Imidazole and *p*-Nitrophenolate Complexes of Oxoiron(IV) Porphyrin π-Cation Radicals as Models for Compounds I of Peroxidase and Catalase

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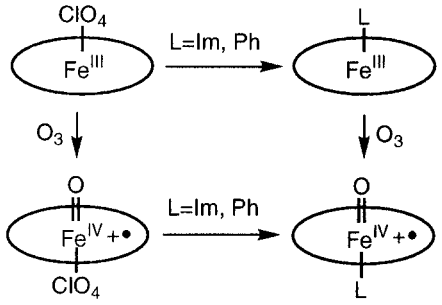
The involvement of oxoiron(IV) porphyrin π-cation radical species as intermediates in catalytic cycles of peroxidases,[1] catalases,[2] and cytochrome P-450s, is well-known.[3] For the case of peroxidases and catalases, an oxoiron(IV) porphyrin π-cation radical, called compound I, has been identified as a reactive intermediate.[1] A compound I species has also been proposed as the reactive oxygenating intermediate of cytochrome P-450.[3] Interestingly, in spite of the fact that compound I is common to a series of enzymes, the reactivity of the compound I species differs from one enzyme to another. In cytochrome P-450, the compound I species catalyzes the direct transfer of a single oxygen atom to a variety of substrates,[3,4] while for the case of peroxidase and catalase, it catalyzes the oxidation of the organic compounds and hydrogen peroxide, respectively.[1,2,5] These diverse functions are generally thought to depend on heme structures, such as, for example, porphyrin peripheral structures and the heme proximal ligand, as well as protein structures in the immediate vicinity of the heme. Indeed, different proximal ligands in these enzymes (e.g., imidazole in peroxidase, phenolate in catalase, and thiolate in cytochrome P-450) suggest that the proximal ligand structure controls the reactivity of compound I species.

While a few reports on the effect of the axial ligand of oxoiron(IV) porphyrin π-cation radicals have appeared,[6] there are no reported oxoiron(IV) porphyrin π-cation radical complexes having imidazole and phenolate as axial ligands. We have prepared imidazole, (P$^{\bullet}$)Fe^{IV}O(Im), and *p*-nitrophenolate, (P$^{\bullet}$)Fe^{IV}O(OAr), complexes of an oxoiron(IV) 2,7,12,17-tetramethyl-3,8,13,18-tetramesitylporphyrin π-cation radical.[7] These radical complexes may be more desirable than those of *meso*-tetraarylporphyrins as spectroscopic models for biological heme enzymes because of similarities in the porphyrin structure to those of naturally occurring compounds.[7a]

We examined the preparation of (P$^{\bullet}$)Fe^{IV}O(Im) by two alternative routes, shown in Scheme 1. We employed perchloratoiron(III) 2,7,12,17-tetramethyl-3,8,13,18-tetramesityporphyrin, (P)Fe^{III}(ClO_4), as the starting complex because the perchlorate ligand is easily displaced by imidazole due to its weak ligand field.[8] Ozone was used as an oxidant, since ozone oxidation of an iron(III) porphyrin forms only an oxoiron(IV) porphyrin π-cation radical complex and dioxygen, which is easily removed by bubbling with nitrogen gas.[6,7e]

Scheme 1



A ferric mono(imidazole) complex, (P)Fe^{III}(Im), was prepared by addition of 1 equiv of imidazole to (P)Fe^{III}(ClO_4) in dichloromethane.[8,9] The absorption spectrum of (P)Fe^{III}(Im), which has peaks at 385, 503, and 626 nm, closely resembled that of the resting form of horseradish peroxidase (HRP).[10] When (P)Fe^{III}(Im) was oxidized in dichloromethane at −80 °C by ozone, a green complex was formed. The absorption spectrum of the green complex showed the characteristic features of the oxoiron(IV) porphyrin π-cation radical, namely a Soret band at 390 nm with decreased intensity and a broad band around 640 nm. The spectral features of the green complex were not identical with those of the previously characterized (P$^{\bullet}$)Fe^{IV}O(mCB) (389 and 624 nm) but were close to those of compound I (402 and 650 nm) of HRP.[10] The absorption spectrum of the green complex was also formed with isosbestic points when 1 equiv of imidazole was titrated into a perchlorate oxoiron(IV) porphyrin π-cation radical complex, (P$^{\bullet}$)Fe^{IV}O(ClO_4), prepared from the oxidation of (P)Fe^{III}(ClO_4) by ozone in dichloromethane at −80 °C. These findings suggest that the green complex is (P$^{\bullet}$)Fe^{IV}O(Im).

