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Electronic structure and reactivity of high-valent oxo iron porphyrins

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Contents

Abstract

High valent oxo iron porphyrins have been prepared and characterized as models for compounds-I and compounds-II in heme enzymes. In this review, we survey studies of high valent oxo iron porphyrin complexes. Spectroscopic properties and reactivities of oxo iron(IV) porphyrin π-cation radical complexes are summarized. Electron-withdrawing effects of *meso*-substituents and pyrrole β-substituents on the electronic structure of oxo iron(IV) porphyrin π-cation radicals are discussed. The effect of the axial ligand is also reviewed. Isoelectronic forms of oxo iron(IV) porphyrin π-cation radical are reviewed. We have summarized the synthesis and characterization of oxo iron(IV) porphyrins as models for compounds-II.

Keywords: Cytochrome P450; Peroxidase; Catalase; Heme; Porphyrin

1. Introduction

High-valent oxo iron porphyrins participate in the biological cycles of several different kinds of heme enzymes [1–5]. For example, peroxidase and catalase, when activated by peroxides or peracids, form an oxo iron(IV) porphyrin π-cation radical intermediate called compound-I [1,4,5]. Compound-I is also believed to be an active intermediate in the reactions of oxygenases, such as cytochrome P450 [2,3]. In spite of the common active intermediates, the reactivity of compound-I differs from enzyme to enzyme. In cytochrome P450 (P450), the compound-I species transfers a single oxygen atom directly to a variety of substrates [2,3], while with peroxidase and catalase, the compound-I species oxidize organic compounds (phenols and amines) and hydrogen peroxide, respectively [4]. An oxo iron(IV) porphyrin intermediate named compound-II has also been characterized in the reaction cycles of peroxidases [4]. Compound-II is produced when compound-I is reduced by a substrate in a one-electron transfer. Compound-II also oxidizes various kinds of substrates to yield a resting state. These diverse functions of compound-I and compound-II have been thought to de-

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pend on heme environmental structures, such as porphyrin peripheral structures, the heme proximal ligand structures, and protein structures in the immediate vicinity of the heme.

Because of its biological significance, the electronic structure and the reactivity of compound-I and compound-II have been studied by a number of groups. However, detailed characterization and mechanistic studies are difficult to perform because of its intrinsic reactivity and lability. As a result, considerable interest has been directed toward model complexes of compound-I and compound-II. The formation of an oxo iron(IV) porphyrin π-cation radical complex was first reported by Groves et al. in 1981, by the oxidation of iron(III) *meso*-tetramesitylporphyrin (TMP) with *m*-chloroperbenzoic acid (*m*-CPBA) at −78 °C [6]. On the other hand, oxo iron(IV) porphyrin complexes have been synthesized by oxygenation of iron(II) porphyrin at low temperature [7,8]. After these reports, the model complexes of compound-I and compound-II have been prepared by many groups from various porphyrins and by various methods, and the electronic structure and reactivity of these model complexes have been studied.

In this review article, the formation and characterization of high-valent oxo iron porphyrins in synthetic model systems are summarized. Especially, effects of *meso*-substitution, pyrrole β-substitution, and axial ligand on the electronic structure and magnetic properties of oxo iron(IV) porphyrin π-cation radicals are discussed in detail.

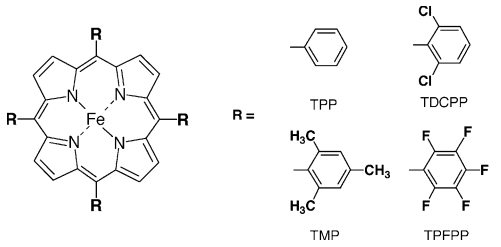

Fig. 1. The structures of *meso*-tetraarylporphyrins employed for synthesis of high valent oxo iron porphyrin complexes.

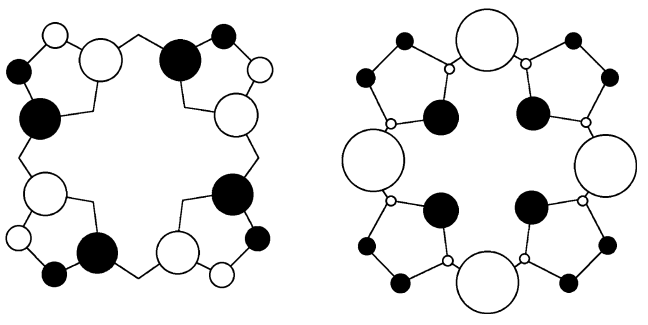

Fig. 2. Electron spin distribution of porphyrin atomic orbitals with a_{1u} (left) and a_{2u} (right) symmetries. Black and white circles represent signs of the upper lobe of the π-AOs.

2. High valent oxo iron porphyrin complexes as models for compounds-I of heme enzymes

A high-valent oxo iron(IV) porphyrin complex was first characterized by Groves et al. [6]. They observed the formation of a green compound by the oxidation of chloro iron(III) TMP (see Fig. 1) with *m*-CPBA in dichloromethane–methanol mixture at −78 °C. The green compound is characterized as an oxo iron(IV) TMP π-cation radical complex, $O{=}Fe^{IV}[TMP]^{+\bullet}$, like compounds-I of peroxidases and catalases. They also reported the formation of a red compound which was assigned as an oxo iron(IV) TMP complex, $O{=}Fe^{IV}[TMP]$, like compounds-II of peroxidase, by the oxidation of the iron(III) TMP complex with iodosobenzene in basic media. Later, the green complex was prepared by one-electron oxidation of the red compound [7]. Instead of *m*-CPBA, ozone can also be used to prepare the oxo iron(IV) porphyrin π-cation radical complex from iron(III) porphyrin [8,9]. Recently, $O{=}Fe^{IV}[TMP]^{+\bullet}$ was formed from the reaction of the iron(III) TMP complex with dimethyldioxirane [10].

