of ^{15}N labeled t-RNA, where, however, an interference between different relaxation mechanisms is discussed.[13]

From the measurements shown partly in Figures 1 and 2, we calculate $k_{ab}^{HH} = 10^{11} \exp(-41(kJmol^{-1})/RT)$ and $(k^{HH}/k^{HH})_{298K} = 9$. These data are not affected by intermolecular proton exchange.[11] Although the chemical shifts depend strongly on concentration and temperature, indicating aggregation of I, preliminary measurements show that the rate constants are not influenced by this phenomenon, which is consistent with the observation of the absence of kinetic solution–solid state effects for *meso*-tetratolylporphine.[5]

We find a surprising coincidence of the rate constants k_{ab}^{HH} derived here for I with the symmetrical rate constants of TPP in solution,[3] where $k^{HH} = 10^{10.9} \exp(-40(kJmol^{-1})/RT)$ and $(k^{HH}/k^{HD})_{298}K = 10$. The acetyl group in I, therefore only decreases the basicity of the adjacent N atom (i.e., increases the energy of the corresponding tautomer) but does affect significantly the barrier of the migration. A similar coincidence was found previously[5] for the solid-state tautomerism of TPP, where only the energy of the dominant tautomer was lowered. As far as the theory is concerned, it seems that the idea of thermally activated hydrogen tunneling in porphyrins[3-5] has been accepted,[6,8] in spite of the absence of a definite tunneling model. However, a coherent tunneling process,[4] where the tunneling rates would be very sensitive to perturbations, can be excluded. The possibility of a nonconcerted hydrogen motion warrants reconsideration. In order to contribute to a better understanding of these processes, we are currently studying the complex kinetic HH/HD/DD isotope effects of the tautomerism in I and of related compounds in solution and in the solid state.

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NMR Studies of Metalloporphyrin Radicals. Iron(II) Oxophlorin Radical Formed from Iron(III) *meso*-Hydroxyoctaethylporphyrin

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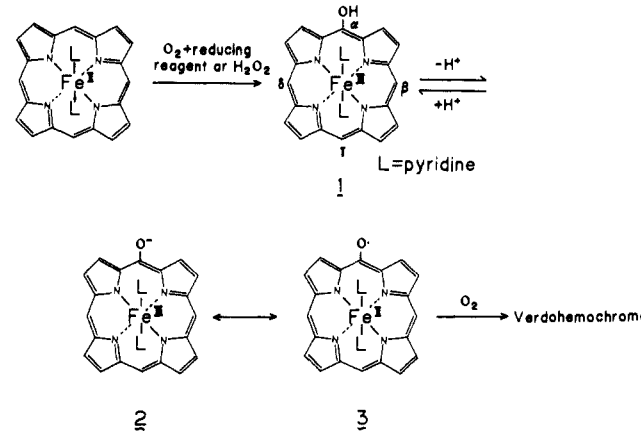
The Fe(II) oxophlorin radical **3**, a redox valence isomer of Fe(III) *meso*-oxophlorin anion **2** formed from Fe(III) *meso*-hydroxyporphyrin **1**, has been a putative key intermediate in the heme catabolism, in which the heme ring is cleaved by the coupled oxidations (Scheme I).[1,2] However, evidence for iron oxophlorin radical **3** has not been obtained[3] until we observed the unusual

Scheme I



hyperfine-shifted proton NMR spectrum of Fe(III) *meso*-hydroxyhemin in aqueous pyridine solution which was attributed to Fe(II) oxophlorin radical rather than the Fe(III) state on the basis of unusually large hyperfine shifts for the meso protons.[4] To further confirm Scheme I and to better characterize the electronic state of the iron oxophlorin radical, we examined here the NMR and UV spectra of the iron oxophlorin radical derived either from Fe(III) *meso*-hydroxyoctaethylporphyrin (OEP) under various solution conditions or directly from Fe(II) porphyrin bis(pyridine) complex plus H_2O_2.

