Preparation and Characterization of Oxoiron(IV) Chlorin Complexes as the First Models for a Reaction Intermediate in the Catalytic Cycle of Cytochrome *d*

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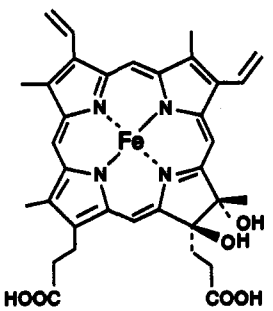
Abstract: As models for a reaction intermediate in the catalytic cycle of cytochrome *d*, two types of oxoferryl chlorin complexes, (TPC)$Fe^{IV}O$(*N*-MeIm) and (TPC)$Fe^{IV}O$ (TPC = tetraphenylchlorin, *N*-MeIm = *N*-methylimidazole), have been prepared for the first time by an autoxidation reaction of (TPC)Fe^{II} with O_2. Oxyiron(II)(TPC) and μ-peroxo-bridged iron(III) dimer, (TPC)$Fe^{III}OOFe^{III}$(TPC), are also detected in the course of the reaction. The six-coordinated (TPC)$Fe^{IV}O$(*N*-MeIm) complex is produced by adding *N*-methylimidazole to the μ-peroxo-bridged iron(III) chlorin dimer at −80 °C. The μ-peroxo-bridged iron(III) dimer, the formation of which has been confirmed by paramagnetic NMR, undergoes homolytic cleavage of the O–O bond upon laser illumination to yield the five-coordinated (TPC)$Fe^{IV}O$ complex. The absorption spectrum of (TPC)$Fe^{IV}O$(*N*-MeIm) shows a characteristic red-shifted band at 630 nm as observed in the case of the oxoferryl intermediate of cytochrome *d*. Proton NMR spectra of (TPC)$Fe^{IV}O$(*N*-MeIm) exhibit a small hyperfine shift of the pyrrole protons, consistent with the oxoferryl formulation. However, the paramagnetic NMR resonances of the saturated pyrrole (pyrroline) ring protons show unusual splitting into upfield and downfield region, suggesting deformation of the pyrroline ring of the chlorin complex. While the unusually high frequency of $\nu(Fe^{IV}{=}O)$ (815 cm^{-1}) and large isotopic shift ($\Delta\nu$ = 46 cm^{-1}) observed for the oxoferryl intermediate of cytochrome *d* have been attributed to the chlorin macrocycle (heme *d*), the five- and six-coordinated oxoferryl chlorin complexes reported here exhibit $\nu(Fe^{IV}{=}O)$ frequencies and isotopic ($^{16}O/^{18}O$) frequency shifts nearly identical to those of the corresponding porphyrin complexes, respectively.

Introduction

Cytochrome *d*, having an chlorin cofactor referred to as heme *d*, is one of two terminal electron acceptors of *Escherichia coli* catalyzing the four-electron reduction of dioxygen to water using ubiquinol as an electron donor.[1-4] 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 illustrated.



Heme *d*

The reduced form of cytochrome *d* complex binds dioxygen reversibly at room temperature to form a stable oxygenated intermediate designated as d_{650} owing to its prominent absorption at ~645 nm.[7] Recently, the $\nu(Fe{-}O_2)$ vibrational frequency of the oxygenated enzyme was reported to be 568 cm^{-1} (542 cm^{-1} for the $^{18}O_2$ derivative), which is nearly identical to those of other oxyheme proteins.[7c] The bound O_2 is then reduced to H_2O via several intermediates, i.e., a putative peroxo intermediate,[7a,8] an oxoferryl intermediate with a characteristic absorption at 680 nm (d_{680}),[9] and a ferric state, as summarized in Scheme 1.[7c,8b,9]

While the peroxo intermediate has been poorly characterized,[7a,8] the oxoferryl intermediate (d_{680}) has been recently identified by resonance Raman (RR) spectroscopic measurements.[9] The RR spectra of the oxoferryl intermediate showed a band at 815 cm^{-1} assigned to the $\nu(Fe^{IV}{=}O)$ mode, which is higher than that of any other oxoferryl-containing heme enzyme.[9] Further, the band

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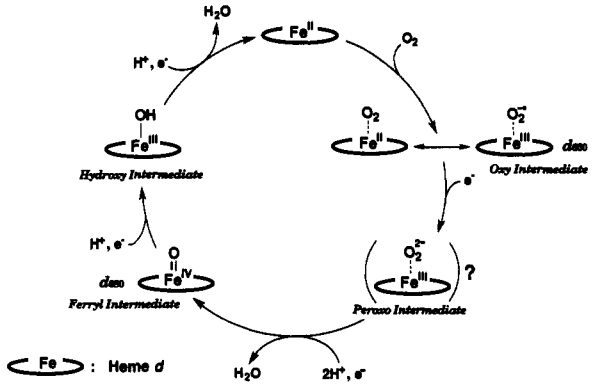
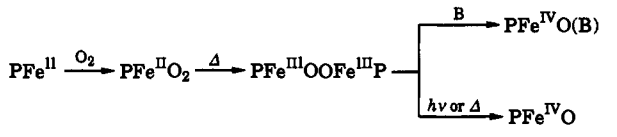
Scheme 1



exhibited an extremely large isotopic ($^{16}O/^{18}O$) frequency shift of 46 cm^{-1}.[9] Although the participation of the chlorin macrocycle has been suggested for the unusual feature of the $\nu(Fe^{IV}{=}O)$ frequencies in RR spectroscopy,[9] the effect of the chlorin macrocycle has not been studied yet. It is therefore of particular importance to prepare and characterize oxoferryl chlorin complexes by using synthetic model complexes to understand the details of the intermediate of the enzyme.

Model complexes of *oxoferryl porphyrins* are readily prepared by an autoxidation reaction of iron(II) porphyrins (PFe^{II}) with O_2 via μ-peroxo-bridged iron(III) porphyrin dimes (PFe^{III}-$OOFe^{III}P$).[10-14] For example, six-coordinated oxoferryl porphyrin complexes ($PFe^{IV}O(B)$) can be synthesized according to homolytic cleavage of the O–O bond by addition of nitrogenous bases (B) to the peroxo-bridged dimers.[10a,b,11,12] On the other hand, the μ-peroxo-bridged dimers are known to give five-coordinated oxoferryl complexes ($PFe^{IV}O$) by thermal[10c] or photo decomposition,[13,14] as summarized below. While *oxoferryl porphyrin*



complexes have been extensively investigated as models for the catalytic intermediate of heme enzymes,[10-14] *oxoferryl chlorin* complexes have not been examined yet.

In this paper, we describe the first models for the oxoferryl intermediate of cytochrome *d*, i.e., five- and six-coordinated oxoiron(IV) tetraphenylchlorin (TPC) complexes, which have been characterized by electronic absorption, NMR, and RR spectroscopies. Further, oxyiron(II) and μ-peroxo-bridged iron(III) dimer complexes of chlorin are detected during the autoxidation reaction of a ferrous chlorin complex with O_2. It is shown here that the six-coordinated oxoferryl chlorin complexes exhibit unusual splitting of the pyrroline proton resonances, suggestive of deformation of the pyrroline ring of the chlorin complex. Further, the five- and six-coordinated oxoferryl chlorin complexes show $\nu(Fe^{IV}{=}O)$ bands at frequencies nearly identical to those of the corresponding porphyrin complexes, in contrast to the results obtained in cytochrome *d*.

