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Five- and Six-Coordinated Nitrosyl Iron(II) Complexes of Tetrakis (*p*-substituted phenyl)porphyrins. Substituent Effects on the EPR Parameters and the NO Stretching Frequencies

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Five- and six-coordinated nitrosyl iron(II) complexes of a series of tetrakis(*p*-substituted phenyl)porphyrins have been prepared. Their EPR and IR spectra have been measured and analyzed in order to elucidate the cis-effect of substituents on the axial nitrosyl ligand. As the first redox potentials of the free base porphyrins in the complexes became positive, the NO stretching frequencies increased in both five-coordinated and six-coordinated nitrosyl(porphyrinato)iron(II) complexes with a nitrogenous base at an axial site trans to the NO group. The positive shift of the redox potentials led to an increase in the EPR *g* values and hyperfine coupling constants at room temperature and at 77 K for the five-coordinated complexes, while it led to a decrease in *g* values and to a slight increase in hyperfine coupling constants for the six-coordinated complexes. These results are rationalized by invoking that, as the electron-withdrawing power of porphyrin peripheral substituents is enhanced, the iron-to-NO bond in both the five- and six-coordinated complexes is weakened and the iron-to-nitrogenous base bond in the six-coordinated complexes is strengthened. The difference in the cis- and trans-effects on the EPR parameters and NO stretching frequencies is also discussed.

The electronic properties of the heme at active centers of various hemoproteins and the biological functions of these proteins are markedly influenced by the electronic states of the equatorial porphyrinato and axial ligands. The porphyrin peripheral substituents and axial ligands serve as intermediaries of the electronic and steric interactions between the heme group(s) in hemoprotein and amino acid residues surrounding the heme group. From this point of view, the cis and trans effects on various properties of metalloporphyrins have been extensively investigated.[1–5)]

Nitrogen monoxide (nitric oxide) is formed as an intermediate in the biological denitrification process[6)] and is the most probable candidate for an endothelium-derived relaxing factor.[7)] Further, nitrogen monoxide has been known as a biological ligand of nitrite reductases[8)] and guanylate cyclase.[9)] Thus, an increasing interest is placed on the physiological significance of nitrogen monoxide.

Because the nitrogen monoxide molecule has an unpaired electron and is coordinated as sixth ligand to the heme iron in hemoproteins, it has so far been employed as a useful electronic probe for elucidating the heme environments and the heme group.[10–13)] For understanding the properties of these nitrosylhemoproteins, studies on a model complex, nitrosyl(porphyrinato)iron(II), under various conditions have yielded useful information.[14–20)] In a preceding paper, we reported EPR, IR, magnetic circular dichroism (MCD), and electronic absorption spectral studies on the five-coordinated nitrosyl(porphyrinato)iron(II) complex and its six-coordinated complexes with N-, O-, and S-donor ligands under various conditions.[20)] These studies on the model nitrosyl(porphyrinato)iron(II) complexes have dealt almost exclusively with elucidating the effects on the properties of an axial ligand trans to a nitrosyl group, because the identification of the axial ligand(s) is critical for hemoproteins. However, differences among the natural hemes (e. g., hemes a, b, and c) in porphyrin peripheral substituents have been demonstrated to cause pronounced changes in the spectral properties of hemoproteins.[1)] In an attempt to elucidate a cis-effect on the axial nitrosyl ligand, we investigated the EPR and IR spectra of five- and six-coordinated nitrosyl iron(II) complexes of a series of tetrakis(*p*-substituted phenyl)porphyrins. The studies yielded more systematic information for the cis-effect than the electronic absorption and MCD studies.[21)]

Experimental

Materials and Methods. Para-substituted tetraphenylporphyrins, $H_2(p\text{-}X)$tpp, where X=NMe_2, OCH_3, F, CO_2CH_3, and NO_2, were purchased from Midcentury Chemicals and their iron(III) chlorides were synthesized as described previously.[22)] Fe(tpp)Cl and Fe[(F_5)tpp]Cl [where (F_5)tpp is the dianion of tetrakis(pentafluorophenyl)porphyrin] were purchased from Aldrich Chemical Co. In the synthesis of Fe[(*p*-NO_2)tpp]Cl, the compound was not obtained in a spectroscopically pure form as described previously.[3c)] Hence a crude material was prepared as follows. After $H_2(p\text{-}NO_2)$tpp and $FeCl_2$ were refluxed in DMF, H_2O and CH_2Cl_2 were added, the CH_2Cl_2 layer was washed three times with water, dried over Na_2SO_4, and then the solvent was evaporated. The solid was redissolved in CH_2Cl_2 and heptane was added. The crude product thus obtained was dried in vacuo and used as an iron(III) complex of (*p*-NO_2)tpp for Sample Preparation.

1-Methylimidazole (*N*-MeIm) and pyridine (Py) were stored over KOH and distilled under an N_2 atmosphere. Nitrogen monoxide (99.5% minimum) purchased from Seitetsu Kagaku Co. was passed through a KOH column to remove higher nitrogen oxides. Gaseous ^{15}NO was generated by the reaction of $Na^{15}NO_2$ (99.2% enrichment, Amersham) with an ascorbic acid aqueous solution and was passed through a cold trap (about −90 °C) and a KOH column. All other chemicals were of the highest available grade and were used without further purification.

EPR measurements at X-band frequency were carried out on a JES-ME-3X or a JES-RE-2X spectrometer with 100-kHz field modulation at room temperature (ca. 23 °C) and at 77 K. For accurate measurements of the EPR parameters, the microwave frequencies were measured with a digital frequency counter (Advantest, Model TR-5211A), and the magnetic field was calibrated with an NMR field meter (Echo Electronics, EMF 2000A).

