obtained. However, in the reactions of **1** and $Os_3(CO)_9(\mu_3\text{-}S)_2$ with $W(CO)_5(PMe_2Ph)$, several products **6**, **7**, and **11** containing tungsten–iron and tungsten–osmium bonds were obtained. In all such cases, the tungsten atom contains a PMe_2Ph ligand. It is believed that the removal of electron density from the metal atoms is unfavorable to metal–metal bond formation;[27] thus, the substitution of phosphine ligands, which are not as effective in electron density withdrawal as CO ligands, may favor the formation of metal–metal bonds.

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Supplementary Material Available: Tables of crystallographic data, positional parameters and B(eq) values, and anisotropic thermal parameters (U values) for **5–8** (15 pages); tables of structure factor amplitudes for all four structural analyses (79 pages). Ordering information is given on any current masthead page.

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Direct Evidence of Heme–*tert*-Butyl Peroxide Adduct Formation Demonstrated by Simultaneous ESR and Optical Measurements

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A possible model of a heme protein–peroxide complex has been obtained by mixing chloro(5,10,15,20-tetraphenylporphyrinato)iron(III) (Fe^{III}(TPP)Cl) with *tert*-butyl hydroperoxide (BHPO) in the presence of alkaline reagents. The ESR and optical absorption spectra were simultaneously measured for the frozen solution at 77 K. The observed optical spectra showed Soret, β, and α band absorption maxima at 420, 543, and 571 nm. The ESR spectra show that this iron complex takes the low-spin ferric state with an anomalously small g anisotropy ($g_1 = 1.96$, $g_2 = 2.15$, and $g_3 = 2.30$). It has been shown from thaw-and-freeze ESR measurements that this complex is very unstable above 5 °C and readily decomposes to non-heme iron type species such as open-chain polypyrrole complexes. On the basis of the ESR spectrometric titration carried out by changing the mixing molar ratio of BHPO and $NaOCH_3$, the coordination structure of the intermediate complex is concluded to be a six-coordinate Fe^{III}-TPP($^-OCH_3$)($^-OOC(CH_3)_3$) complex. The crystal-field parameters estimated by Bohan's treatment indicate that the peroxide anion causes a strong axial distortion toward the ferric ion, compared with the usual nitrogenous and oxygenous donors. The present complex would be the first example of a low-spin six-coordinate ferric alkyl peroxide complex.

Introduction

Mechanisms of the reactions occurring between peroxides and porphyrin–iron complexes have attracted extensive attention from researchers of heme chemistry, since some classes of heme enzymes showed their enzymatic activities in the presence of peroxides.[2] For example, (1) in the oxidation process of some organic molecules in the presence of horseradish peroxidase (HRP), the addition of hydrogen peroxide starts the enzymatic action of HRP,[3] and (2) alkyl hydroperoxides or acyl peroxides readily activate the monooxygenases such as cytochrome P-450 in the absence of oxygen and reductase.[4] One oxygen atom of the peroxide moiety in acyl peroxides is inserted into a wide variety of substrate molecules, and (3) the catalases[5] promote the decomposition of hydrogen peroxide to water and oxygen to inhibit the biological damages caused by hydrogen peroxide. These heme enzymes have distinctively different biological functions, but it has been concluded that the heme chromophores commonly form heme–peroxide adducts as an intermediate in the earlier reaction stage of their enzymatic actions.[4] Such intermediate peroxide adducts have been regarded as an important species in catalytic reaction cycles of these heme enzymes, since higher valent oxoiron complexes called Compound I[2] are successively generated from the iron peroxide complexes. Model systems composed of synthetic porphyrin–iron complexes and several oxidants such as alkyl hydroperoxides and acyl peroxides[6] have extensively been studied to understand the peculiar electronic structure and chemical reactivity of heme–oxo or heme–peroxo complexes. For example, Groves et al.[7] reported that the (5,10,15,20-tetrakis(2,4,6-trimethylphenyl)porphyrinato)iron(III) complex reacts with *m*-chloroperbenzoic acid to form an oxoiron(IV) π cation radical species. However, the profile for the coordination structures of iron peroxide complexes has not fully been established in spite of the important relevance to such intermediate species generated in the reaction processes of heme enzymes such as monooxygenase, peroxidase, and catalase.

Recently, we have proposed that an intermediate formed in the reaction system composed of the tetraphenylporphyrin–iron complex (Fe^{III}(TPP)Cl) and *tert*-butyl hydroperoxide (BHPO) in the presence of an alkaline reagent such as tetramethylammonium hydroxide ((TMA)OH) can be regarded as a possible model complex[8] of these heme–peroxide intermediates.[9] The ESR

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spectra observed at 77 K showed a typical line shape ascribable to the ferric low-spin complex with an anomalously small g anisotropy. Time-dependent ESR measurements carried out for the low-spin ferric complex indicated that this complex was highly reactive and readily decomposed to non-heme iron complexes characterized by ESR signals appearing at $g = 4.3$. The ligation of the *tert*-butyl peroxide anion at an axial position on $Fe^{III}TPP$ was postulated to interpret the ESR results, but a detailed coordination structure of the iron complex was still unknown.

In the present study, the reaction between $Fe^{III}(TPP)Cl$ and BHPO in the presence of various types of alkaline reagents has been investigated by means of simultaneous measurements of ESR and optical absorption spectra.[10] A possible coordination structure and the electronic structure of the heme–peroxide adducts are proposed based on the obtained results. Finally, some similarities in the coordination structure between this peroxide adduct complex and the naturally occurring oxygenases are discussed from the viewpoint of bioinorganic chemistry.

Experimental Section

Optical and ESR Measurements. Absorption spectra at 25 °C were measured by a JASCO UV-1000 spectrophotometer. Quartz cells with optical path length of 0.1, 0.2, 0.5, and 1.0 cm were used as required. Optical spectra of the frozen solutions at 77 K were recorded by using an MCPD-100 spectrophotometer, Ohtsuka Electronic Co. Ltd., with wavelengths ranging from 450 to 800 nm. ESR spectra were recorded at 77 K by using a JEOL FE2-XG X-band spectrometer operating with a 100-kHz field modulation of 6.3 G. The microwave frequency was monitored by an Advantest TR-5212 frequency counter. The magnetic field strength was calibrated by the hyperfine coupling constant (hfcc) of the Mn(II) ion doped in MgO powder (86.9 G). The g values of the observed ESR spectra were estimated based on the g value of the Li–TCNQ radical salt ($g = 2.0025$) as a standard.