Experimental Section

Spectral Measurements. Electronic absorption spectral measurements were made on a Hitachi 330 spectrometer and a Shimadzu UV-2200 spectrometer. Low-temperature absorption spectra were obtained by using a DN1704 variable-temperature liquid nitrogen cryostat equipped with digital temperature controller DTC2 (Oxford Instruments). Proton NMR spectra at 500 MHz were recorded with a GE omega 500 spectrometer, and deuterium NMR spectra at 46.1 MHz were measured on a Nicolet NT-300 spectrometer equipped with a 1280 computer system. Chemical shifts were referenced to a solvent signal (toluene, 2.1 ppm), and downfield shifts are given a positive sign. Raman scattering was excited at 406.7 nm with a Kr^+ ion laser (Spectra Physics, model 2016) and detected with a photodiode array (PARC OMA III) attached to a double monochrometer (Spex 1404). The laser power on the sample was kept at ~6 mW. Raman shifts were calibrated with indene as the standard with accuracy of ±1 cm^{-1}. All Raman measurements were carried out with a spinning cell (2-cm diameter) which was spun at 1600 rpm in a cryostat that was cooled to −100 °C by cold N_2 gas.

Materials. Toluene (Wako) was shaken with concentrated H_2SO_4 and then water to remove sulfur-containing impurities. The resultant solvent was dried with Na_2SO_4 and refluxed over calcium hydride, followed by distillation from sodium (solid) or sodium–benzophenone ketyl under vacuum. Toluene-d_8 was obtained from Aldrich and distilled from sodium (solid) under vacuum just before use. *N*-Methylimidazole (Aldrich) and sodium dithionite were used without further purification.

Tetraphenylchlorin (TPC) was synthesized by diimide reduction of tetraphenylporphyrin (TPP),[15] and iron was inserted into the free base by an acetic acid method.[16] The saturated pyrrole ring-d_2-pyrrole-d_6 TPC derivative was prepared by using pyrrole-d_8 TPP. Selective deuteration of the saturated pyrrole ring was accomplished by a published procedure.[17] The phenyl-d_{20} TPC complex was synthesized from phenyl-d_{20} TPP.

Synthesis of an Unligated Iron(II) Chlorin Complex. (TPC)Fe^{II} was prepared by reduction of (TPC)$Fe^{III}Cl$ in toluene solution with an aqueous sodium dithionite solution. Typically 1 mg of (TPC)$Fe^{III}Cl$ and ca. 10 mg of sodium dithionite were dissolved in a mixture of 3 mL of toluene and 1.5 mL of water. After the solution was degassed by repeated freeze–pump–thaw cycles, the solution was shaken to mix the two layers. During the reduction, the green solution of (TPC)$Fe^{III}Cl$ changed into a blue-green solution of (TPC)Fe^{II}. After the reduction was completed, the solvent was evaporated from the sample under vacuum and the sample was dried under continuous vacuum. (TPC)Fe^{II} was then dissolved in deoxygenated toluene and transferred to appropriate cuvettes for spectral measurements.

Alternatively, (TPC)$Fe^{III}Cl$ dissolved in degassed toluene was reduced to (TPC)Fe^{II} by contact with a sodium mirror. The resultant toluene solution was used for spectral measurements. Several isosbestic points were observed in electronic absorption spectral changes due to reduction.

Formation of an Oxoferryl Chlorin Complex. In a typical reaction, a degassed toluene solution of (TPC)Fe^{II} (0.02 mM, 4 mL) in a 1-cm UV cuvette was placed in a cryostat unit of the absorption spectrophotometer. After the equilibrium of the solution at the desired temperature (−100 or −80 °C) was reached, O_2 was introduced into the solution through a syringe needle, followed by addition of a toluene solution of 1 equiv of *N*-methylimidazole (*N*-MeIm) in one portion. Preparations of samples for NMR and RR spectral measurements were carried out in the same way.

Results

Reaction of an Iron(II) Chlorin Complex and Dioxygen. An oxygenated form of (TPC)Fe^{II} ((TPC)$Fe^{II}O_2$, **1**) was prepared by introduction of O_2 into a degassed toluene solution of iron(II) tetraphenylchlorin (TPC) at −100 °C. The formation of **1** accompanies a decreased intensity of the Soret band and a red

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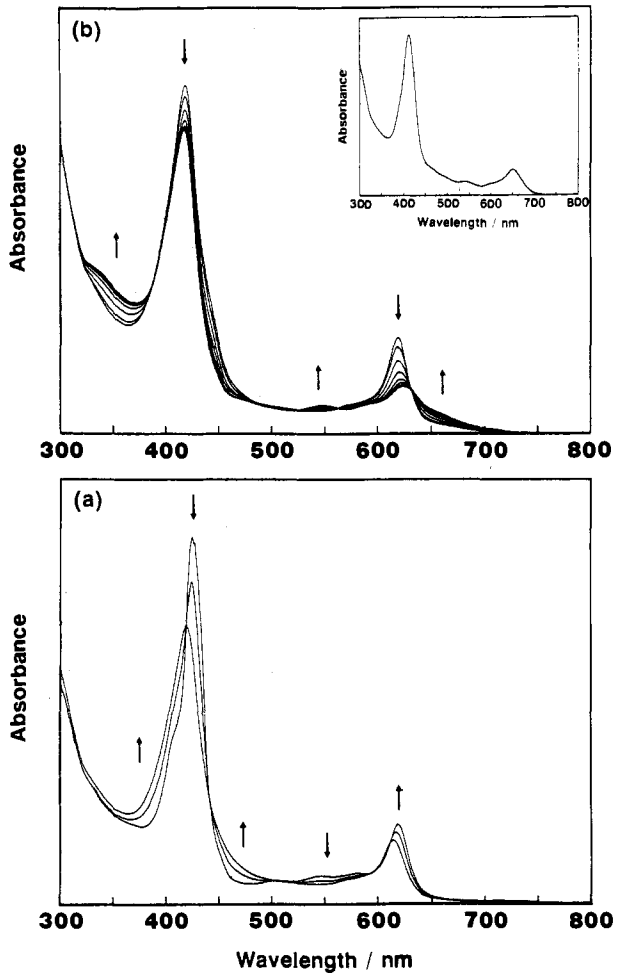

Figure 1. Electronic absorption spectral changes in toluene (0.02 mM) (a) upon introducing O_2 to (TPC)Fe^{II} at −100 °C and (b) upon warming the solution of a to −80 °C. Inset: the resultant spectrum of b at 25 °C.

shift of the characteristic band for chlorin complexes as shown in Figure 1a.[18] Repetitive evacuation and introduction of argon did not cause any changes in the spectrum of **1**, showing the irreversible formation of **1** under the condition. Thus, **1** is relatively stable at −100 °C under UV concentrations (~10^{-5} M). Upon warming the solution of **1** to −80 °C, the spectrum of **1** was altered to that of a new intermediate **2** with several isosbestic points (Figure 1b). The spectral changes upon the transformation of **1** to **2** cannot be reversed by lowering the temperature to −100 °C. Alternatively, introduction of O_2 into the toluene solution of (TPC)Fe^{II} at −80 °C also yielded the intermediate **2**. When the solution of **2** was warmed to 25 °C, the spectrum of a well-known μ-oxo-bridged dimer, (TPC)-$Fe^{III}OFe^{III}$(TPC), was obtained (Figure 1b, inset). The formation of **2** from the oxy form of (TPC)Fe^{II} (**1**) and its thermal decomposition to the μ-oxo-bridged dimer are indicative of the formulation of **2** being a μ-peroxo-bridged dimer, (TPC)Fe^{III}-$OOFe^{III}$(TPC). This assignment is confirmed both by hyperfine-shifted NMR spectra of **2** and by its conversion to (TPC)$Fe^{IV}O$(*N*-MeIm) as described below.

