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Electron Paramagnetic Resonance Study of the Interaction of Nitrosylprotoheme Dimethyl Ester with Nitrogenous Bases

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The interaction of nitrosylprotoheme dimethyl ester with various nitrogenous bases has been studied by electron paramagnetic resonance (EPR) measurements at room temperature and 77 K. Through the analysis of the dependence of EPR spectra on the concentration of base at room temperature, it was found that the pyridine and the imidazole derivatives differ in exchange rate among the five- and six-coordinate species in reaction with the nitrosylprotoheme dimethyl ester. The EPR parameters of the systems with an unhindered base vary linearly with the basicity of the base. The effect on EPR spectra of the steric interaction of the base with the porphyrin core is also discussed.

Introduction

The EPR spectroscopy for the nitrosylhemoproteins has afforded useful information concerning the stereochemistry of the heme environment, the characterization of the axial ligand *trans* to a nitrosyl group and the equilibrium of the quaternary structure between the oxy- and deoxy forms for nitrosylhemoglobin [1]. The analysis of the EPR spectra of the nitrosylporphyrinatoiron(II) complexes with various conditions has been shown to be applicable to the understanding of structure–function relationships in hemoproteins [2].

In a preceding paper [2f] dealing with the EPR of model systems, nitrosylprotoheme dimethyl ester complexes with imidazole derivatives, it was found that two molecular species can exist in the model systems and probably in the nitrosylhemoproteins. It was also reported that the imidazole and the pyridine as an axial base of the nitrosylprotoheme dimethyl ester differ in the dependence of EPR spectra on the base concentration at room temperature.

In this paper the result of further studies for the EPR of the systems with the nitrosylprotoheme dimethyl ester and the nitrogenous base is presented. It can be shown that the pyridine and imidazole derivatives differ in exchange rate among the five- and six-coordinate species in reaction with the nitrosylprotoheme dimethyl ester and that the EPR parameters of the systems with an unhindered base vary linearly with the basicity of the base.

The abbreviations used are as follows: nitrosyl(protoporphyrin IX dimethyl ester)iron(II), ONFe(PPDME); pyridine, Py; 2-methylpyridine, 2MePy; 3-methylpyridine, 3MePy; 3-chloropyridine, 3ClPy; 4-methylpyridine, 4MePy; 4-acetylpyridine, 4AcPy; 4-cyanopyridine, 4CNPy; 2,6-dimethylpyridine, 2,6-DMePy; 3,4-dimethylpyridine, 3,4DMePy; 3,5-dimethylpyridine, 3,5DMePy; 2,4,6-trimethylpyridine, 2,4,6TMePy; 4,4′-bipyridine, 4,4′BPy; imidazole, Im; 1-methylimidazole, NMeIm; 1-acetylimidazole, NAcIm; 4-methylimidazole, 4MeIm; histamine, Him; 2-methylimidazole, 2MeIm; pyrazine, Pyz; isoquinoline, Isoq; aniline, Anil; *p*-toluidine, *p*-Tol; piperidine, Pip.

Experimental

Materials

Nitrosyl(protoporphyrin IX dimethyl ester)iron(II) was prepared as described previously [2e]. The nitrogenous bases were obtained commercially. The liquid bases were stored over molecular sieves (4A) for several days before being distilled by flowing N_2 under reduced pressure. They were then stored under N_2 until used. The solid bases were recrystallized from benzene. Chloroform (ethanol-free) and acetone were dried, distilled, and stored under N_2. The solvents and the liquid bases were deoxidized by bubbling pure N_2 prior to use.

EPR Measurements

EPR measurements were carried out using a JES-ME-3X (X-band) spectrometer with 100 kHz field modulation at room temperature (about 20 °C) and at 77 K. The second-derivative display was obtained by the use of 80 Hz field modulation. As a standard, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) powder and MgO powder doped with Mn^{2+} were used. EPR samples, in which the concentration of ONFe(PPDME) was 0.015 *M*, were prepared under N_2 atmosphere.