$O{=}Fe^{IV}[TMP]^{+\bullet}$ has been characterized by various spectroscopic methods such as UV–visible, NMR, EPR, Mössbauer, EXAFS, and resonance Raman spectroscopies. The absorption spectrum of $O{=}Fe^{IV}[TMP]^{+\bullet}$ is close to that of compound-I of catalase (CAT); a broad and weak Soret band at 405 nm and a new absorption around 660 nm [11]. The ^{1}H-NMR spectrum of $O{=}Fe^{IV}[TMP]^{+\bullet}$ shows an upfield shift of pyrrole protons and downfield shifts of *meso*-mesityl protons in dichloromethane–methanol at −80 °C; pyrrole (−27 ppm), *o*-methyl (26 ppm), *m*-H (68 ppm), *p*-methyl (11 ppm) [6]. While two types (a_{1u} and a_{2u}) of porphyrin π-cation radical states have been characterized from EPR and theoretical studies of metalloporphyrin π-cation radical complexes (Fig. 2), these ^{1}H-NMR shifts indicate an a_{2u} radical state, an unpaired electron in an a_{2u} porphyrin orbital, for $O{=}Fe^{IV}[TMP]^{+\bullet}$. The Mössbauer study of $O{=}Fe^{IV}[TMP]^{+\bullet}$ indicates the $S = 3/2$ ground state; ferromagnetic coupling between ferryl iron spins ($S = 1$) and TMP π-cation radical spin ($S = 1/2$) [12]. The ferromagnetic interaction is further supported by the EPR spectrum of $O{=}Fe^{IV}[TMP]^{+\bullet}$. The EPR signals at $g = 4.3$, 3.9, and 1.99 at 4 K suggest strong ferromagnetic coupling between ferryl iron spins ($S = 1$) and the porphyrin π-cation radical spin ($S = 1/2$) at $J > +40$ cm^{-1} [13]. The EXAFS of $O{=}Fe^{IV}[TMP]^{+\bullet}$ shows the Fe=O distance to be 1.6 Å, identical to that of compound-I and compound-II of horseradish peroxidase (HRP) [14]. The resonance Raman bands for ν(Fe=O) of $O{=}Fe^{IV}[TMP]^{+\bullet}$ are observed at 828 and 792 cm^{-1} for ^{16}O- and ^{18}O-derivatives, respectively [15]. In addition, a ^{54}Fe substituted sample gives the resonance Raman band at 832 cm^{-1}. These isotope shifts are

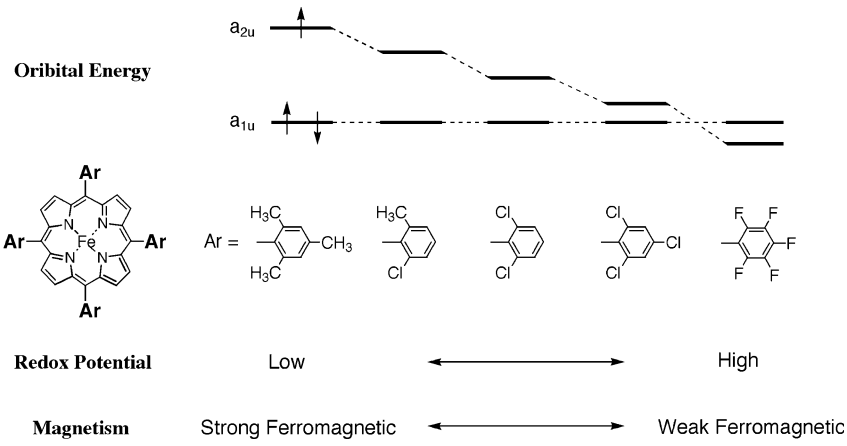

Fig. 3. Electron-withdrawing effect of the *meso*-substituent on the electronic structure of oxo iron(IV) porphyrin π-cation radical.

consistent with theoretical isotope frequency shifts expected for an Fe=O oscillator. The ν(Fe=O) band of O=Fe^{IV}[TMP]$^{+\bullet}$ is sensitive to the absence and presence of methanol [16]. The ν(Fe=O) band of O=Fe^{IV}[TMP]$^{+\bullet}$ is observed at 801 cm^{-1} in dichloromethane [17]. In the presence of methanol, methanol seems to bind O=Fe^{IV}[TMP]$^{+\bullet}$ as an axial ligand while, in the absence of methanol, a chloride counter anion coordinates at the axial position.

2.1. Effect of porphyrin structure on the electronic structure and reactivity of oxo iron(IV) porphyrin π-cation radical complex

A porphyrin *meso*-substituent changes the reactivity and electronic state of the oxo iron(IV) porphyrin π-cation radical complex. Iron porphyrins with electron-withdrawing *meso*-substituents, such as *meso*-tetra-2,6-dichlorophenylporphyrin (TDCPP) and *meso*-tetra-pentafluorophenylporphyrin (TPFPP) (see Fig. 1), are shown to be efficient catalysts of oxygen atom transfer reactions from iodosobenzene and hydrogen peroxide [18]. The oxo iron(IV) porphyrin π-cation radical complex of TDCPP, O=Fe^{IV}[TDCPP]$^{+\bullet}$, was first prepared by the oxidation of perchlorate iron(III) TDCPP complex, Fe^{III}[TDCPP](ClO$_4$), with ozone in acetonitrile at −35 °C [8].

The electron-withdrawing effect of *meso*-substituent on the electronic state and reactivity of oxo iron(IV) porphyrin π-cation radical has been studied by using mesityl, 2-chloro-6-methylphenyl, 2,6-dichlorophenyl, and 2,4,6-trichlorophenyl substituents (Fig. 3) [19]. The electron-withdrawing effect of the *meso*-substituent is increased by replacing the methyl group of mesityl group to chlorine one by one. While the overall absorption spectral features of these oxo iron(IV) porphyrin π-cation radicals are unchanged, a blue shift of the Soret bands and red shift of the peaks around 650 nm are observed with an increase in the electron-withdrawing effect. As observed for O=Fe^{IV}[TMP]$^{+\bullet}$, the EPR spectra of these oxo iron(IV) porphyrin π-cation radicals exhibit the $S = 3/2$ EPR signals at 4 K, suggesting ferromagnetic ground states of these π-cation radical complexes [20]. The magnetic interaction between ferryl iron and porphyrin π-cation radical spins is not drastically changed with an increase in the electron-withdrawing effect of *meso*-substituent. However, the EPR signals indicate a decrease in E/D value of the oxo iron(IV) porphyrin π-radical complex with an increase in the electron-withdrawing effect of *meso*-substituent (Fig. 4).

