Spectroscopic Characterization of Peroxo–Iron(III) Chlorin Complexes. The First Model for a Reaction Intermediate of Cytochrome *d*

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As reaction intermediate proposed in the catalytic cycle of cytochrome *d*, peroxo–iron(III) chlorin complexes are formed and characterized by using synthetic models. The peroxo complexes have been successfully formed by the reaction of chloro–iron(III) complexes of tetramesitylchlorin (H_2TMC) and octaethylchlorin (H_2OEC) with 2 equiv of the superoxide anion in acetonitrile under an argon atmosphere. The peroxo complexes of iron(III) chlorins afford characteristic electronic absorption spectra, in which the Soret band and the characteristic band for chlorin complexes are largely red-shifted, as compared with those of the parent ferric chlorin complexes. ESR spectra of the peroxo chlorin complexes exhibit resonances at $g \approx 9$ and 4.2, indicative of rhombic high-spin ferric states. Further, the oxygen–oxygen stretching mode of the peroxo complexes is observed at 806 cm^{-1}, implying that the peroxide ligands are bound to the iron in a bidentate fashion. On the basis of these spectral features, the peroxo–iron(III) chlorin complexes are suggested to have electronic and molecular structures similar to those of the corresponding porphyrin complexes. On the contrary, the paramagnetic deuterium NMR signals of the saturated pyrrole (pyrroline) rings of the peroxo chlorin complexes exhibited an unusually large splitting into downfield and upfield regions. The unusual splitting of the hyperfine-shifted pyrroline deuterium resonances could be explained by the large deformation of the pyrroline rings in the peroxo chlorin complexes.

Introduction

Cytochrome *d* is a terminal electron acceptor of *Escherichia coli*, catalyzing the four-electron reduction of dioxygen to water using ubiquinol as an electron donor.[1] The enzyme contains no copper, but instead, it contains two *b* hemes and one or two chlorin cofactors, heme *d*, which is fundamentally different from cytochrome *c* oxidase.[2–4] The structure of heme *d* has been postulated to be a dihydroxychlorin,[5,6] derived from protoheme IX by reductive dihydroxylation of the pyrrole ring C, as depicted in Figure 1. Optical and ESR studies have shown that the heme *d* component of the enzyme binds O_2 and CO and is directly involved in the activation of O_2 and its reduction to H_2O.[2,7]

During the conversion of O_2 into H_2O, several intermediates have been observed. Starting with the chlorin in the ferrous state, heme *d* reversibly binds O_2 to form the oxygenated species having an intense absorption at 650 nm (d_{650}).[8] On the other hand, oxygen oxidation of the dithionite-reduced cytochrome *d* complex gives a species with a characteristic absorption at 680 nm (d_{680}),[9] which is also obtained in the reaction of the ferricyanide-oxidized enzyme with excess hydrogen peroxide.[8a,10] The species d_{680} has been recently shown to be an oxo–ferryl intermediate with the ν(Fe=O) frequency at 815 cm^{-1}.[9] Moreover, a ferric peroxo complex has been proposed as a catalytic intermediate in the reaction of the reduced enzyme with O_2 by kinetic[11] and ESR[12] studies. Lorence and Gennis also suggested[8a] the formation of the peroxo intermediate upon addition of hydrogen peroxide to the oxygenated form of the enzyme. On the basis of these observations, a possible catalytic cycle for cytochrome *d* has been proposed[9,12] as shown in Scheme 1.

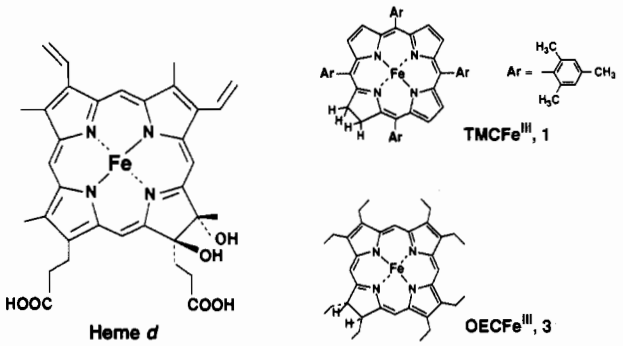

Figure 1. Structures of heme *d* and model complexes used in this study.

In contrast to those of the oxygenated and oxo–ferryl intermediates, the structure and spectroscopic properties of the peroxo intermediate have been obscure. It is therefore quite important to prepare and characterize the peroxo–iron(III) chlorin species by using synthetic model complexes.

Valentine and co-workers prepared[13] ferric porphyrin–peroxo complexes by the reaction of ferric porphyrins with 2 equiv of the superoxide anion, O_2^-. The first equivalent reduces iron(III) porphyrin to iron(II) porphyrin. Addition of the second equivalent

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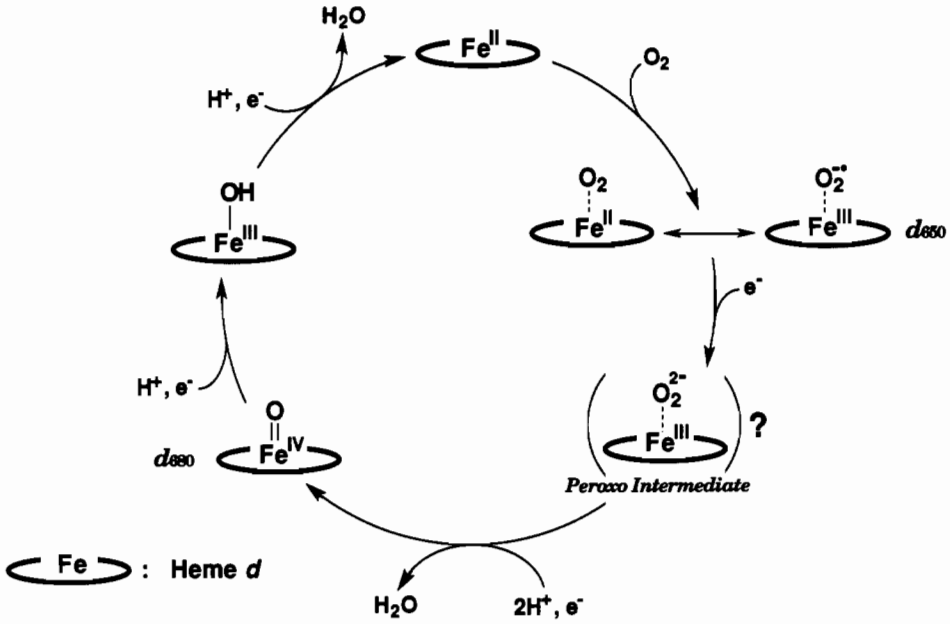
Scheme 1



produces the peroxo–iron(III) complex formulated as $[Fe^{III}O_2]^-$.[13] The dioxygen ligand has been thought to be peroxide, bound in a side-on (bidentate) fashion, from the results of spectroscopic measurements.[13–15] A very similar result was also reported for peroxo–manganese(III) porphyrin complexes,[16] whose X-ray structures have been determined as

Peroxo–iron(III) porphyrin complexes were also prepared by the reaction of iron(I) porphyrin with O_2[15,17,18] and by electrochemical[19] and chemical[20] reduction of the oxygenated iron(II) porphyrin complexes. Although synthetic models of the peroxo–iron(III) porphyrin complexes were well-characterized by magnetic and spectrosopic measurements,[13–20] the peroxo–iron(III) chlorin complex has never been examined.