Results

Dependence of EPR Spectra on the Base Concentration

The reaction of ONFe(PPDME) with nitrogenous bases (B) in solution takes place according to the following equilibrium:

$$\text{ONFe(PPDME)} + \text{B} \underset{}{\overset{\text{K}}{\rightleftharpoons}} \text{ONFe(PPDME)B} \quad (1)$$

$$\text{K} = \frac{[\text{ONFe(PPDME)B}]}{[\text{ONFe(PPDME)}]\,[\text{B}]} \quad (2)$$

Both the five-coordinate ONFe(PPDME) and the six-coordinate ONFe(PPDME)B in eqn. 1 are well known to be EPR positive [2f]. Thus, if the rate of exchange among the two species in eqn. 1 is slow on the EPR time scale, two signals assignable to the two species can be observed. On the contrary, if the rate is fast on the EPR time scale, only one signal is observed at the equilibrium position dependent upon the base concentration, as the two species cannot be distinguished [3].

At room temperature, only one signal (triplet) was observed in chloroform solution of ONFe(PPDME)–Py system and was shifted to a higher magnetic field side with an increase in molar ratio, [Py]/[ONFe(PPDME)] (Fig. 1); then the g value and the coupling

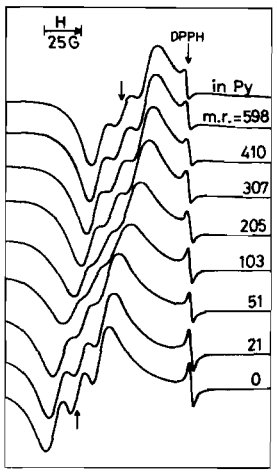

Fig. 1. Dependence on the base concentration of EPR spectra at room temperature for the ONFe(PPDME)–Py system in chloroform. Molar ratio (m.r.) = [Py]/[ONFe(PPDME)]. [ONFe(PPDME)] = 15 mM.

constant of the triplet become smaller with the molar ratio. At 77 K, the EPR spectra of the systems with the molar ratio greater than 23 were essentially identical to the characteristic spectrum (Fig. 4a) of six-coordinate species. It is considered from these results in the case of B = Py that both the five- and six-coordinate species are rapidly exchanging on EPR time scale at room temperature. Consequently, the concentration of the two species can be determined by using the g value (at the center of the triplet) or the coupling constant for each given concentration of the base. In the case of using the g value,

$$[\text{ONFe(PPDME)}] = \frac{g_c - g}{g_c - g_0}\,[\text{ONFe(PPDME)}]_T \quad (3)$$

$$[\text{ONFe(PPDME)B}] = \frac{g - g_0}{g_c - g_0}\,[\text{ONFe(PPDME)}]_T \quad (4)$$

where g_0 and g_c are the g values of the five-coordinate ONFe(PPDME) (2.052 in chloroform) and the six-coordinate ONFe(PPDME)B respectively, g is the observed g value, and $[\text{ONFe(PPDME)}]_T$ is the total concentration of ONFe(PPDME). From eqns. 2, 3, and 4,

$$\text{K} = \frac{g_0 - g}{(g - g_c)[\text{B}]} \quad (5)$$

From eqn. 5, the following equation is derived:

$$\frac{1}{g_0 - g} = \frac{1}{g_0 - g_c} + \frac{1}{g_0 - g_c} \cdot \frac{1}{\text{K}} \cdot \frac{1}{[\text{B}]} \quad (6)$$

It is possible to evaluate both K and g_c from the intercept and slope of plots of $1/(g_0 - g)$ against $1/[\text{B}]$.

Similarly, K and A_c (the coupling constant of the six-coordinate species) can also be evaluated from the change of coupling constant. These plots of the data obtained from Fig. 1 are shown in Fig. 2. Then drawing a straight line by use of the least-squares method, K = 0.29 (M^{-1}) and g_c = 2.024 are obtained from the change of g value; K = 0.49 (M^{-1}) and A_c = 15.0 (G) from that of coupling constant.

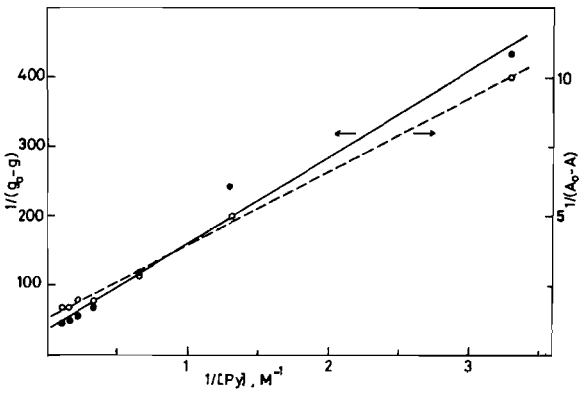

Fig. 2. Plot of $1/(g_0 - g)$ (●——●) and $1/(A_0 - A)$ (○– – –○) against the reciprocal of the base concentration for ONFe(PPDME)–Py system.