The ^{1}H-NMR spectra of these oxo iron(IV) porphyrin π-cation radicals are drastically changed by the

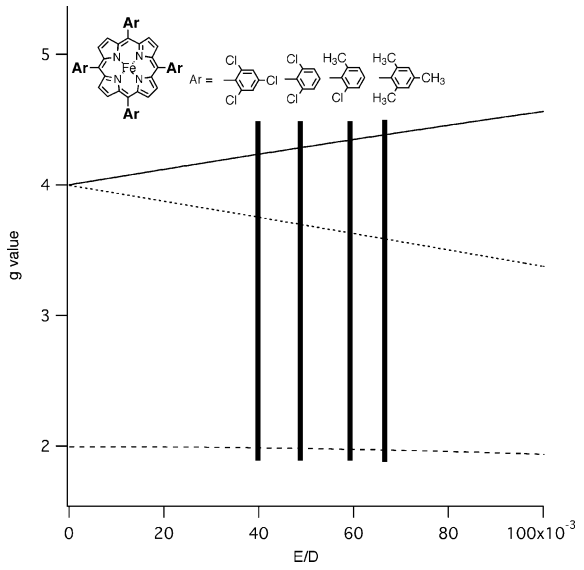

Fig. 4. The g values for $S = 3/2$ paramagnet as a function of E/D. The solid line represents the y component, the dotted line represents the x component, and the broken line represents the z component. The vertical lines in the plot show the g values and E/D values for oxo iron(IV) porphyrin π-cation radical complexes with different electron-withdrawing *meso*-substituents.

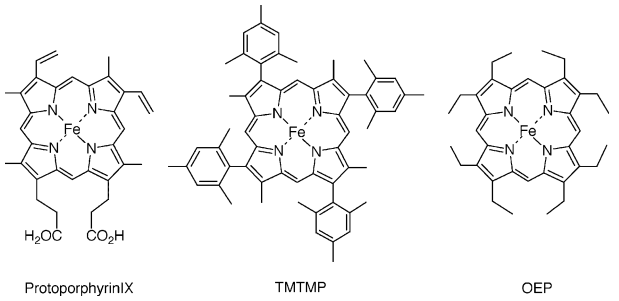

Fig. 5. The structures of pyrrole β-substituted porphyrins employed for synthesis of high valent oxo iron porphyrin complexes.

meso-substituents. With an increase in electronegativity of *meso*-substituent; 2,4,6-trichlorophenyl > 2,6-dichlorophenyl > 2-methyl-6-chlorophenyl > mesityl, the pyrrole proton signals shift upfield and the meta phenyl proton signals decrease their paramagnetic shift [19]. These NMR spectral changes can be interpreted by mixing of the a_{1u} radical state into the original a_{2u} radical state via a vibronic coupling. The ^{1}H-NMR shift can be related to the π-spin density at the carbon to which the proton is attached. Hence, a typical a_{2u} radical state should give a small upfield shift of the pyrrole β-proton and large downfield shifts of *meso*-mesityl protons (Fig. 2) [21,22]. On the other hand, generation of a typical a_{1u} radical state can be expected to produce a large upfield shift of the pyrrole β-proton and small shifts of the *meso*-mesityl protons (Fig. 2) [21,22]. The ^{1}H-NMR spectrum of O=Fe^{IV}[TMP]$^{+\bullet}$ clearly indicates the a_{2u} radical state. With an increase in an electron-withdrawing effect of *meso*-substituent, the energy of the a_{2u} state would be stabilized relative to the a_{1u} state via the interaction of the aryl and porphyrin π-orbitals because of the large spin density at the meso position in the a_{2u} orbital but a node in the a_{1u} orbital (see Figs. 2 and 3). Therefore, the energy separation between the a_{1u} and a_{2u} states would be decreased and stronger mixing of the a_{1u} radical state into the a_{2u} state would be expected with an increase in the electron-withdrawing effect of *meso*-substituent, as shown in Fig. 3. After all, with an increase in the mixing of the a_{1u} radical state, the pyrrole proton and the meta proton would shift upfield and downfield, respectively, as observed.

When the *meso*-substituent is much more electronegative, such as pentafluorophenyl, the orbital energy of the a_{2u} orbital would be lower than that of the a_{1u} orbital, leading the a_{1u} porphyrin π-cation radical state (see Fig. 3). In fact, the pyrrole proton signal of oxo iron(IV) porphyrin π-cation radical complex of TPFPP, O=Fe^{IV}[TPFPP]$^{+\bullet}$, shows a large upfield shift at −96 ppm, which is consistent with the ^{1}H-NMR shift expected from the a_{1u} radical state [23]. By changing the porphyrin radical state, magnetic interaction between ferryl iron and porphyrin radical spins is also altered (see Fig. 3). The EPR spectrum of O=Fe^{IV}[TPFPP]$^{+\bullet}$ exhibits a broad signal around $g = 2$ at 4 K [23], suggesting very weak magnetic interaction.

