistent with the proposal above that when the spin–spin interaction is relatively strong, the iron relaxation rate is not fast enough to fully average the signals for nitroxyl interacting with iron $m_s = \pm^{1/2}$ with signals for nitroxyl that is interacting with iron $m_s = \pm^{3/2}, \pm^{5/2}$.

Interaction with slowly relaxing $S = 5/2$ iron(III) in a rigid lattice splits the nitroxyl signal into a multiplet. The positions and relative intensities of the lines in the multiplet are determined by the magnitude of the spin–spin interaction. As the magnitude of the exchange interaction increases, the multiplet splitting increases and more of the nitroxyl intensity is shifted to lower field.^{7,46} Thus the direction of the g -value shift in fluid solution is also consistent with the calculations for rigid-lattice spectra.

These spectra demonstrate that if iron–nitroxyl interaction in fluid solution is sufficiently strong, broadening and shifting of the nitroxyl signals can be observed.

Nitroxyl Signal in Fe(P)X. The nitroxyl signals for Fe(P)X were broadened by interaction with the iron and shifted to higher g values than the $g = 2.0059$ that is characteristic of noninteracting nitroxyl radicals (Figure 3, Table I). The g values correlated

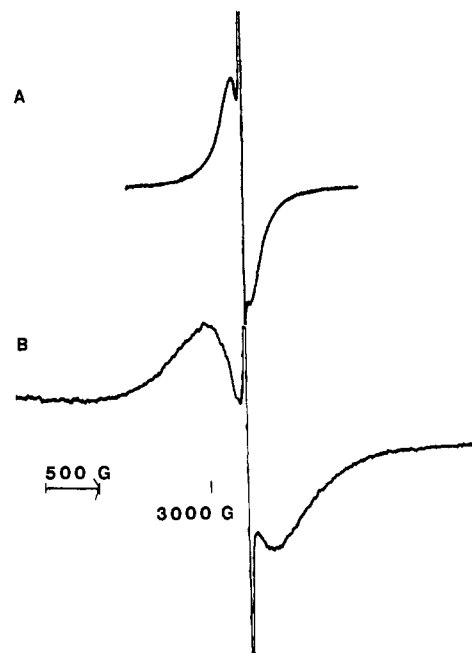


Figure 3. X-Band (9.10-GHz) EPR spectra of the nitroxyl signals in Fe(P)X (P = I) in toluene solution: (A) 2000-G scan for X = F (2 mM) at 22 °C obtained with 10-G modulation amplitude and 100-mW microwave power; (B) 4000-G scan for X = Cl (5 mM) at 60 °C obtained with 25-G modulation amplitude and 100-mW microwave power.

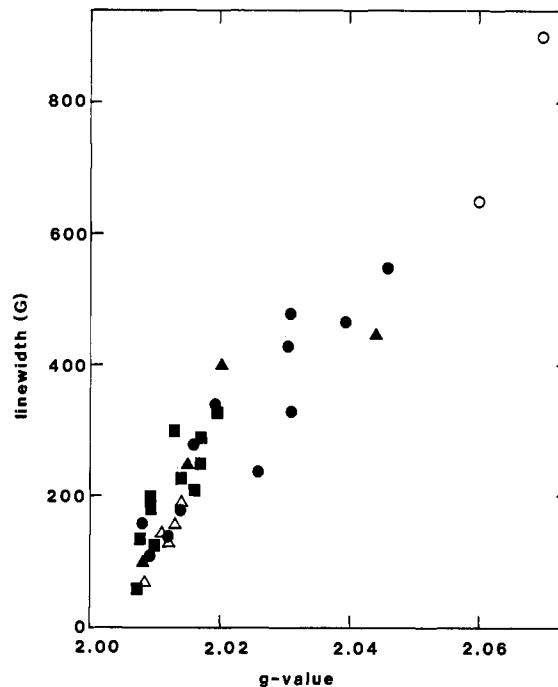


Figure 4. Plot of the nitroxyl line widths as a function of g value in fluid solution for Fe(P)X (P = I, X = F, Cl, Br, I (●); P = III, X = F, Cl (▲); P = IV, X = F, Cl (△); P = V, X = F, Cl, Br, I (■)) and axially spin labeled iron porphyrins IX and X (○). Temperature-dependent data (−40 to +60 °C) are included.

with the line widths as shown in Figure 4. The data for the porphyrins with axial nitroxyl ligands were also included in Figure 4. The correlation between the shifts in the g values and the increases in line width indicate that both were due to spin–spin interaction with the iron(III).