TABLE I. EPR Parameters of the ONFe(PPDME)–Nitrogenous Base at Room Temperature[a].

Base	pK_a[b] (BH^+)	In Acetone[c]		In Chloroform[c]		In Neat Base	
		g_{iso}	A_{iso}	g_{iso}	A_{iso}	g_{iso}	A_{iso}
None		2.050_9	16.1	2.052_0	15.8		
Unhindered Base							
4CNPy	1.90	2.041_3	15.3	2.042_5	15.4		
3ClPy	2.84	2.039_4	15.6	2.041_9	15.6	2.038_2	15.5
4AcPy	3.51	2.034_8	14.9	2.037	15	2.034	
4,4'BPy	4.82	2.032	15				
Py	5.23	2.031_2	15.3	2.034_0	15.3	2.029_2	15.3
3MePy	5.68	2.032_7	15.4	2.036_7	15.3	2.030_9	15.5
4MePy	6.02	2.029_6	15.1	2.032	15	2.028_5	15.1
3,5DMePy	6.15	2.034_7	15.4	2.038_8	15.3	2.033_7	15.4
3,4DMePy	6.46	2.030_8	15.0	2.033	15	2.030	15
Isoq	5.40	2.036	15	2.038			
Pyz	0.65	2.037_8	15.7	2.038_8	15.8		
Hindered Base							
2MePy	5.97	2.049_4	15.7	2.050_6	15.7	2.049_8	15.7
2,6DMePy	6.75	2.049_5	15.3	2.050_9	15.8	2.050_5	15.8
2,4,6TMePy	7.43	2.050_3	15.9	2.051_3	15.9	2.050_8	15.9
2MeIm	7.86	2.050_0	16.1	2.052_2	16.1		
Anil	4.60	2.047_2	15.9	2.045_6	15.7	2.042_8	15.4
p-Tol	5.12	2.046_8	15.9	2.046_5	15.6		

[a]Abbreviations are as given in the Introduction. g_{iso}, isotropic g value; A_{iso}, isotropic coupling constant in gauss. [b]A. Albert, *Phys. Methods Heterocycl. Chem.*, *1*, 1 (1963) and D. M. Smith, 'Rodd's Chemistry of Carbon Compounds', Vol. IV-F, ed. by S. Coffey, Elsevier, Amsterdam (1976), Chap. 24. [c][B]/[ONFe(PPDME)] ≈ 300.

Since the value of g_c (2.022) in the system ONFe(PPDME)–Ims is known to be almost insensitive to the kind of imidazole derivatives [2f], it is reasonably assumed that the value of g_c in the system with all pyridine derivatives used is 2.024. Then, from the g value observed in chloroform (Table I), the values of g_0 (2.052) and g_c (2.024) and eqn. 5, K was estimated as follows: 0.1 for 4-cyanopyridine, 0.6 for 4-methylpyridine, and 0.01 for 2-methylpyridine. It is obvious from eqn. 5 that at a given concentration of base the farther the g value is from the g_0 one and the closer it is to the g_c one, the larger the equilibrium constant becomes.

EPR Spectra at Room Temperature of ONFe(PPDME)–Nitrogenous Base System in Solution

At room temperature, the systems with all bases except those with 4,4'-bipyridine (in chloroform) and with piperidine exhibited the EPR spectrum with a triplet structure as shown in Fig. 1, though the resolution of the triplet was lower in the systems with 4-acetylpyridine, 3,4-dimethylpyridine, 4,4'-bipyridine (in acetone), and isoquinoline. Both the g value (at the center of the triplet) and the coupling constant, which were evaluated from the second-derivative curve, are given in Table I. The EPR parameters of ONFe(PPDME)–nitrogenous base systems differ slightly with different bases and with different solvents and are smaller than those of free ONFe(PPDME). The unhindered- and hindered bases in Table I refer to the bases without and with a steric interaction between the porphyrin core and the substituent (or the atom) adjacent to the bonding nitrogen, respectively.