Most heme enzymes contain iron protoporphyrinIX, or its modifications, as prothestic groups (Fig. 5). These porphyrins contain substituents at the pyrrole β-position, but not at the meso position. Thus, the oxo iron(IV) porphyrin π-cation radical complex of the pyrrole β-substituted porphyrin would be more desirable than that of the *meso*-substituted porphyrin as spectroscopic models for biological heme enzymes because of similarity in the porphyrin structure to that of naturally occurring compounds. However, as discussed above, all compound-I model complexes were prepared from *meso*-substituted porphyrin derivatives. The oxo iron(IV) porphyrin π-cation radical complex of pyrrole β-substituted porphyrin was first characterized by using 2,7,12,17 - tetramethyl - 3,8,13,18 - tetramesityporphyrin (TMTMP) (see Fig. 5) [11,19]. By changing from the *meso*-substituted porphyrin to pyrrole β-substituted porphyrin, the electronic state and magnetic property of oxo iron(IV) porphyrin π-cation radical are drastically altered. Furthermore, oxo iron(IV) porphyrin π-cation radical of TMTMP, O=Fe^{IV}[TMTMP]$^{+\bullet}$, can mimic the electronic states of the compounds-I of peroxidases and catalases. The absorption spectrum of O=Fe^{IV}[TMTMP]$^{+\bullet}$ is similar to that of the compound-I of HRP. The ^{1}H-NMR spectrum of O=Fe^{IV}[TMTMP]$^{+\bullet}$, which shows large downfield shift of heme methyl proton, is also close to that of the compound-I of HRP [24]. Although the a_{2u} radical state has been assigned for O=Fe^{IV}[TMP]$^{+\bullet}$, the small paramagnetic shift of the meso proton signal is consistent with the a_{1u} porphyrin π-cation radical state for O=Fe^{IV}[TMTMP]$^{+\bullet}$ [11,19]. The EPR spectrum of O=Fe^{IV}[TMTMP]$^{+\bullet}$ shows signals at $g = 3.6$ and 2.0, which is close to that of compounds I of *Micrococcus luteus* catalase and ascorbate peroxidase [20,25]. The EPR spectrum of O=Fe^{IV}[TMTMP]$^{+\bullet}$ indicates a weak ferromagnetic interaction between ferryl iron ($S = 1$) and porphyrin π-cation radical ($S = 1/2$) spins [20]. This is in contrast to the strong ferromagnetic coupling for O=Fe^{IV}[TMP]$^{+\bullet}$. The bond strength of Fe=O bond of oxo iron(IV) porphyrin π-cation radical is insensitive to the a_{1u}–a_{2u} radical type. The Raman spectrum of O=Fe^{IV}[TMTMP]$^{+\bullet}$(ClO_4) with the a_{1u} radical state shows the ν(Fe=O) band at 801 cm^{-1}, which is the same position as that of O=Fe^{IV}[TMP]$^{+\bullet}$(ClO_4) with the a_{2u} radical state [26].

The electron-withdrawing effect of the pyrrole β-substituent has also been studied by introducing a series of pyrrole β-substituents: mesityl, 2-chloro-6-methylphenyl, 2,6-dichlorophenyl, and 2,4,6-trichlorophenyl substituents (Fig. 6) [19]. As observed for the meso substituted porphyrins, a blue shift of the

Soret band and red shift of the peaks around 630 nm are observed with an increase in the electron-withdrawing effect of the pyrrole β-substituents. ^{1}H-NMR spectra of oxo iron(IV) porphyrin π-cation radicals of the pyrrole β-substituted porphyrins are unchanged with an increase in the electron-withdrawing effect, indicating the a_{1u} radical states. Although the electron-withdrawing effect of the meso substituent alters the a_{2u} radical state to the a_{1u} radical state, that of the pyrrole β-substituent does not change the a_{1u} radical state of oxo iron(IV) porphyrin π-cation radical. This can be also explained by the spin distributions of the a_{1u} and a_{2u} orbitals, as discussed in the above paragraph. Since the a_{1u} and a_{2u} orbitals have some spin density at the pyrrole β-position (see Fig. 2), the a_{1u} and a_{2u} orbitals are stabilized to the same extent by the electron-withdrawing substituent, as shown in Fig. 6. Thus, the pyrrole β-substituent does not change the energy separation between the a_{1u} and a_{2u} states, leading to the same electronic state. Furthermore, these results imply to us that an oxo iron(IV) porphyrin π-cation radical of protoporphyrinIX (compounds-I) in heme enzymes would be the a_{1u} porphyrin π-cation radical state. The EPR spectra of oxo iron(IV) porphyrin π-cation radical complexes of the pyrrole β-substituted porphyrins are also insensitive to the electron-withdrawing effect. As observed for $O{=}Fe^{IV}[TMTMP]^{+\bullet}$, the EPR spectra of these complexes show signals at $g = 3.6$ and 2.0 [20], indicating a weak ferromagnetic interaction between ferryl iron and porphyrin π-cation radical spins. The weak ferromagnetic interaction of the pyrrole β-substituted porphyrins is in contrast to the strong ferromagnetic interaction of the *meso*-substituted porphyrins. The drastic change of the spin coupling can be related to the porphyrin π-cation radical state, which is discussed in the next section.

The magnetic properties of the compound-I model complexes, introduced in the above paragraphs, would be explained by their porphyrin π-cation radical states. The EPR spectra of oxo iron(IV) porphyrin π-cation radical complexes indicate that the ferromagnetic coupling between the ferryl iron and porphyrin radical spins is strong for the a_{2u} radical state and weak for the a_{1u} radical state. The same change in the magnetic interaction with going from the a_{2u} radical to the a_{1u} radical is also observed in copper(II) porphyrin π-cation radical complexes [27]. As indicated by Reed et al. [28], a net overlap between the d_{xz} and d_{yz} orbitals (e_g) of ferryl iron and π-orbital (a_{1u} and a_{2u}) of the porphyrin ring would not be allowed even if symmetry of the porphyrin ring is reduced from 4- to 2-fold symmetry. Therefore, ferromagnetic coupling is expected for both a_{1u} and a_{2u} radical complexes. To have an antiferromagnetic coupling, as observed for the compound-I of chloroperoxidase (CPO) [29], the distortion of the porphyrin ring must include a further lowering in symmetry, for example, a buckling of the ring. The fact that the spin coupling of the a_{2u} radical complex is stronger than that of the a_{1u} radical complex is explained by the spin densities of pyrrole nitrogen atoms in the a_{1u} and a_{2u} porphyrin radicals (see Fig. 2). In the a_{2u} radical, a large spin density exists at the pyrrole nitrogen atom, which allows large orbital overlap with ferryl d_{xz} and d_{yz} orbitals. This results in the strong ferromagnetic interaction. On the other hand, a magnetic interaction of the a_{1u} radical state would be weak because the spin density at the pyrrole nitrogen atom is a node. Furthermore, flexible porphyrin plane of pyrrole β-substituted porphyrin (the a_{1u} radical complex) shifts the magnetic interaction to an antiferromagnetic. For these reasons, the magnetic coupling of the a_{1u} radical complex would be small. The magnetic coupling of oxo iron(IV) porphyrin π-cation radical complexes and compounds-I, determined from the EPR spectra, are summarized in Fig. 7.