The structure of (P$^{\bullet}$)Fe^{IV}O(Im) was further confirmed by ^{1}H- and ^{2}H-NMR measurements. Figure 1 shows ^{1}H-NMR spectral changes on titration of (P$^{\bullet}$)Fe^{IV}O(ClO_4) with imidazole in dichloromethane-d_2 at −80 °C. The assignments are based on selectively deuterated (meso-d_4 and imidazole-d_3) samples and the intensities of the signals. As shown in Figure 1a, the pyrrole β-methyl and meso proton signals of (P$^{\bullet}$)Fe^{IV}O(ClO_4) are observed at 132 and 54 ppm, respectively. The splitting of the meta proton (15 ppm) and *o*-methyl (11 ppm) signals is indicative of two different axial ligands in (P$^{\bullet}$)Fe^{IV}O(ClO_4): oxo

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(9) Although the formation of (P)Fe^{III}(Im) was confirmed by absorption and ^{1}H-NMR measurements, the isolation of (P)Fe^{III}(Im) resulted in a mixture of (P)Fe^{III}(Im)$_2$ and (P)Fe^{III}ClO_4. The absorption and ^{1}H-NMR spectra of (P)Fe^{III}(Im) are shown in the Supporting Information.
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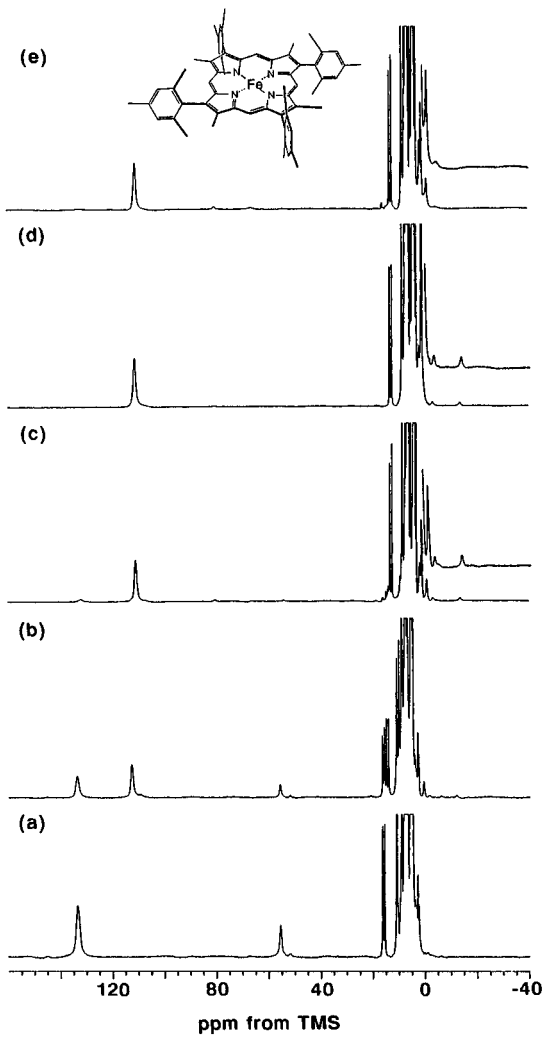

Figure 1. ^{1}H-NMR spectra for the titration of (P$^{\bullet}$)Fe^{IV}O(ClO$_4$) with imidazole at −80 °C in dichloromethane-d_2. Concentration of iron porphyrin: 5.3 mM. Imidazole added: (a) 0.0 equiv; (b) 0.5 equiv; (c) 1.0 equiv; (d) meso-d_4 (P$^{\bullet}$)Fe^{IV}O(ClO$_4$) with 1.0 equiv of imidazole; (e) (P$^{\bullet}$)Fe^{IV}O(ClO$_4$) with 1.0 equiv of imidazole-d_3.

and perchlorate anion ligands.[11] With the addition of imidazole, the signals for (P$^{\bullet}$)Fe^{IV}O(ClO$_4$) decreased in intensity and new signals appeared (Figure 1b,c). The signals for (P$^{\bullet}$)Fe^{IV}O(ClO$_4$) were nearly displaced by the new signals when 1 equiv of imidazole was added, and further addition of imidazole caused reduction to an oxoiron(IV) porphyrin complex. As shown in Figure 1c,d, the meso proton signal of (P$^{\bullet}$)Fe^{IV}O(Im) is observed at −1 ppm. The pyrrole β-methyl proton was assigned the signal at 111 ppm. The *m*-proton and *o*-methyl signals of the pyrrole β-mesityl group were observed at 12 and 7 ppm, respectively. The splitting of meta proton and *o*-methyl signals is also indicative of two different axial ligands: oxo and imidazole coordination. More unambiguous evidence of (P$^{\bullet}$)-Fe^{IV}O(Im) is the iron-bound-imidazole signals at −3 and −14 ppm, which was confirmed by using imidazole-d_3 as the titrant (Figure 1e). The assignment of these imidazole signals is also supported by ^{2}H-NMR measurements. All of these results indicate the formation of (P$^{\bullet}$)Fe^{IV}O(Im) with the addition of 1 equiv of imidazole to (P$^{\bullet}$)Fe^{IV}O(ClO$_4$). As observed for absorption spectral measurements, the ^{1}H-NMR spectrum of (P$^{\bullet}$)Fe^{IV}O(Im) was also observed when (P)Fe^{III}(Im) was oxidized by ozone in dichloromethane-d_2 at −80 °C.