The intensity of the nitroxyl signal for Fe(P)X was about 30–40% of that expected for P = II, V, VI, VII and 60–80% of that expected for P = I, III, IV. These integrals are sufficiently different from 100% that the discrepancy cannot be attributed to uncertainties in the measurements. Apparently only part of the nitroxyl in the sample was contributing to the observed signals.

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Table I. Line Widths and *g* Values for Nitroxyl Signals in Fluid Solution^a

P	parameter	Fe(P)- (Me ₂ SO) ₂ ⁺ (Fe(P)) ₂ O			
		Fe(P)F	Fe(P)Cl	(Fe(P)) ₂ O	(Fe(P)) ₂ O
I	line width	140	480	12	14
	<i>g</i> value	2.012	2.031		
II	line width	72	105	12	4
	<i>g</i> value	2.007	2.009		
III	line width	135	450	15	9
	<i>g</i> value	2.011	2.045		
IV	line width	65	160	15	20
	<i>g</i> value	2.008	2.013		
V	line width	203	210	5	15
	<i>g</i> value	2.014	2.016		
VI	line width	48	100	5	10
	<i>g</i> value	2.0065	2.008		
VII	line width	38	55	5	8

^a Values are at room temperature in toluene solution. Peak to peak line widths are in gauss. *g* values that differed significantly from 2.0059 are listed. The uncertainty in the *g* values is ± 0.001 .

Two possible explanations for the decreased intensities are discussed in the following paragraphs.

Interaction with different iron spin states may give rise to different line widths of the nitroxyl signals. Detection of broad signals is difficult in the presence of sharper signals. At room temperature kT is much greater than the ZFS so all six iron energy levels are approximately equally populated. The observation of more than one-third of the expected nitroxyl signal intensity for many of the complexes indicates that the nitroxyl signal is not due just to the component that is interacting with the iron $\pm 1/2$ energy levels. Iron relaxation rates may be fast enough to average (or partially average) the nitroxyl signals due to weaker interaction but not fast enough to average the nitroxyl signals due to stronger interactions observed for IX and X.

Two isomers were observed in the EPR spectra of the low-spin analogues of these complexes.¹¹ The nitroxyl signal for the isomer with stronger spin-spin interaction was not detected in fluid solution due to the difficulty in observing broad EPR signals that are superimposed on sharper signals. EPR spectra of the high-spin complexes in frozen solution also indicate the presence of two or more isomers with substantially different magnitudes of spin-spin interaction.¹² The observed nitroxyl signal may be due only to the isomer(s) with the weaker spin-spin interaction. The populations of the isomers were different for the various ortho-substituted porphyrins, which is consistent with the observation of different fractions of the signal for different porphyrins.

In frozen solution the iron relaxation rate was sufficiently fast at 90 K to largely collapse the splitting of the nitroxyl signal in Fe(P)X (P = V, X = Cl, Br) for the conformation (about 50% of the complex) with an iron-nitroxyl interaction of about 0.04 cm⁻¹.¹² The spectrum of this conformation in fluid solution would therefore be expected to be a sharp three-line signal. We have previously observed examples in which weak iron-nitroxyl interaction in frozen solution was collapsed to a "normal" three-line nitroxyl spectrum in fluid solution by rapid iron relaxation.¹⁰ Since the only sharp nitroxyl signal in the fluid-solution spectrum of Fe(P)X (P = V) is due to a trace of nitroxyl impurity, there is not a simple correspondence between the signals observed in the fluid and frozen solutions. The observation of broadened and shifted signals in fluid solution must be due to stronger iron-nitroxyl interaction than was observed for a large fraction of the molecules in frozen solution. Either the signal observed in fluid solution is due to averaging of conformations with different magnitudes of spin-spin interaction or the conformations of the molecules are different in fluid and frozen solution.

Between -40 and +60 °C the line widths of the nitroxyl signals in Fe(P)F (P = I, IV, VI, VII) showed little change, the nitroxyl line widths for Fe(P)F (P = II, III), Fe(P)Cl (P = II, III, IV, VI, VII), and Fe(P)X (P = II, X = Br, I) increased by about a factor of 2, and the nitroxyl line widths for Fe(P)X (P = I, X = Cl, Br, I; P = V, X = F, Cl, Br, I) increased by factors of 3-5. The line-width changes as a function of temperature were ac-

companied by *g*-value shifts. The variable-temperature data are included in Figure 4. If the line widths of these signals were dominated by incomplete motional averaging of anisotropic splittings, the line widths would be expected to decrease as tumbling rates increased at higher temperatures. The iron electron spin relaxation rate is expected to increase with increasing temperature, which would also cause a narrowing of the nitroxyl signals. The observation that the line widths did not narrow at higher temperatures indicates that these effects were not as large as other factors that caused broadening of the nitroxyl lines.