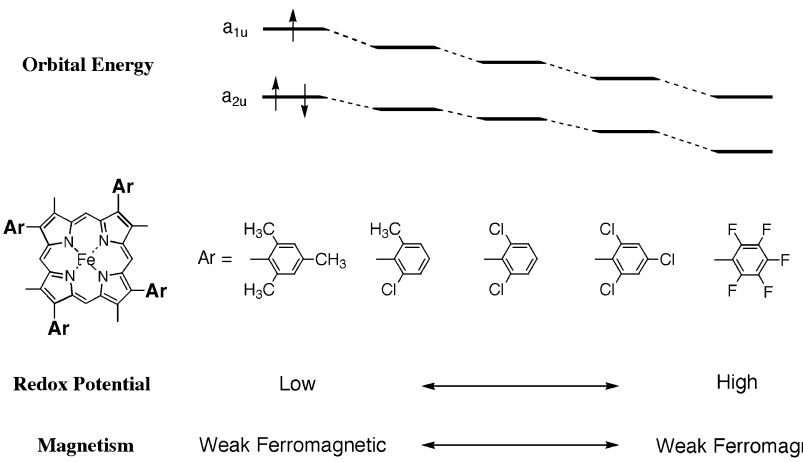

Fig. 6. Electron-withdrawing effect of the pyrrole β-substituent on the electronic structure of oxo iron(IV) porphyrin π-cation radical.

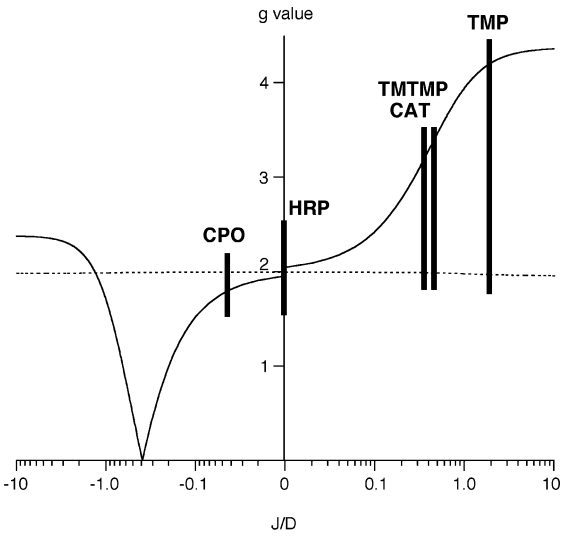

Fig. 7. The effect g values obtained from the spin coupling system of $S = 1$ and $S = 1/2$ as a function of J/D [20]. The solid line represents the g perpendicular component and the dotted line represents the g parallel component. The positive sign of J/D indicates ferromagnetic ground state and the negative sign of J/D means antiferromagnetic ground state. The vertical lines in the plot show the g values and J/D values for oxo iron(IV) porphyrin π-cation radical complexes and compounds-I of heme enzymes.

Successful formation of oxo iron(IV) porphyrin π-cation radical complexes with different porphyrin radical states or with different redox potentials allows us to study the relationship between the electronic state and reactivity of oxo iron(IV) porphyrin π-cation radical complexes [19]. The competitive epoxidation reaction of cyclohexene indicates that O=Fe^{IV}[TMP]$^{+\bullet}$ is almost as reactive as O=Fe^{IV}[TMTMP]$^{+\bullet}$. Since O=Fe^{IV}[TMP]$^{+\bullet}$ and O=Fe^{IV}[TMTMP]$^{+\bullet}$ have the a_{2u} and a_{1u} radical states, respectively, the a_{1u} and a_{2u} porphyrin radical states do not affect the epoxidation activity of cyclohexene. On the other hand, oxo iron(IV) porphyrin π-cation radical complexes with electron-withdrawing substituent are more reactive than those with electron-releasing substituents. All of these results indicate that the reactivity of the oxygen atom of an oxo iron(IV) porphyrin π-cation radical depends on the redox potential of the complex, regardless of the a_{1u} and a_{2u} radical states.

2.2. Effect of axial ligand on the electronic structure and reactivity of oxo iron(IV) porphyrin π-cation radical complex

Cytochromes P450 are known to have a cysteine thiolate as the axial ligand, whereas peroxidases and catalases, in general, contain a histidine imidazole and a tyrosine phenolate, respectively (Fig. 8). The nature of the axial ligand present in the heme enzymes has been reported to be essential to the actual type of chemistry catalyzed. Thus, the effect of an axial ligand on the electronic structure of oxo iron(IV) porphyrin π-cation radical has been studied in connection to various axial ligands in heme enzymes. A pronounced axial ligand effect was reported on styrene epoxidation by O=Fe^{IV}[TMP]$^{+\bullet}$ [30]. While O=Fe^{IV}[TMP]$^{+\bullet}$(F) rapidly reacted with styrene to form styrene oxide, O=Fe^{IV}[TMP]$^{+\bullet}$(Cl), O=Fe^{IV}[TMP]$^{+\bullet}$(CH_3OH), and O=Fe^{IV}[TMP]$^{+\bullet}$(CH_3CO_2) were less reactive than O=Fe^{IV}[TMP]$^{+\bullet}$(F). O=Fe^{IV}[TMP]$^{+\bullet}$(ClO_4) did not oxidize styrene to styrene oxide. There was no obvious correlation between the rate constant of the epoxidation reaction and ^{1}H-NMR and EPR spectroscopic features of these complexes. However, the resonance Raman ν(Fe=O) band was sensitive to the nature of the trans axial ligand; near 835 cm^{-1} for the perchlorate and triflate complexes and near 801 cm^{-1} for the