The simultaneous ESR and optical measurements were carried out for the frozen solutions prepared in a 5.0-mm-o.d. ESR quartz tube by using a JEOL quartz Dewar. All of the measurements were performed at the Advanced Instrumental Center for Chemical Analysis, Ehime University.

Materials. Tetraphenylporphyrin ($TPPH_2$) and its iron complex $Fe^{III}(TPP)Cl$ were synthesized in our laboratory by the usual procedures described by Adler et al.[11] *tert*-Butyl hydroperoxide (BHPO) supplied by Nippon Oil & Fats Co., Ltd., was used after drying with $MgSO_4$ and distillation under reduced pressure. The purity of $Fe^{III}(TPP)Cl$ and BHPO was checked by ^1H NMR spectra and elemental analyses. Anal. Found (calcd) for $Fe^{III}(TPP)Cl$ ($C_{44}H_{28}N_4FeCl$): C, 74.56 (75.06); H, 4.14 (4.01); N, 8.11 (7.96). Anal. Found (calcd) for BHPO ($C_4H_{10}O_2$): C, 53.03 (53.31); H, 11.33 (11.19).

Methanol solutions of alkaline reagents such as choline and tetrabutylammonium hydroxide ((TBA)OH) obtained from Wako Junyaku Co., Ltd., were used without further purification. These solutions were stored under nitrogen atmosphere at temperatures below −20 °C. A methanol solution of sodium methoxide was prepared by addition of sodium metal to dry methanol and was also stored below −20 °C. The concentration of the Na^+ ion in sodium methoxide was determined to be 1.0 M by the usual pH titration. Tri-*n*-propylamine (TPA) was obtained from Wako Junyaku Co., Ltd., and supplied for measurements after drying with Na_2SO_4 and distillation. The organic solvents used here, dichloromethane and methanol, obtained from Merk Co., Ltd., were used after drying and distillation under nitrogen atmosphere. Sample solutions were prepared just before use for each measurement.

Preparation of the Iron–*tert*-Butyl Peroxide Complex. The $Fe^{III}TPP$–*tert*-butyl peroxide complex (C) was preared in a dry ice–acetone bath at −78 °C by using a quartz ESR tube. The frozen dichloromethane solution composed of $Fe^{III}(TPP)Cl$ (1.0 mM, 0.4 mL) and BHPO (1.0 M, 0.02 mL) at 77 K was gradually thawed at −78 °C, and a previously cooled methanol solution of choline (0.1 M, 0.01 mL) was added. The resulting mixture was rapidly frozen in liquid nitrogen. Formation of complex C was mainly monitored by observing the ESR spectrum at 77 K. Complex C was obtained even when the order of mixing of $Fe^{III}(TPP)Cl$, BHPO, and alkaline reagent was changed. In fact, Complex C was produced by mixing BHPO with a solution composed of choline and $Fe^{III}(TPP)Cl$. Complex C was also prepared by using a methanol solution of (TBA)OH or sodium methoxide instead of choline. However, an ethanol solution of $NaOC_2H_5$ failed to generate complex C.

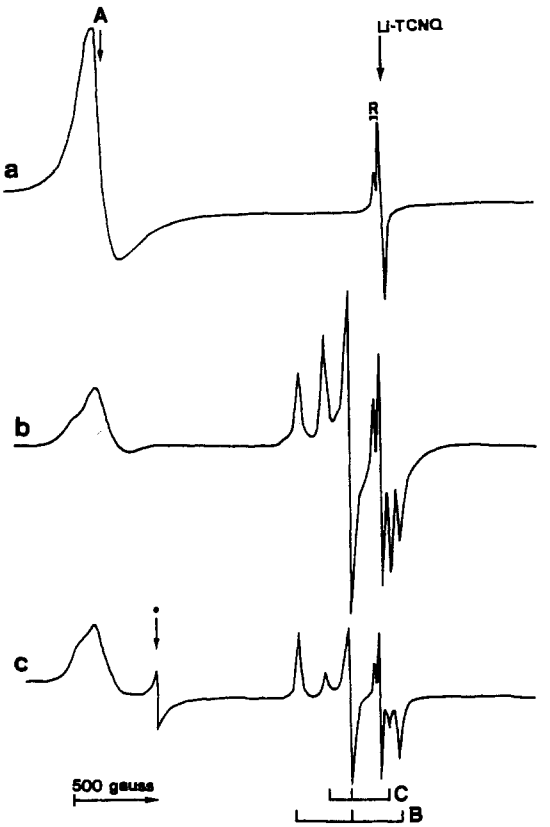

Figure 1. (a) ESR spectrum observed for a dichloromethane solution composed of $Fe^{III}(TPP)Cl$ (1.0 mM, 0.4 mL) and BHPO (1.0 M, 0.04 mL) at 77 K. (b) ESR spectrum of the mixture in part a recorded after addition of choline (0.1 mM) in methanol (0.01 mL); (c) ESR spectrum of the reaction mixture from part b kept at −5 °C for 30 s with the spectrum again recorded at 77 K. The asterisk in the figure shows the ESR signal of the non-heme iron complex at $g = 4.3$. The extra splittings seen at $g = 6.0$ and 5.8 may be due to a small amount of ferric high-spin complexes, $Fe^{III}(TPP)Cl$ and $Fe^{III}TPP(HOCH_3)(^-OCH_3)$. See ref 17.