Increasing frequency of collisions between paramagnetic centers at higher temperatures causes line broadening that is concentration-dependent. The line widths of the nitroxyl signals for Fe(P)X were not concentration-dependent for approximately 0.5-5.0 mM solutions, so intermolecular collisions do not appear to make a significant contribution to the line widths. Increasing rates of exchange between free and coordinated X⁻ at higher temperatures might also contribute to the broadening of the nitroxyl signal. Excess Cl⁻ accelerates the rate of axial ligand exchange for Fe(TTP)Cl.⁴⁷ Since the addition of a 20-fold excess of tetrabutylammonium chloride to solutions of Fe(P)Cl (P = IV, VI) caused no observable change in the line width of the nitroxyl signal between -10 and +40 °C, the rate of halide exchange does not appear to have a significant impact on the line width of the nitroxyl signal.

The line widths of the nitroxyl signals for Fe(P)Cl (P = I-VII) in di-*o*-xylylene solution were between 10 and 20 G for all of the ortho substituents. These line widths are substantially smaller than the values obtained in toluene solution (Table I). The large effect of viscosity on the line widths at constant temperature suggests that increasing motion of the flexible metal-nitroxyl linkages caused a significant increase in the magnitude of the spin-spin interaction. Motions may permit increased population of conformations with intermittent strong overlap of the orbitals of the ortho substituent with the porphyrin π orbitals or with the metal orbitals. This effect may be related to the effects of solvent on the exchange interaction in Cu(II) complexes of ortho-spin-labeled porphyrins.⁴⁸

Increasing motion of flexible metal-nitroxyl linkages at higher temperatures and population of conformations with greater spin-spin interaction was suggested to explain the increasing line widths at higher temperatures in the EPR spectra of spin-labeled complexes of Ni(II).⁴⁹ In these complexes the line widths of the nitroxyl signals were also substantially smaller for spectra obtained in the viscous solvent di-*o*-xylylene than for spectra obtained in toluene solution.

Nitroxyl Signal in Fe(P)(Me₂SO)₂⁺. The line widths of the iron EPR signals indicated that the iron relaxation rate was about the same for Fe(P)Cl and Fe(P)(Me₂SO)₂⁺. Therefore, if the spin-spin interactions were similar for the two axial ligands, similar nitroxyl line widths would be expected. However, the line widths for the nitroxyl signals in Fe(P)(Me₂SO)₂⁺ were between 5 and 15 G (Table I), which is much smaller than for Fe(P)X. Unlike the case for the nitroxyl signals in Fe(P)X there were negligible shifts in the *g* values of the nitroxyl signals in Fe(P)(Me₂SO)₂⁺. Therefore, these signals were due to conformations of the six-coordinate complexes in which the average iron-nitroxyl spin-spin interaction was much weaker than in the five-coordinate complexes. The line widths of the nitroxyl signals increased slightly as the temperature was increased. Integration of the spectra indicated that these signals accounted for 45% to 75%, depending on P, of the expected nitroxyl intensity. Since these lines are relatively narrow, the integrals are not subject to the same uncertainties as the integrals of the broad signals for the five-coordinate complexes. Since the nitroxyl line widths were much greater for Fe(P)X than for Fe(P)(Me₂SO)₂⁺, incomplete conversion of Fe(P)X to Fe(P)(Me₂SO)₂⁺ might contribute to low

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values of the integrals of the nitroxyl signals. There was sufficient overlap of the bands in the visible spectra that it was difficult to determine whether all of the sample was converted to the Me_2SO adduct. However, the visible spectra did not vary from one porphyrin to another, so differences in the extent of coordination of Me_2SO is not a plausible explanation for the variations in the integrals of the nitroxyl signals as a function of porphyrin. It is probable that the high-spin $\text{Fe}(\text{P})(\text{Me}_2\text{SO})_2^+$ complexes existed as two isomers with substantially different magnitudes of the spin-spin interaction and that the observed nitroxyl signal was due only to the isomer with the weaker spin-spin interaction. The smaller line widths for the nitroxyl signals in $\text{Fe}(\text{P})(\text{Me}_2\text{SO})_2^+$ than in $\text{Fe}(\text{P})\text{X}$ suggest that the presence of axial ligands on both sides of the porphyrin plane may restrict the motion of the ortho substituents and decrease the range of values of J for the conformations that are accessible to the isomers with weak spin-spin interaction compared with the range of conformations accessible for $\text{Fe}(\text{P})\text{X}$.