The temperature dependence of NMR signals of (P$^{\bullet}$)Fe^{IV}O-(Im) showed normal Curie law behavior from −100 to −40 °C, suggesting that the axial imidazole binds tightly with the porphyrin iron. From the Curie plots for (P$^{\bullet}$)Fe^{IV}O(Im), we estimated the pyrrole methyl signals to be 71 ppm at 25 °C, which is close to the methyl signals (average; 64 ppm at 25 °C) of compound I of HRP.[12]

The small paramagnetic shift of the meso proton signal of (P$^{\bullet}$)Fe^{IV}O(Im) indicates an a_{1u} radical state, as has been observed for (P$^{\bullet}$)Fe^{IV}O(ClO$_4$) and (P$^{\bullet}$)Fe^{IV}O(mCB).[7] The NMR spectral change with imidazole coordination may be explained by a minor change in spin distribution in the a_{1u} orbital in (P$^{\bullet}$)Fe^{IV}O-(Im) resulting from vibrational mixing[7,13] and/or a withdrawal of iron(IV) into the porphyrin plane with imidazole coordination. Although it has been proposed that the HOMO of the compound I species is altered with coordination of strong donor imidazole and oxo ligands on the basis of the a_{2u} assignment of compound I of HRP,[14] the present study demonstrates that the HOMO remains unchanged on coordination with imidazole. Furthermore, all of the present results for (P$^{\bullet}$)Fe^{IV}O(Im) indicate the a_{1u} radical state of compound I of HRP.[7]

We also examined the oxidation of (P)Fe^{III}(OAr) with ozone in dichloromethane at −95 °C. The absorption spectrum of (P)-Fe^{III}(OAr) (405, 493, 617 nm) is similar to that of the resting form of catalase (405, 505 and 625 nm).[1a,15] On oxidation by ozone, the absorption spectrum of (P)Fe^{III}(OAr) quickly changed to a spectrum which had absorption peaks at 392 and 655 nm. The absorption spectrum was different from those of (P$^{\bullet}$)Fe^{IV}O-(ClO$_4$), (P$^{\bullet}$)Fe^{IV}O(mCB), and (P$^{\bullet}$)Fe^{IV}O(Im) but closely resembled that of compound I of catalase, which has absorption peaks at 400 and 662 nm.[1a,15] While (P$^{\bullet}$)Fe^{IV}O(Im) was stable at −80 °C for 1 h, the oxidized complex of (P)Fe^{III}(OAr) was quite unstable even at −95 °C and decomposed to a ferric complex within a few minutes showing a new spectrum with absorption peaks at 387 and 640 nm. We attempted to obtain an ^{1}H-NMR spectrum of the oxidized complex but failed because of its short lifetime. Although further work will be needed to define the structure of the oxidized complex of (P)-Fe^{III}(OAr), the absorption spectral features imply the formation of the *p*-nitrophenolate complex of the oxoiron(IV) porphyrin π-cation radical, (P)Fe^{IV}O(OAr).

We have prepared imidazole and *p*-nitrophenolate complexes of oxoiron(IV) porphyrin π-cation radicals and characterized them using absorption and NMR spectroscopies. Further work, including stabilization of the phenolate complex and the reactivity of these complexes, is currently in progress.

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Supporting Information Available: Figure S1, showing absorption spectra of (P$^{\bullet}$)Fe^{IV}O(Im) and (P$^{\bullet}$)Fe^{IV}O(OAr), Figure S2, showing absorption spectral changes during the titration of (P)Fe^{III}ClO$_4$ with imidazole in dichloromethane, and Figure S3, showing ^{1}H-NMR spectra for the titration of (P)Fe^{III}ClO$_4$ with imidazole in CD_2Cl_2 at 24 °C (3 pages). Ordering information is given on any current masthead page.

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