Results

Simultaneous ESR and Optical Absorption Measurements at 77 K. The ESR spectrum observed for the frozen dichloromethane solution composed of $Fe^{III}(TPP)Cl$ (1.0 mM, 0.4 mL) of BHPO (1.0 M, 0.04 mL) showed the presence of a high-spin ferric complex (A) ($g_\perp = 6$ and $g_\parallel = 2$), and a free-radical species (R) ($g_\parallel = 2.03$ and $g_\perp = 2.008$), as shown in Figure 1a. According to the previous literature,[12] the free-radical species R was assigned as the *tert*-butyl peroxide radical derived from BHPO. After addition of the methanol solution of choline (0.1 M, 0.01 mL) at −78 °C, the ESR spectrum was again recorded at 77 K. Figure 1b shows the formation of two types of low-spin ferric complexes (B, with $g_1 = 1.92$, $g_2 = 2.16$, and $g_3 = 2.50$; C, with $g_1 = 1.96$, $g_2 = 2.16$, and $g_3 = 2.30$). A small amount of the remaining complex A is also detected in the lower magnetic field. The frozen solution was thawed at −78 °C and placed in ice–salt bath at −5 °C for about 2 min. Then the reaction mixture was rapidly frozen in liquid nitrogen, and the ESR spectrum was again recorded. The ESR signal intensity of complex C showed an abrupt decrease (Figure 1c) with concomitant growth of a new ESR signal observed at $g = 4.3$ (D). This means that complex C is very unstable at −5 °C and is easily converted to the ferric iron complex with $g = 4.3$. The high-spin species appeared at $g = 4.3$ with an almost isotropic line shape and was recently assigned to be an iron complex of open-chain tetrapyrrole derivatives.[13] In fact, the Soret

(9) Abbreviations: ESR, electron spin resonance; ENDOR, electron nuclear double resonance; $Fe^{III}(TPP)Cl$, chloro(5,10,15,20-tetraphenylporphyrinato)iron(III); BHPO, *tert*-butyl hydroperoxide; (TMA)OH or (TBA)OH, tetramethyl- or tetrabutylammonium hydroxide; TPA, tri-*n*-propylamine; CCP, cytochrome *c* peroxidase; HRP, horseradish peroxidase, P-450, cytochrome P-450.

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Table I. Results of the Simultaneous ESR and Optical Measurements for Iron Porphyrin Peroxide Complexes and Related Complexes

complex	solvent	base	peroxide	temp,[a] K	λ_{max}, nm					g values[b]	ref
$Fe^{III}(TPP)Cl$	CH_2Cl_2	none	none	77	425	508	575	655	700	6.0, 2.0	*e*
		none	BHPO		423	508	576	652	698	6.0, 2.0	*e*
$Fe^{III}TPP(^-OCH_3)_2$	CH_2Cl_2	$NaOCH_3$	none	77	430	546	588	652		1.921, 2.166, 2.497	*e*
		(TBA)OH	none		431	548	588	652		1.921, 2.166, 2.497	*e*
		choline	none		430	546	586	654		1.921, 2.166, 2.497	*e*
$Fe^{III}TPP(^-OCH_3)_2$	DMSO	$NaOCH_3$	none	RT	438	550	597	638		1.92, 2.16, 2.45	16
$Fe^{III}TPP$-basket-handle porphyrin	$CHCl_3$	TBA[c]	none							1.92, 2.16, 2.45	17
$(Fe^{III}TPP)_2O$	CH_2Cl_2	none	none	RT	406		569	612			15
$Fe^{III}TPP(^-OCH_3)(^-OOC(CH_3)_3)$	CH_2Cl_2	$NaOCH_3$	BHPO	77	420		543	571		1.952, 2.157, 2.316	*e*
		(TBA)OH	BHPO		420		543	573		1.953, 2.150, 2.316	*e*
		choline	BHPO		420		544	573		1.955, 2.154, 2.303	*e*
$[Fe^{II}Hb-O_2]^-$	H_2O	none	none[d]							1.967, 2.148, 2.265	21
$[Fe^{II}Mb-O_2]^-$		none	none[d]							1.941, 2.176, 2.295	21
$Fe^{III}BLM-^-O_2H$		none	H_2O_2							1.937, 2.171, 2.254	20
$Fe^{III}TPP(^-OOC(CH_3)_3)_2$	CH_2Cl_2	$NaOCH_3$	BHPO							1.964, 2.157, 2.242	*e*
		(TBA)OH	BHPO							1.964, 2.150, 2.242	*e*
		choline	BHPO							1.964, 2.154, 2.243	*e*
		TPA	BHPO							1.964, 2.148, 2.248	*e*

[a] Temperature for the optical measurements. [b] ESR spectra were recorded at 77 K. [c] TBA means tri-*n*-butylamine. [d] UV irradiation to oxy-Hb and oxy-Mb at 77 K. [e] This work.

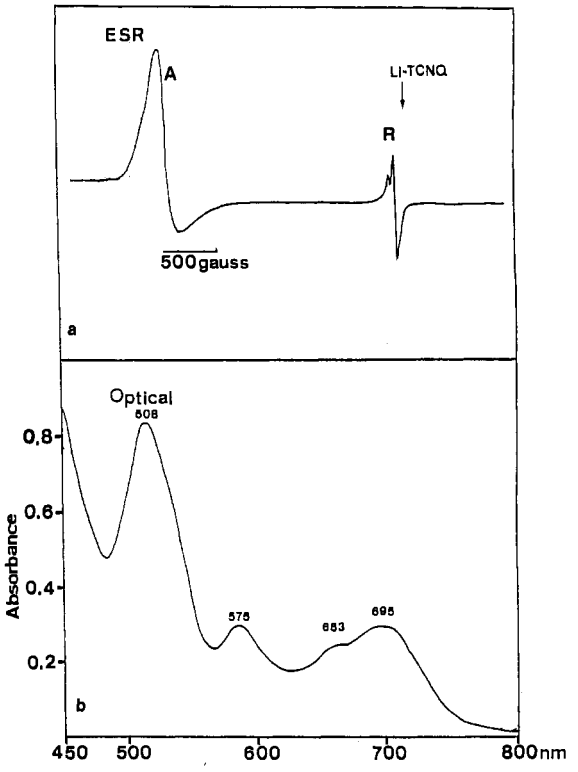

Figure 2. Simultaneous measurements of ESR and optical spectra (77 K) observed for a dichloromethane solution of $Fe^{III}(TPP)Cl$ (1.0 mM, 0.4 mL) and BHPO (1.0 M, 0.02 mL): (a) ESR spectrum showing the formation of a high-spin ferric iron porphyrin complex (A) and a small amount of a free radical species (R); (b) optical spectrum recorded at 77 K.