Figure 3 shows the EPR spectra of the acetone and chloroform solutions of ONFe(PPDME)–4,4'BPy system with both first- and second-derivative display. It is more distinct in the second-derivative display that the spectrum in acetone, which is slightly resolved into a triplet, is different from that in chloroform The second-derivative display of the spectrum in chloroform resembles that of ONFe(PPDME)–unhindered imidazoles system in chloroform which exhibits overlapping of two components [2f]. This suggests that two signals assignable to two species can be observed in the chloroform solution of ONFe(PPDME)–4,4'BPy system. The ONFe(PPDME)–Pip systems in acetone, chloroform, and neat piperidine also exhibited a similar spectrum to the ONFe(PPDME)–4,4'BPy system in chloroform.

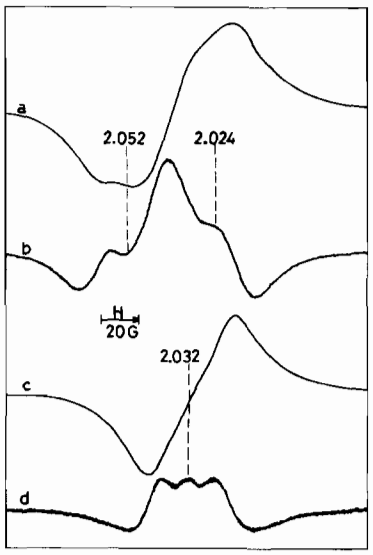

Fig. 3. The EPR spectra for ONFe(PPDMe)–4,4'BPy system at room temperature: (a) first- and (b) second-derivative displays in chloroform; (c) first- and (d) second-derivative displays in acetone.

EPR Spectra at 77 K of ONFe(PPDME)–nitrogenous Base System in Solution

Figure 4 shows the EPR spectra (first- and second-derivative display) of ONFe(PPDME)–Py and –2,4,6-TMePy systems ([B]/[ONFe(PPDME)] ≈ 300) in frozen acetone glass at 77 K. The system with 2,6-dimethylpyridine exhibited a similar spectrum to that with 2,4,6-trimethylpyridine (Fig. 4 c, d). The systems with the other bases exhibited a similar spectrum to that with pyridine (Fig. 4 a, b), though the resolution of the hyperfine structure at g_z component was lower in the system with 2-methylimidazole.

The spectrum of the system with pyridine, as well as that with 1-ethylimidazole [2f], was characterized by the line shape with three effective g values and its g_z component exhibited a hyperfine structure of a triplet of triplets, which is originated from the hyperfine interaction with both ^{14}N nuclei of the NO group (the coupling constant, A_1) and the *trans* axial base (A_2).

The EPR parameters of the systems with various nitrogenous bases are shown in Tables II and III. The g_y and g_A values and the value of axial coupling constants were evaluated from the second-derivative curve; thus the g_y and g_A values obtained should be considered as rough estimates. The absorptions termed I and II in Tables II and III are probably derived from the two molecular species (species I and II), as described previously [2f], which differ in the structure of the Fe–N–O unit. At X-band frequency one component (g_A) of II absorption was present as a shoulder and another component (g_B) was hidden in the g_y and g_z components of I absorption, whereas

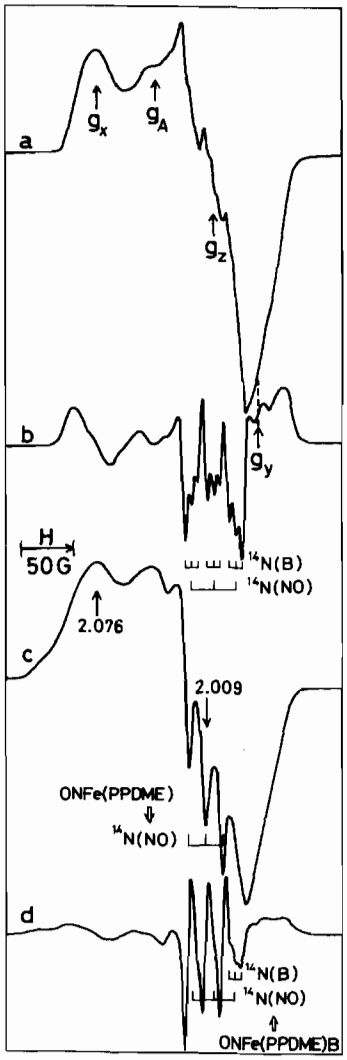

Fig. 4. The EPR spectra in frozen acetone glass at 77 K: (a) first- and (b) second-derivative displays of ONFe(PPDME)–Py system; (c) first- and (d) second-derivative displays of ONFe(PPDME)–2,4,6TMePy system.