The IR absorption spectra of chloroform or 1-methylimidazole solutions of the complexes were measured in a region 1550—1750 cm^{-1}, using CaF_2 windows with pathlength of 0.1 mm. They were recorded on a JASCO A-302 apparatus attached with a DP-A300 data processor for data accumulation and manipulation. In the IR spectrum of the complex with (p-CO_2CH_3)tpp, since the bands of the CO bond at the side chain and the NO bond partially overlapped, the frequencies of the NO band were determined from the difference spectrum of Fe(P)(NO) minus Fe(P)Cl with the same concentration.

Sample Preparation. Nitrosyl(porphyrinato)iron(II) complexes were prepared by a modified method of Kon and Kataoka.[14b,c] The reaction with NO was carried out in a Thunberg-type tube with a side arm on a vacuum line. Five-coordinated complexes were prepared as follows. Pyridine–toluene (1:1, v/v) solution of Fe(P)Cl (P=porphyrin dianion) was degassed by three successive freeze-pump-thaw cycles. Then the solution was equilibrated with NO gas; a tube containing the solution was shaken gently for about 1 h until the solution changed from dark brown to red; the solution was freed of excess NO, pyridine, and solvent in vacuo. The solid thus obtained was dissolved in degassed toluene and the solvent was evaporated. This dissolution–evaporation cycle was repeated and then the product was dried in vacuo for a week in order to completely remove the pyridine ligand. In EPR sample preparations, the product was again dissolved in degassed toluene; the solution was transferred into an EPR tube and sealed on a vacuum line. In IR sample preparations, it was dissolved in chloroform; the solution was transferred into an IR cell under an N_2 atmosphere.

The six-coordinated complexes as EPR or IR samples were prepared by the following methods. (a)EPR samples were prepared by reductive nitrosylation of iron(III) porphyrins with NO. The solution containing Fe(P)Cl and nitrogenous base in toluene was carefully degassed by freeze-pump-thaw cycles. Then the solution was equilibrated with NO gas at a pressure slightly below 1 atm, followed by standing for 1—2 h, transferred into an EPR tube, and sealed. (b)IR samples were prepared by dissolving the five-coordinated nitrosyl iron(II) complexes, obtained by the above-mentioned method, into 1-methylimidazole. The solution was transferred into an IR cell under an N_2 atmosphere.

The concentrations of Fe(P)(NO), N-MeIm, and Py in EPR samples were 2—3 mM, ca. 4 M, and ca. 4 M, respectively (1 M=1 mol dm^{-3}); those of Fe(P)(NO) in IR samples were 5—7 mM.

Results

EPR Spectra of Five-Coordinated Nitrosyl(porphyrinato)iron(II) Complexes. The EPR spectra of five-coordinated nitrosyl(porphyrinato)iron(II) complexes in toluene exhibited a well-resolved triplet for ^{14}NO complexes or a doublet for ^{15}NO complexes (Fig. 1), originated from an ^{14}N (I=1) or ^{15}N (I=1/2) of the NO ligand, respectively. Table 1 lists the g values (g_{iso}) at the central position of the triplet or doublet and the average hyperfine coupling constants (A_{iso}) in the second-derivative curve, together with the first redox potentials of free base porphyrin.

EPR spectral results of five-coordinated Fe(P)(NO) complexes at low temperatures have been utilized in elucidating the stereochemistry of the heme environment for nitrosylhemoproteins such as the NO adducts of

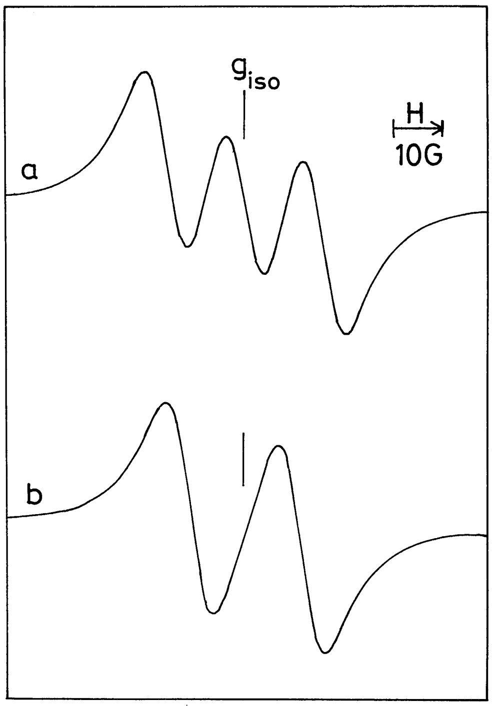

Fig. 1. EPR spectra of (a) Fe(tpp)(^{14}NO) and (b) Fe(tpp)(^{15}NO) in toluene at room temperature. Instrument settings: modulation frequency and amplitude, 100 kHz and 2G; microwave frequency and power, (a) 9.4419 GHz, (b) 9.4481 GHz and 5 mW.

Table 1. EPR Parameters of Five-Coordinated Nitrosyl(porphyrinato)iron(II) Complexes in Toluene at Room Temperature

Porphyrinato ligand	$E_{1/2}$(I)$^{a)}$/V	g_{iso}	A_{iso}
^{14}NO (p-NMe_2)tpp	−1.660	2.0506	16.45
(p-OCH_3)tpp	−1.593	2.0504	16.49
(p-H)tpp	−1.557	2.0507	16.45
(p-F)tpp	−1.520	2.0508	16.42
(p-CO_2CH_3)tpp	−1.435	2.0513	16.55
(F_5)tpp	−1.205	2.0555	16.81
^{15}NO (p-H)tpp	−1.557	2.0506	23.05
(F_5)tpp	−1.205	2.0555	24.31

a) First redox potential of free base porphyrin. Ref. 31.