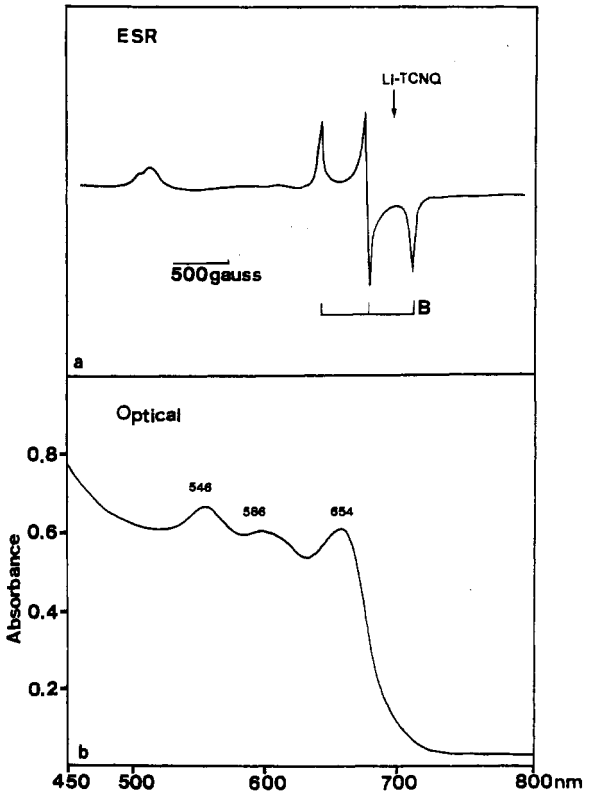

Figure 3. Simultaneous measurements of ESR and optical absorption spectra (77 K) of the low-spin ferric dimethoxide complex B prepared with $Fe^{III}(TPP)Cl$ (1.0 mM, 0.4 mL) and a methanol solution of choline (0.1 M, 0.02 mL): (a) ESR spectrum revealing the typical line shape ascribable to the low-spin ferric complex B; (b) optical spectra showing three Q-band absorption maxima. The line shape of the optical spectrum was drastically changed from that of $Fe^{III}(TPP)Cl$ (Figure 2).

band absorption maxima of $Fe^{III}TPP$ disappeared after the reaction mixture was allowed to stand for about 30 min at 25 °C.[14] These findings indicate that complex C is a key intermediate complex in the processes of oxidative degradation of iron porphyrin to the non-heme type complex. The detailed reaction mechanism of the heme breakdown reaction that occurred with complex C will be published elsewhere.

Further measurements were continued to identify the coordination structure of complex C with the aid of simultaneous ESR and optical measurements at 77 K. For comparison, the complexation reaction occurring between $Fe^{III}(TPP)Cl$ and BHPO was first monitored. The ESR spectrum observed for a dichloromethane solution of $Fe^{III}(TPP)Cl$ (0.1 mM, 0.4 mL) and BHPO (0.1 M, 0.02 mL) showed a typical line shape ascribable to be the high-spin ferric complex (A, with $g_\perp = 6.0$ and $g_\parallel = 2.0$). Formation of complex C was not detected at this reaction stage. The observed ESR line intensity and *g* parameters were completely coincident with those of the $Fe^{III}(TPP)Cl$ complex (Table I). The optical spectra recorded for the frozen solution (Figure 2) showed absorption maxima at 508, 575 (sh), 653, and

(14) Optical spectral parameters of the non-heme (D) iron complex generated in our reaction system are as follows: 375 nm (ϵ = 22 000), 450 nm (sh), 500 nm (sh), 640 nm (ϵ = 1800), and 720 nm (ϵ = 1100).

698 nm. Optical parameters of Fe^{III}(TPP)Cl recorded in the absence and presence of BHPO were summarized in Table I. These results indicate that both spectral parameters of complex A are not affected by addition of BHPO.

Next simultaneous ESR and optical measurements for complex B were carried out. As shown in Figure 3, the optical spectrum observed for the mixture of Fe^{III}(TPP)Cl (1.0 mM, 0.4 mL) and the methanol solution of choline (0.1 M, 0.02 mL) clearly showed new absorption maxima at 546, 586, and 654 nm. The observed optical spectrum was different from those observed for Fe^{III}(TPP)Cl and for the μ-oxo dimer complex,[15] which was often produced under alkaline conditions (Table I). The ESR spectrum showed formation of a ferric complex in the low-spin state (g_1 = 1.921, g_2 = 2.166, and g_3 = 2.497) along with a small amount of the remaining high-spin ferric complex. Similar ESR and optical spectra of complex B were successfully recorded even in the cases in which the alkaline reagent was changed from a methanol solution of choline to a methanol solution of (TBA)OH or $NaOCH_3$. As summarized in Table I, the spectroscopic parameters of complex B are less dependent on the chemical structure of the alkaline reagents used. Since the common anion present in these alkaline solutions is the $^-OCH_3$ anion, this anion is probably ligated to the axial position of complex B.

Analogous ESR and optical spectra were independently reported by two groups: (1) Ohtsuka et al.[16] reported ESR and optical spectra of a low-spin ferric complex prepared by mixing $^-OCH_3$ and Fe^{III}(TPP)Cl in DMSO; (2) Schaeffer et al.[17] reported ESR spectra observed for basket-handle porphyrin derivatives in which two alkoxide groups are tightly locked at both axial positions of the iron chromophore. From the comparison of our ESR and optical parameters with those of related complexes (Table I), the present complex (B) formed at 77 K is indeed concluded to be the low-spin ferric six-coordinate $Fe^{III}TPP-(^-OCH_3)_2$ complex.