Addition of 2 equiv (1 equiv per iron) of *N*-MeIm to the solution of **2** at −80 °C results in the complete formation of a new species **3** as illustrated in Figure 2. The absorption spectrum of **3** remained unchanged by further addition of *N*-MeIm, inferring that only one molecule of *N*-MeIm coordinates to the iron in **3**. When the temperature of the solution was raised to 25 °C, **3** was also converted to (TPC)$Fe^{III}OFe^{III}$(TPC). In order to examine the

(18) The five-coordinated oxy form (**1**) is converted into the six-coordinated oxygenated complex, (TPC)$Fe^{II}(O_2)$(*N*-MeIm), upon addition of 1 equiv of *N*-MeIm to the solution of **1** at −100 °C.

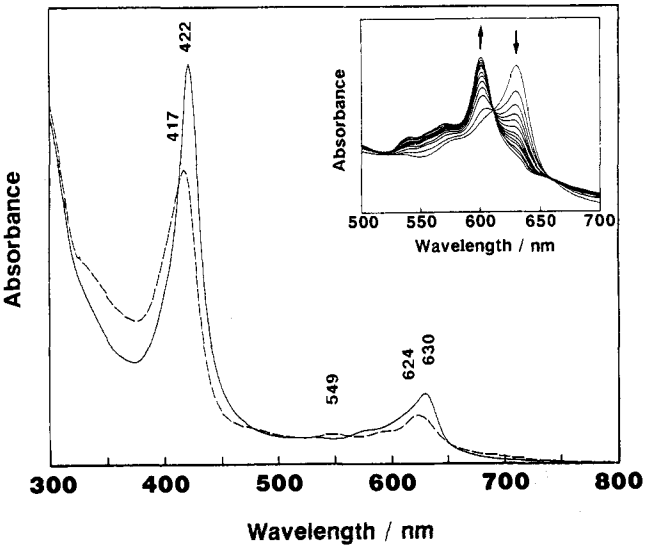

Figure 2. Electronic absorption spectral changes before (- - -, **2**) and after (—, **3**) addition of 1 equiv (per iron) of *N*-MeIm to **2** (0.02 mM per iron) in toluene at −80 °C. Inset: absorption spectral changes upon addition of 1000 equiv of triphenylphosphine to **3** at −80 °C.

reactivity of **3**, 1000 equiv of triphenylphosphine was added to the solution of **3** at −80 °C. Upon addition of triphenylphosphine, the spectrum of **3** was altered to that of (TPC)Fe^{II}(*N*-MeIm)$_2$ in 5 h (Figure 2, inset). A very similar reaction was reported to proceed when an oxoferryl porphyrin complex was employed.[19] The formation of (TPC)Fe^{II}(*N*-MeIm)$_2$ in the oxidation of triphenylphosphine by **3** suggests the formulation of **3** to be (TPC)-$Fe^{IV}O$(*N*-MeIm).

Characterization of 2 and 3 by NMR Spectroscopies. To gain further insight into the formulation of **2** and **3**, we followed the reaction of (TPC)Fe^{II} and O_2 by proton and deuterium NMR spectroscopies. Upon introduction of O_2 to (TPC)Fe^{II} at −80 °C, proton NMR resonances of (TPC)Fe^{II} (spectrum not shown) were replaced by new resonances of **2** as demonstrated in Figure 3. The assignments of the signals for **2** were made by using selectively deuterated derivatives (Figure 4). Three nonequivalent pyrrole proton signals for **2** are clearly observed at 16.5, 16.1 and 15.4 ppm (Figure 3). The hyperfine shifts for the pyrrole proton resonances of **2** are indicative of antiferromagnetic dimers as found for the μ-oxo-bridged dimer, (TPC)$Fe^{III}OFe^{III}$(TPC) (12.5 and 12.2 ppm at −80 °C). Larger hyperfine shifts for the pyrrole proton resonances of **2** suggest a weaker antiferromagnetic coupling in **2** than (TPC)$Fe^{III}OFe^{III}$(TPC) possibly due to the longer bridge in **2**. Non-Curie law behavior for the pyrrole resonances of **2** is also a clear indication of antiferromagnetic dimer formation (Figure 3, inset).[20] Furthermore, these NMR characteristics of **2** are very similar to those of previously characterized μ-peroxo-bridged dimers of iron(III) porphyrin complexes.[21] Therefore, **2** is identified as a μ-peroxo-bridged iron(III) dimer, (TPC)$Fe^{III}OOFe^{III}$(TPC). The pyrrole proton NMR shift values of **2** and the corresponding prophyrin complexes are summarized in Table 1.

When an excess amount of *N*-MeIm was added to a solution of **2** at −80 °C, the resonances of **3** replaced those of **2**. Though the proton NMR spectrum of **3** is much more complicated (Figure 5), identification of the resonances for **3** was accomplished by using selectively deuterated samples (Figure 6). Pyrrole proton

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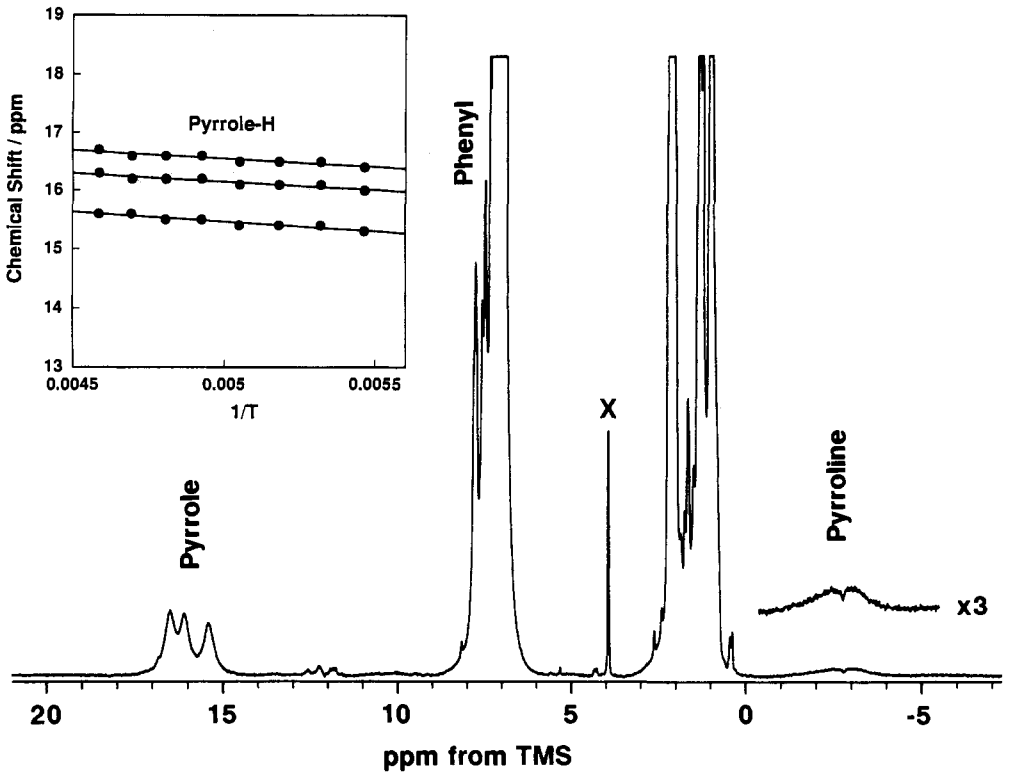


Figure 3. 500-MHz proton NMR spectrum of **2** (3 mM per iron) in toluene-d_8 at −80 °C. Inset: temperature dependence of the proton NMR signals of **2**. The chemical shifts are plotted against $1/T$ (Curie law plot).