In this paper, we present the formation of the peroxo–iron(III) chlorin complexes in the reaction of ferric chlorins with potassium superoxide. The complexes have been characterized by a variety of methods, including electronic absorption, ESR, NMR, and IR spectroscopies. It is shown here that the peroxo–iron(III) chlorin complexes have spectroscopic characteristics similar to those of peroxo–iron(III) porphyrin complexes. However, the peroxo complexes of iron(III) chlorins exhibit unusual deuterium NMR spectra, which is discussed in relation to a local structure of the saturated pyrrole ring of the chlorin macrocycle.

Experimental Section

Spectral Measurements. The sample preparations and manipulation were carried out in a glovebox under an argon atmosphere. Electronic absorption spectral measurements were made on a Shimadzu UV-2200 spectrometer. ESR spectra were obtained on a JEOL PE-2X spectrometer operating at X-band frequencies. FT-IR spectra of acetonitrile solutions in demountable cells with NaCl windows were recorded on a Bio-Rad FTS-30 spectrometer at 1.0-cm^{-1} resolution. Deuterium NMR spectra at 46.1 MHz were recorded on a Nicolet NT-300 spectrometer equipped with a 1280 computer system. Chemical shifts were referenced to a solvent signal (acetonitrile: 2.0 ppm), and downfield shifts are given positive signs.

Materials. Acetonitrile was refluxed over calcium hydride and then distilled under an argon atmosphere. The distilled solvent was treated with potassium superoxide (KO_2) overnight to ensure the complete removal of water, followed by distillation in vacuo and storage over 4-Å molecular sieves in the glovebox. 18-Crown-6 was purchased from Aldrich and dried under vacuum in the glovebox. KO_2 was obtained from Aldrich and used without further purification.

Iron(III) chlorin complexes were prepared by published procedures.[21–23] Deuterated chlorin derivatives were synthesized as described previously.[23,24]

Peroxo–Iron(III) Chlorin Complexes. To a dry acetonitrile solution containing a chloro–iron(III) chlorin complex ($\sim 10^{-5}$ M for electronic absorption spectra and $\sim 10^{-3}$ M for ESR, IR, and NMR spectra) and 2–3 equiv of 18-crown-6 was added an excess amount of KO_2 (solid), and the mixture was stirred for ca. 5 min. The resultant solution was filtered through a disposable filter, and the filtrate was used for spectral measurements.

Results

Formation of Peroxo–Iron(III) Chlorin Complexes. The reaction of a chloro–iron(III) complex of tetramesitylchlorin ($(TMC)Fe^{III}Cl$, **1**)[25] with 2 equiv of superoxide anion (2 equiv of 18-crown-6 and excess KO_2) was performed in dry acetonitrile at room temperature under an argon athosphere. During the

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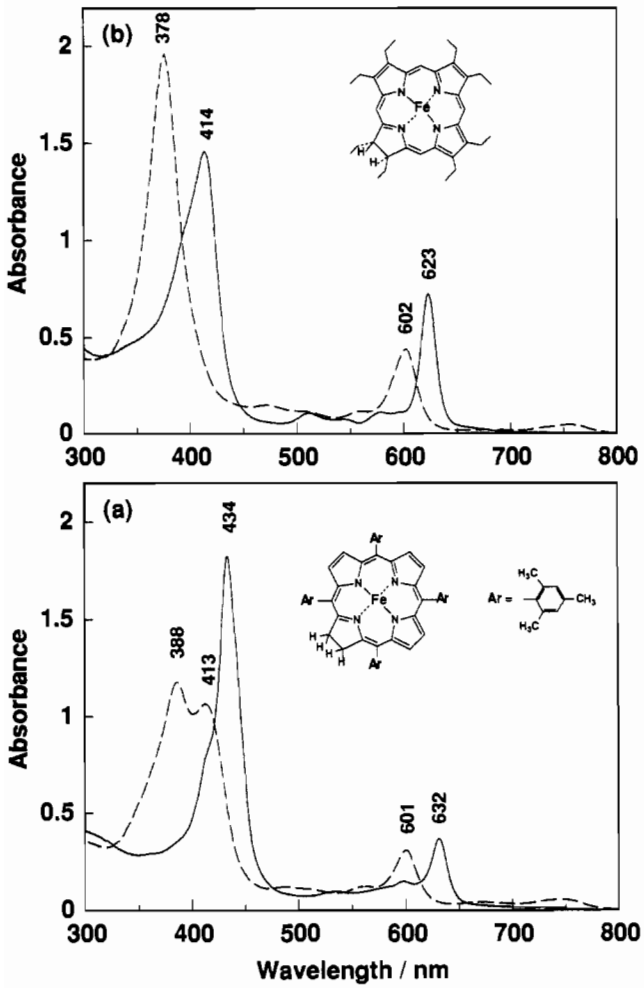

Figure 2. Electronic absorption spectra in acetonitrile (0.02 mM) at room temperature: (a) (TMC)$Fe^{III}Cl$ (**1**) (- - -) and the reaction product, **2** (—), (b) (OEC)$Fe^{III}Cl$ (**3**) (- - -) and the reaction product, **4** (—).

reaction, the green solution of **1** changed into a blue-green solution to give a new species (**2**). As shown in Figure 2a, **2** exhibits an absorption spectrum distinct from that of the parent complex (**1**); i.e., the Soret band (434 nm) and the characteristic band for chlorin complexes (632 nm) of **2** show large red shifts from those of **1** (388, 413, and 601 nm), respectively. The reaction of a chloro–iron(III) *trans*-octaethylchlorin ((OEC)$Fe^{III}Cl$, **3**) with superoxide showed similar spectral changes to yield a new complex, **4** (Figure 2b). In order to examine the reactivity (stability) of **2** and **4**, air (moisture) was introduced into the solutions of **2** and **4**. Upon introduction of air (moisture), alteration of the absorption spectra of **2** and **4** occurred immediately, to give those of the hydroxy–iron(III) complex and the μ-oxo-bridged dimer, respectively.[26] The large Soret band shift and reactivity with water resemble the previous results for the peroxo–iron(III) porphyrin complexes.[13]

