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Chemistry

Stabilization of higher-valent states of iron porphyrin by hydroxide and methoxide ligands: Electrochemical generation of iron(IV)-oxo porphyrins

(cytochrome P-450/peroxidases)

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ABSTRACT **An electrochemical study of hydroxide- and methoxide-ligated iron(III) tetraphenylporphyrins possessing *ortho*-phenyl substituents that block μ-oxo dimer formation has been carried out. Ligation by these strongly basic oxyanions promotes the formation of iron(IV)-oxo porphyrins upon one-electron oxidation. Further one-electron oxidation of the latter provides the iron(IV)-oxo porphyrin π-cation radical. These results are discussed in terms of chemical model studies and the enzymatic intermediate compounds I and II of the peroxidases.**

The mechanism of substrate oxidation by the peroxidase enzymes involves (1, 2) the formation of an enzyme-bound higher valent iron-oxo protoporphyrin IX. The chemistry is well understood in the peroxidases, where the oxidizing intermediates have been isolated and characterized. The iron(III) porphyrin moiety of the peroxidases undergoes a two-electron oxidation upon reaction with hydrogen peroxide, alkyl hydroperoxides (3), or peroxycarboxylic acids (4) to yield an intermediate known as compound I (5). On the basis of visible (6), Mössbauer (7), ESR (8), and electron nuclear double resonance (ENDOR) (9) spectral data, compound I has been assigned the structure of a low-spin oxo-ligated iron(IV) porphyrin π-cation radical. This same entity is suggested to serve as the oxygen transfer agent formed at the active site of the cytochrome P-450 enzymes (10, 11). One-electron reduction of the π-cation radical of compound I results in the formation of an iron(IV)-oxo species termed compound II (12). Interestingly, compounds I and II of the peroxidases exhibit almost identical redox potentials (13).

Only a few well-characterized model analogs of compounds I and II exist. These iron(IV) porphyrins have all been generated chemically at low temperatures. Until now, an iron(IV)-oxo porphyrin species has not been generated electrochemically. In the cyclic voltammogram of simple iron porphyrins, anodic sweeps beyond the iron(II)/iron(III) couple yield two reversible one-electron oxidation waves (32). Originally it was proposed that the first wave pertained to the iron(III)/iron(IV) couple and the second wave was due to porphyrin π-cation radical formation (14, 15). This was shown to be incorrect in a report (16) which demonstrated that the redox potential of the first oxidation was independent of the many axial ligands employed (16) and that the physical properties of the oxidized species were consistent with an iron(III) porphyrin π-cation radical structure (17). The second wave was attributed to an iron(III) porphyrin dication.

In the literature, there is no mention of electrochemical investigations involving HO^-, CH_3O^-, etc. ligated iron porphyrins. This does not of course indicate an oversight (17) but simply reflects the instability, due to μ-oxo dimer formation, of oxo-ligated iron(III) porphyrins. The inability to electrochemically generate a compound I or compound II analog could be due to the importance of oxo ligation to the stabilization of high-valent iron porphyrin species. It is well established that metals in their higher-valent oxidation states are generally stabilized by oxo ligation (18). Using sterically hindered porphyrins that are capable of hydroxide ligation, we have now been able to observe the electrochemical generation of iron(IV)-oxo porphyrin species. In addition, the similarity of the iron(IV)-oxo and the porphyrin π-cation redox potentials provides a rationale for the redox potentials of compounds I and II of the peroxidases. In the present study we describe the electrochemistry of porphyrins **1** to **5** when ligated to Cl^-, HO^-, and CH_3O^- and discuss these results as they relate to studies involving the chemical generation of oxo-ligated higher-valent iron porphyrins.

MATERIALS AND METHODS

Reagents. Dichloromethane, used as the solvent for the electrochemical studies, was obtained from Burdick and Jackson (Muskegon, MI) as their "distilled-in-glass" grade. Tetrabutylammonium perchlorate from G. Fredrick Smith was used as the supporting electrolyte. Argon (Linde Gas, Danbury, CT) that had been passed through an Oxiclear Gas Purifier (Labclear) was used to provide an inert atmosphere for the electrochemical experiments.