hemoglobins in the presence of inositol hexaphosphate (IHP)[23] or sodium dodecyl sulfate,[14a] those of mutant hemoglobin M,[24] and those of cytochrome c′,[13] which contain five-coordinated nitrosylheme. Five-coordinated Fe(P)(NO) complexes in nondonor solvents at low temperatures exhibit characteristic EPR spectra with an intense triplet in the g_3 (or g_z) absorption.[14c,15,20a,l]

The EPR spectra of Fe(tpp)(^{14}NO) and Fe(tpp)(^{15}NO) in glassy toluene at 77 K (Fig. 2) indicate the existence of three g values (g_1, g_2, and g_3). The higher field part of the g_2 absorption is hidden in the intense g_3, absorption. The g_1, g_2, and g_3 absorptions split into a triplet for ^{14}NO complexes or a doublet for ^{15}NO complexes. Thus, the g_2' absorption in Fig. 2 can be identified as a lowest field line of the triplet or doublet of the g_2 absorption. The absorption between g_1 and g_2 which is called an unidentified $g_?$ absorption[20m] is probably related to the coordinated NO group because it splits into a triplet or a doublet depending on the nuclear spin of the NO nitrogen. The EPR spectra of six-coordinated complexes at low temperatures also have such a $g_?$ absorption (vide infra). These $g_?$ absorptions most probably arise from another species.[10,20b,25,26] It is known that the intensity of these absorptions depends on the nature of the porphyrinato ligand, medium or solvent, temperature, and other factors.[10,14c,20b,20l,25–27] Because of the presence of small amounts of another species, there is not a good agreement between the experimental and simulated EPR spectra of nitrosylheme.

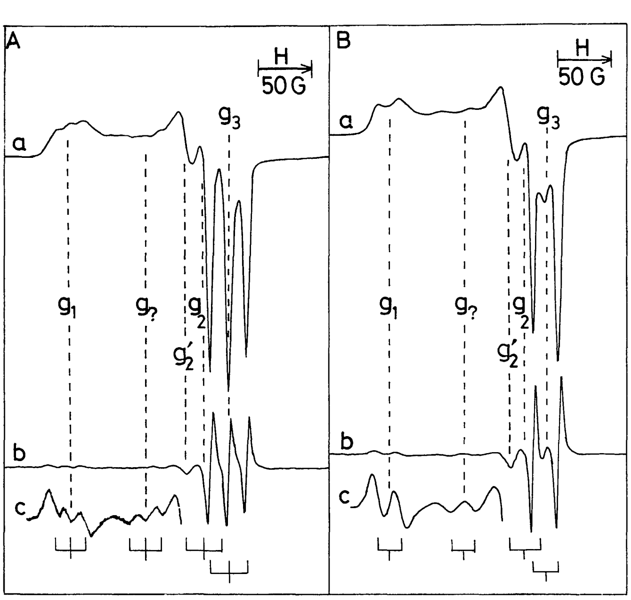

Fig. 2. EPR spectra of (A) Fe(tpp)(^{14}NO) and (B) Fe(tpp)(^{15}NO) in frozen toluene glass at 77 K; (a) first- and (b, c) second-derivative display, where (c) is the expansion of the ordinate of (b). Instrument settings: modulation frequency and amplitude, 100 kHz and 2 G; microwave frequency and power, (A) 9.1811 GHz, (B) 9.1871 GHz and 10 mW.

Table 2 lists the EPR parameters of five-coordinated nitrosyl(porphyrinato)iron(II) complexes at 77 K. The g_3 value was determined from the central line position of triplet or the center of doublet in the first-derivative display, while the g_1, g_2, g_2', and $g_?$ values were determined from those in the second derivative because of the poor resolution in the first derivative. Similarly, the A_3 value was obtained as an average of intervals in the first derivative, while the A_1, A_2, and $A_?$ values were obtained from those in the second derivative. However, the A_2 value for ^{14}NO complexes can not be determined because the two lines at the higher field side of the g_2 absorption were hidden into the g_3 absorption. Thus, the A_2 values of Fe(tpp)(NO) and Fe[(F$_5$)tpp](NO) were estimated from the equation $A_2(^{14}\mathrm{NO})=A_2(^{15}\mathrm{NO})\text{-}[A_3(^{14}\mathrm{NO})/A_3(^{15}\mathrm{NO})]$.

EPR Spectra of Six-Coordinated Nitrosyl(porphyrinato)iron(II) Complxes. Figure 3 shows the first- and second-derivative spectra of the Fe[(F$_5$)tpp](^{14}NO) complex with *N*-MeIm in toluene at room temperature; the spectra of the complexes with ^{15}NO and with the other porphyrinato ligands were virtually identical with Fig. 3 in the line shape. The first-derivative curve was slightly asymmetric and its second-derivative curve indicated overlapping of two components. These spectral features resemble those of the nitrosyl iron(II) complex of protoporphyrin IX dimethyl ester (Fe(ppixdme)(NO)) with *N*-MeIm.[20b] In contrast with the *N*-MeIm complexes, the Py complexes exhibited EPR spectra with a well-resolved triplet for ^{14}NO complexes, being similar to those of the five-coordinated complexes.

Table 2. EPR Parameters of Five-Coordinated Nitrosyl(porphyrinato)iron(II) Complexes in Toluene at 77 K

Porphyrinato ligand	$E_{2/1}$(I)[a]	g values					Hyperfine coupling const.			
	V	g_1	$g_?$	g_2'	g_2	g_3	A_1/G	$A_?$/G	A_2/G	A_3/G
^{14}NO (*p*-NMe$_2$)tpp	−1.660	2.104	2.060	2.035		2.0100	15.0			16.6
(*p*-OCH$_3$)tpp	−1.593	2.104	2.060	2.035		2.0101	15.0	15.0		16.6
(*p*-H)tpp	−1.557	2.107	2.060	2.035	2.024	2.0100	14.5	15.5	19.3	16.7
(*p*-F)tpp	−1.520	2.107	2.059	2.035		2.0102	15.0	15.6		16.9
(*p*-CO$_2$CH$_3$)tpp	−1.435	2.106	2.059	2.034		2.0103	14.7	15.9		17.0
(F$_5$)tpp	−1.205	2.108	2.063	2.040	2.028	2.0109	15.0	16.0	20.2	17.2
^{15}NO (*p*-H)tpp	−1.557	2.107	2.060	2.032	2.024	2.0102	20.5	21.5	26.8	23.2
(F$_5$)tpp	−1.205	2.108	2.061	2.037	2.028	2.0108	21.0	22.6	27.8	23.7

a) See footnote a of Table 1.