To confirm the peroxo chlorin complex formation, ESR spectral measurements of **2** and **4** were carried out in acetonitrile at 77 K. The ESR spectra of **2** and **4** show intense signals at $g = 4.24$ and 4.23 and weak transitions at $g = 8.8$ and 8.5, respectively (Figure 3). These spectra are characteristic of rhombic high-spin ferric complexes, such as peroxo–iron(III) complexes of

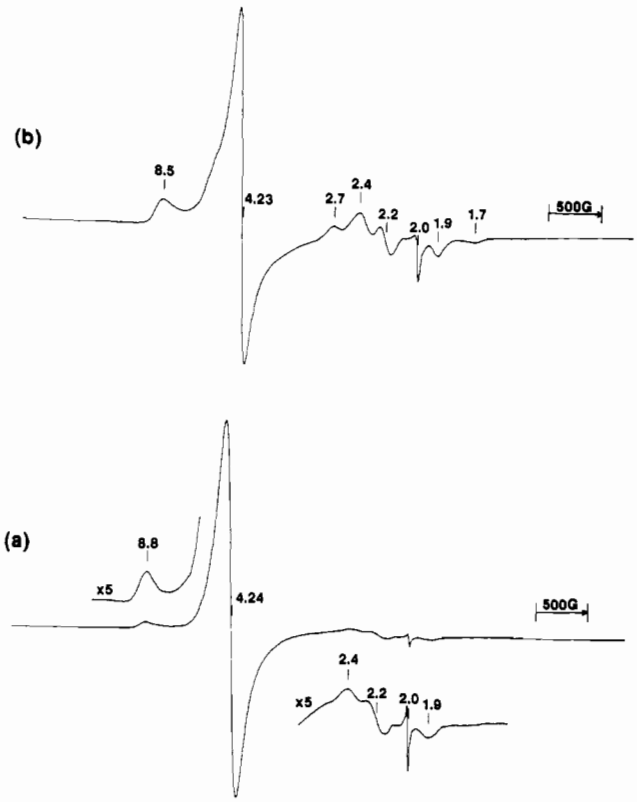

Figure 3. ESR spectra of the reaction products of TMC, **2** (a), and OEC, **4** (b), complexes in acetonitrile (2 mM) at 77 K. Apparent g values are indicated.

EDTA[27,28] and porphyrins.[13] Although a highly rhombic system is expected to have a transition below $g = 2$, which arises from the lowest doublet,[29] we could not detect this transition at 77 K, possibly due to its low intensity and large line width and the appearance of several ESR signals around $g = 2$ for low-spin impurities.[30] The similarities of electronic absorption and ESR spectral features for **2** and **4** to those of peroxo–iron(III) porphyrin complexes suggest that **2** and **4** would be peroxo–iron(III) chlorin complexes with peroxide bound in a bidentate fashion.

Further evidence for the formation of the peroxo–iron(III) chlorin complexes was obtained by IR spectroscopy. As shown in Figure 4a, **2** exhibits infrared bands at 806 and 793 cm^{-1}, which disappear when **2** is converted into the hydroxy form ((TMC)Fe^{III}(OH)) by introducing a trace amount of water.[31] Pyrrole-d_8 and meta-d_8 derivatives of **2** also show the intense band at 806 cm^{-1} (Figure 4b,c), suggesting that this band is not due to the chlorin macrocycle or substituted mesityl modes, but rather due to an oxygen-oxygen stretching mode. This band falls near the low end, but within the range of known O–O vibrations for a metal-bound peroxide ligand.[32] It was previously reported[13a] that the IR spectrum of the peroxo–iron(III) OEP complex in DMSO exhibited a single absorption at 806 cm^{-1} with a small shoulder in the same region. Thus, the 806-cm^{-1} band of **2** can be assigned to the O–O stretching mode. On the other hand, the

(25) Abbreviations: H_2TMC, 7,8-dihydro-5,10,15,20-tetrakis(2,4,6-trimethylphenyl)porphyrin (tetramesitylchlorin); H_2TPC, 7,8-dihydro-5,10,15,20-tetraphenylporphyrin (tetraphenylchlorin); H_2TPP, 5,10,15,20-tetraphenylporphyrin; H_2OEC, *trans*-7,8-dihydro-2,3,7,8,12,13,17,18-octaethylporphyrin (*trans*-octaethylchlorin); H_2OEP, 2,3,7,8,12,13,17,18-octaethylporphyrin.

(26) While **2** is stable at room temperature under UV concentrations ($\sim 10^{-5}$ M) without additional moisture, **4** partially changes into the ((OEC)Fe^{III})$_2$O complex in 1 h.

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(30) Although the minor components for low-spin signals could not be characterized, these impurities would be bis(hydroxy) complexes under basic conditions.
(31) The IR bands labeled X at 842 and 837 cm^{-1}, which are due to 18-crown-6, did not change upon introduction of air to the solution of **2**.
(32) (a) Vaska, L. *Acc. Chem. Res.* **1976**, *9*, 175–183. (b) Jones, R. D.; Budge, J. R.; Ellis, P. E., Jr.; Linard, J. E.; Summerville, D. A.; Basolo, F. *J. Organomet. Chem.* **1979**, *181*, 151–158.

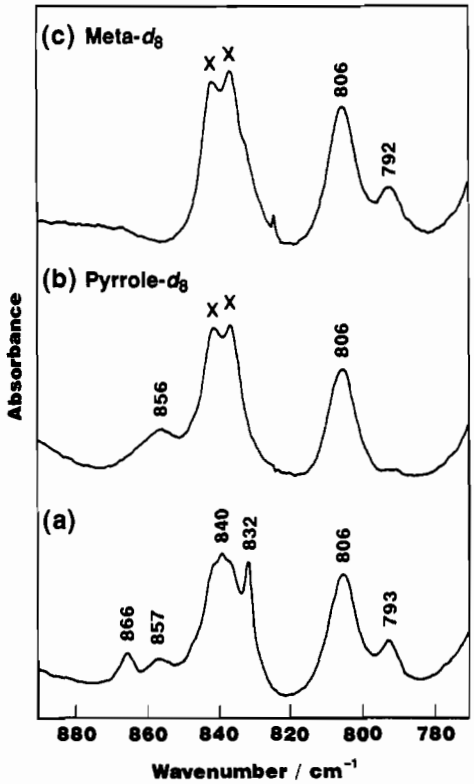

Figure 4. FT-IR spectra (890-770 cm^{-1}) of **2** (a) and its saturated pyrrole ring-d_2–pyrrole-d_6 (b) and meta-d_8 (c) derivatives in acetonitrile solutions (~6 mM) at room temperature. The bands labeled × at 842 and 837 cm^{-1} are due to 18-crown-6.