at Q-band frequency both components were resolved [2f]. From the EPR spectrum at Q-band frequency of ONFe(PPDME)–Py system in frozen chloroform glass at 77 K, the values of g_A and g_B have been evaluated to be 2.036 and 1.992, respectively [2f].

At 77 K the spectra in chloroform were essentially identical with those in acetone, whereas those in some liquid bases were lower in resolution of the hyperfine structure. As shown in Table III, the EPR parameters of the systems with pyridine and 3-chloropyridine are almost insensitive to the solvent.

Discussion

In solution at room temperature, the EPR spectra of ONFe(PPDME)–nitrogenous base system can

TABLE II. EPR Parameters of the ONFe(PPDME)–Nitrogenous Base Systems at 77 K[a].

Base	pK_a[b] (BH^+)	I Absorption[c]			II Absorption[c]		Axial Coupling Constant (G)	
		g_x	g_y	g_z	g_A	g_B	A_1	A_2
Unhindered Base								
4CNPy	1.09	2.078	1.98_2	2.005_9	2.04_2		21.6	6
3ClPy	2.84	2.080	1.98_1	2.005_5	2.04_2		21.1	6
4AcPy	3.51	2.078	1.97_9	2.005_4	2.04_1		21.3	6.2
4,4'BPy	4.82	2.077	1.98_7	2.005_1	2.04_0		21.4	6.2
Py	5.23	2.076	1.97_8	2.004_9	2.04_0		21.4	6.2
3MePy	5.68	2.076	1.97_7	2.004_9	2.04_0		21.4	6
4MePy	6.02	2.076	1.97_8	2.004_6	2.03_8		21.5	6.3
3,5DMePy	6.15	2.078	1.97_8	2.005_2	2.03_9		21.7	6.4
3,4DMePy	6.46	2.075	1.97_8	2.004_6	2.03_9		21.7	6.1
NAcIm[d]	3.6	2.077	1.977	2.004_9	2.031	1.987	21.8	7.1
Him[d]	6.03	2.073	1.974	2.004_5	2.032	1.987	21.5	5.5
Im[d]	6.95	2.072	1.971	2.004_0	2.030	1.987	21.7	6.9
NMeIm[d]	7.33	2.074	1.971	2.004_1	2.031	1.988	21.5	6.9
4MeIm[d]	7.52	2.071	1.970	2.003_8	2.031	1.987	21.9	7.0
Isoq	5.40	2.075	1.98	2.004	2.04		~21	~6
Pyz	0.65	2.081	1.98_2	2.005_8	2.04_3		20.8	6.4
Hindered Base								
2MePy	5.97	2.078	1.97_9	2.005_4	2.04_1		21.0	6.3
2MeIm	7.86	2.079	1.97_7	2.004_4	2.03_7		21.6	6.2
Anil	4.60	2.084	1.98_4	2.006_6	2.04_9		20.5	4.4
p-Tol	5.12	2.082	1.98_3	2.006_2	2.04_8		20.5	4.6
Pip	11.12	2.078	1.97_6	2.004_8	2.03_9		21.0	6.2

[a] Abbreviations are as given in the Introduction. The solvent is acetone for pyridine derivatives and 2MeIm and chloroform for the other bases. [B]/[ONFe(PPDME)] ≈ 200 for Im and NMeIm and ≈300 for the other bases. [b] See footnote b of Table I. [c] See text. [d] The data in ref. 2f were reevaluated in this study.

TABLE III. EPR Parameters of the ONFe(PPDME)–3-Chloropyridine and –Pyridine Systems in Various Solvents at 77 K[a].