The g values at the zero-crossing in the first-derivative spectra of *N*-MeIm complexes and those at the central peak of the triplet in the second derivative spectra of Py complexes at room temperature are listed in Table 3, together with the first redox potentials of free base porphyrin.

The nitrosyl derivatives of many hemoproteins at low temperatures exhibited EPR spectra similar to those of six-coordinated nitrosyl(porhyrinato)iron(II) complexes with a nitrogenous base. These results have been utilized in identifying the fifth heme ligand of which the most probable candidate is an imidazolyl group of the histidine residue.10–12) The EPR spectra of six-coordinated nitrosyl(porphyrinato)iron(II) complexes with nitrogenous bases at low temperatures exhibit a line shape characteristic of randomly oriented systems with a rhombic symmetry and its central g_2 (or g_z) absorption has mostly nine hyperfine lines or a triplet of triplets, originating from the hyperfine interaction of an unpaired electron of the NO ligand with two axial ^{14}N nuclei.14,15,20)

The EPR spectra of Fe[(F$_5$)tpp](NO) complexes with *N*-MeIm (Fig. 4) and with Py (Fig. 5) in glassy toluene at 77 K exhibited distinct three g_1, g_2, and g_3 absorptions and an additional $g_{\text{II-1}}$ absorption. The $g_{\text{II-1}}$ absorption which was called the $g_?$ absorption10) has been observed in the EPR spectra of various nitrosylhemoproteins and their model complexes.10,11,15,20) This absorption was interpreted as a part of signal originating from another species (II), which is different in the structure of Fe–N–O unit from the dominant species (I) showing g_1, g_2, and g_3 absorptions.20b,25,26) Another part of the g_{II} absorption which is hidden into the g_2 and g_3 absorptions could be resolved in the spectrum at a Q-band frequency.20b) As shown in the second derivative, the g_2 absorption was highly resolved into a triplet of triplets for ^{14}NO

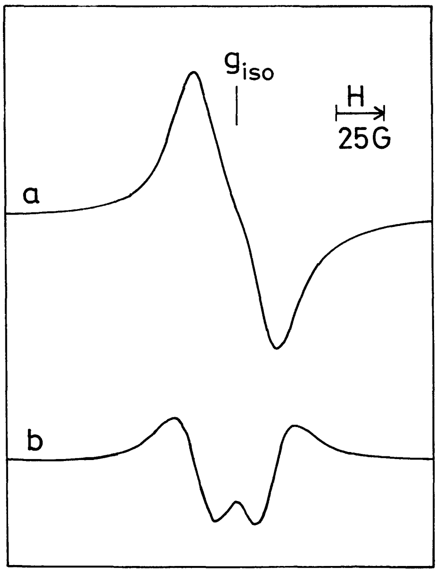

Fig. 3. EPR spectra of Fe[(F$_5$)tpp](^{14}NO)(*N*-MeIm) in toluene at room temperature; (a) first- and (b) second-derivative display. Instrument settings are similar to those in Fig. 1.

Table 3. EPR Parameters of Six-Coordinated Nitrosyl(porphyrinato)iron(II) Complexes with 1-Methylimidazole and Pyridine in Toluene at Room Temperature

Porphyrinato ligand	$E_{1/2}$(I)a)	g_{iso}	
	V	*N*-MeIm	Py
(*p*-NMe$_2$)tpp	−1.660	2.021	2.0445
(*p*-OCH$_3$)tpp	−1.593	2.019	2.0366
(*p*-H)tpp	−1.557	2.017	2.0349
(*p*-F)tpp	−1.520	2.017	2.0328
(*p*-CO$_2$CH$_3$)tpp	−1.435	2.016	2.0316
(*p*-NO$_2$)tpp	−1.340	2.015	2.0299
(F$_5$)tpp	−1.205	2.014	2.0263

a) See footnote a of Table 1.

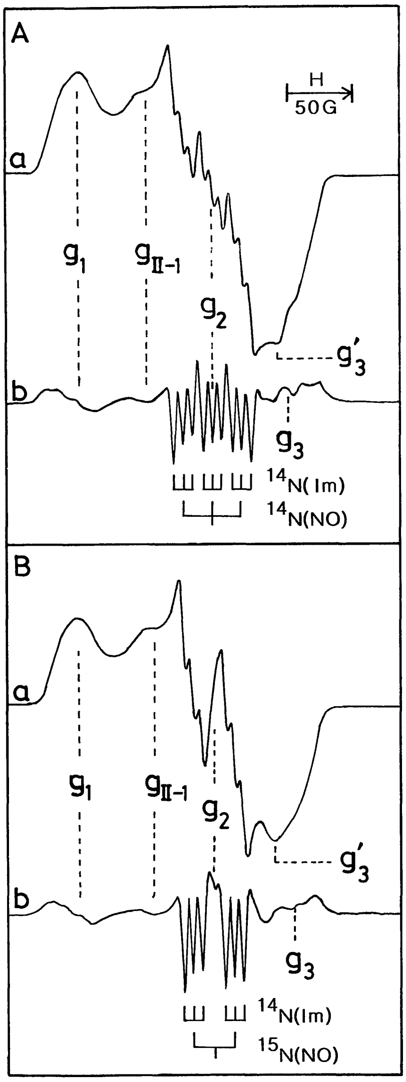

Fig. 4. EPR spectra of (A) Fe[(F$_5$)tpp](^{14}NO)(*N*-MeIm) and Fe[(F$_5$)tpp](^{15}NO)(*N*-MeIm) in frozen toluene glass at 77 K; (a) first- and (b) second-derivative display. Instrument settings are similar to those in Fig. 2.