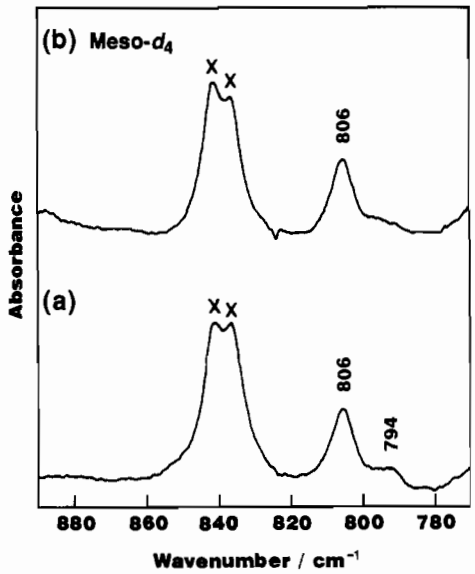

Figure 5. FT-IR spectra (890-770 cm^{-1}) of **4** (a) and its meso-d_4 derivative (b) in acetonitrile solutions (~6 mM) at room temperature. The bands labeled × at 842 and 837 cm^{-1} are due to 18-crown-6.

less intense band at 793 cm^{-1} for **2** disappears in the spectrum of the pyrrole-d_8 complex, as shown in Figure 4b. The results allow us to assign the 793-cm^{-1} band to a C_β–H deformation mode, as previously reported for a Zn complex of tetraphenylchlorin, H_2TPC (792 cm^{-1}).[33]

As illustrated in Figure 5, **4** exhibits a similar IR spectrum in the peroxide region. The 806-cm^{-1} band for **4**, which was not altered by deuterium substitution at the meso positions, is also assigned to the oxygen–oxygen stretching mode.[34] Further, **4**

(33) Andersson, L. A.; Loehr, T. M.; Thompson, R. G.; Strauss, S. H. *Inorg. Chem.* **1990**, *29*, 2142–2147.

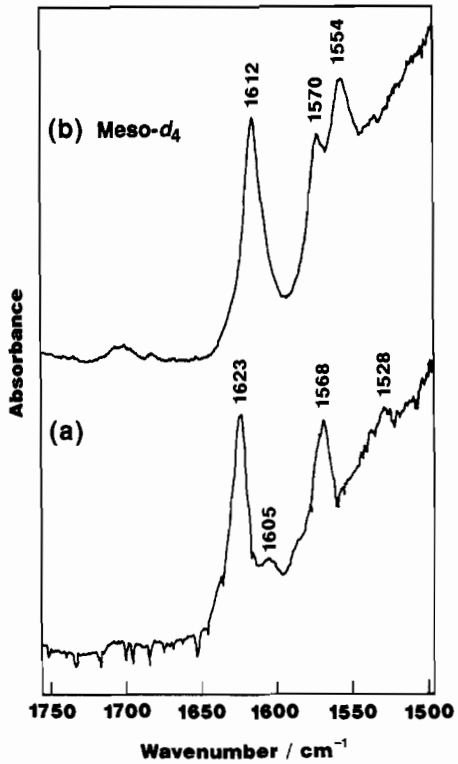

Figure 6. FT-IR spectra of **4** (a) and its meso-d_4 derivative (b) in the 1750–1500-cm^{-1} region.

has strong and characteristic IR bands at 1623 and 1568 cm^{-1}, as depicted in Figure 6. That meso deuteration lowered these bands by 11 and 14 cm^{-1} (Figure 6b), respectively, indicates the bands to be associated with the $C_\alpha C_m$ stretching modes. It has been shown that metallochlorins exhibit a strong band (ν_{10}-equivalent) above 1600 cm^{-1} in both IR and resonance Raman spectra and that the band is sensitive to spin, coordination, and oxidation-state changes at the metal center.[35,36] For the ferric high-spin state, the characteristic band is observed around 1630 cm^{-1} for five-coordinate complexes and at 1610–1620 cm^{-1} for six-coordinate complexes.[36b] Accordingly, the 1623-cm^{-1} band observed here suggests the coordination of a solvent (acetonitrile) to the second axial site in **4**.

It is therefore concluded that the formulation of **2** and **4** is peroxo–iron(III) chlorin with the peroxide ligand bound in a side-on (bidentate) fashion, similar to the structures of the corresponding porphyrin complexes based on the results of electronic absorption, ESR, and IR measurements.

NMR Spectra of the Peroxo Complexes. To gain further insight into the molecular and electronic structures of the peroxo chlorin complexes, deuterium NMR spectra of **2** and **4** in acetonitrile were examined. Deuterium NMR spectra of **2** and **4** exhibited well-resolved hyperfine-shifted deuterium NMR resonances, and signal assignments were made by using selectively deuterated derivatives. Three nonequivalent pyrrole deuterium resonances for **2** are clearly observed in a downfield region at 79, 72, and 67 ppm at –25 °C (Figure 7a), consistent with the ferric high-spin (i.e., the presence of singly occupied $d_{x^2-y^2}$) configuration.[14] As illustrated in Figure 7b, two meta deuterium signals are slightly split into doublets, suggesting placement of the iron atom out of the chlorin macrocycle plane toward the peroxide ligand.