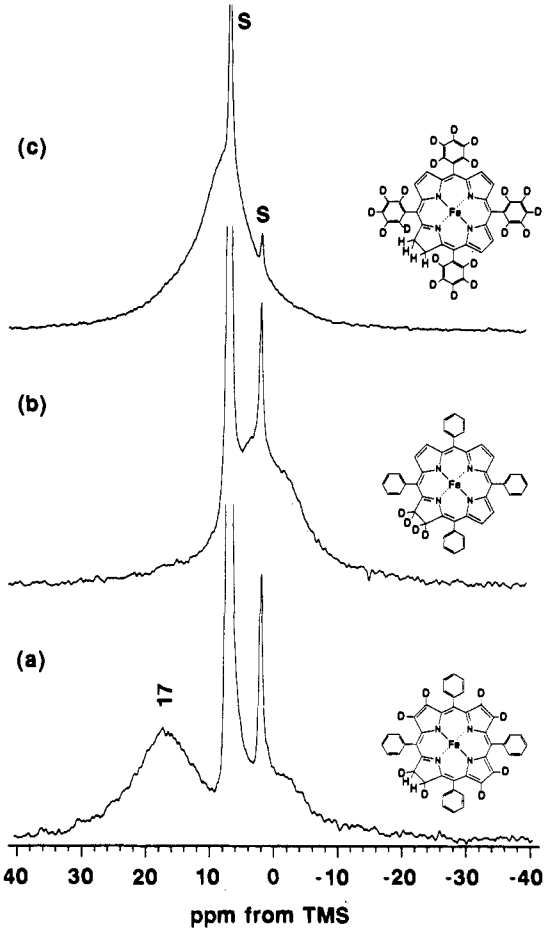


Figure 4. 46.1-MHz deuterium NMR spectra of selectively deuterated complexes of **2** (3.5 mM per iron) in toluene-h_8 at −80 °C: (a) saturated pyrrole ring-d_2-pyrrole-d_6, (b) saturated pyrrole ring-d_4, and (c) phenyl-d_{20} complexes.

Table 1. Pyrrole Proton Resonances for $PFe^{III}OOFe^{III}P$ in Toluene-d_8 at −80 °C[a]

porphyrin	chemical shift, ppm	ref
TmTP	16.2	21b
TPP	16.0	21b
TMP	17.7[b]	10c
TPC (**2**)	16.5, 16.1, 15.4	this work

[a] Abbreviations: TmTP, tetra-*m*-tolylporphyrin; TPP, tetraphenylporphyrin; TMP, tetramesitylporphyrin; TPC, tetraphenylchlorin. [b] At −70 °C.

resonances appear at ~4 ppm (overlapped with impurity peaks), and signals for phenyl protons are observed at 8~9 ppm. The saturated pyrrole ring (pyrroline) proton resonances exhibit downfield and upfield shifts at 14.6 and −14.2 ppm, respectively, showing that the two pyrroline protons of **3** are nonequivalent. A proton signal of the coordinated *N*-MeIm (5-H) is observed at −3.9 ppm,[22] as shown in Figure 5. The identity of the resonance for 5-H has been determined by its relative intensity and line width. The proton NMR resonances of the other two protons of the axial *N*-MeIm were not observed, possibly due to much broadening of these signals. The NMR resonances of the pyrroline and coordinated *N*-MeIm protons of **3** follow the Curie law (Figure 5, inset), which demonstrates that **3** exists as a monomeric and well-defined paramagnetic species. Moreover, the small hyperfine shifts of the pyrrole proton resonances are consistent with the oxoferryl formulation of **3**, as noted in oxoferryl porphyrin complexes.[10,11]

Resonance Raman Spectra of Oxoferryl Chlorin Complexes. In order to gain further information on the structure of the oxoferryl chlorin complexes, we have examined resonance Raman (RR) spectra of the intermediates, **2** and **3**. Spectra A and B in Figure 7 were obtained for $^{16}O_2$ and $^{18}O_2$ derivatives of **2** in toluene-h_8 at −100 °C, respectively. It is observed that the band at 846

(22) Previously, proton NMR spectra of (tetra-*m*-tolylporphyrin)$Fe^{IV}O$(*N*-MeIm) were reported to exhibit a 5-H proton resonance of *N*-MeIm at −4 ppm at −80 °C.[10b,11]

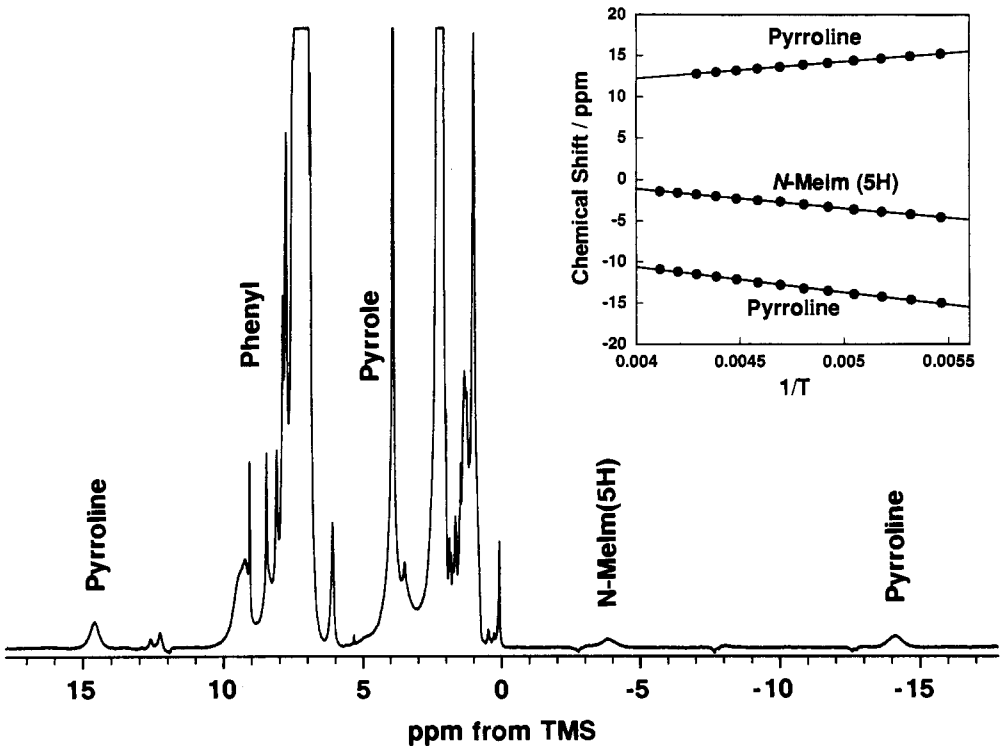


Figure 5. 500-MHz proton NMR spectrum of 3 (3 mM) in toluene-d_8 at −80 °C. Inset: temperature dependence of the proton NMR signals of 3. The chemical shifts are plotted against $1/T$ (Curie law plot).