The porphyrins used in this investigation were as follows: 5,10,15,20-tetraphenylporphyrin (**1**); 5,10,15,20-tetra(2,4,6-trimethylphenyl)porphyrin (**2**); 5,10,15,20-tetra(2,6-dimethylphenyl)porphyrin (**3**); 5,10,15,20-tetra(2,6-dichlorophenyl)porphyrin (**4**); 5,10,15,20-tetra(2,6-difluorophenyl)porphyrin (**5**). 5,10,15,20-Tetraphenylporphyrin was purchased from Aldrich. Literature procedures were followed in the synthesis of porphyrins **2** (19), **3** (20), and **5** (21); porphyrin **4** was synthesized by T. C. Woon, using procedures that will be described elsewhere. The iron was inserted into the porphyrins by refluxing with ferrous chloride in dimethylformamide (22). After work-up the iron porphyrins were purified by column chromatography on silica gel, eluting with $CHCl_3/CH_3OH$ (95:5, vol/vol). The purified iron porphyrin was then dissolved in CH_2Cl_2 and dry HCl was bubbled through the solution to exchange any extraneous axial ligands for Cl^-. The hydroxide ligand was introduced by stirring a toluene solution of the iron(III) porphyrin chloride with an aqueous sodium hydroxide solution for 1 day as previously reported (23). Contrary to the results with 5,10,15,20-tetra(pentafluorophenyl)porphyrin iron(III) hydroxide (23), NMR and UV-visible characterization indicate that hydroxy-ligated **5** does not form a μ-oxo

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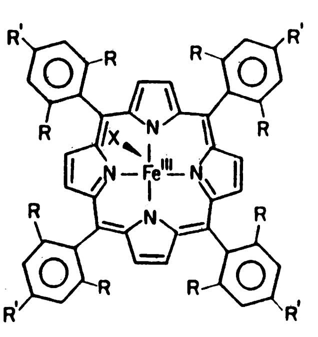
Abbreviations: Abbreviations for the iron(III) porphyrins employed in this study are provided in Fig. 1.



X	R	R'
1—Cl	H	H
1—OMe	H	H
2—Cl	Me	Me
2—OH	Me	Me
2—OMe	Me	Me
3—Cl	Me	H
3—OH	Me	H
4—Cl	Cl	H
4—OH	Cl	H
4—OMe	Cl	H
5—Cl	F	H
5—OH	F	H
5—OMe	F	H

FIG. 1. Structures of the 5,10,15,20-tetraphenylporphyrin iron(III) salts employed in this study.

dimer species. The synthesis of methoxide-ligated **1** (i.e., 1-OCH_3) has been reported (24).

Instrumentation. A three-electrode potentiostat (Bioanalytical Systems model CV-27 voltammograph) was used for the cyclic voltammetry and controlled-potential electrolysis experiments. Cyclic voltammograms were recorded on a Houston Instruments model 100 *x–y* recorder. The scans were initiated at the rest potential of each solution. The electrochemical cells were equipped with a Bioanalytical Systems platinum-inlay electrode, a platinum-flag auxiliary electrode that was separated from the main compartment by a medium glass frit, and an Ag/AgCl reference electrode filled with aqueous tetramethylammonium chloride and adjusted to 0.00 V vs. saturated calomel electrode. The reference electrode was placed in a Luggin capillary. For controlled-potential electrolysis, a platinum-mesh working electrode was employed. Visible and ultraviolet spectra were recorded on Perkin–Elmer 553 and Cary 15 spectrophotometers.

RESULTS

Electrochemical studies have been carried out on the iron(III) prophyrin salts **1**—X, **2**—X, **3**—X, **4**—X, and **5**—X (Fig. 1), in which X represents the axial ligated anions Cl^-, HO^-, and CH_3O^-. Cyclic voltammetry of **2**, **3**, **4**, and **5** ligated with hydroxide or methoxide shows a new oxidation peak that is not observed with the chloride salts. The effect of hydroxide ligation versus chloride ligation on the cyclic voltammogram for **2** is shown in Fig. 2. The new oxidation peak observed with hydroxide as a ligand is approximately 100 mV less positive than the first oxidation peak observed with chloride as ligand. The first and second oxidation waves are fully reversible at scan rates between 25 and 400 mV/sec. Repetitive scans at 100 mV/sec also demonstrated the reversibility of the redox couples. The redox potentials obtained by cyclic voltammetry for the iron(III) porphyrin salts used in this study are listed in Table 1. All potentials are relative to the saturated calomel electrode.