The simultaneous ESR and optical measurements were continued for the frozen solution of complex C prepared as described in the Experimental Section. When a cooled solution of complex B was treated with BHPO (0.1 M, 0.02 mL), the color of the resulting mixture turned bright red, similar to that of oxyhemoglobin. As shown in Figure 4, the ESR spectrum revealed the formation of complex C (g_1 = 1.955, g_2 = 2.154, and g_3 = 2.303) with a strong signal intensity, in addition to a small amount of a free radical (R) ($g_\parallel$ = 2.033 and $g_\perp$ = 2.008), and complex B remained. ESR spectra of this frozen solution were also recorded at 4.2 K; however, no paramagnetic iron complex was detected besides complexes A–C described above. From the ESR spectrum recorded at 4.2 K, the content of complex C was thus estimated to be above 85%. These results of the ESR measuements indicate clearly that complex C is the major iron complex involved in this frozen solution. The optical spectrum recorded at 77 K showed two strong absorption maxima at 544 and 573 nm and a weak absorption maximum at 654 nm. In comparison with the optical spectra before and after addition of BHPO, the weak absorption band (marked with an asterisk) was explained to be due to small amount of complex B or high-spin complex A. It is appropriate to conclude that the iron porphyrin complex characterized by a pair of absorption maxima (544 and 573 nm) is the same as complex C detected by ESR. Analogous ESR and optical spectra were also detected for the mixture composed of Fe^{III}(TPP)Cl and BHPO in the presence of other alkaline reagents. Even if the alkaline reagent is changed from choline to (TBA)OH or $NaOCH_3$, the observed g and λ_{max} values of complex C coincide with each other, as is shown in Table I. It is noted here that the ESR signal at g = 4.3 due to the non-heme iron complex D is not seen in the ESR spectrum (Figure 4). This indicates that the oxidative decomposition of the porphyrin molecule is completely inhibited when the reaction temperature was kept below −78 °C.

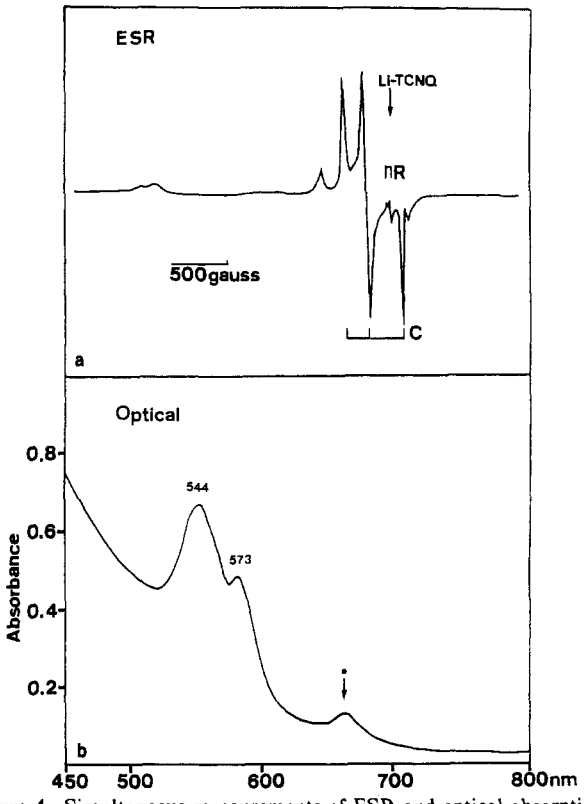

Figure 4. Simultaneous measurements of ESR and optical absorption spectra (77 K) recorded for the frozen solution composed of Fe^{III}(TPP)Cl (1.0 mM, 0.4 mL) and BHPO (1.0 M, 0.02 mL) in the presence of a methanol solution of choline (0.1 M, 0.02 mL): (a) ESR spectrum showing the presence of the low-spin ferric complex C and the *tert*-butyl peroxide radical (R) and the small signal due to the dimethoxide complex B; (b) optical spectrum showing a pair of absorption maxima clearly observed after addition of BHPO. The asterisk in the part b at 654 nm shows the small amount of the dimethoxide complex B and the high-spin complex A.

These observations confirm that the rapid mixing and freezing method is an efficient procedure to carry out optical and ESR detection for highly reactive intermediates such as complex C.

ESR Spectrometric Titration. Complex B was titrated by BHPO at −78 °C (Figure 5) by monitoring the ESR signal height of low-spin ferric complexes. On addition of BHPO (1.0 M, 0.01 mL) to complex B, the small amount of complex C obtained was clearly detected as shown in Figure 5b. The ESR signal height of complex C increased by addition of BHPO with concomitant decrease of the signal due to complex B. When BHPO (1.0 M, 0.02 mL) was added to a solution of complex B, the ESR signal height corresponding to complex C reached a maximum, followed by substantial decrease of the peak due to complex B, as shown in Figure 5c. The ESR intensity of both low-spin complexes B and C were reversibly changed by addition of the methanol solution of choline (0.1 M, 0.01 mL) to the reaction mixture was shown in Figure 5d. The ESR signal due to complex B was successively changed to that of complex C, depending on the mixing ratio of alkaline reagents and BHPO.

On the further addition of a 250 times excess amount (by mole) of BHPO (1.0 M, 0.1 mL) to a solution of complex C, a new ferric low-spin complex with a smaller g separation (E, with g_1 = 1.964, g_2 = 2.154, and g_3 = 2.243) compared with that of complex C was detected as shown in Figure 5e. ESR spectra of this low-spin complex (E) were always observed when a large amount of BHPO (about 200-fold excess relative to the amount of Fe^{III}(TPP)Cl present) was added to complex B. The same ESR spectrum was also detected by addition of BHPO (1.0 M, 0.02 mL) to a solution composed of Fe^{III}(TPP)Cl (1.0 mM, 0.4 mL) and tri-*n*-propylamine (TPA) (0.1 M, 0.02 mL) instead of choline base (Figure 5f). Contrary to the case for complex C, this low-spin complex (E) was produced in the absence of methoxide anion in the reaction system. This implies that complex E would be generated by the