cm^{-1} (A) shifts to 812 cm^{-1} (B) by $^{16}O/^{18}O$ isotopic substitution, which is unambiguously confirmed by subtraction of B from A as shown in Figure 7C. The magnitude of the observed isotopic frequency shift ($\Delta\nu$ = 34 cm^{-1}) is closer to that expected for an Fe–O diatomic oscillator ($\Delta\nu$ = 37 cm^{-1}) than that for an O–O diatomic oscillator ($\Delta\nu$ = 48 cm^{-1}). Furthermore, the observed shift is in good agreement with the corresponding data for five-coordinated *oxoferryl porphyrin* complexes obtained in toluene solutions ($\Delta\nu$ = 33 cm^{-1})[13,14] and in an O_2 matrix ($\Delta\nu$ = 34 cm^{-1}).[23] Thus, we assign the 846-cm^{-1} band to the ν(Fe^{IV}=O) mode of a five-coordinated oxoferryl chlorin complex, (TPC)Fe^{IV}O (**4**). Previously, Paeng and Nakamoto[13b] observed an oxygen isotope sensitive band at 577 cm^{-1} for the symmetric Fe–O stretching mode of a μ-peroxo-bridged dimer, (TPP)Fe^{III}OOFe^{III}(TPP). The corresponding band is weakly seen at 569 cm^{-1}. It is therefore most likely that the μ-peroxo-bridged dimer of the chlorin complex **2** is extremely photolabile at this excitation wavelength and rather undergoes rapid homolytic cleavage of the O–O bond upon laser illumination to yield the five-coordinated oxoferryl chlorin complex **4**.

Spectra A and B in Figure 8 were observed in toluene-h_8 at −100 °C for $^{16}O_2$ and $^{18}O_2$ derivatives of **3**, respectively. A band at 817 cm^{-1} (A) was apparently missing in spectrum B possibly due to overlapping with a strong solvent band. However, when the difference between spectra A and B was calculated as shown by spectrum C, the presence of a band at 783 cm^{-1} became apparent. To confirm the isotopic shift of the band, RR spectral measurements were performed in toluene-d_8 solutions. As seen in the spectra D and E in Figure 8, the band at 817 cm^{-1} (D) shifts to 783 cm^{-1} (E) by isotopic substitution, established by subtraction of E from D as illustrated in Figure 8F. The magnitude of the observed isotopic shift ($\Delta\nu$ = 34 cm^{-1}) is compatible with an Fe–O diatomic oscillator ($\Delta\nu$ = 36 cm^{-1}) and values obtained for six-coordinated *oxoferryl porphyrin* complexes ($\Delta\nu$ = 36 cm^{-1}).[12] It is therefore reasonable to assign the band at 817 cm^{-1} to the

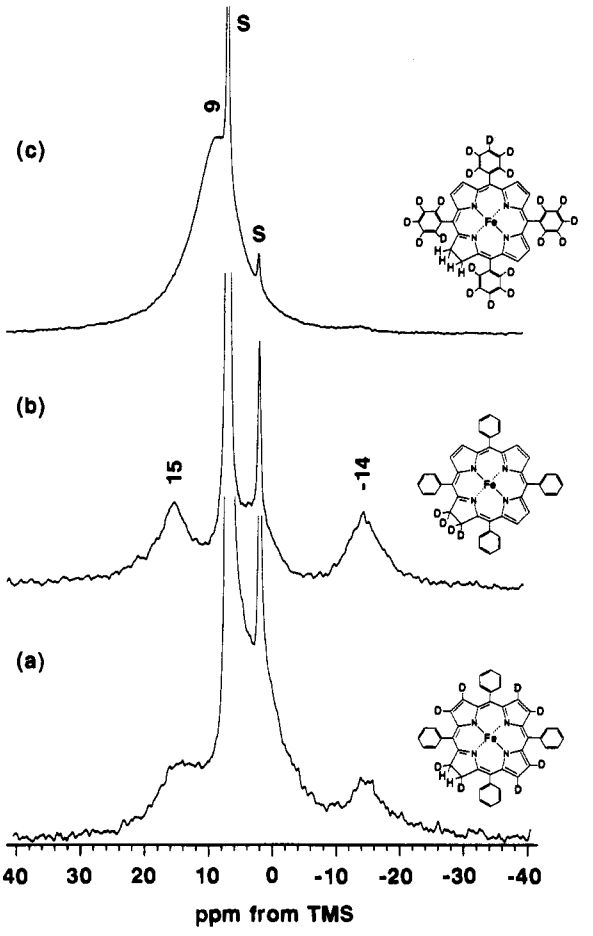


Figure 6. 46.1-MHz deuterium NMR spectra of selectively deuterated complexes of 3 (3.5 mM) in toluene-h_8 at −80 °C: (a) saturated pyrrole ring-d_2-pyrrole-d_6, (b) saturated pyrrole ring-d_4, and (c) phenyl-d_{20} complexes.

(23) (a) Bajdor, K.; Nakamoto, K. *J. Am. Chem. Soc.* **1984**, *106*, 3045–3046. (b) Proniewicz, L. M.; Bajdor, K.; Nakamoto, K. *J. Phys. Chem.* **1986**, *90*, 1760–1766.

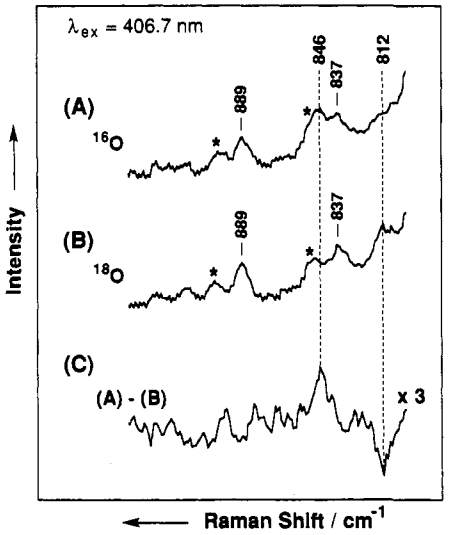


Figure 7. Isotopic substitution effect of **2** (**4**, see text) on RR spectra in the 950-800-cm^{-1} region in toluene-h_8 at −100 °C (0.2 mM per iron): (A) ^{16}O derivative; (B) ^{18}O derivative; (C) difference spectrum (A − B). Asterisks denote Raman bands of solvent.