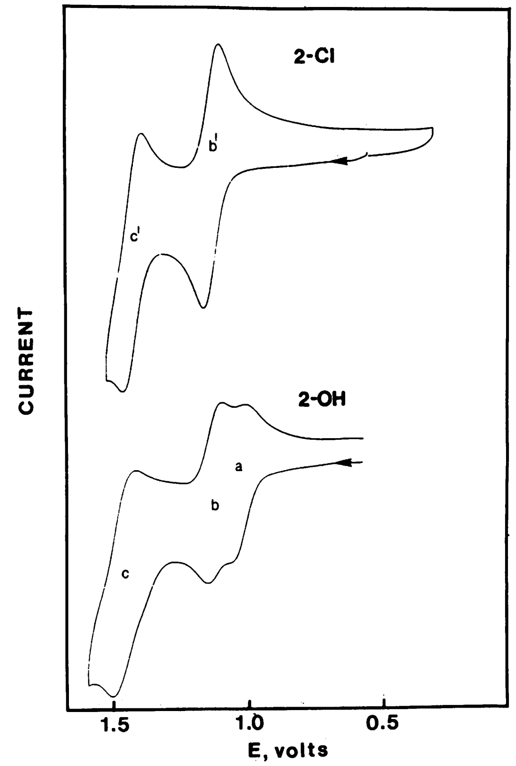


FIG. 2. Cyclic voltammograms (dichloromethane solvent with 0.1 M tetrabutylammonium perchlorate) at a platinum electrode for **2**—Cl (2.0×10^{-4} M) and **2**—OH (2.0×10^{-4} M) where *a* is the redox couple for Fe(III) porphyrin/Fe(IV) porphyrin, *b* is the redox couple for Fe(IV) porphyrin/Fe(IV) porphyrin π-cation radical, *b'* is the redox couple for Fe(III) porphyrin/Fe(III) porphyrin π-cation radical, *c* is the redox couple Fe(IV) porphyrin π-cation radical/Fe(IV) porphyrin dication, and *c'* is the redox couple for Fe(III) porphyrin π-cation radical/Fe(III) porphyrin dication. Scans were initiated from the rest potential; scan rate, 0.1 V/sec.

Controlled-potential coulometry of the hydroxide and methoxide salts of the iron(III) porphyrins shows that both the first and the second oxidations are one-electron processes. At room temperature, no stable one-electron oxidation product was detectable. Instead, spectro-electrochemical experiments show that **2**—OH is converted to **2**—Cl by the removal of one electron at room temperature. The changes in the visible spectrum are shown in Fig. 3. The

Table 1. Redox potentials (V) of the iron(III) porphyrin salts

Porphyrin	Fe^{III}P/Fe^{IV}P	Fe^{III}P/Fe^{III}P$^{\cdot+}$	Fe^{IV}P/Fe^{IV}P$^{\cdot+}$	Fe^{III}P$^{\cdot+}$/Fe^{III}P^{2+}	Fe^{IV}P$^{\cdot+}$/Fe^{IV}P^{2+}
1—Cl	—	1.24	—	1.55	—
1—OMe	1.11	—	1.16	—	1.64
2—Cl	—	1.13	—	1.49	—
2—OH	1.01	—	1.14	—	1.58
2—OMe	1.01	—	1.13	—	1.56
3—Cl	—	1.18	—	1.51	—
3—OH	1.07	—	1.19	—	1.60
4—Cl	—	1.43	—	1.75	—
4—OH	1.26	—	1.44	—	1.73
4—OMe	1.26	—	1.44	—	1.73
5—Cl	—	1.50	—	—	—
5—OH	1.38	—	1.52	—	—
5—OMe	1.38	—	1.52	—	—

P, porphyrin.

formation of 2—Cl was also evidenced by the cyclic voltammogram of the oxidation product. This suggests that the oxidized iron porphyrin species decomposes the solvent, yielding both iron(III) porphyrin and Cl^- at room temperature.

The cyclic voltammogram of 1—OCH_3 shows an additional oxidation peak at 1.0 V similar to that of 2—OCH_3. This oxidation wave is not reversible and the cathodic scan yields a new reduction wave at 0.88 V. A second repetitive scan, as shown in Fig. 4, results in a cyclic voltammogram identical to the μ-oxo dimer of **1**. Controlled-potential coulometry on the first oxidation peak of 1—OCH_3 resulted in the removal of 0.5 electron per equivalent of iron porphyrin and the formation of the porphyrin π-cation radical of the μ-oxo dimer as the sole oxidation product (Fig. 4, trace B).