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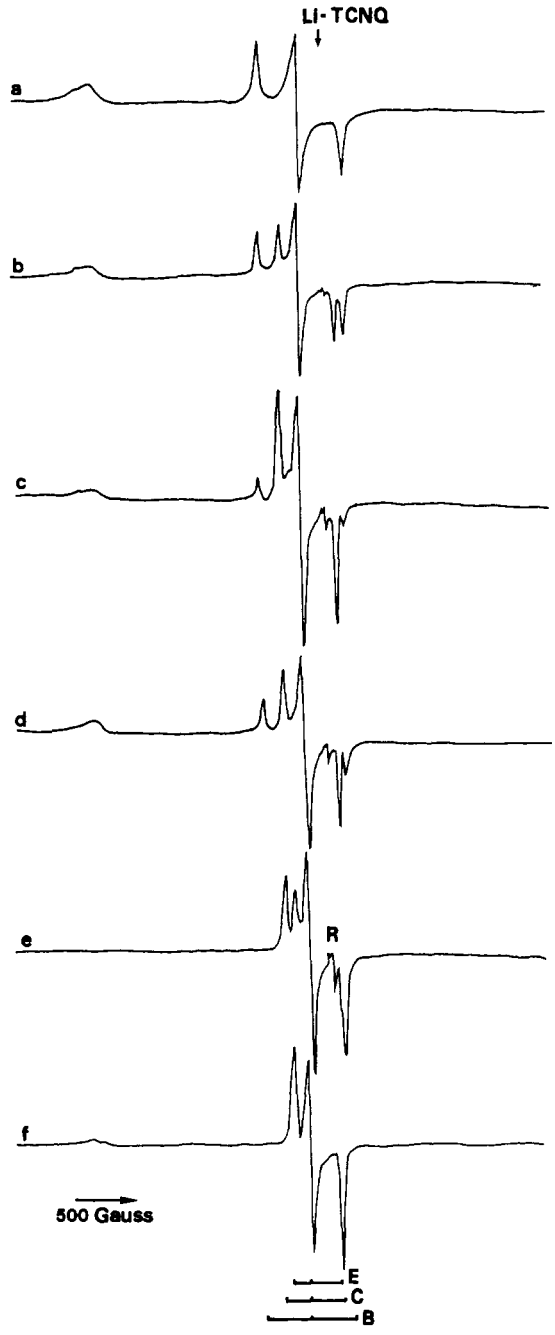


Figure 5. ESR spectrometric titration performed by changing the mixing molar ratios of complex B and BHPO: (a) spectrum before addition of BHPO, with complex B prepared by addition of choline (0.1 M, 0.02 mL) to a dichloromethane solution of Fe^{III}(TPP)Cl (1.0 mM, 0.4 mL); (b) spectrum after addition of a 25 times excess amount (by mole) of BHPO (1.0 M, 0.01 mL); (c) spectrum after addition of a 50 times excess amount (by mole) of BHPO (1.0 M, 0.02 mL); (d) after addition of a 2.5 times excess amount (by mole) of methanol solution of choline (0.1 M, 0.01 mL) the solution from part c, noting that the ESR signal intensity of the dimethoxide complex again increases; (e) spectrum of a solution of complex B treated with a 200-fold excess amount of BHPO (1.0 M, 0.1 mL), with new ESR signals ascribable to the low-spin ferric complex E observed; (f) ESR spectrum observed for a dichloromethane solution composed of Fe^{III}(TPP)Cl (1.0 mM, 0.4 mL), tri-*n*-propylamine (1.0 M, 0.04 mL) and BHPO (1 M, 0.04 mL). The ESR spectrum of complex E was clearly observed.

reaction occurring between Fe^{III}TPP and peroxide anion formed in the presence of TPA base. At present, the axial ligands of complex E were presumed to be the two *tert*-butyl peroxide anions derived from BHPO.

Discussion

The application of ESR spectroscopy has widely been established as a useful physicochemical tool to characterize the detailed

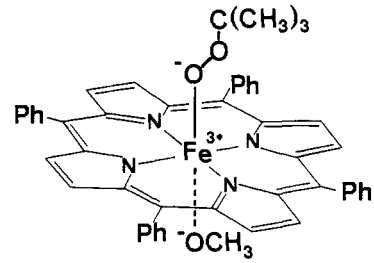
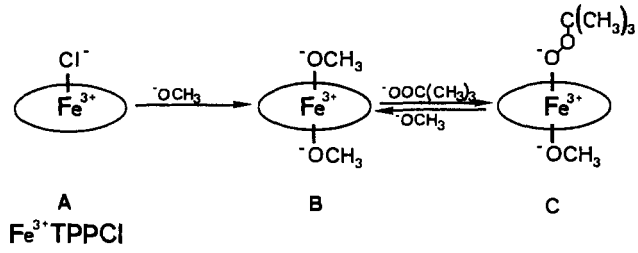


Figure 6. Coordination structure of complex C, $Fe^{III}TPP(^{-}OCH_3)(^{-}OO\text{-}C(CH_3)_3)$.

Scheme I. Equilibrium Reaction between Complex B and Complex C



coordination mode of paramagnetic iron complexes.[18,19] The ESR spectral change observed during the ESR spectrometric titration (Figure 5) provides valuable information for identification of the axial ligands of complex C. The intensity of the ESR signal ascribable to complex C increased with addition of BHPO, while the intensity due to complex B decreased successively. This spectral change indicates that complex B is converted to complex C in the presence of the BHPO anion. Addition of the $^{-}OCH_3$ ion to complex C resulted in reappearance of complex B. These behaviors are reasonably interpreted by taking into account an equilibrium between complex C and complex B as shown in Scheme I. This equilibrium is a reversible axial ligand exchange reaction occurring between $^{-}OCH_3$ and the *tert*-butyl peroxide anion. The addition of $^{-}OCH_3$ tends to shift the equilibrium to the left-hand side, which results in an increase of the concentration of complex B. The coordination structure of complex C is, therefore, concluded to be six-coordinate $Fe^{III}TPP(^{-}OCH_3)(^{-}O\text{-}O\text{-}C(CH_3)_3)$, as schematically shown in Figure 6.

In a communication of ours,[8] one of the axial ligands was postulated to be a nitrogenous ligand derived from tetramethylammonium hydroxide ((TMA)OH). However, ESR and optical parameters of complex C prepared by using the methanol solution of sodium methoxide agreed well with those prepared by using the nitrogenous bases (Table I). Therefore, the present study demonstrates that the fifth ligand of complex C is the $^{-}OCH_3$ anion.