Base (pK_a)[b]	Solvent	I Absorption[c]			II Absorption[c] g_A	Axial Coupling Constant (G)	
		g_x	g_y	g_z		A_1	A_2
3ClPy	acetone	2.080	1.98_1	2.005_5	2.04_2	21.1	6
(2.84)	chloroform	2.081	1.98_3	2.005_8	2.04_3	21.2	6.2
	neat	2.081	1.98_0	2.005_4	2.04_3	21.0	6.0
Py	acetone	2.076	1.97_8	2.004_9	2.04_0	21.4	6.2
(5.23)	chloroform	2.079	1.97_7	2.004_9	2.04_0	21.0	6.5
	neat	2.075		2.004_9	2.04	~22	~6

[a,b,c] See footnotes a, b, and c of Table II, respectively.

apparently be divided into two types. In the first type (Im type) two signals assignable to the two species of five-coordinate ONFe(PPDME) and six-coordinate ONFe(PPDME)B can be observed as in the systems with unhindered imidazoles [2f]. In the EPR spectra of this type, the relative intensity of the ONFe(PPDME) to ONFe(PPDME)B signal decreased with an increase in base concentration. In the second type (Py type) only one signal can be observed at the equilibrium position dependent upon the base con-

centration as in the systems with pyridine derivatives. Such dependence of EPR spectra on the base concentration is clearly shown in Fig. 1. These two types differ in rate of exchange among the five- and six-coordinate species in eqn. 1. In the Py-type spectra the rate of exchange can be fast on the EPR time scale, whereas in the Im-type spectra the rate can be slow. Thus, the difference ($\approx$ 50 G) between the ONFe(PPDME) and ONFe(PPDME)B signals in resonant field strength may allow us to expect that the exchange reaction in eqn. 1 occurs in the time range shorter than 10^{-9} sec for Py type and longer than 10^{-9} sec for Im type.

In the dependence of EPR spectra on the base concentration at room temperature, the equilibrium constant (K) in eqn. 1 is evaluated from the relative intensity change of two signals in the case of Im type and from the change of the signal position in the case of Py type. The value of the equilibrium constant thus obtained was about 2 for 1-methylimidazole (Im type) [2f] and about 0.3 for pyridine (Py type).

The fact that the exchange rate for Py type faster than for Im type suggests that the nitrogenous bases of Py type are more labile than those of Im type. Such results have ben obtained also by NMR study for axial lability of nitrogenous bases in $[PFe(III)B_2]^+X^-$ (P, *meso*-tetraarylporphyrin or octaethylporphyrin; B, imidazoles or 4-methylpyridine; X, halide ions) [4].

The system with piperidine which is assumed to be a labile base on account of the steric interactions between the hydrogen atom but the porphyrin core did not exhibit the Py-type and Im-type spectrum. This may be based on the high basicity (pK_a, 11.12) of piperidine.

The system with 4,4′-bipyridine exhibited the spectrum of Im type in chloroform and that of Py type in acetone. This suggests that the axial lability of the base in ONFe(PPDME)–nitrogenous base system is sensitive to solvent.

The g values of ONFe(PPDME)–nitrogenous base system in acetone at room temperature (Table I) are plotted against the pK_a values of bases in Fig. 5, in which the straight line was drawn by the use of the least-squares method for unhindered pyridines. The g_x and g_z values at 77 K (Table II) are plotted against the pK_a values of the bases in Figs. 6 and 7, respectively, in which the solid and dashed lines were drawn by the use of the least-squares method for unhindered pyridines and imidazoles, respectively. Although it has been reported [2a, f] that the EPR parameters of

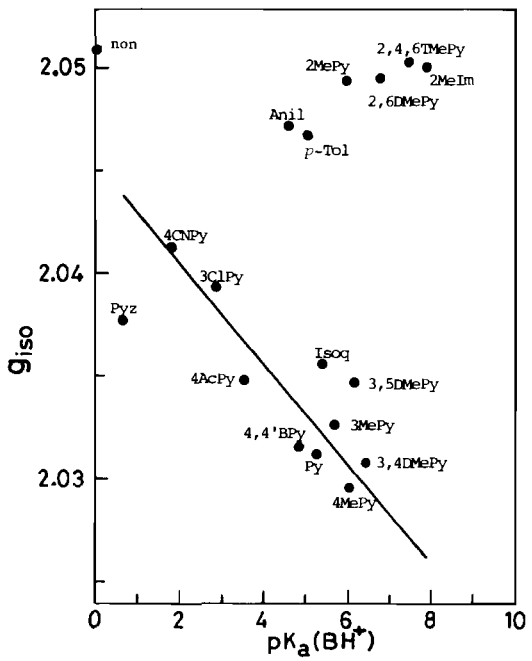

Fig. 5. Relationships between the isotropic g value in acetone and the basicity of the bases at room temperature: the solid line is for unhindered pyridines. Abbreviations are as given in the Introduction.