Low-temperature electrochemistry was employed in an effort to generate a stable oxidized species. At −78°C, the cyclic voltammogram of 2—OH showed greater separation between the first and second redox couples. Upon removal of one electron from 2—OH at −78°C, an oxidized species is formed that can be observed electrochemically by cyclic voltammetry. Warming the solution above −50°C results in the conversion of the oxidized species to 2—Cl. All attempts to isolate the oxidized species have so far been unsuccessful.

Combining electrochemical and chemical methods, an iron(IV)—OCH_3 species was produced at −78°C by adding 1 equivalent of $NaOCH_3$ to the π-cation radical of 2—Cl that had been generated at room temperature by controlled-potential electrolysis. The low-temperature cyclic voltammogram of the π-cation radical and the species produced upon addition of 1 equivalent of $NaOCH_3$ are shown in Fig. 5. Addition of $NaOCH_3$ causes the rest potential to shift from 1.15 V to 1.08 V. The cyclic voltammogram of the product shows a new oxidation wave, which matches that obtained for 2—OCH_3. The visible spectrum for the species produced by $NaOCH_3$ addition to the π-cation radical of 2—Cl has a λ_{max} at 545 nm with a shoulder at 575 nm as reported (25) for a similar species generated chemically.

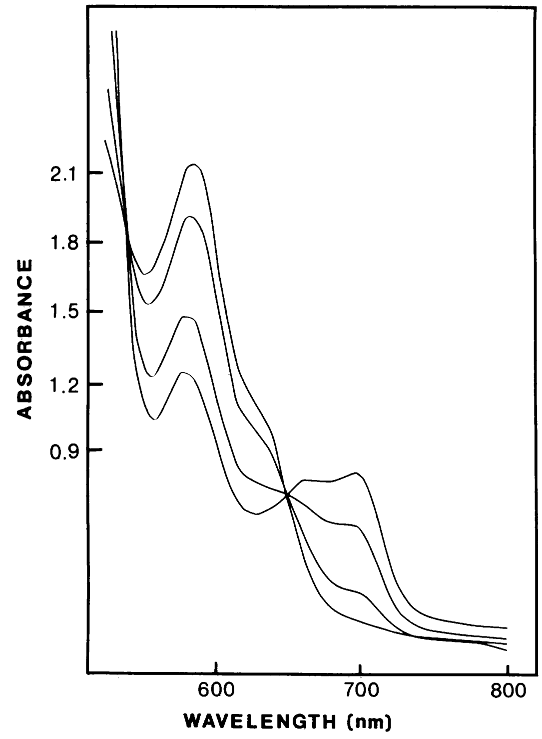


FIG. 3. Spectro-coulometric titration of 2—OH (2.8 × 10^{-4} M). Species showing major absorbance at 580 nm is 2—OH and the absorbing species at 690 nm is 2—Cl. Scans for conversion of 2—OH to 2—Cl were made at 0.05, 0.1, and 0.15 coulombs, respectively (initial scan was of 2—OH, and controlled-potential electrolysis was carried out at 1.03 V in ethylene dichloride with 0.1 M tetrabutylammonium perchlorate).

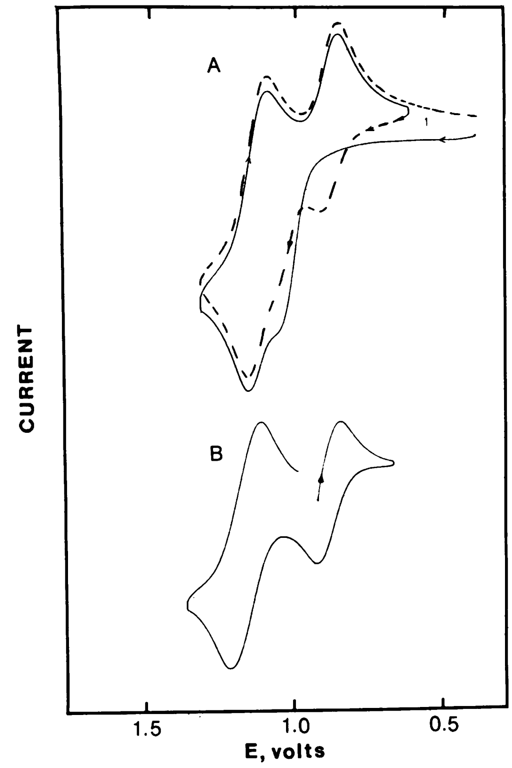


FIG. 4. Cyclic voltammograms (conditions as in Fig. 2). Trace A, 1—OCH_3 (5.7 × 10^{-4} M) (initial scan is shown as a solid line and the second repetitive scan is shown with a dashed line). Trace B, product solution of 1—OCH_3 after 0.5 electron equivalent of oxidation.