Fe(III) *meso*-hydroxyOEP was obtained by hydrolysis of the corresponding *meso*-benzoyloxyOEP.[1] When we dissolved it in pure pyridine under anaerobic condition, we obtained the sharp hyperfine-shifted ^1H NMR spectrum in the upfield and downfield regions (Figure 1a). The two proton peaks in the far upfield region at −154 and −111 ppm were readily assigned to the meso protons by utilizing the deuterium-labeled compound. The four methylene proton resonances, one at 32 ppm and others in the 6 to −5 ppm region, are also quite unique in their shift positions compared with those established for Fe(II) or Fe(III) paramagnetic porphyrin complexes.[5] The ESR was silent at the temperatures employed for the NMR measurements,[6] but the magnetic susceptibility measurement by the NMR Evans method showed a single electron spin ($\mu_{eff} = 2.4\ \mu_B$ at 23 °C). All of the NMR peaks exhibited an unusual temperature-dependent shift without obeying the Cuire law (Figure 2A). Its electronic spectrum with absorption maxima at 606 and 649 nm (Figure 1c) which is quite different from those for Fe(II) or Fe(III) porphyrin complexes did not experience any significant changes with temperature variation from 20 to −30 °C except for slight intensity changes having isosbestic points at 662 and 710 nm. Upon addition of acid, the meso proton resonances drastically shifted downfield (Figure 1b), depending on the acid concentration. This is most likely due to an equilibrium shift toward the protonated ferric low spin form (**1**) (Scheme I), which was further confirmed

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(3) It has been reported that oxophlorine radical is formed on oxidation of oxophlorin (the predominant keto form of *meso*-hydroxyporphyrin) either in its metal-free or zinc-complex form; Bonnett, R.; Dimsdale, M. J.; Sales, K. D. *J. Chem. Soc.* **1970**, 962–963. Fuhrhop, J.-H.; Besecke, S.; Subramanian, J. *J. Chem. Soc., Chem. Commun.* **1973**, 1–2. Fuhrhop, J.-H.; Besecke, S.; Subramanian, J.; Mengersen, Chr.; Riesner, D. *J. Am. Chem. Soc.* **1975**, *97*, 7141–7152.

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(5) We examined paramagnetic NMR spectral characterization of a variety of Fe(III) *meso*-benzoyloxyOEP complexes in high- and low-spin states. No unusual hyperfine shifts as compared with Fe(III) OEP analogues were noticed.

(6) In our previous report,[4b] we described the ESR spectrum ($g = 2.31$, 2.002, 1.78) obtained at 77 K for **3** derived from Fe(III) *meso*-hydroxyhemin in aqueous pyridine, which was attributed to the low-spin Fe(I) state formed from **3** via an intramolecular reduction at low temperature. The similar ESR spectrum was obtained for the present OEP analogue at 77 K, but no ESR signal was detected at temperatures above 180 K. Thus, we tentatively interpret these findings in a way that oxophlorin radical **3** with an enhanced electron spin relaxation predominates at room temperature.

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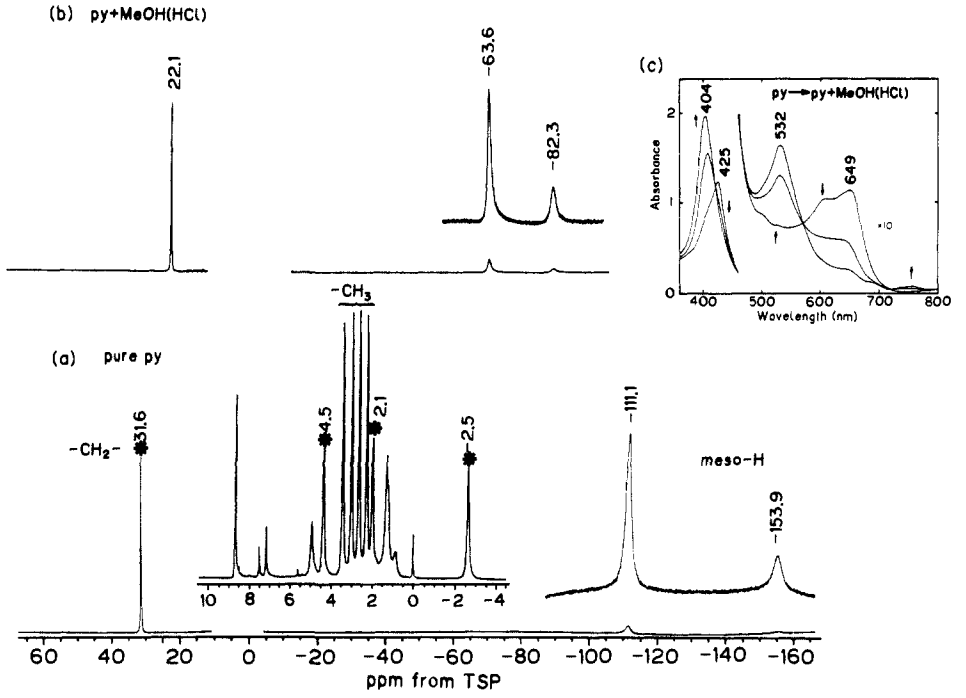