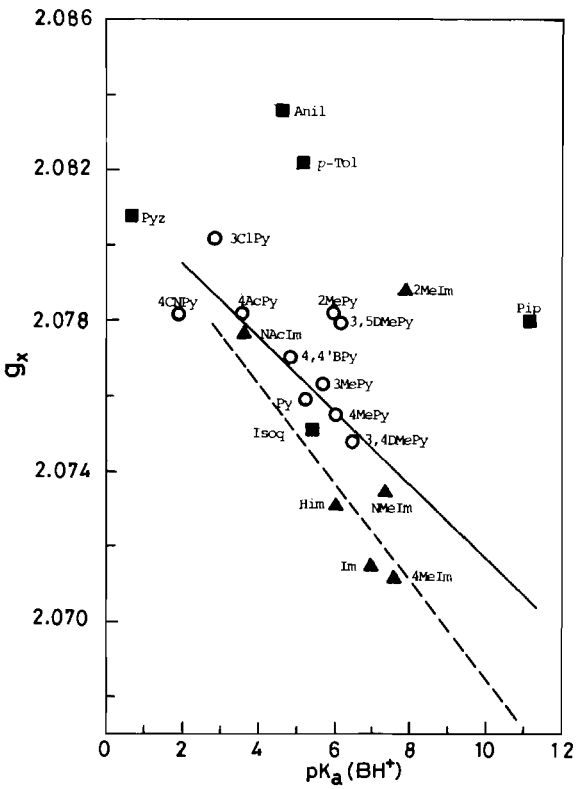

Fig. 6. Relationships between the g_x value and the basicity of the bases at 77 K: the solid line is for unhindered pyridines, the dashed line for unhindered imidazoles. Abbreviations are as given in the Introduction. ○, pyridines; ▲, imidazoles; ■, the other bases.

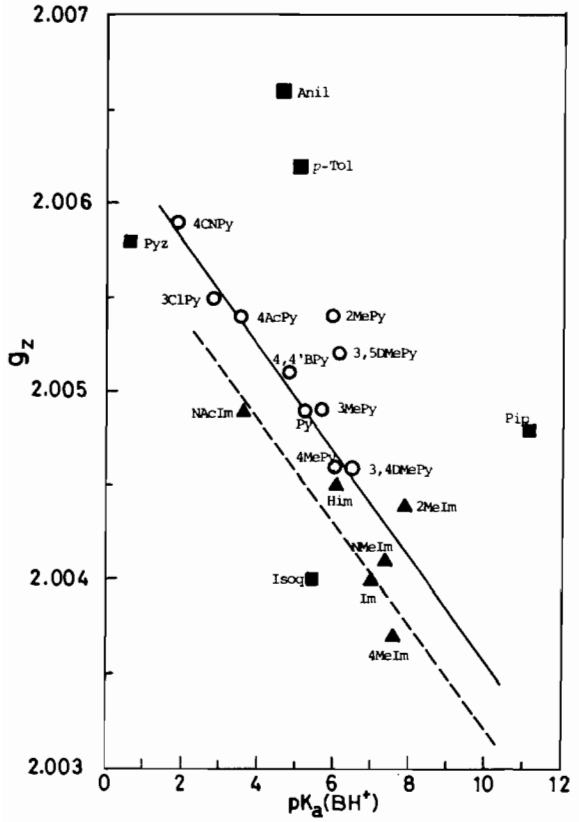

Fig. 7. Relationships between the g_z value and the basicity of the bases at 77 K: the solid line is for unhindered pyridines, the dashed line for unhindered imidazoles. Abbreviations are as given in the Introduction. ○, pyridines; ▲, imidazoles; ■, the other bases.

the systems with unhindered bases were not highly sensitive to the kind of base; the distinct relationship between the g values and the basicity of the base can be found in Figs. 5–7.