Nitroxyl Signal in $(\text{Fe}(\text{P}))_2\text{O}$. The line widths of the nitroxyl signals in the μ -oxo-bridged dimers were 4–20 G (Table I). The integrated intensities of the nitroxyl signals in the fluid-solution EPR spectra of $(\text{Fe}(\text{P}))_2\text{O}$ were between 80 and 100% of the expected values. Since the nitroxyl signals were much broader in $\text{Fe}(\text{P})\text{OH}$ than in $(\text{Fe}(\text{P}))_2\text{O}$, the presence of small amounts of $\text{Fe}(\text{P})\text{OH}$ that had not been converted to the μ -oxo dimer may contribute to values of the integrals of the nitroxyl signals for $(\text{Fe}(\text{P}))_2\text{O}$ that are less than 100%. The relatively high values of the integrals indicate that the nitroxyl signal was probably observed for all conformations of $(\text{Fe}(\text{P}))_2\text{O}$. The narrow nitroxyl lines in $(\text{Fe}(\text{P}))_2\text{O}$ may arise from several factors. The strong antiferromagnetic interaction between the two iron atoms in the dimer causes rapid relaxation of the iron unpaired electrons and "decouples" the iron spin from the nitroxyl spin. The strong coupling between the unpaired electrons on the two irons also causes large separations between the resulting energy levels, and almost all of the dimers have $S = 0$ or $S = 1$ for the Fe_2O unit at room temperature.⁵⁰ This reduces the magnitude of the average multiplet splitting from that expected for $S = 5/2$. The conformations for the molecules may also be such that the iron-nitroxyl spin-spin interaction is weak. It is also significant that there was no evidence of distinguishable nitroxyl signals arising from $S =$

0 and $S = 1$ spin states of the Fe_2O unit. The iron relaxation is probably sufficiently fast to average the signals from these species.

The possibility that nitroxyl-nitroxyl interaction might also contribute to the line widths in the dimers should also be considered. The bulky nitroxyl groups are likely to be trans to the oxo bridge linking the two porphyrins, so strong nitroxyl-nitroxyl interaction is not expected. In the spectra of $(\text{Fe}(\text{P}))_2\text{O}$ in which the nitroxyl line widths were less than 10 G, the spectra were sufficiently well-resolved that it was evident that there was no five-line nitroxyl pattern arising from strong nitroxyl-nitroxyl interaction and there were no lines arising from weaker nitroxyl-nitroxyl interaction. Therefore, it seems unlikely that nitroxyl-nitroxyl interaction made a large contribution to the line widths in the μ -oxo dimers.

Conclusion

EPR spectra were observed in fluid solution for the iron $-1/2$ to $+1/2$ transition. The fluid-solution EPR spectra for this series of spin-labeled iron porphyrins provide examples of iron-nitroxyl interaction ranging from weak to relatively strong. As the strength of the interaction increases, the nitroxyl line broadens and shifts downfield (Table I, Figure 4). For stronger interactions an averaged iron and nitroxyl signal is observed (Figure 2C). The observation of these signals is particularly significant since previous examples of spin-labeled iron(III) had sufficiently weak interaction that the iron relaxation collapsed the splitting at room temperature and "normal" nitroxyl spectra were observed.^{2,10} For $\text{Fe}(\text{P})\text{X}$ ($\text{P} = \text{I-VII}$) the EPR spectra can be interpreted in terms of rapid interconversion between conformations with different values of J . The population of conformations with stronger interaction is decreased in the viscous solvent di-*o*-xylylethane. In $\text{Fe}(\text{P})(\text{Me}_2\text{SO})_2^+$ ($\text{P} = \text{I-VII}$) part of the nitroxyl signal was too broad to detect in fluid solution. The presence of isomers that differ substantially in the magnitude of the spin-spin interaction is consistent with the behavior of the low-spin $\text{Fe}(\text{III})$ complexes of these spin-labeled porphyrins.¹¹

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