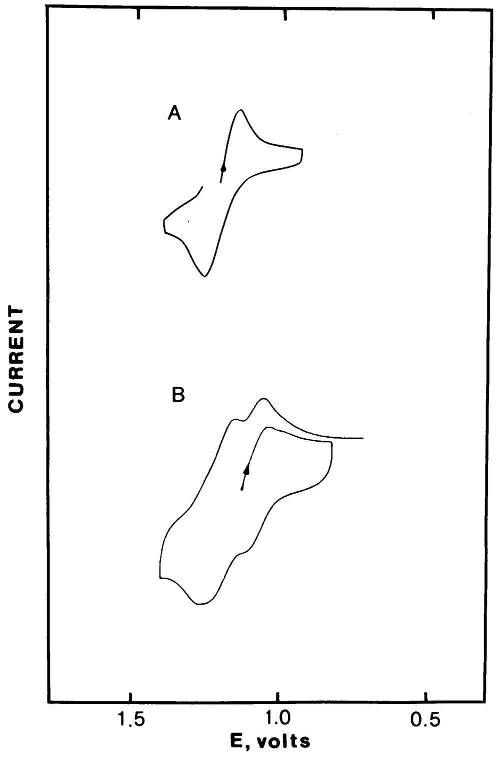


FIG. 5. Cyclic voltammograms at −78°C. Porphyrin π-cation radical of 2—Cl (4.2 × 10^{-4} M) (trace A) and product solution after the addition of 1 equivalent of $NaOCH_3$ to the porphyrin π-cation radical of 2—Cl (trace B).

DISCUSSION

It is known that the one-electron oxidation of iron(III) porphyrins when ligated to Cl^-, ClO_4^-, etc. results in a porphyrin-centered oxidation and not a metal-centered oxidation (17). Extensive physical characterization of the one-electron oxidation product suggests it is best formulated as an iron(III) porphyrin π-cation radical. This is in contrast to the well-characterized iron(IV) structure of the peroxidase intermediates (6–9). Considering their biological significance and their importance as synthetic oxygen-transfer catalysts, very few examples of iron(IV)-oxo porphyrins are known. Those examples that do exist have all been generated chemically in oxygen-transfer experiments. An iron(IV) porphyrin π-cation radical has been isolated and characterized in the reaction of *m*-chloroperoxybenzoic acid or iodosylbenzene with **2**—Cl at low temperature (26). Oxygenation of iron(II) porphyrins at low temperature results in a μ-peroxo dimer species, which decomposes to a monomeric iron(IV)-oxo porphyrin species upon addition of imidazole (27). More recently it has been shown that the iron(III) porphyrin π-cation radical could be converted to the iron(IV) porphyrin by axial substitution of two methoxide anions for two perchlorate anions (25).

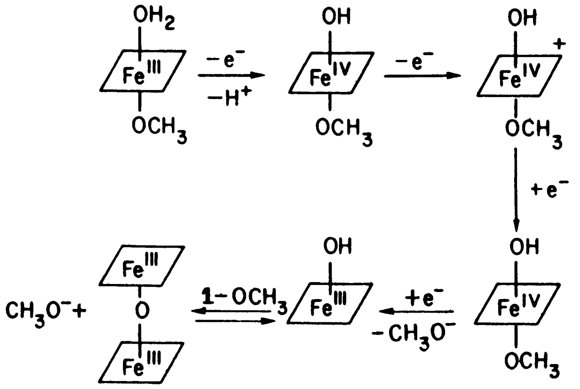
It has long been recognized that hard ligands tend to stabilize high-valent metal species (18). Attempts to form hydroxide-ligated iron(III) porphyrins results in the formation of very stable μ-oxo iron(III) porphyrin dimers (29). Electrochemical oxidation of the μ-oxo dimer shows only porphyrin-centered oxidations (30). We have prepared a series of iron(III) tetraphenylporphyrin derivatives (Fig. 1) in which substitution at the *ortho* positions of the phenyl groups effectively prevents μ-oxo dimer formation. This has allowed the preparation of stable hydroxide-ligated species (23) as well as the chloride- and methoxide-ligated analogs.