Figure 1. (a) Proton NMR spectrum (at 300 MHz, 23 °C) of Fe(III) *meso*-oxyOEP under anaerobic condition in pure pyridine-d_5 and (b) in pyridine + methanol + 20%HCl (69:30:1). (c; inset) The absorption spectral change of Fe(III) *meso*-oxyOEP upon addition of methanol-HCl. When the pyridine solution of **1** was acidified, the UV-visible absorption peak appeared at 532 nm, characteristic of the Fe(III) low-spin porphyrin complex.

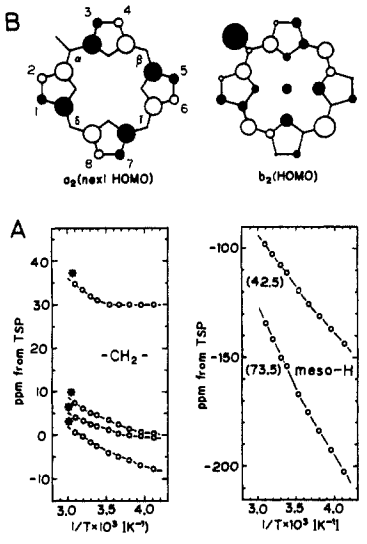

Figure 2. (A) Temperature dependences (Curie plot) of the proton NMR resonances in Figure 1a. The numbers in the parenthesis for the meso proton resonances are the limiting shifts extrapolated to an infinite temperature. (B) The MO-calculated (INDO) electron distribution in the b_2 (HOMO) and a_2 (next HOMO) orbitals for Fe(II) oxophlorin radical **3**.

by the UV-visible spectra (Figure 1c).

All these spectral observations for Fe(III) *meso*-hydroxyOEP in aqueous or pure pyridine solution can be best explained by Scheme I[7] in which the hydroxy proton is deprotonated to afford the enolate form, thereby electron transfer occurring from the enolate anion to the ferric iron to form the bis(pyridine) complex of Fe(II) low-spin oxophlorin radical **3**.[8,9] **1** in various amounts of aqueous pyridine solution afforded the meso proton resonances at about −100 to −140 ppm, depending on the H_2O content, which could be due to incomplete deprotonation of the meso-hydroxy group. The use of DMF or *N*-methylimidazole in place of pyridine also afforded **3**, suggesting that pyridine did not serve as the reducing reagent to form **3**. Aeration of these solutions yielded the absorption spectra characteristic of verdohemochrome, indicating that the free radical species **3** is attacked by oxygen to form the heme-cleaved species.[10]

Since the metal center of **3** is in a diagmagnetic state (ferrous low spin), the unusually large upfield meso proton hyperfine shifts for **3** must be solely due to extended electron spin delocalization in the porphyrin π-system (positive π spin density at the meso carbon). The MO calculations (INDO level) for oxophlorin radical (C_{2v}) predict b_2 and a_2 π orbitals as HOMO and next HOMO, respectively (Figure 2B). The spin density at the γ-meso carbon is substantially larger than that at the β- or δ-position in the b_2 orbital, which appears to be parallel with observed results. The preferential downfield hyperfine shift for one of the four methylene groups is also substantiated by the MO-calculated spin distribution in the b_2 orbital (Figure 2B).