(34) The IR spectrum of **4** has a shoulder at 794 cm^{-1}, which is not assigned.
(35) (a) Fonda, H. N.; Oertling, W. A.; Salehi, A.; Chang, C. K.; Babcock, G. T. *J. Am. Chem. Soc.* **1990**, *112*, 9497–9507. (b) Prendergast, K.; Spiro, T. G. *J. Phys. Chem.* **1991**, *95*, 1555–1563 and references therein.
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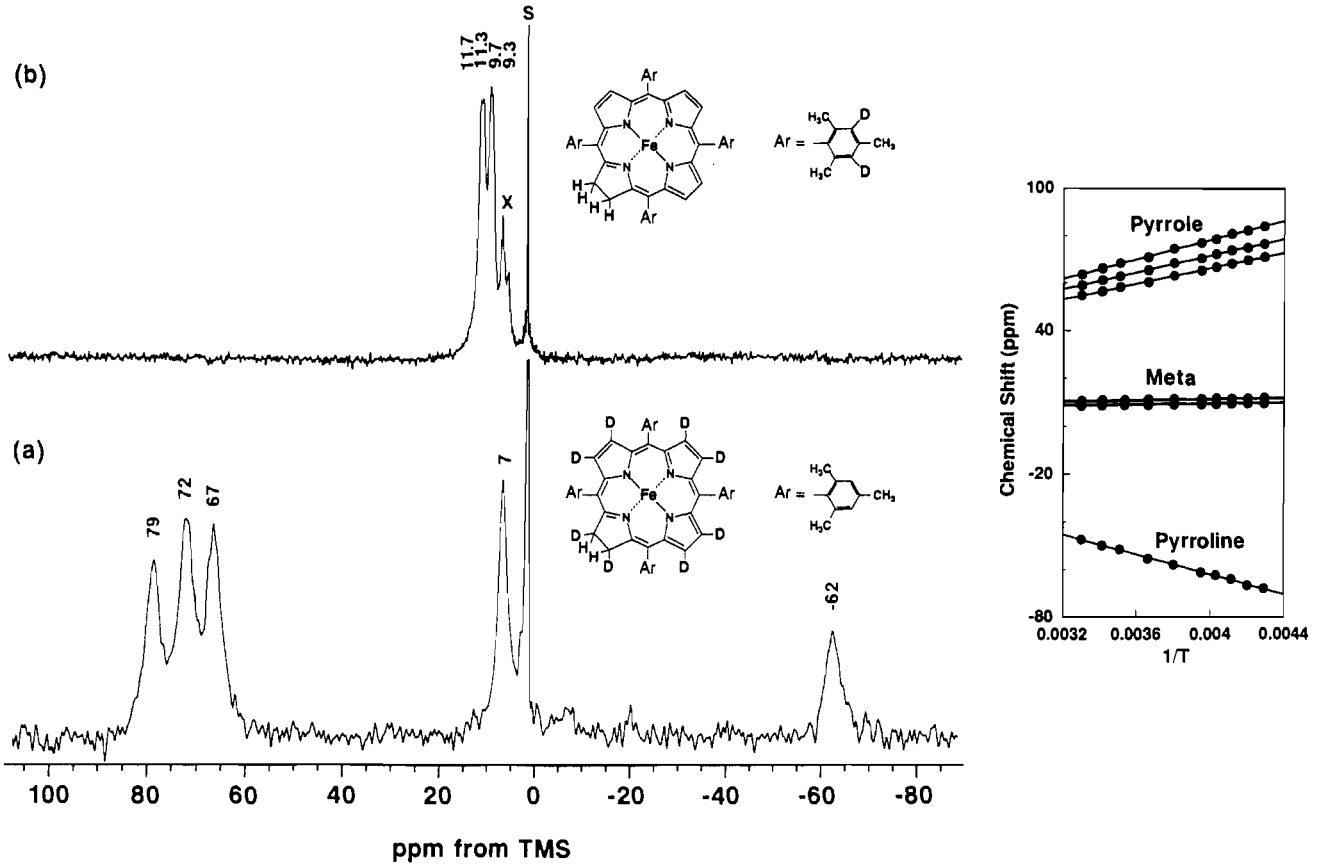


Figure 7. Deuterium NMR spectra of the selectively deuterated peroxo complex of TMC, **2**, in acetonitrile (3 mM) at −25 °C: (a) saturated pyrrole ring-d_2–pyrrole-d_6 and (b) meta-d_8 complexes. Curie law plots for the deuterium NMR resonances of **2** are included (right side).

Deuterium NMR signals for the saturated pyrrole (pyrroline) ring exhibit downfield and upfield shifts at 7 and −62 ppm at −25 °C. To establish unambiguous assignment of the pyrroline deuterium signals in **2**, deuterium NMR spectra of the peroxo complex of (TPC)Fe^{III} were also examined. As shown in Figure 8, deuterium NMR resonances for the pyrroline ring of TPC derivatives are observed at 14 and −60 ppm at −25 °C. Thus, the peroxo–iron(III) complexes of TMC and TPC exhibit a large splitting of the deuterium signals for the pyrroline rings, indicating that two pyrroline deuteriums are nonequivalent. While other iron(III) complexes of TPC also exhibit splitting of the pyrroline proton resonances,[37] a large separation and appearance in the opposite direction for the peroxo chlorin complexes have not been observed to date. The unusual splitting of the pyrroline deuterium resonances is hardly due to placement of the iron atom out of the chlorin plane, but rather results from large deformation of the pyrroline ring of **2** (*vide infra*).

As shown in Figure 9a, meso deuterium resonances of **4** exhibit upfield shifts at −37 and −68 ppm at −25 °C. Further, deuterium NMR signals for the pyrroline ring of **4** are observed in downfield and upfield regions at 32 and −49 ppm at −25 °C (Figure 9b). Methylene deuterium resonances of **4** are seen in the downfield region (10–30 ppm) and are less intense than pyrroline ring or meso deuterium resonances owing to their partial deuteration (<40%). These deuterium NMR spectral features of **4** are similar to those of **2**. The deuterium NMR chemical shift values for the peroxo chlorin complexes (including the TPC derivative) and the chloro–iron(III) chlorin complexes at 20 °C are summarized in Table 1.

Variable-Temperature Measurements. Variable-temperature deuterium NMR measurements for **2** and **4** were examined in the range from −40 to +30 °C. As illustrated in Figures 7 and 9 (right sides), temperature dependence of the deuterium NMR signals for **2** and **4** revealed Curie law behavior, implying monomeric structures for **2** and **4**. Furthermore, the electronic absorption spectrum of **2** did not show any significant change when the temperature of the solution was lowered to −35 °C.