ESR parameters of a number of ferric low-spin complexes have extensively been reported by many workers. However, such iron porphyrin complexes with anomalously small g anisotropy like complex C have rarely been reported. ESR parameters of an activated form of Fe(III)-bleomycin (BLM), $Fe^{III}(BLM)\text{-}^{-}OOH$,[20] were comparable to that of complex C (Table II). Symons and co-workers[21] measured the ESR spectra of hemoprotein-peroxide complexes, $[Fe^{II}Hb\text{-}OO]^{-}$ or $Fe^{II}Mb\text{-}OO]^{-}$, generated by the γ-ray or UV irradiation of oxyhemoglobin (Hb) or oxymyoglobin (Mb) at 77 K. In a recent paper,[22] the presence of a hydrogen bond between the axially ligating O_2^{-} and the amino acid residue closely located around the heme chromophore was well documented by ENDOR spectroscopy. The coordination

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Table II. ESR Parameters for Low-Spin Ferric Six-Coordinate Iron Complexes

complex	g_1	g_2	g_3	$\|R/\mu\|$	$\|\mu/\lambda\|$	label	ref
		N–Fe–N					
$Fe^{III}TPP(ImH)_2$	1.56	2.30	2.92	0.65	3.2	a	27
$Fe^{III}TPP(4MeImH)_2$	1.54	2.29	2.87	0.65	3.1	b	27
$Fe^{III}TPP(Im)(ImH)$	1.74	2.28	2.73	0.65	4.0	c	27
		N–Fe–O					
$Fe^{III}PPIXDBE(OAr)(py)$	1.84	2.19	2.61	0.51	6.2	d	28
$Fe^{III}PPIXDBE(OAr)(1MeIm)$	1.85	2.21	2.56	0.56	5.7	e	28
Fe^{III}(basket-handle porphyrin)($^-$OCR)(1MeIm)	1.83	2.21	2.65	0.53	5.8	f	17
		O–Fe–O					
$Fe^{III}TPP(^-OCH_3)_2$	1.921	2.166	2.497	0.49	8.5	**1**	*a*
$Fe^{III}TPP(^-OCH_3)_2$	1.92	2.16	2.45	0.54	8.5	g	16
$Fe^{III}TPP$–basket-handle porphyrin	1.92	2.16	2.45	0.54	8.5	h	17
	1.92	2.15	2.44	0.54	8.3	i	17
		O–Fe–OOR					
$Fe^{III}TPP(^-OCH_3)(^-OOC(CH_3)_3)$	1.952	2.157	2.316	0.59	9.0	**2**	*a*
	1.953	2.150	2.316	0.63	9.3	**3**	*a*
	1.955	2.154	2.303	0.58	9.3	**4**	*a*

[a] This work.

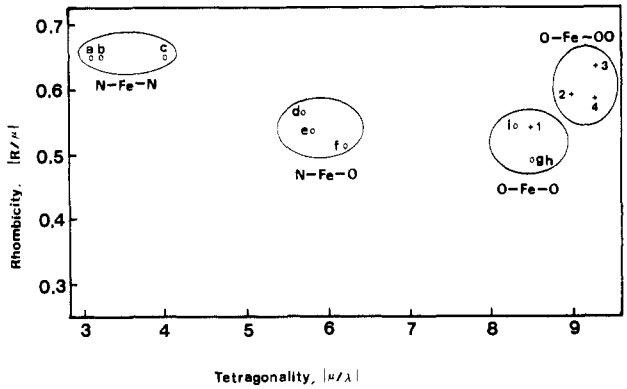

Figure 7. Plot of rhombicity vs tetragonality for representative iron complexes; Labels are given in Table II.

structure of the hemoprotein–peroxide adducts was confirmed to be six-coordinate $[Fe^{II}$–Hb–(His)–OO$]^-H^+$. At present, however, detailed electronic structures of these low-spin complexes with anomalously small g anisotropy are still equivocal. The present complex, C, consists of conventional chemicals and can be easily prepared. Complex C would be a useful model complex to deduce the electronic structure of these iron–peroxide complexes.

Crystal-field analyses of the ESR g parameters for low-spin ferric iron porphyrin complexes have been developed by Griffith[23] and Kotani.[24] Blunberg and Peisach[25] and Bohan[26] proposed two crystal-field parameters, rhombicity (R/μ) and tetragonality (μ/λ), calculated from g values in various types of low-spin ferric hemoproteins and their model complexes. Here, the crystal-field parameters of complex C were calculated according to the Bohan treatment. The crystal-field parameters of complexes B and C were tabulated in Table II along with those previously reported for model iron complexes. In Figure 7 the crystal-field parameters of these complexes are plotted in a rhombicity–tetragonality diagram. These iron complexes were classified into four groups having N–Fe–N, O–Fe–N, O–Fe–O, and O–Fe–OOR axial modes of coordination. The region of the N–Fe–N complexes in the figure is far away from that of other three groups. The O–Fe–O and O–Fe–OOR groups are located very close to each other, but they can be distinctively classified. The $|R/\mu|$ values of four groups were comparable with each other, while the $|\mu/\lambda|$ values show a stepwise increase with the number of oxygenous donors ligating at the axial positions instead of nitrogenous ligands. In particular, the data points for the O–Fe–OOR complexes are located at the extreme right-hand side in the crystal-field diagram. This implies that the iron chromophore of complex C has a strong axial perturbation due to the ligating peroxide anion. In fact, the estimated $|\mu/\lambda|$ value of complex C (9.3) is the largest one among those of the iron model complexes previously reported (Table II). Therefore, it is concluded that the *tert*-butyl peroxide anion causes a strong axial perturbation toward the iron chromophore compared with the other ligands.

Recently, Arasasingham et al.[29] reported an $Fe^{III}TPP$–alkyl peroxide complex generated by the reaction of $Fe^{III}TPP$–CH_3 or $Fe^{III}TPP$–C_2H_5 complexes and oxygen at −80 °C. The accurate NMR measurements showed that the peroxide complex takes the five-coordinated ferric high-spin state in which the methyl or ethyl peroxide moiety binds at the axial position of heme. Although these complexes and complex C commonly possess the peroxide moiety at the axial position, the spin state was evidently different. The six-coordinate peroxide complex C takes the ferric low-spin state, but this five-coordinated peroxide complex prefers the ferric high-spin state. The spin state of the $Fe^{III}TPP$–peroxide complex strongly depends on the axial ligand at the fifth position. The methoxide anion located at the fifth position would be indispensable to generate complex C. Several types of model iron–peroxide[29] or iron–acyl peroxide[7] complexes have been proposed, but the present complex (complex C) is a unique example of a low-spin ferric six-coordinate peroxide complex, to our present knowledges.