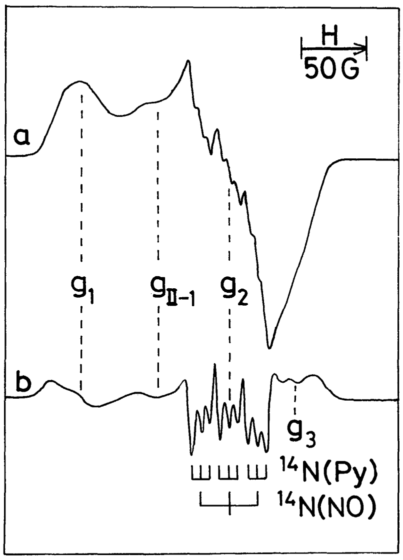

Fig. 5. EPR spectra of Fe[(F$_5$)tpp](^{14}NO)(Py) in frozen toluene glass at 77 K; (a) first- and (b) second-derivative display. Instrument settings are similar to those in Fig. 1.

complexes and a doublet of triplets for ^{15}NO complexes, while the g_1 and g_3 absorptions were poorly resolved into four lines for ^{14}NO complexes and three lines for ^{15}NO complexes. Thus, although the line shapes of g_1, g_2, and g_3 absorptions were affected by the isotopic substitution, the determination of hyperfine coupling constants in the g_1 and g_3 absorptions was not possible because of the poor resolution and complicated patterns. It has been demonstrated that the lack of resolved hyperfine splittings in the in-plane g_1 and g_3 absorptions results from both the inhomogeneous broadening based on unresolved hyperfine interaction with pyrrole nitrogens and the poor resolution derived from weak hyperfine interaction with two axial nitrogens.[10] Although nitrosyl cytochrome P-450 contains a six-coordinated nitrosyl iron(II) complex, the resolved hyperfine structure derived from an NO nitrogen has been detected on the in-plane absorptions because the axial site trans to NO is occupied by a thiolate sulfur (I=0).[28,29]

Tables 4 and 5 list the EPR parameters of six-coordinated nitrosyl(porphyrinato)iron(II) complexes with 1-methylimidazole and pyridine at 77 K, respectively, together with the first redox potential of free base porphyrins. The g_2 value was determined from the central line position of nine hyperfine lines for ^{14}NO

Table 4. EPR Parameters of Six-Coordinated Nitrosyl(porphyrinato)iron(II) Complexes with 1-Methylimidazole in Toluene at 77 K

Porphyrinato ligand	$E_{2/1}$(I)$^{a)}$	g values					Hyperfine coupling const.	
	V	g_1	$g_{\text{II-1}}$	g_2	g_3	g_3'	A_2/G	a_2/G
^{14}NO (p-NMe$_2$)tpp	−1.660	2.073	2.035	2.0041	1.968	1.974	22.1	6.90
(p-OCH$_3$)tpp	−1.593	2.072	2.035	2.0038	1.968	1.973	22.1	6.88
(p-H)tpp	−1.557	2.072	2.036	2.0036	1.968	1.974	22.3	7.00
(p-F)tpp	−1.520	2.072	2.035	2.0035	1.966	1.972	22.6	7.03
(p-CO$_2$CH$_3$)tpp	−1.435	2.072	2.034	2.0033	1.965	1.972	22.5	7.01
(p-NO$_2$)tpp	−1.340	2.070	2.033	2.0032	1.965	1.972	22.9	7.12
(F$_5$)tpp	−1.205	2.067	2.034	2.0027	1.964	1.973	22.8	7.10
^{15}NO (p-H)tpp	−1.660	2.072	2.033	2.0040	1.968	1.975	30.7	6.92
(p-OCH$_3$)tpp	−1.593	2.072	2.033	2.0039	1.969	1.975	30.6	7.00
(p-H)tpp	−1.557	2.072	2.032	2.0038	1.968	1.975	30.9	7.03
(F$_5$)tpp	−1.205	2.068	2.032	2.0027	1.965	1.975	31.7	7.10

a) See footnote a of Table 1.

Table 5. EPR Parameters of Six-Coordinated Nitrosyl(porphyrinato)iron(II) Complexes with Pyridine in Toluene at 77 K

Porphyrinato ligand	$E_{2/1}$(I)$^{a)}$	g values				Hyperfine coupling const.	
	V	g_1	$g_{\text{II-1}}$	g_2	g_3	A_2/G	a_2/G
^{14}NO (p-NMe$_2$)tpp	−1.660	2.079	2.041	2.0046	1.977	22.0	6.50
(p-OCH$_3$)tpp	−1.593	2.078	2.041	2.0043	1.978	21.9	6.50
(p-H)tpp	−1.557	2.078	2.039	2.0042	1.977	21.9	6.58
(p-F)tpp	−1.520	2.079	2.038	2.0041	1.975	22.1	6.66
(p-CO$_2$CH$_3$)tpp	−1.435	2.077	2.040	2.0040	1.972	22.1	6.67
(p-NO$_2$)tpp	−1.340	2.076	2.039	2.0039	1.971	22.0	6.67
(F$_5$)tpp	−1.205	2.075	2.039	2.0038	1.971	22.1	6.73

a) See footnote a of Table 1.