The observed non-Curie law behavior of the temperature-dependent NMR shifts and temperature-dependent UV-visible spectra could be plausibly explained if Fe(II) oxophlorin radical is thermally admixed between nearly degenerate b_2 and a_2 radical states[11] as were the case for Ru(II) and Co(III) porphyrin π-cation radicals,[12] where the a_{1u} and a_{2u} radical states are nearly degen-

(7) To further confirm Scheme I, we also examined the NMR spectrum of the reaction mixture of bispyridine complex of Fe(II) OEP and H_2O_2 in aqueous pyridine solution under anaerobic condition. We observed the same spectrum as shown in Figure 1, contaminated with those for the unreacted and ferric low-spin OEP species.

(8) Bonnett et al.[1] found that one-electron reduction of **3** with dithionite yields hemochrome having a characteristic absorption spectrum (519 and 547 nm), which is similar to that of OEP·$Fe^{II}(py)_2$ (517 and 545 nm). This may suggest that two pyridine molecules are coordinated to ferrous low-spin iron in **3**.

(9) Similar intramolecular electron transfer between the central metal and the porphyrin ring has been found for the nickel porphyrin complex (Dolphin, D.; Niem, T.; Felton, R. H.; Fujita, I. *J. Am. Chem. Soc.* **1975**, *97*, 5288–5290) and for the iron porphyrin complex (Traylor, T. G.; Nolan, K. B.; Hildreth, R.; Evans, T. A. *J. Am. Chem. Soc.* **1983**, *105*, 6149–6151).

(10) The reaction mechanism and conditions were described in detail in our previous report.[4b]

(11) The curvature in the Curie plot (Figure 2A) could be alternatively explained in terms of the temperature-dependent equilibrium between Fe(II) oxophlorin radical **3** and either Fe(III) *meso*-hydroxyhemin **1** or Fe(I) porphyrins, which was suggested in our previous report[4b] on the basis of the ESR spectrum at 77 K.[6] However, the present study including temperature dependence and acid titration features (Figure 1c) of absorption spectra appears to rule out these possibilities.

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erate. As the temperature raises, the a_2 radical state is more admixed, thereby allowing an increase in spin density at the β-pyrrole carbons. This could be responsible for the observed non-Curie law behavior of the CH_2 resonances in Fe(II) oxophlorin radical which experiences substantial spin density changes in going from the b_2 to a_2 radical state. The unusual limiting shifts of the meso proton resonances could be also explained in terms of the temperature-dependent a_2–b_2 mixing state.[12] Bearing in mind that the metal-free oxophlorin radical gives the ESR signal with the meso proton hyperfine coupling a_H = 4.6 G[3] which is translated into 334 ppm contact shift at 23 °C, the much smaller value of the observed shift for **3** could be also attributed to mixing of the two radical states and perhaps to metal and ligand (pyridine) effects as well. The small changes in the UV–visible spectra of **3** at varying temperatures appear to be in accordance with this b_2–a_2 mixing mechanism. The accidental near degenerate states of b_2 and a_2 radicals in **3** may allow the mixing of the two states through vibronic coupling and result in enhanced electron spin relaxation,[13] as the case for a_{1u} and a_{2u} radicals in Ru(II) or Co(III) porphyrin π-cation radicals. Full details of these and related studies will be published in the near future.[15]

Acknowledgment. This work is supported by grants from Ministry of Education, Science and Culture, Japan (60540285, 60790122) and from Yamada Science Foundation.

(13) The neutral radical state of **3** should demand enhanced electron spin relaxation, favorable for the unbroadened NMR spectrum. It has been suggested[14] that degenerate or near degenerate radicals may exhibit enhanced electron spin relaxation caused by modulation of the spin–orbit coupling between the electron spin and its orbital motion about the system and of the hyperfine interaction.