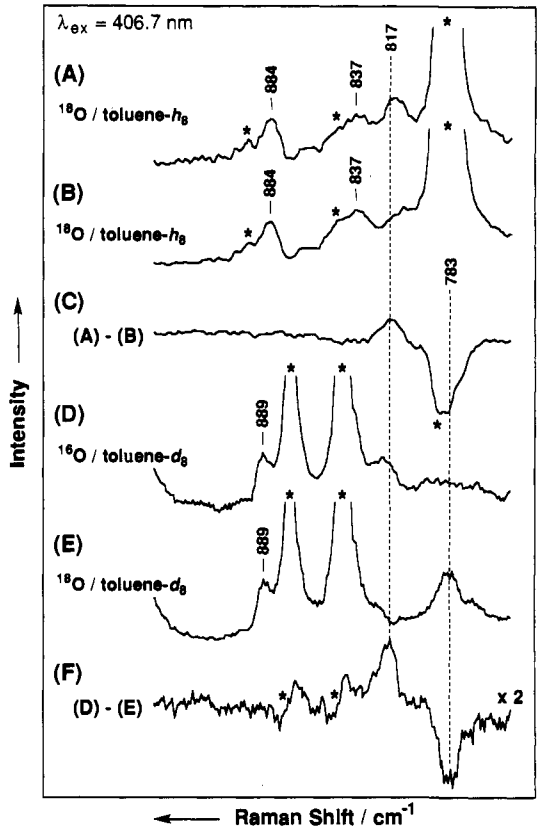


Figure 8. Isotopic substitution effect of **3** on RR spectra in the 950–750-cm^{-1} region at −100 °C (0.1 mM): (A) ^{16}O derivative in toluene-h_8; (B) ^{18}O derivative in toluene-h_8; (C) difference spectrum (A − B); (D) ^{16}O derivative in toluene-d_8; (E) ^{18}O derivative in toluene-d_8; (F) dif ference spectrum (D - E). Asterisks denote Raman bands of solvent.

ν(Fe^{IV}=O) mode for the six-coordinated oxoferryl complex (TPC)Fe^{IV}O(*N*-MeIm) (**3**). The Fe–O stretching frequencies of the five- and six-coordinated oxoferryl porphyrin and chlorin complexes are compiled in Table 2.

RR spectra in high-frequency regions for the oxoferryl (**3** and **4**) and ferric chlorin complexes are presented in Figure 9. As noted for metallochlorin complexes,[24] the RR spectra of the chlorin complexes reported here are more complicated than those of the corresponding prophyrin derivatives, owing to the lowered

Table 2. Iron–Oxygen Stretching Frequencies of Five- and Six-Coordinated Oxoferryl Porphyrin and Chlorin Complexes[a]

complex	ν(Fe^{IV}=O), cm^{-1}	$\Delta\nu$, cm^{-1}	temp, °C	ref
(TMP)Fe^{IV}O	845 (812)[b]	33	−78	13a
	843 (810)	33	−70	14
(TPP)Fe^{IV}O	845 (812)	33	−80	13b
(TPC)Fe^{IV}O (**4**)	846 (812)	34	−100	this work
(OEP)Fe^{IV}O(*N*-MeIm)	820 (784)	36	−120	12
(TPP)Fe^{IV}O(*N*-MeIm)	820 (784)	36	−120	12
(TPC)Fe^{IV}O(*N*-MeIm) (**3**)	817 (783)	34	−100	this work

[a] All work was done in toluene solution. [b] The numbers in parentheses indicate the frequencies of the ^{18}O species.

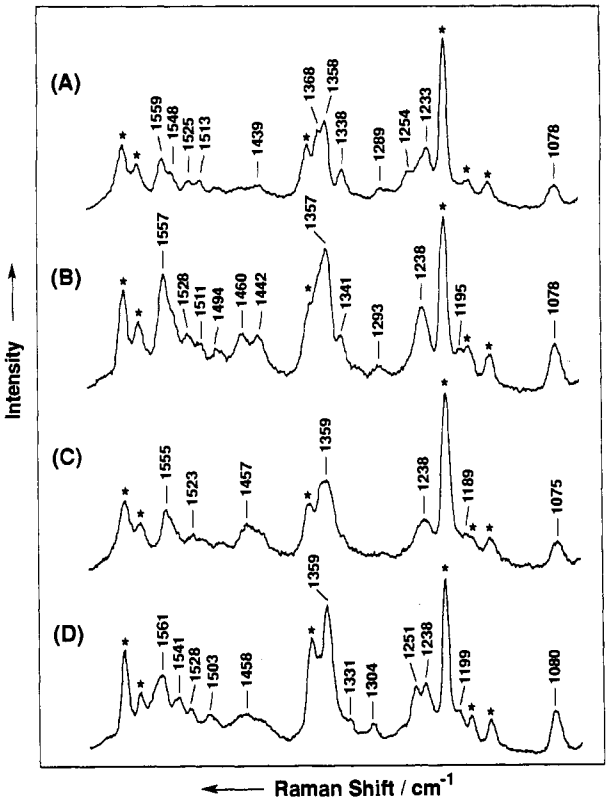


Figure 9. High-frequency RR spectra (1650–1050 cm^{-1}) of (TPC)Fe^{III}-Cl (A), (TPC)Fe^{III}OFe^{III}(TPC) (B), **4** (C), and **3** (D) in toluene-h_8. Spectra A and B were obtained at room temperature. Asterisks denote Raman bands of solvent.

symmetry of the chlorin macrocycle. This feature is clearly observed in the 1450–1550-cm^{-1} regions. Furthermore, two ν_4 bands are split at 1368 and 1358 cm^{-1} for (TPC)Fe^{III}Cl in spectrum A, in contrast to a single band for (TPP)Fe^{III}Cl (1366 cm^{-1}).[25] The bands at 1555 and 1359 cm^{-1} for **4** in spectrum C and 1561 and 1359 cm^{-1} for **3** in spectrum D are reasonably assigned to the ν_2 and ν_4 modes, respectively, on the basis of comparison with those of the porphyrin complexes.[12b,14] These frequencies are rather similar between the Fe^{III} and Fe^{IV} states, indicative of similar π delocalization of d_π electrons to the macrocycle.

(24) (a) Ozaki, Y.; Kitagawa, T.; Ogoshi, H. *Inorg. Chem.* **1979**, *18*, 1772–1776. (b) Ozaki, Y.; Iriyama, K.; Ogoshi, H.; Ochiai, T.; Kitagawa, T. *J. Phys. Chem.* **1986**, *90*, 6105–6112. (c) Andersson, L. A.; Loehr, T. M.; Chang, C. K.; Mauk, A. G. *J. Am. Chem. Soc.* **1985**, *107*, 182–191. (d) Andersson, L. A.; Loehr, T. M.; Sotiriou, C.; Wu, W.; Chang, C. K. *J. Am. Chem. Soc.* **1986**, *108*, 2908–2916. (e) Boldt, N. J.; Bocian, D. F. *J. Phys. Chem.* **1988**, *92*, 581–586. (f) Andersson, L. A.; Loehr, T. M.; Thompson, R. G.; Strauss, S. H. *Inorg. Chem.* **1990**, *29*, 2142–2147. (g) Fonda, H. N.; Oertling, W. A.; Salehi, A.; Chang, C. K.; Babcock, G. T. *J. Am. Chem. Soc.* **1990**, *112*, 9497–9507. (h) Prendergast, K.; Spiro, T. G. *J. Phys. Chem.* **1991**, *95*, 1555–1563. (i) Perng, J.-H.; Bocian, D. F. *J. Phys. Chem.* **1992**, *96*, 10234–10240.