Table 6. NO Stretching Frequencies of Five-Coordinated Nitrosyl(porphyrinato)iron(II) Complexes and the Six-Coordinated Complexes with 1-Methylimidazole at Room Temperature in cm^{-1}

Porphyrinato ligand	$E_{1/2}(I)^{a)}$	Five-coordinates$^{b)}$	Six-coordinates$^{c)}$
(*p*-H)tpp	−1.557	1678	1625
(*p*-CO_2CH_3)tpp	−1.435	1687	1636
(F_5)tpp	−1.205	1703	1650

a) See footnote a of Table 1. b) Solvent; chloroform. c) Solvent; 1-methylimidazole.

complexes or the center of six hyperfine lines for ^{15}NO complexes in the second derivative. The g_3 value was also determined from the center of the g_3 absorption in the second derivative. On the other hand, the g_1 and g_3' values were determined from the extreme position of corresponding absorptions in the first derivative. Because the g_3 absorption overlapped significantly with that of g_2 appearing to be a slight hump or a shoulder, the second-derivative center may be preferable, for the determination of *g*-value, to the extreme position of the first derivative. The hyperfine coupling constants A_2 and a_2 correspond to hyperfine splittings due to the nitrogen nucleus of the NO ligand and the trans axial nitrogenous base, respectively. Both values were obtained from averaged intervals in the second derivative.

NO Stretching Frequencies. It has been demonstrated that the NO stretching frequencies in the IR spectra for five-coordinated Fe(ppixdme)(NO) and the six-coordinated complexes are comparable to those for NO-hemoglobin A in the presence of IHP and in the absence of IHP, respectively, and the binding of IHP to the protein results in cleavage of the proximal histidine to iron bonds in two of four subunits.[16] The NO stretching frequencies of the complexes vary linearly with the solvent polarity[20g] and the basicity of the axial ligand trans to NO.[20h] Thus, the NO stretching frequencies in the IR spectra of nitrosylhemoproteins and those model complexes are a useful measure of the iron-to-NO bond strength and the polarity of heme environments.

Table 6 lists the NO stretching frequencies of three nitrosyl(porphyrinato)iron(II) complexes in chloroform and in 1-methylimidazole. The IR spectra of the complexes in 1-methylimidazole showed a single NO band assigned to that of six-coordinated complexes, though those in neat liquid bases such as pyridine derivatives sometimes show both NO bands of five- and six-coordinated complexes which coexist at equilibrium.[20g] The NO stretching frequencies of five- and six-coordinated complexes with tpp agreed closely with those in KBr pellets (1670 and 1625 cm^{-1})[17] and were slightly higher than those with ppixdme (1675 and 1618 cm^{-1}, respectively).[20g]

Discussion

Introduction of electron-withdrawing or -donating groups at the periphery of a porphyrin results in the shift to positive or negative side, respectively, of the redox potentials of the porphyrin and the corresponding metalloporphyrins.[1,4,30,31] In what follows, the first redox potentials of the free base porphyrin $[E_{1/2}(I)]$[31] are used as a measure of the electron-withdrawing or -donating power of the porphyrin peripheral substituents.

Alben and Caughey investigated the cis-effect of porphyrin peripheral substituents on the axial carbonyl ligand in carbonyl(2,4-substituted deuteroporphyrin IX dimethyl esterato)iron(II) complexes with pyridine.[32] They demonstrated that the CO stretching frequency increases with an increase in electron-withdrawing character of the 2,4-substituents. Similarly, the NO stretching frequencies of both five- and six-coordinated complexes in Table 6 markedly and linearly increase as the electron-withdrawing power of the substituents becomes greater. This cis-effect can be explained as follows, in the same manner as that on carbonyl complexes. An increse in the electron-withdrawing power of the porphyrin periphery results in weakening in iron-to-NO π back bonding, followed by a decrease in the electron density of iron d_π and NO π^* orbitals. Consequently, the decreased antibonding character of the NO bond induces the observed increase in the NO stretching frequencies. It is reasonable to predict that this weakening or lengthening of the iron-to-NO bond decreases the displacement of iron from the porphyrin plane toward the NO group, as is generally found in the molecular structure of nitrosyl(porphyrinato)iron(II) complexes.[17]

The changes ($\Delta\nu$) in these stretching frequencies per unit volt in the redox potential of free base porphyrins were estimated by a least-squares method from the linear relation of the NO stretching frequencies in Table 6 and the CO stretching frequencies of carbonyl(2,4-substituted deuteroporphyrin IX dimethyl esterato)iron(II) complexes[32] to $E_{1/2}(I)$ values.[31] The $\Delta\nu$ values thus obtained were 71 cm^{-1} in the five-coordinated NO complexes, 70 cm^{-1} in the six-coordinated NO complexes, and 31 cm^{-1} in the CO complexes. The $\Delta\nu$ values for the NO complexes are about twice that for the CO complexes, suggesting that the π back-bonding of the iron-to-NO ligand is more sensitive to the substitution at porphyrin peripheral side chains than that of the iron-to-

CO ligand.

On the basis of the interpretations for NO stretching frequencies of nitrosyl iron(II) porphyrin complexes, the EPR spectral results of the five- and six-coordinated complexes are analyzed as follows.

Five-Coordinated Complexes. Figures 6 and 7 illustrate the $E_{1/2}$(I) dependence of g_{iso} and A_{iso} values at room temperature and g_3 (or g_z) values at 77 K of five-coordinated nitrosyl(porphyrinato)iron(II) complexes. These g and A_{iso} values increase as $E_{1/2}$(I) becomes positive. (Other g values in Table 2 also exhibit similar tendencies).