Discussion

Spectroscopic Properties of the Peroxo–Iron(III) Chlorin Complexes. It has been demonstrated here that the peroxo–iron(III) chlorin complexes are successfully formed by the reaction of chloro–iron(III) chlorin complexes with the superoxide anion. The chlorin peroxo complexes exhibit electronic absorption, ESR, and IR spectral features similar to those of the corresponding porphyrin complexes. These observations led us to conclude that the peroxo complexes of iron(III) chlorins have electronic and molecular structures similar to those of the peroxo porphyrin complexes, except for the pyrroline ring. The absorption spectra of the peroxo chlorin complexes show large red shifts of both the characteristic chlorin band and the Soret band, as observed for the porphyrin complexes.[13] The positions of the optical absorption bands in the spectra of zinc porphyrins are known to correlate with the charge and polarizability of the axial ligand.[38] A large negative charge on the ligand leads to a decrease in the energy of the transition.[38] Thus, the presence of a dianionic ligand such as O_2^{2-} is likely to cause a substantial decrease in the transition energy (a large red shift of the absorption band), as suggested by Nappa and Valentine.[38] The ESR spectra of the chlorin peroxo complexes exhibit the resonances at $g \approx 9$ and 4.2 characteristic of rhombic high-spin ferric complexes, such as peroxo–iron(III) porphyrin complexes,[13] but very different from the resonances of five- and six-coordinated hydro- or alkylperoxo iron(III) porphyrin

(37) (a) Strauss, S. H.; Pawlik, M. J.; Skowyra, J.; Kennedy, J. R.; Anderson, O. P.; Spartalian, K.; Dye, J. L. *Inorg. Chem.* **1987**, *26*, 724–730. (b) Pawlik, M. J.; Miller, P. K.; Sullivan, E. P., Jr.; Levstik, M. A.; Almond, D. A.; Strauss, S. H. *J. Am. Chem. Soc.* **1988**, *110*, 3007–3012.

(38) Nappa, M.; Valentine, J. S. *J. Am. Chem. Soc.* **1978**, *100*, 5075–5080.

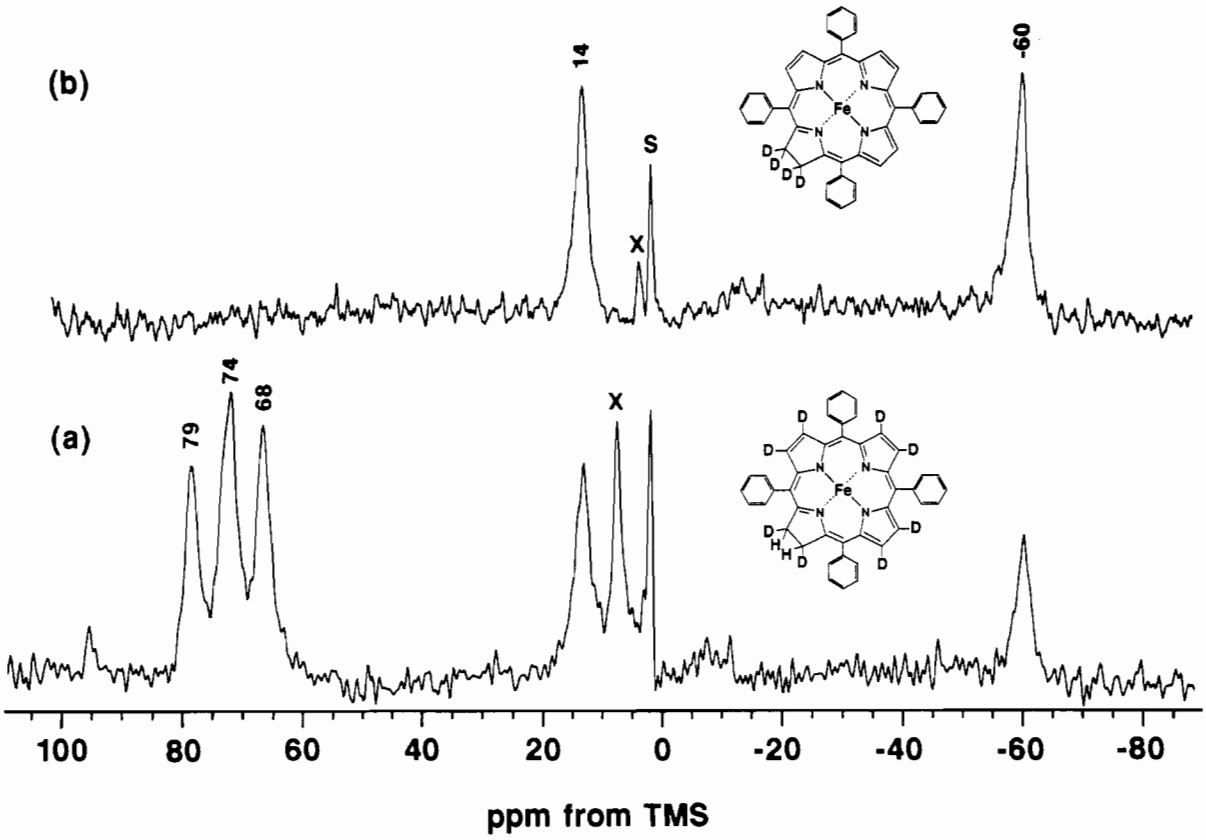
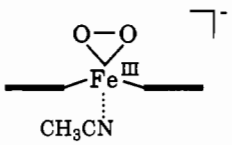


Figure 8. Deuterium NMR spectra of the selectively deuterated peroxo complex of TPC in acetonitrile (3 mM) at −25 °C: (a) saturated pyrrole ring-d_2–pyrrole-d_6 and (b) saturated pyrrole ring-d_4 complexes.

complexes.[39] Further, the oxygen–oxygen stretching mode (806 cm^{-1}) of the peroxo complexes of iron(III) chlorins is also compatible with that of the porphyrin peroxo complexes.[13] While the lowering of symmetry from porphyrin to chlorin could afford a drastic change in the electronic structure of the macrocycle, it did not alter the spectral features.

Deuterium NMR measurements presented here provide further insight into the molecular structure of the peroxo chlorin complexes. Though the observed downfield shifts for the pyrrole deuteriums of **2** are consistent with the ferric high-spin configuration, these signals (66, 61, and 57 ppm at 20 °C) experienced smaller downfield shifts than those of the five-coordinated complex (**1**: 87, 79, and 69 ppm at 20 °C), as shown in Table 1. Similar deuterium NMR results were also observed for **4**, for which meso deuterium resonances (−29, −54, and −55 ppm at 20 °C) are downfield from the resonances for meso deuteriums of the five-coordinated complex, **3** (−48, −59, −84, and −94 ppm at 20 °C). The contact-shift contributions appear as the result of unpaired spin delocalization through either σ or π molecular orbitals or both.[40] A large downfield shift for pyrrole substituents in ferric high-spin five-coordinated complexes is well rationalized by predominant σ-spin delocalization.[40b,c,41] On the other hand, larger π delocalization in six-coordinated complexes due to coplanarity of the metal and the macrocycle causes a smaller downfield shift for the pyrrole substituents.[14,40b,42] Therefore, the smaller downfield shifts for the pyrrole deuteriums of **2** observed here imply a six-coordinated structure for **2** in acetonitrile solution. Further, it was previously reported by Sullivan et al.[43] that the proton NMR shifts of meso protons for high-spin iron(III) OEC complexes were largely changed from those for five- (−44.3, −53.2, −82.0, and −91.4 ppm) to those for six-coordination (31.7 and 29.6 ppm). Thus, the downfield bias of the meso deuterium shifts for **4** as compared with those for a typical five-coordinated OEC complex (**3**) (Table 1) suggest that a solvent (acetonitrile) molecule could weakly coordinate to the iron in **4**. The six-coordinated formulation of **4** is further supported by the observation of the characteristic IR band of 1623 cm^{-1} in the range for six-coordinated iron(III) chlorin complexes.[36b] On the basis of these results, a plausible structure of the peroxo–iron chlorin complexes is proposed:



Deformation of the Pyrroline Ring of the Peroxo Chlorin Complexes. The deuterium NMR resonances of the saturated pyrrole (pyrroline) rings of **2** and **4** exhibited downfield and upfield shifts. The splitting of the pyrroline deuterium signals was also observed for the peroxo complex of (TPC)Fe^{III}. The magnitudes of splitting of the pyrroline deuterium signals are surprisingly large (60–70 ppm at 20 °C) with opposite directions (downfield and upfield regions). To our knowlege, the unusual deuterium NMR features for the peroxo complexes have never been reported.

(39) (a) Tajima, K.; Jinno, J.; Ishizu, K.; Sakurai, H.; Ohya-Nishiguchi, H. *Inorg. Chem.* **1989**, *28*, 709–715. (b) Tajima, K.; Shigematsu, M.; Jinno, J.; Ishizu, K.; Ohya-Nishiguchi, H. *J. Chem. Soc., Chem. Commun.* **1990**, *144*–145. (c) Yamaguchi, K.; Watanabe, Y.; Morishima, I. *J. Am. Chem. Soc.* **1993**, *115*, 4058–4065.

(40) (a) La Mar, G. N. In *NMR of Paramagnetic Molecules*; La Mar, G. N., Horrocks, W. D.; Holm, R. H., Eds.; Academic Press: New York, 1973; pp 85–126. (b) Goff, H. M. In *Iron Porphyrins*; Lever, A. B. P., Gray, H. B., Eds.; Addison-Wesley: Reading, MA, 1983; Part I, pp 237–281. (c) La Mar, G. N.; Walker, F. A. In *The Porphyrins*; Dolphin, D., Ed.; Academic Press: New York, 1979; Vol. 4, pp 61–157.

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(42) Budd, D. L.; La Mar, G. N.; Laugry, K. C.; Smith, K. M.; Nayyir-Mazhir, R. *J. Am. Chem. Soc.* **1979**, *101*, 6091–6096.

(43) Sullivan, E. P., Jr.; Grantham, J. D.; Thomas, C. S.; Strauss, S. H. *J. Am. Chem Soc.* **1991**, *113*, 5264–5270.

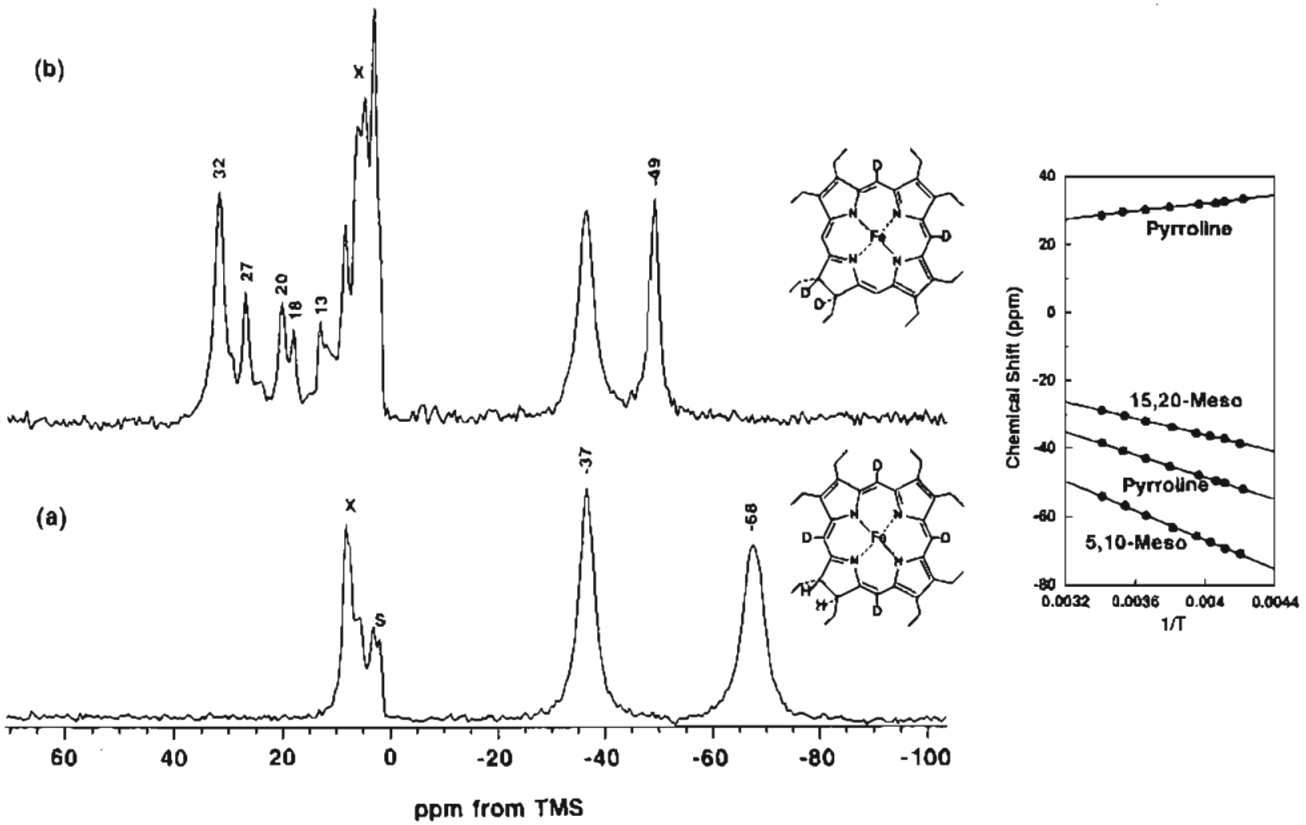


Figure 9. Deuterium NMR spectra of the selectively deuterated peroxo complex of OEC, **4**, in acetonitrile (3 mM) at −25 °C: (a) meso-d_4 and (b) saturated pyrrole ring-d_2 and methylene deuterated complexes. The methylene groups are partially deuterated (<40%). Curie law plots for the deuterium NMR resonances of **4** are included (right side).