Ligation of hydroxide or methoxide results in a previously unseen one-electron oxidation wave that is anodic to the iron(II)/iron(III) couple observed with chloride as a ligand. This observation is general for all of the octa-*ortho*-substituted porphyrins (i.e., **2** to **5**). We suggest that this new oxidation wave is consistent with the presence of an iron(III)/iron(IV) couple. This being so, oxo ligation has changed the chemistry of one-electron oxidation of the iron(III) porphyrin from porphyrin-centered to metal-centered. This is most certainly due to the increased stability of high-valent metals when bound to highly basic oxo ligands. The reactivity of the iron(IV)-oxo species generated electrochemically is very similar to that reported for the iron(IV)-oxo species produced chemically (25–28). The decomposition of the iron(IV)-oxo porphyrin at temperatures above −50°C shows that it is more chemically reactive than the iron(III) porphyrin π-cation radical, even though the redox potential of the latter is more positive. We have also demonstrated that the iron(IV) porphyrin generated from the porphyrin π-cation radical of **2**—Cl by exchange of methoxide for chloride exhibits a cyclic voltammogram identical to that obtained directly by the electrochemical oxidation of **2**—OCH_3 (Eqs. **1** and **2**, in which P = porphyrin).

$$PFe^{III}\text{—}OCH_3 \xrightarrow{-1\,e^-} PFe^{IV}\text{—}OCH_3 \qquad [1]$$

$$PFe^{III}\text{—}Cl \xrightarrow{-1\,e^-} {}^{+\cdot}PFe^{III}\text{—}Cl \xrightarrow[-Cl^-]{+CH_3O^-} PFe^{IV}\text{—}OCH_3 \qquad [2]$$

The variation in the redox potentials for the iron(IV)-oxo porphyrin and the porphyrin π-cation radical (Table 1) reflects the electronic nature of the substituents on the phenyl moieties of the iron *meso*-tetraphenylporphyrins **2** to **5**. For **4** and **5** the iron(IV)-oxo potentials are shifted anodically 0.25 V and 0.37 V relative to **2**—OH, while the second porphyrin oxidation of **5** is anodic of the solvent window (*ca.* 1.8 V). The similar potentials for the hydroxide- and methoxide-ligated compounds (**2** to **5**) indicate that both oxo ligands confer comparable stabilization to the iron(IV) species. Interestingly, the first oxidation peak for **1**—OCH_3 is consistent with the formation of an iron(IV) species. The corresponding reduction is not reversible and a new peak is observed cathodic of the porphyrin π-cation reduction. A second repetitive scan results in the cyclic voltammogram of the μ-oxo dimer of **1** (Fig. 4). This suggests that reduction of the oxidized species of **1**—OCH_3 results in a hydroxide ligand exchange followed by subsequent μ-oxo dimer formation. Dimerization and ligand exchange are required to be fast since scan rates of up to 500 mV/sec have no effect on the appearance of the μ-oxo dimer reduction wave. Scheme 1 is consistent with these observations.



SCHEME 1.

Alternatively, H_2O ligation can occur to either of the oxidized species preceding reduction. The observation that 0.5 electron equivalent is required to convert **1**—OCH_3 to the mono π-cation radical of the μ-oxo dimer supports Scheme 1.

Finally, the similarity in the redox potentials of the iron(III) porphyrin/iron(IV)-oxo porphyrin and the iron(III) porphyrin/iron(III) porphyrin π-cation radical partially explains the reported redox potentials for compounds I and II of the peroxidase enzymes. Using kinetic and equilibrium data from the oxidation and reduction of compounds I and II of horseradish peroxidase C by K_2IrCl_6/K_3IrCl_6, Hayashi and Yamazaki showed that the redox potentials for the intermediates at pH 6.3 are 0.71 and 0.73 V, respectively (13). This was surprising for several reasons. First, in iron(III) porphyrins with non-oxo ligands, redox potentials for the higher oxidation states are separated by 400 mV. Second, the redox potential of compound II is greater than that of compound I, while the rate constants for substrate oxidation are greater for compound I. The latter result has recently been explained to be due to the spin change of the iron upon reduction of compound II to the resting ferric enzyme (31). Our electrochemical results (Table 1) provide an explanation for the first anomaly. The previously reported redox potentials for Cl^-, ClO_4^-, etc. ligated iron porphyrins are porphyrin-centered oxidations and our results show that the redox potentials of iron(IV)-oxo porphyrin and iron(IV)-oxo porphyrin π-cation radical species are similar in model compounds as well as in the peroxidases.

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