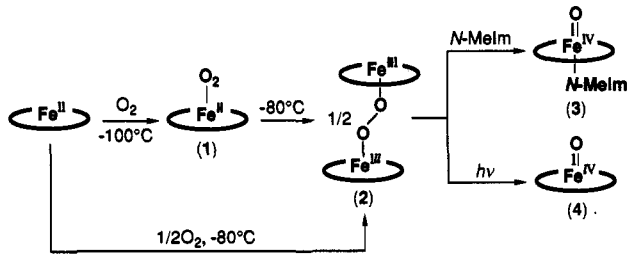
(25) Burke, J. M.; Kincaid, J. R.; Spiro, T. G. *J. Am. Chem. Soc.* **1978**, *100*, 6077–6083.

Table 3. Electronic Absorption Spectral Characteristics of Iron Chlorin Complexes in Toluene Solutions

complex	temp, °C	λ_{max}, nm (relative intensity)		
(TPC)Fe^{II}	−100	614 (0.173)	580 (0.083)	546 (0.076)
		506 (0.064)	425 (1.000)	
(TPC)$Fe^{II}(O_2)$ (1)	−100	618 (0.285)	418 (1.000)	
((TPC)$Fe^{III}O)_2$ (2)	−80	624 (0.163)	549 (0.101)	417 (1.000)
(TPC)$Fe^{IV}O$-(*N*-MeIm) (3)	−80	630 (0.174)	422 (1.000)	

Discussion

Detection of Oxygenated Intermediates of Iron Chlorin Complexes. It has been demonstrated here that oxoferryl chlorin complexes are successfully prepared by an autoxidation reaction of (TPC)Fe^{II} with O_2. Furthermore, oxyiron(II) and μ-peroxo-bridged iron(III) dimer complexes of chlorin are also detected in the course of the reaction at low temperature. Thus, the experimental observations reported here allow us to depict the reaction scheme of (TPC)Fe^{II} and O_2 shown below.



The five-coordinated oxyiron(II) chlorin complex (**1**) is formed by the reaction of (TPC)Fe^{II} and O_2 at −100 °C with a red shift of the characteristic band for chlorin complexes (from 614 to 618 nm) as shown in Figure 1a. The absorption spectral characteristics of the chlorin complexes obtained here are summarized in Table 3. While **1** converts to the μ-peroxo-bridged dimer (**2**) upon warming to −80 °C, **1** is relatively stable at −100 °C under concentrations for UV spectral measurements ($\sim 10^{-5}$ M). On the other hand, an oxyiron(II) tetraphenylporphyrin (TPP) complex is not observable even at −100 °C, while a μ-peroxo-bridged (TPP)Fe^{III} dimer complex can be detected under similar conditions.[14] These results imply that the five-coordinated oxygenated form of chlorin is more stable than that of porphyrin derivatives.[26]

Characteristic Spectral Features for Oxoferryl Chlorin Complexes. The six-coordinated oxoferryl chlorin complex **3** is produced by adding *N*-MeIm to the μ-peroxo-bridged dimer **2** at −80 °C. On the other hand, the five-coordinated oxoferryl chlorin complex **4** is formed only upon laser illumination (RR measurements), and therefore other spectroscopic characterization of **4** could not be made. The absorption spectrum of **3** shows a largely red-shifted characteristic band (630 nm) in comparison with those of the oxygenated species (**1**, 618 nm). A similar red shift of the band was also observed for a peroxide-bound iron(III) tetramesitylchlorin (TMC) complex (632 nm).[27] Thus, the red shift of the absorption band relative to that of the oxygenated form would be characteristic for oxoferryl and peroxide-bound iron(III) chlorin complexes. In fact, the oxoferryl intermediate of cytochrome *d* exhibits a significantly red-shifted absorption at ~680 nm as compared with that of the oxygenated form (~645 nm).[28]

(26) On the contrary, iron chlorin-reconstituted myoglobins were reported to exhibit enhanced rates of autoxidation of oxy adducts at room temperature as compared with porphyrin-containing myoblobin: Takeda, M.; Aoyagi, K.; Aoyama, Y.; Ogoshi, H. *Chem. Lett.* **1983**, 1393–1396.

(27) Ozawa, S.; Watanabe, Y.; Morishima, I. *Inorg. Chem.*, in press.

(28) Reference 7c and references therein.

It is also quite important to inspect the proton NMR spectrum of **3**. The pyrrole proton resonance of **3** exhibits a small hyperfine shift (~4 ppm). Very similar small contact shifts of the pyrrole protons were also reported for oxoferryl porphyrin complexes.[10,11] The small contact shift of the ferryl species was proposed to be an indicator of the strong oxo π bonding.[11] Accordingly, the hyperfine shift of the pyrrole protons of **3** is consistent with the oxoferryl formulation. On the contrary, an anomalous behavior has been observed for the hyperfine shifts of the saturated pyrrole (pyrroline) ring protons of **3**. The signals of the pyrroline protons appear at 14.6 and −14.7 ppm as seen in Figures 5 and 6, suggestive of nonequivalency of the pyrroline ring protons in **3**. The unusually large splitting of the pyrroline proton resonances for **3** cannot be ascribed to the out of plane displacement of the iron atom but is presumed to result from a deformation of the pyrroline ring in **3**.[29]

RR spectral measurements for **2** and **3** have provided solid evidence for the formation of the five- and six-coordinated oxoferryl chlorin complexes **4** and **3**, although the five-coordinated oxoferryl chlorin complex **4** is a photoconverted product of **2**. The $\nu(Fe^{IV}{=}O)$ modes for **3** and **4** were observed at 817 and 846 cm^{-1}, respectively. The lower frequency of the six-coordinated species is owed to donation from the trans ligand which makes the Fe–O σ bond weaker. These frequencies are very close to those of the corresponding porphyrin derivatives,[12-14] and the magnitudes of the $^{16}O/^{18}O$ isotopic shifts for **3** and **4** ($\Delta\nu$ = 34 cm^{-1}) are nearly identical with those observed for oxoferryl porphyrins,[12-14] as noted in Table 3. On the contrary to these results, the oxoferryl intermediate of cytochrome d^9 showed RR spectral features distinct from those of porphyrin-containing heme proteins (vide infra).

Oxoferryl Intermediate of Cytochrome *d*. The formation of the oxoferryl intermediate of cytochrome *d* was reported in its reaction with an excess amount of hydrogen peroxide.[9] The same intermediate was also obtained by aerobic oxidation of the fully reduced enzyme.[9] RR spectra of the intermediate exhibited a band assignable to $\nu(Fe^{IV}{=}O)$ at 815 cm^{-1} (pH 7.5 at 5 °C),[9] which is higher than that observed for any oxoferryl species of heme enzymes, for example, cytochrome *c* oxidase (805 cm^{-1}, pH 7.2 at 5 °C)[30] and myoglobin (797 cm^{-1}, pH 8.6 at 20 °C).[31]

The $\nu(Fe^{IV}{=}O)$ frequencies of model oxoferryl porphyrin complexes show a large trans ligand effect with inverse depedency for the electron-donating ability of the axial ligand.[12] In the present results, in fact, the $\nu(Fe^{IV}{=}O)$ frequency for the five-coordinated oxoferryl chlorin (**4**, 846 cm^{-1}) is higher than that for the six-coordinated complex (**3**, 817 cm^{-1}). Thus, one factor contributing to the high ferryl frequency of cytochrome *d* complex was proposed to be a proximal ligand with lower electron-donating ability than ligands such as histidine.[9] However, recent RR studies[7c] have demonstrated that the stretching frequency of the oxygenated form of cytochrome *d* ($\nu(Fe^{II}{-}O_2)$ = 568 cm^{-1}) is remarkably close to those of hemoglobin (567 cm^{-1}, pH 7.4 at 10 °C,[32] and 572 cm^{-1}, pH 8.5 at 10 °C[33]), myoglobin (569 cm^{-1}, pH 6.8 at −15 °C),[34] and cytochrome *c* oxidase (569 cm^{-1}, pH 7.2 at 5 °C,[35] and 571 cm^{-1}, pH 7.4 at 10 °C[36]). These observations suggest that the oxy form of cytochrome *d* adopts a structure similar to that of these proteins and therefore the

(29) A similar large splitting of pyrroline deuterium resonances was observed in peroxide-bound iron(III) chlorin complexes.[27]

(30) Ogura, T.; Takahashi, S.; Shinzawa-Itoh, K.; Yoshikawa, S.; Kitagawa, T. *J. Biol. Chem.* **1990**, *265*, 14721–14723.