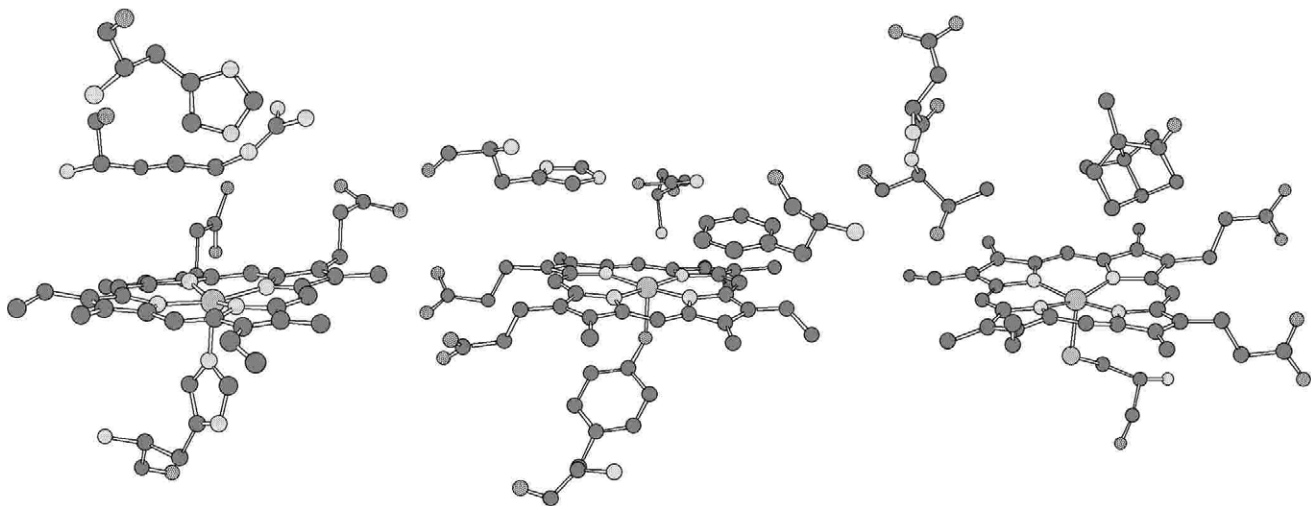

Fig. 8. Active site structures of peroxidase (left; from PDB accession code 1CCP), catalase (middle; from PDB accession code 1DGF), and cytochrome P450 (right; from PDB accession code 5CP4).

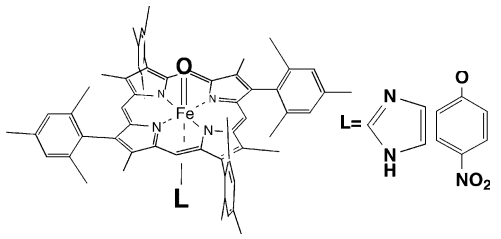

Fig. 9. Synthetic model complexes of compounds-I of peroxidase and catalase.

m-chlorobenzoate, fluoride and chloride complexes [31]. These studies explored the effect of non-biomimetic axial ligands, such as alcohol, halides, and several weakly coordinating anions. Recently, oxo iron(IV) TMTMP π-cation radical complexes bearing a biomimetic axial ligand such as imidazole or *p*-nitrophenol were prepared and characterized by absorption and ^{1}H-NMR spectroscopies (Fig. 9) [9]. These are legitimate model complexes for compounds-I of peroxidases and catalases. Successful formation of the imidazole and *p*-nitrophenolate complexes result from a weak ligand field of perchlorate anion and use of the clean oxidant ozone. O=Fe^{IV}[TMTMP]$^{+\bullet}$(ImH) shows absorption peaks at 390 and 640 nm and the spectrum is similar to that of compound-I of HRP. O=Fe^{IV}[TMTMP]$^{+\bullet}$(*p*-NO$_2$-PhO) exhibits absorption peaks at 392 and 655 nm and the spectrum is close to that of compound-I of CAT. The absorption peak around 650 nm shows a red shift with increase in the push effect from the axial ligand. This explains a difference in the absorption spectra of compounds-I of HRP, CAT and CPO; the push effect of the axial ligand would be increased in the order of imidazolate (HRP) < phenolate (CAT) < thiolate (CPO). ^{1}H-NMR spectrum of O=Fe^{IV}[TMTMP]$^{+\bullet}$(ImH) also exhibits a large paramagnetic shift of pyrrole β-methyl proton similar to that of compound-I of HRP. From the ^{1}H-NMR shift, O=Fe^{IV}[TMTMP]$^{+\bullet}$(ImH) is assigned as the a_{1u} radical state. The porphyrin radical state of oxo iron(IV) porphyrin π-cation radical is not changed by coordination of imidazole as an axial ligand. This is also true of the *meso*-substituted porphyrin complex; O=Fe^{IV}[TMP]$^{+\bullet}$(ImH) is the a_{2u} radical state. The resonance Raman spectrum of O=Fe^{IV}[TMP]$^{+\bullet}$(ImH) shows ν(Fe=O) vibration at 810 cm^{-1}, which is close to that (814 cm^{-1}) of imidazole complex of oxo iron(IV) porphyrin, O=Fe^{IV}[TMP](ImH) [32]. The Raman band shows only a 4 cm^{-1} downshift with formation of porphyrin π-cation radical. Although it has been thought that the Fe=O bond would weaken with formation of the porphyrin π-cation radical because compound-I is much more reactive than compound-II, the result demonstrates that the Fe(IV)=O bond strength is insensitive to the porphyrin π-cation radical formation [33,34].