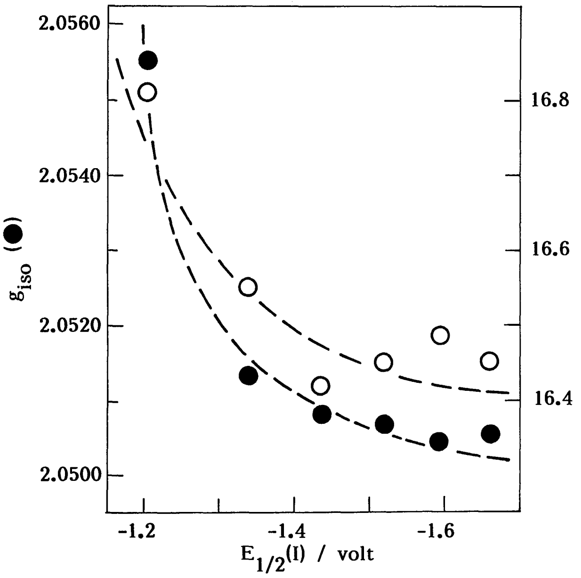

Fig. 6. Relationship between the g_{iso} values of five-coordinated Fe[(p-X)tpp](NO) complexes at room temperature and the $E_{1/2}$(I) values of (p-X)tpp. The trend in the data is indicated by the dashed lines.

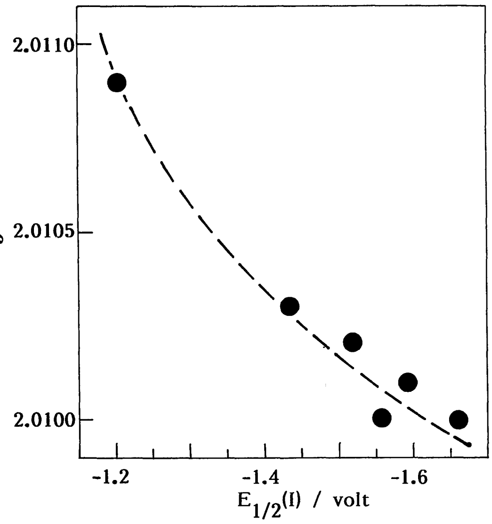

Fig. 7. Relationship between the g_3 values of five-coordinated Fe[(p-X)tpp](NO) complexes at 77 K and the $E_{1/2}$(I) values of (p-X)tpp. The trend in the data is indicated by the dashed line.

The spin density on the nitrogen of NO was estimated to be 2—3% from three hyperfine coupling constants at 77 K according to the method of McNeil et al.[33] Thus, the unpaired electron of an NO group can considerably delocalize to an iron d orbital through the d_{z^2} orbital; the spin-orbit coupling between the iron d orbitals is responsible for the deviation of the g value from a free spin value.[14b,15,34-36]

When the electron density on the iron d_π and NO π^* orbitals decreases with increasing electron-withdrawing character of peripheral substituents as described above, the unpaired electron on d_{z^2} orbital can shift toward the NO nitrogen. This slight increase of the spin density on the NO nitrogen can cause an increase in the hyperfine coupling constants, A_{iso} (Table 1 and Fig. 6) and A_3 (Table 2).

The decrease of the unpaired electron density in the d_{z^2} orbital would be accompanied by a stabilization of the d_{z^2} orbital. The consequent decrease in the separation between the d_{z^2} and d_{xy} levels leads to further spin-orbit coupling,[35] as reflected by an increase in the g values (Figs. 6 and 7).

Six-Coordinated Complexes. Figures 8 and 9 illustrate the $E_{1/2}$(I) dependence of the g_{iso} (at room temperature) and g_2 (at 77 K) values of six-coordinated

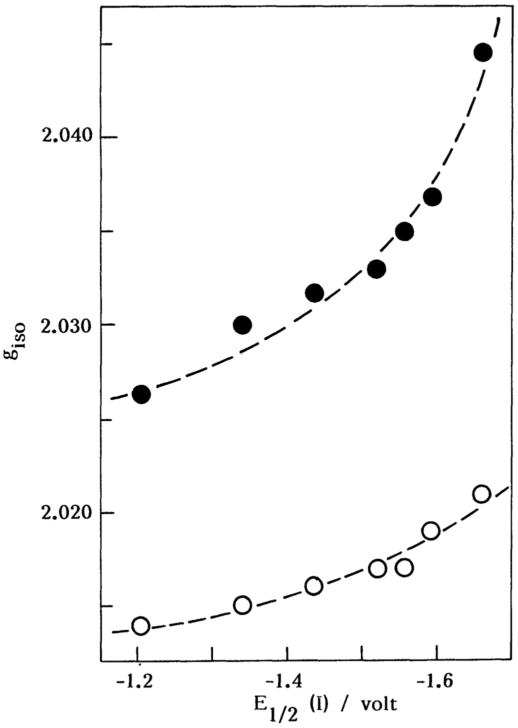

Fig. 8. Relationship between the g_{iso} values of six-coordinated Fe[(p-X)tpp](NO)(Base) complexes at room temperature and the $E_{1/2}$(I) values of (p-X)tpp. Base: ●, pyridine; ○, 1-methylimidazole. The trend in the data is indicated by the dashed lines.

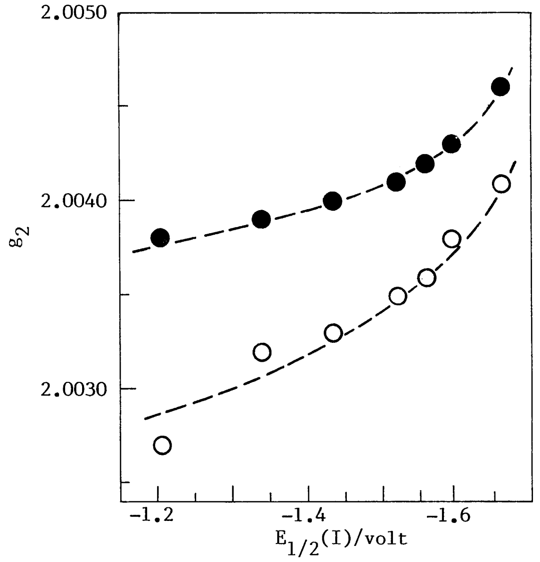

Fig. 9. Relationship between the g_2 values of six-coordinated Fe[(*p*-X)tpp](NO)(Base) complexes at 77 K and the $E_{1/2}$(I) values of (*p*-X)tpp. Base: ●, pyridine; ○, 1-methylimidazole. The trend in the data is indicated by the dashed lines.