(31) Sitter, A. J.; Reczek, C. M.; Terner, J. *Biochim. Biophys. Acta* **1985**, *828*, 229–235.

(32) Brunner, H. *Naturwissenschaften* **1974**, *61*, 129.

(33) Nagai, K.; Kitagawa, T.; Morimoto, H. *J. Mol. Biol.* **1980**, *136*, 271–289.

(34) Van Wart, H. E.; Zimmer, J. *J. Biol. Chem.* **1985**, *260*, 8372–8377.

(35) Ogura, T.; Takahashi, S.; Shinzawa-Itoh, K.; Yoshikawa, S.; Kitagawa, T. *J. Am. Chem. Soc.* **1990**, *112*, 5630–5631 and references therein.

(36) Varotsis, C.; Woodruff, W. H.; Babcock, G. T. *J. Am. Chem. Soc.* **1989**, *111*, 6439–6440; **1990**, *112*, 1297.

proximal ligand of heme *d* might be similar to that of these proteins (histidine)[37] since the $\nu(Fe^{II}-O_2)$ frequencies are also to be affected by the trans ligand.[12b,34] Assuming that the coordination environment of the oxoferryl state is the same as that of the oxy state, the trans ligand effect may not be the major factor for the high frequency of the $\nu(Fe^{IV}=O)$ band in cytochrome *d*.

The dihydroxychlorin macrocycle of heme *d* was also proposed to be a possible factor for the high oxoferryl stretching frequency.[9] As reported here, however, the chlorin macrocycle of the oxoferryl complexes *does not* alter their $\nu(Fe^{IV}=O)$ frequencies compared with those of the corresponding porphyrin derivatives for the five- and six-coordinated complexes. Furthermore, the electron-withdrawing ability of the hydroxy groups bound to the pyrroline ring is not enough to decrease the basicity of the chlorin macrocycle beyond that of porphyrin one.[38] Thus, the unusually high stretching frequency observed for the oxoferryl form of cytochrome *d* should not be attributed to the chlorin macrocycle.

Hydrogen bonding to the oxoferryl oxygen causes a decrease in the $\nu(Fe^{IV}=O)$ frequency.[12b,39] For example, the $\nu(Fe^{IV}=O)$ of horseradish peroxidase increases in frequency by 13 cm^{-1} upon raising the pH to 11 (787 cm^{-1}) due to deprotonation of the distal histidine that serves as a hydrogen bond donor at pH 7 (774 cm^{-1}).[39] It is possible that the high $\nu(Fe^{IV}=O)$ frequency in cytochrome *d* is caused by a lack of hydrogen bonding to the oxoferryl oxygen from a distal amino acid group, as previously suggested by Kahlow et al.[9,40] However, the $\nu(Fe^{IV}=O)$ band of the oxoferryl intermediate of cytochrome *d* is reported to exhibit an $^{16}O/^{18}O$ isotopic frequency shift of 46 cm^{-1}, which is distinctly larger than those of other oxoferryl heme-containing enzymes[30,31,39] and that of an isolated Fe–O diatomic oscillator (36 cm^{-1}). It would mean that the reported band should not be assigned to $\nu(Fe^{IV}=O)$ if the observed isotopic shift were correct. Although Kahlow et al.[9] assumed that the iron of cytochrome *d* is so tightly bound to the macrocycle that the effective mass of the iron ion is infinite, it is quite unlikely since the restraining force against the out-of-plane displacement of the iron ion is generally extremely weak.[41] Furthermore, as mentioned above, the magnitude of the $^{16}O/^{18}O$ isotopic shift observed for the oxoferryl chlorin complexes is comparable to those of the corresponding porphyrin complexes.[12-14,23] We rather suggest that the reported oxygen isotope sensitive band would arise from the O–O stretching mode of the peroxo intermediate, if the observed value were correct. In fact, the presence of a hydroperoxy intermediate has been demonstrated for the bovine cytochrome oxidase as an intermediate following to the dioxygen complex.[42] Therefore, more careful and complete examination of RR spectra of the oxoferryl intermediate of cytochrome *d* is required.

In conclusion, the oxoferryl chlorin complexes as synthetic models of the oxoferryl intermediate of cytochrome *d* are successfully prepared by the reaction of the iron(II) chlorin with O_2. In addition, oxyiron(II) and μ-peroxo-bridged iron(III) chlorin complexes have been detected in the course of the reaction as intermediates. The oxoferryl chlorin complex exhibits NMR spectral characteristics similar to those of the oxoferryl porphyrin complexes except for the unusual hyperfine shifts of the pyrroline proton resonances of the chlorin complex. The five- and six-coordinated oxoferryl chlorin complexes exhibit $\nu(Fe^{IV}=O)$ frequencies and isotopic ($^{16}O/^{18}O$) frequency shifts nearly identical to those of the corresponding porphyrin complexes. Therefore, the unusually high frequency of $\nu(Fe^{IV}=O)$ and large isotopic shift ($\Delta\nu$) observed for the oxoferryl intermediate of cytochrome *d* should not be attributed to the chlorin macrocycle (heme *d*), contrary to the previous suggestion.

(37) It was suggested that the proximal ligand of heme *d* is not a histidine.[9]

(38) Ozawa, S.; Watanabe, Y.; Morishima, I. Submitted for publication.

(39) (a) Hashimoto, S.; Tatsuno, Y.; Kitagawa, T. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 2417–2421. (b) Sitter, A. J.; Reczek, C. M.; Terner, J. *J. Biol. Chem.* **1985**, *260*, 7515–7522.

(40) Apparently, the high ferryl stretching frequency in cytochrome *d* could not be rationalized by a lack of hydrogen bonding only. The $\nu(Fe^{IV}=O)$ frequency of cytochrome *d* is still larger than that of myoglobin, in which no hydrogen bonding of the ferryl moiety has been demonstrated.[31,39b]

(41) (a) Saito, M.; Kashiwagi, H. *J. Chem. Phys.* **1985**, *82*, 848–855. (b) Yamamoto, S.; Teraoka, J.; Kashiwagi, H. *J. Chem. Phys.* **1988**, *88*, 303–312.

(42) Ogura, T.; Takahashi, S.; Hirota, S.; Shinzawa-Itoh, K.; Yoshikawa, S.; Appelman, E. H.; Kitagawa, T. *J. Am. Chem. Soc.* **1993**, *115*, 8527–8536.