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trans-Diamminedichloroplatinum(II) Can Chelate d(GpTpG) via Both Guanines in a Similar Fashion as the Cis Isomer

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In contrast to the widely applied antitumor drug *cis*-$PtCl_2(NH_3)_2$ (cis-Pt),[1,2] the trans isomer (trans-Pt) exhibits marginal or no antineoplastic activity.[3,4] Both platinum compounds do react with DNA "in vitro" and "in vivo"[2,5,6] and for both isomers a kinetic preference for the nucleobase guanine is observed.[2,7,8] Since the antineoplastic activity of cis-Pt is ascribed to interactions with the cellular DNA,[1,2] it is tempting to ascribe the differences in biological activity between both isomers to the formation of different platinum–DNA adducts.

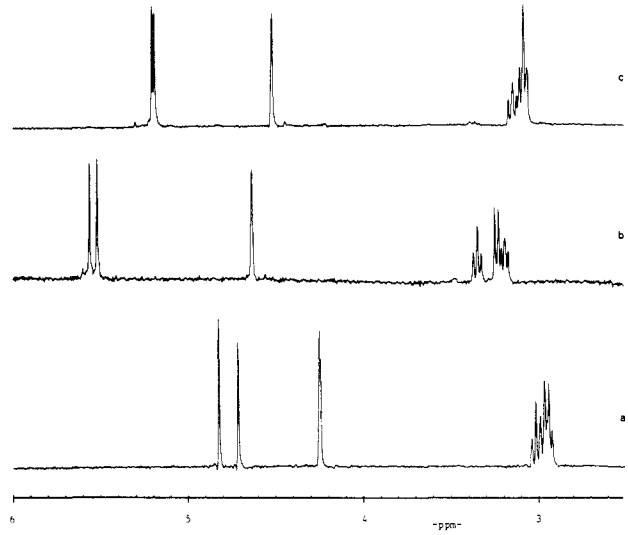

Figure 1. Downfield part of the 300-MHz NMR spectrum of unreacted d(GpTpG) (a), *trans*-$Pt(NH_3)_2$[d(GpTpG)-*N*7(1),*N*7(3)] (b), and *cis*-$Pt(NH_3)_2$[d(GpTpG)-*N*7(1),*N*7(3)] (c). The spectra were recorded at 300 K and pH′ 5.0 (pH′ denotes meter reading uncorrected for ^2H_2O). Chemical shifts are relative to tetramethylammonium chloride (TMA, 3.18 ppm downfield from DSS), which was added as an internal reference.

Cis-Pt preferently chelates neighboring purines,[9] and both GG and AG (but no GA) chelation has been reported in several studies.[9-12] To a smaller extent, also next-neighboring guanines (the so-called GNG chelates[13]) as well as guanines positioned in opposite strands (interstrand cross-links) can be bound by cis-Pt. Due to the stereochemistry of trans-Pt, this compound cannot chelate neighboring purines[14] but is thought to form "in vitro" mainly interstrand cross-links, long-range intrastrand cross-links, and monofunctionally bound adducts. Very recently, however, Pinto et al. reported to have indications for the formation of GNG chelates by trans-Pt, found in a "replication mapping" assay.[15] This prompted us to investigate the reaction of trans-Pt with the DNA trimer d(GpTpG) and to compare the product with the earlier investigated adduct of cis-Pt and d(GpTpG).

d(GpTpG) (disodium salt, to prevent the partial inactivation of the platinum compounds that occurs when the diammonium salt is used[16]) was synthesized via an improved phosphotriester method.[17] An equimolar reaction of cis- and trans-Pt with this trimer was performed at 37 °C for 2 weeks in the dark at room temperature (concentration about 4×10^{-6} M; pH 6–7). Gel permeation (Sephadex G25, Pharmacia, using the volatile salt triethylammonium bicarbonate TEAB as eluent) revealed in both cases only one well-defined major adduct. In the case of the trans isomer, also a variable but substantial amount of high molecular, UV-absorbing products was observed, which are most likely oligomers of d(GpTpG) cross-linked by trans-Pt. The major products for both isomers, however, appeared to be monomeric GNG chelates, in which the $Pt(NH_3)_2$ moiety is chelated via N7 to both terminal guanines, i.e., $Pt(NH_3)_2$[d(GpTpG)-*N*7(1),*N*7-

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