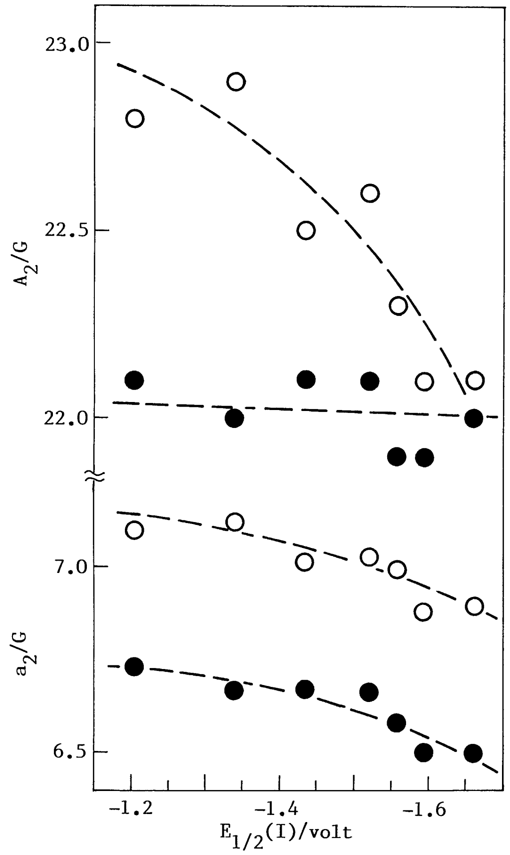

Fig. 10. Relationship between the A_2 and a_2 values of six-coordinated Fe[(*p*-X)tpp](NO)(Base) complexes at 77 K and the $E_{1/2}$(I) values of (*p*-X)tpp. Base: ●, pyridine; ○, 1-methylimidazole. The trend in the data is indicated by the dashed lines.

nitrosyl(porphyrinato)iron(II) complexes with 1-methylimidazole and pyridine. (Other g values in Table 4 also exhibit similar tendencies). As $E_{1/2}$(I) becomes positive, these g values decrease in the complexes with 1-methylimidazole and pyridine, and at the same time, both the g_{iso} value at room temperature and the $(g_1+g_2+g_3)/3$ values at 77 K approach the free spin value. As shown in Fig. 10, the hyperfine coupling constants, A_2 and a_2, increase with a shift of the $E_{1/2}$(I) to the positive side, though the A_2 values of the complexes with pyridine lie within 22.0±0.1 G. Thus, the dependencies of these EPR parameters on $E_{1/2}$(I) for the six-coordinated nitrosyl complexes are in contrast to those for the five-coordinated complexes.

In the six-coordinated nitrosyl complexes as well as the five-coordinated complexes, an enhancement in the electron-withdrawing power of porphyrin peripheral substituents results in a decrease in the electron density on the iron d_π and NO π^* orbitals and the concomitant shift of unpaired electron population on d_{z^2} orbital toward the NO nitrogen. The increase in the unpaired electron population at the NO nitrogen leads to an increase in hyperfine coupling constants. An axial position trans to NO group in the six-coordinated complexes is occupied by 1-methylimidazole or pyridine, which is primarily a σ-donor ligand.[20h,j] Therefore, the above-mentioned decrease in the electron density on iron(II) can be compensated by electron donation from the nitrogenous bases through the d_{z^2} orbital. This donation results in destabilizing the d_{z^2} orbital, which can induce the observed decrease in the g_{iso} (at room temperature) and $(g_1+g_2+g_3)/3$ (at 77 K) values toward the free spin value.

The g_{iso} (at room temperature) and g_2 values (at 77 K) for Fe[(F$_5$)tpp](NO) complex with 1-methylimidazole are very close to those for the Fe(ppixdme)(NO) complex with imidazolate, which is formed by deprotonation of imidazole and has greater σ-donor ability than the imidazole.[20j] This suggests that the 1-methylimidazole ligand with no dissociable proton in the former complex profoundly donates the σ electron toward iron(II) in a manner similar to the imidazolate in the latter complex.

Consequently, as the electron-withdrawing power of the substituents becomes greater, the Fe–N(nitrogenous base) bond can be strengthened (or shortened) in the six-coordinated complexes, accompanying an increase in NO stretching frequencies as described above. This is inconsistent with the result obtained by the X-ray structural and IR spectral study for six-coordinated nitrosyl(tetraphenylporphyrinato)iron(II) complexes with nitrogenous base that the Fe–N(nitrogenous base) bond lengths decrease with a decrease in the NO stretching frequencies.[17d] Further, when the trans axial ligand in the six-coordinated nitrosyl(porphyrinato)-iron(II) complexes is successively substituted, the g

values increased with an increase in the NO stretching frequencies.[20g)] On the other hand, when the side chains of porphyrin periphery in the complexes are also successively substituted, the g values increased with a decrease in the NO stretching frequencies. Such reverse relations between the EPR g values and NO stretching frequencies present a striking contrast between the cis- and trans-effects. This inconsistency or contrast would arise from the fact that the cis-effect on the axial nitrosyl ligand in nitrosyl(porphyrinato)iron(II) complexes is transferred primarily through the porphyrin π and iron d_π orbitals, while the trans-effect is transferred primarily through the iron d_{z^2} orbital.

These findings provide a clue to evaluate the extent of contribution of the cis- and trans-effects to the EPR and IR parmeters in nitrosylhemoproteins and would improve the availability of a nitrosyl ligand as an electronic probe for clarifying the electronic structure and the stereochemistry around the heme.

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