As shown in Fig. 5, the g values of the systems with unhindered bases at room temperature decrease with the basicity and approach that of six-coordinate ONFe(PPDME)B ($g_c \approx 2.024$). Equation 5 shows that the equilibrium constants at a given concentration of base increase as the g values approach the g_c one. These results indicate that the equilibrium constants or the Fe–N(base) bonding strength can increase with the basicity in the systems with unhindered pyridines. Then, the g values for almost all bases in Table I decrease in the following order; in chloroform, in acetone, and in neat base and thus, the equilibrium constants may increase in that order. Both the g_x (Fig. 6) and g_z values (Fig. 7) of the systems with unhindered pyridines and imidazoles at 77 K decrease with the basicity.

Table II shows that the g_y and g_A values also exhibit a slight tendency to decrease with the basicity. The systems with hindered base, the Fe–N(base) bonding of which can be weakened by the steric interaction with the porphyrin core, exhibit larger g values than those with unhindered base as shown in Figs. 6 and 7; thus, the g values at 77 K for these systems appear to become larger with a decrease in the strength of Fe–N(base) bonding. The lone-pair electrons on the nitrogen atom of the base are available for σ bonding of the base with the iron and thus, the basicity of the base is a measure of σ-bonding ability [5, 6]. The above-mentioned findings, accordingly, imply that the Fe–N(base) bonding strength increases with a σ-bonding ability of the base. This suggests that the σ bonding is relatively important in the Fe–N(base) bonding of the systems.

As shown in Figs. 6 and 7, the systems with unhindered pyridines have slightly larger g values than those with unhindered imidazoles of similar basicity, indicating that the former systems are weaker in Fe–N(base) bonding. Further, pyridines are more labile than imidazoles as described above. Such difference between both bases seems to be derived from a different degree of π back-bonding from Fe d_π to base π^* orbital. Thus, in this case, imidazoles may be slightly better π acceptors than pyridines, which is consistent with the results of the study of porphyrin cobalt(II) complexes with some amines [7].

It is noted that the II absorption is sensitive to basicity, though it has been described [2f] that the Fe–N(base) bonding in the species II is weaker than in the species I.

As shown in Figs. 5 to 7, the g values of the systems with hindered bases vary widely from system to system, being sensitive not to basicity but to degree of steric interaction with the porphyrin core. The g values of the system with 3,5-dimethylpyridine are larger than those which would be predicted from the straight line of the system with unhindered pyridines, which suggests that the substituents on the β carbons also interact sterically with the porphyrin core. It has previously been shown [2f] that the EPR spectra at 77 K of chloroform solution of the system with a [2MeIm]/[ONFe(PPDME)] molar ratio of 200 are essentially identical to those of free ONFe(PPDME). In this study the characteristic spectrum of six-coordinate species was observed in acetone solution of this system with the molar ratio of 300 at 77 K, whereas the chloroform solution of the molar ratio of 400 exhibited only the spectrum similar to free ONFe(PPDME). This sensitivity to solvent remains to be clarified. Then, the spectra at 77 K of the systems with 2,6-dimethyl- and 2,4,6-trimethylpyridine (Fig. 4 b) exhibit overlapping of the signals of five- and six-coordinate species. Accordingly, it seems likely that the hindered bases can coordinate to iron(II) of ONFe(PPDME) to form a six-coordinate species.

In the systems with aniline and *p*-toluidine which show the largest g_z values at 77 K, the coupling constant A_1 due to NO nitrogen is similar to that of the other systems and the A_2 due to base nitrogen is 30% less than that of the other systems, indicating the existence of larger steric interactions. In the other systems, the coupling constants appear to be almost insensitive to the degree of interactions and the bonding between the iron and the base.

The systems with 4,4′-bipyridine and pyrazine, which are known as bridging ligands, exhibit essentially identical line shape and parameters to those with the other bases. Thus, EPR spectral change based on dimer formation and exchange interaction was not observed in these systems.

As mentioned above, an approximate linear relationship exists between the g values and the basicity of the base in ONFe(PPDME)–nitrogenous base systems both at room temperature and at 77 K. Accordingly, the evaluation of the g values for the ONFe(PPDME) complex with unknown *trans* axial base may lead to a rough estimation of the basicity of the base. It seems likely that the application of this relationship to nitrosylhemoproteins leads to the characterization of the *trans* axial ligand.

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