Table 1. Deuterium NMR Chemical Shift Values (δ_{obs}) for Iron(III) Chlorin Complexes[a]

complex	pyrrole	pyrroline	*meta* (mesityl)
$[(TMC)Fe^{III}(O_2)]^-$ (**2**)	66, 61, 57	7, −50	11.1, 10.5, 9.4, 9.0
$[(TPC)Fe^{III}(O_2)]^-$	67, 62, 58	12, −48	
$(TMC)Fe^{III}Cl$[c] (**1**)	87, 79, 69	−6	17.4, 16.2 15.3, 14.0
$(TPC)Fe^{III}Cl$[c]	89, 79, 69	−1	

complex	15,20-meso	5,10-meso	pyrroline
$[(OEC)Fe^{III}(O_2)]^-$ (**4**)	−29	−54, −55[b]	29, −38
$(OEC)Fe^{III}Cl$[c] (**3**)	−48, −59	−84, −94	30, 25

[a] In CH_3CN (3 mM) at 20 °C; chemical shifts are referenced to a solvent signal (acetonitrile: 2.0 ppm) and are given in ppm. [b] These resonances are not resolved below −20 °C. [c] At ~10^{-4} M.

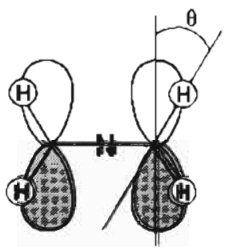
The isotropic NMR hyperfine shifts are composed of contact and dipolar (pseudocontact) terms.[40,41] The metal-centered dipolar term resulting from zero-field splitting is shown to exhibit T^{-2} dependence for a high-spin d^5 ion, unlike the usual reciprocal temperature dependence of the contact shift.[40] Inspection of Figures 7 and 9 (right sides) shows normal Curie law behavior (T^{-1} dependence) of the hyperfine-shifted NMR signals for **2** and **4**, implying that any dipolar shifts for the deuteriums in these complexes are substantially small relative to the contact shifts.[44]

The contact shift for the pyrroline proton is related to the π-spin density at the α-carbons of the pyrroline ring through eqs 1 and 2.[40,41,45] Equation 1 relates the contact shift $(\Delta H/H)_{con}$ to

$$\left(\frac{\Delta H}{H}\right)_{con} = A^{H}\frac{\gamma_e}{\gamma_H}\frac{g\beta S(S+1)}{3kT} \quad (1)$$

$$A^{H} = (B_0 + B_2\cos^2\theta)\rho_{C\text{-}CH} \quad (2)$$

the proton hyperfine coupling constant (A^H). Equation 2 is the McConnell relation which connects A^H with the π-spin density on the α-carbons of the pyrroline ring. The term B_0 in eq 2 is often equated with the magnitude of spin polarization, whereas the $B_2\cos^2\theta$ term is proportional to the magnitude of the hyperconjugation interaction ($B_0 < B_2$). θ is the dihedral angle between the $2p_z$ orbital of the α-carbon and the plane defined by the α- and β-carbons and the proton, depicted as follows:



The observed contact shifts are dependent on the extent of spin polarization and hyperconjugation effects (θ values). The large splitting (downfield and upfield) of the deuterium signals in the spectra of the peroxo complexes is possibly caused by the deformation of the pyrroline ring to give different θ values for each deuterium. According to this idea, two possible conformations of the pyrroline ring are proposed as illustrated in Figure 10. In the twist form (a), the protons at the axial positions could largely overlap with the $2p_z$ orbital ($\theta \approx 0°$), resulting in the predominant hyperconjugation interaction (this direct spin delocalization induces a downfield shift), whereas the equatorial protons would be preferentially affected by the spin polarization, affording the upfield shift, due to less extent of the overlap with the $2p_z$ orbital ($\theta \approx 90°$). In the half-chair form (b), on the other hand, the upside protons could experience larger overlap with the $2p_z$ orbital than the protons underneath the five-membered

(44) Further, the *large* upfield shift for the pyrroline deuteriums should not result from predominant dipolar contribution.
(45) Kreilick, R. W. In ref 40a, pp 595–626.

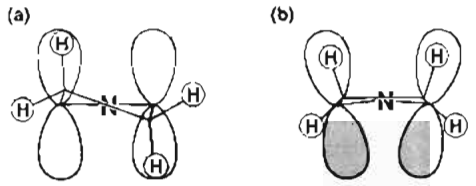


Figure 10. Possible conformations of the pyrroline ring in the peroxo chlorin complexes: (a) twist form; (b) half-chair form.

pyrroline ring. Thus, the NMR signals for each proton in these conformations should be observed in downfield and upfield regions. The splitting of the deuterium signals in the spectrum of **4** (*trans*-OEC) having deuterium only at the *trans* position implies that the pyrroline deuterium at the *trans* position is nonequivalent. Therefore, conformation b would be more plausible for the peroxo chlorin complexes, assuming the similar deformation of the pyrroline rings in **2** and **4**.[46] We have no explanation for the deformation of the pyrroline ring in the peroxo chlorin complexes at this time.[47] The pyrroline ring deformation may control the stability or reactivity of the putative reaction intermediate.

In summary, peroxo–iron(III) chlorin complexes have been successfully formed by the reaction of iron(III) chlorin complexes with superoxide anion. The peroxo chlorin complexes have electronic and molecular structures similar to those of the corresponding porphyrin complexes. However, unusual features have been observed in the deuterium NMR spectra of the peroxo complexes, rationalized by the large deformation of the pyrroline ring of the complexes.

Acknowledgment. We thank Dr. H. Hori and Mr. F. Masuya for ESR measurements.

(46) Normal Curie law behavior of the NMR resonances for **2** and **4** could rule out the possibility of dynamic conformational changes of the pyrroline ring. Moreover, relative intensities (1:1) of the pyrroline deuterium signals in downfield and upfield regions did not change in the course of the variable-temperature measurements.

(47) Previously, Stolzenberg and Stershic also suggested pyrroline ring deformations in *trans*- and *cis*-Ni^{II}OEC complexes from the results of NMR measurements.[48]

(48) Stolzenberg, A. M.; Stershic, M. T. *Inorg. Chem.* **1987**, *26*, 1970–1977.