The presence of similar six-coordinate heme–peroxide intermediates has often been speculated upon in the reaction processes of naturally occurring heme enzymes such as monooxygenases,[4] peroxidases,[2] and catalases.[5] An endogenous ligand derived from cysteine, tyrosine, or histidine binds at the fifth position, and the exogenous peroxide ligand was thought to bind at the sixth position of the heme chromophore. The endogenous ligands are believed to play important roles in the regulation of the chemical reactivity of these heme enzymes. Thus it is expected that complex C with a six-coordinate geometry can be a noble model complex to understand the electronic structures of these heme enzyme–peroxide complexes, provided a biologically significant ligand such as imidazole, thiolate, or phenolate is chosen for an axial ligand at the fifth position instead of the present $^-OCH_3$ anion. Further investigations on these possibilities for complex C are now in progress.

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In conclusion, complex C formed in the processes of the heme breakdown reaction is a six-coordinate low-spin ferric complex with an anomalously small g anisotropy. The coordination structure of complex C is schematically formulated as six-coordinate $Fe^{III}TPP(^{-}OCH_3)(^{-}OOC(CH_3)_3)$ based on the data of ESR and optical spectroscopies. Complex C is a possible model complex for transient intermediate species generated in the reaction processes of heme enzymes such as the monooxygenases, peroxidases, and catalases.

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Metal Chelation with Natural Products: Isomaltol Complexes of Aluminum, Gallium, and Indium

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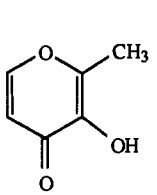
The Al, Ga, and In complexes ($M(ima)_3$) of the naturally occurring starch byproduct isomaltol (Hima) have been prepared in, and isolated from, aqueous solution. They have been characterized by IR and NMR spectroscopy and mass spectrometry; the ^{27}Al NMR spectrum of the Al complex (2.9 ppm, $W_{1/2}$ = 200 Hz) suggested that the three ligands are coordinated in a facial arrangement around the Al center, at least in aqueous solution. Solution equilibrium studies (μ = 0.15 M (NaCl), 25 °C) show that isomaltol is a relatively weak complexing agent for the group 13 (IIIA) metal ions (log β_3 = 14.45 (5) (Al), 16.36 (1) (Ga), 14.80 (2) (In)). The complex $Al(C_6H_5O_3)_3$ has been studied by single-crystal X-ray diffraction. Considerable disorder (two out of three ligands) was found. It crystallized in the monoclinic space group $C2/c$ with the crystal parameters a = 29.215 (2) Å, b = 8.2211 (7) Å, c = 15.052 (2) Å, β = 98.775 (7)°, and Z = 8. The data were refined by using 2091 reflections with $I \geq 3[\sigma(I)]$ to R and R_w values of 0.065 and 0.080, respectively.

Introduction

Recently, a number of low molecular weight complexes of aluminum, gallium, and indium have been prepared with properties that have recommended these species to in vivo examination.[1-3] These complexes contained as ligands various pyrones (e.g. maltol (**1**))[1,3] and pyridinones (**2**),[2,3] and the properties of interest were



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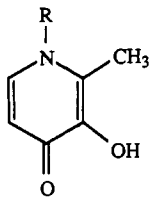

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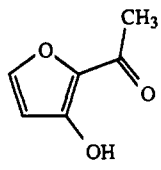

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hydrolytic stability, water solubility, neutral charge, and lipophilicity, all occurring simultaneously. Some of these ligands have very high affinities for the group IIIA (or 13) ions,[4] and certain of the complexes have formed unusual solid-state hydrogen-bonding arrays.[2,3,5] The metal complexes are now studied in various laboratories as agents of in vivo ion transport,[6,7] and this work should shed some light on the mechanism of transport of the ions M^{3+} (M = Al, Ga, In) in vivo. This latter interest stems from the involvement of Al in neurological disorders and of Ga and In in diagnostic nuclear medicine procedures.

In efforts to probe further the coexistence of these four properties, to examine closely the aqueous coordination chemistry of these metals, and to search for more extensive hydrogen-bonding arrays, we are examining new binding groups for the Al^{3+}, Ga^{3+}, and In^{3+} ions. We are also interested in ways to incorporate these groups into multidentate ligand systems. Potential ligands that occur in nature and/or that are likely to prevent the hydrolysis characteristic of these metal ions are being examined in our search for candidate binding groups. The hydrolytic instability[8] of aluminum, gallium, and indium is an important factor in the aqueous chemistry of their trivalent ions and must be compensated for in the design of their chelates. In the absence of suitable ligands, the M^{3+} ions rapidly hydrolyze in neutral (or pH 7.4) solutions to form insoluble hydroxides.

This work examines the potential as an M^{3+} binding group of the natural product isomaltol (**3**). Isomaltol (1-(3-hydroxy-2-furanyl)ethanone, abbreviated as Hima), which was first isolated from a bread distillate almost 80 years ago by Backe,[9] was found to be a structural isomer of maltol (**1**) only 20 years ago.[10,11] We were originally attracted to isomaltol because it has a β-hydroxyeneone group (it is a natural product acetylacetone analogue). Because it is well-known that Al^{3+} will displace even aliphatic hydroxyl protons if they are in positions favorable for metal ion coordination,[12] we were sure that **3** would form a stable Al(III) complex.

Isomaltol is found in nature as a byproduct of the enzymatic degradation of starch in breads, and it contributes to the aroma of freshly baked bread. It is a prototype for new ligands that are currently under active investigation in our laboratory: the 2-acyl-3-hydroxyfurans. Isomaltol has been reported cursorily in the literature as iron,[9] copper,[9,10] or sodium salts.[13,14]

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