The preparation of high valent iron porphyrin bearing thiolate ligand as a model for cytochrome P-450 has been hampered by the high reactivity of thiolate ligand with air and oxidant. Recently, air-stable iron(III) porphyrin complexes carrying a thiolate ligand have been synthesized by connecting the thiolate ligands with porphyrin substituent through a covalent bond and/or by surrounding the thiolate ligand with pivaroyl groups [35–37]. Because of the strong push-effect from the trans axial thiolate ligand, the oxygenation activities of these thiolate complexes are much higher than those of non-thiolate complexes. More recently, an intermediate bearing absorption peak at 388 nm is characterized and tentatively assigned as oxo iron(IV) porphyrin π-cation radical of the thiolate axial ligand complex [38].

3. Isoelectronic forms of oxo iron(IV) porphyrin π-cation radical

Oxo iron(V) porphyrin, iron(III) porphyrin *N*-oxide, and iron(III) porphyrin dications are known as isoelectronic forms of oxo iron(IV) porphyrin π-cation radical and proposed as reactive intermediates of some heme enzymes (Fig. 10).

Oxo iron(V) porphyrin was prepared by introducing methanol into O=Fe^{IV}[TDCPP]$^{+\bullet}$ as an axial ligand at −90 °C [39,40]. The iron(V) oxidation state would be realized by introducing a strong electron-withdrawing substituent on the porphyrin ring and by the coordination of methoxide. Interestingly, the absorption spectrum of O=Fe^{V}[TDCPP] is similar to that of iron(IV) TDCPP bis-methoxide complex, Fe^{IV}[TDCPP](CH$_3$O)$_2$. The two-electron higher state of the complex is confirmed by iodometric titration. Oxo iron(V) porphyrin is reactive toward olefin. At −90 °C, O=Fe^{V}TDCPP oxidizes norborene to norborene oxide at 50% yield. Although these results imply the formation of oxo iron(V) porphyrin state, the spectral similarity of O=Fe^{V}[TDCPP] to that of Fe^{IV}[TDCPP](CH$_3$O)$_2$ suggests that further study including Mössbauer spectroscopy, will be needed to better define oxo iron(V) porphyrin structure.

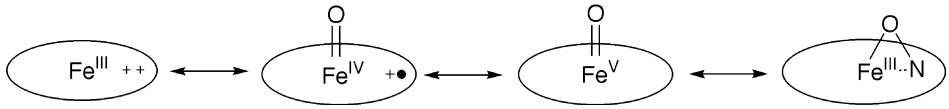

Fig. 10. Isoelectronic forms of oxo iron(IV) porphyrin π-cation radical.

Iron(III) porphyrin *N*-oxide is also prepared from the reaction of iron(III) TMP complex with *m*-CPBA in toluene at 0 °C [41]. The absorption spectrum of Fe^{III}[TMP] *N*-oxide shows a dramatically red-shifted Soret peak at 441 nm. The EPR spectrum of Fe^{III}[TMP] *N*-oxide exhibits strong signals around $g = 4.3$, resulting from a rhombically distorted ferric high-spin state. The EPR spectrum indicates that Fe^{III}[TMP] *N*-oxide porphyrin plane is highly distorted by the *N*-oxide bridge. Fe^{III}[TMP] *N*-oxide does not have monooxygenase activity. The Fe–O stretching mode of Fe^{III}[TMP] *N*-oxide is observed at 506 cm^{-1}, which is close to that of ferric hydroxide complexes (490–495 cm^{-1}) [42]. The Fe–O bond of Fe^{III}[TMP] *N*-oxide is much weaker than that of $O{=}Fe^{IV}[TMP]^{+\bullet}$.

Iron(III) porphyrin dication complexes are prepared by two electron-oxidation of chloro iron(III) porphyrins [21]. Iron(III) porphyrin dication is also prepared by the reaction of Fe^{III}[TMP] *N*-oxide with trifluoroacetic acid in toluene at low temperature [43]. The complex has been prepared and characterized by absorption, ^{1}H-NMR, EPR spectroscopies and assigned as ferric high-spin with rhombic symmetry.

4. Oxo iron(IV) porphyrins as models for compound-II

Oxo iron(IV) porphyrin is known as compound-II in the catalytic cycle of peroxidase. Oxo iron(IV) porphyrin complexes were first prepared by oxygenation of iron(II) porphyrin [44,45]. Through the work on the autoxidation of iron(II) porphyrin complexes, Balch et al. showed the transient formation of μ-peroxo bis iron(III) porphyrin, Fe(III)–O–O–Fe(III), species [46]. More importantly, they found that the introduction of *N*-methylimidazole (Me–Im) to the Fe(III)–O–O–Fe(III) complex afforded oxo iron(IV) porphyrin complex, $O{=}Fe^{IV}$(Por)Me–Im [44,45]. $O{=}Fe^{IV}$(Por)-Me–Im is an unstable intermediate that has been characterized spectroscopically in solution over the temperature range -90 to -30 °C. The ^{1}H-NMR spectrum of $O{=}Fe^{IV}$(Por)Me–Im does not show large paramagnetic shifts of heme peripheral protons and resembles that of compound-II of HRP [44,45]. $O{=}Fe^{IV}$(Por)-Me–Im is also EPR silent like compound-II of HRP. $O{=}Fe^{IV}$(Por)Me–Im is able to oxidize triphenylphosphine even at -80 °C in toluene over a period of several hours to give triphenylphosphine oxide quantitatively [44,45].

Later, oxo iron(IV) porphyrin complex is synthesized by the one-electrochemical oxidation of Fe^{III}[TMP]-(OH) [46,47]. Although the first electrochemical oxidation of chloro iron(III) porphyrins generally affords porphyrin centered π-cation radicals, the same oxidation of Fe^{III}[TMP](OH) gives $O{=}Fe^{IV}$[TMP]. The $E_{1/2}$ values of eight Fe^{III}(Por)(OH) were plotted against the $E_{1/2}$ values of the corresponding Fe^{III}(Por)(Cl), indicating that the first oxidation of Fe^{III}(Por)(OH) proceeds through $Fe^{III}(Por)^{+\bullet}(OH)$ to afford $O{=}Fe^{IV}$(Por). Very recently, $O{=}Fe^{IV}$[TMP] was prepared from Fe^{III}[TMP]-$(ClO_4)_2$ by passing through basic alumina [48]. Though O=Fe(IV) porphyrins are believed to be a poor oxidant for olefin oxidation, they found the epoxidation of substituted styrenes by isolated $O{=}Fe^{IV}$[TMP]. These mechanistic features are quite different from the results by $O{=}Fe^{IV}$[TMP].

As observed for oxo iron(IV) porphyrin π-cation radicals, the resonance Raman ν(Fe=O) band of an oxo iron(IV) porphyrin is sensitive to the axial ligand. The ν(Fe=O) Raman bands of oxo iron(IV) porphyrins are observed around 850 cm^{-1} for five-coordinated forms (no axial ligand) and around 820 cm^{-1} for six-coordinated forms; 818 cm^{-1} (1-Me–Im), 828 cm^{-1} (DMF), 841 cm^{-1} (THF) [49].

Oxo iron(IV) porphyrin complexes are converted quantitatively to the dimethoxide complex of iron(IV) porphyrin, Fe^{IV}[Por]$(CH_3O)_2$, by treatment with sodium methoxide [50]. Thus, Fe^{IV}[Por]$(CH_3O)_2$ may be formed in $O{=}Fe^{IV}$[Por] solution when methanol is present. Since the absorption spectrum of Fe^{IV}[TMP]-$(CH_3O)_2$ is similar to that of $O{=}Fe^{IV}$[TMP], it is difficult to distinguish by the absorption spectrum. Fe^{IV}(TMP)$(CH_3O)_2$ and $O{=}Fe^{IV}$[TMP] can be distinguished by their ^{1}H-NMR, in which the former shows a large upfield shift of pyrrole proton signal but the latter does not.

5. Summary and biological relevance

These are the main results obtained from the studies of electronic structure and reactivity of compound-I model complexes.

1. The radical state of oxo iron(IV) porphyrin π-cation radical complex is, in general, determined by the porphyrin structure; the *meso*-substituted porphyrin, like $O{=}Fe^{IV}[TMP]^{+\bullet}$, has an a_{2u} radical state while the pyrrole-β substituted porphyrin, like $O{=}Fe^{IV}[TMTMP]^{+\bullet}$, has an a_{1u} radical state.
2. With an increase in the electron-withdrawing effect of substituent, the a_{2u} radical state of the *meso*-substituted porphyrin is switched to the a_{1u} radical state, however, the a_{1u} radical state of the pyrrole β-substituted porphyrin is not changed to the a_{2u} radical state.
3. The magnetic coupling between ferryl iron spin and porphyrin π-cation radical spin of oxo iron(IV) porphyrin π-cation radical is mainly controlled by the symmetry of the porphyrin radical orbital; a strong ferromagnetic interaction for an a_{2u} radical state and a weak (ferromagnetic) interaction for an a_{1u} radical state.

4. The nature of the axial ligand opposite to the oxo ligand does not change the porphyrin π-cation radical state, but does modify the absorption spectra feature.
5. The Fe=O bond of oxo iron(IV) porphyrin is not weakened drastically even if the porphyrin ring is oxidized to the porphyrin π-cation radical. Therefore, the fact that the monooxygenation activity of oxo iron(IV) porphyrin π-cation radical is much higher than that of oxo iron(IV) porphyrin cannot be explained by the Fe=O bond strength.
6. The resonance Raman band for ν(Fe=O) of oxo iron(IV) porphyrin π-cation radical complex is not sensitive to the a_{1u}/a_{2u} porphyrin π-cation radical state, but is sensitive to the nature of the axial ligand opposite to the oxo ligand.
7. The reactivity of epoxidation reaction by oxo iron(IV) porphyrin π-cation radical is modulated by the redox potential of the complex, but not by the a_{1u}/a_{2u} radical state.

As summarized above, these compound-I model studies allow us to comment on the electronic state and reactivity of compounds-I of heme enzymes. Previously, the radical states of compound-I species were assigned on the basis of their absorption spectra, and compound-I of HRP was thought to be an a_{2u} radical state and that of CAT was an a_{1u} radical state. However, as discussed in this review, the compound-I model complexes clearly show that the absorption spectra do not reflect its radical state, but the nature of the axial ligand. Furthermore, the compound-I model complexes also indicate that oxo iron(IV) porphyrin π-cation radical of protoporphyrinIX is an a_{1u} radical state. It is thus reasonable to propose that all compounds-I of heme enzymes with *meso*-unsubstituted iron porphyrin, including the compound-I of HRP, have predominantly an a_{1u} radical state. Indeed, the weak magnetic interactions of compound-I species of heme enzymes are reasonably associated with the a_{1u} radical state (Fig. 7).

The compound-I model complexes also demonstrated the effect of the radical state and redox potential on the reactivity of epoxidation reaction by oxo iron(IV) porphyrin π-cation radical complexes. Complexes with higher redox potentials are more reactive. Interestingly, in spite of a common prosthetic group (protoporphyrinIX), the redox potential of HRP, CAT, and P450 differ because of the differences in the heme axial ligand (see Fig. 8); ferric/ferrous couples for P450, HRP, and CAT are -170, -250, and > -500 mV, respectively [51–53]. Oxygen atom transfer reaction conducted by P450 is reasonable given the high oxidation potential of this enzyme. On the other hand, the lowest redox potential of CAT in these enzymes is enough to oxidize hydrogen peroxide. Furthermore, the effect of the redox potential explains the difference in the reactivity of compound-I and compound-II. In general, the redox potential of compound-II is lower than that of compound-I because compound-II is formed by the one-electron reduction of compound-I. The lower redox potential may remove monooxygenation activity from compound-II.

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