

Resonance Raman Spectra of Legitimate Models for the Ubiquitous Compound I Intermediates of Oxidative Heme Enzymes

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In the catalytic cycles of many oxidative heme enzymes, such as peroxidases and catalases, among others, arise the so-called Compound I intermediates. These are two oxidizing equivalents above the ferric heme resting state and are thought to contain an oxo-iron(IV) porphyrin π -cation radical, which adopts an ${}^2A_{1u}$ or ${}^2A_{2u}$ electronic configuration, depending on structural features of the heme active site.¹ Though Resonance Raman (RR) spectroscopy has emerged as a particularly promising probe of these intermediates,^{2–4} to date no RR studies have been reported for model compounds which possess a coordination environment similar to that present in the protein systems. However, the strength and reactivity of the ferryl fragment, as reflected in the frequency of the key $\nu(\text{Fe}=\text{O})$ stretching mode, are expected to be sensitive to the nature and disposition of the trans-axial ligands provided by the associated protein, as well to direct H-bonding or polar interactions with distal pocket side chains of histidine, arginine, or other amino acid residues.²

To provide a valid framework for reliable structural interpretation of spectral parameters observed for the protein systems, we and others have extensively studied the RR spectra of many model compounds, including the π -cation radicals of both ferryl and vanadyl porphyrins.^{2,5–8} While such studies have been generally fruitful in revealing characteristic core mode patterns for the ${}^2A_{1u}$ vs ${}^2A_{2u}$ ground-state electronic configurations and the response of the $\nu(\text{Fe}=\text{O})$ and $\nu(\text{V}=\text{O})$ modes to distal (environmental) conditions, systematic model compound studies of trans-axial ligand effects on the $\nu(\text{Fe}=\text{O})$ mode of ferryl porphyrin π -cation radicals have been quite scarce. Though studies involving various nonphysiological ligands, such as alcohols, halides, and several other weakly coordinating anions have been reported,^{5,6,8} there

have been no reports of the RR spectra of models which are expected to be faithful mimics of the enzymatic intermediates, that is, a ferrylheme π -cation radical bearing a physiologically relevant trans-axial ligand. In fact, it was only very recently that it was shown that solutions of such species, wherein the trans-axial ligand is imidazole or phenolate, can be prepared and stabilized in dichloromethane at $-80\text{ }^\circ\text{C}$.⁹ This important development facilitates systematic spectroscopic studies of the factors which dictate the stability of such species. Herein are reported, for the first time, the RR spectra of two legitimate Compound I models, namely, the imidazole (Im) and 2-methylimidazole (2-MeIm)¹⁰ complexes of the ferryl π -cation radical derivative of iron(5,10,15,20-tetramesitylporphyrin, $[\text{O}=\text{Fe}(\text{TMP}^+)(\text{Im})]^+$ and $[\text{O}=\text{Fe}(\text{TMP}^+)(2\text{-MeIm})]^+$.

The samples were prepared using methods described previously,⁹ briefly stated as follows: $\text{Fe(III)TMP}(\text{ClO}_4)$ was dissolved in dichloromethane, cooled to $-80\text{ }^\circ\text{C}$, and oxidized with ozone^{6b,c} to generate $\text{O}=\text{Fe(IV)(TMP}^+)(\text{ClO}_4)$, which is a green-colored solution. Inasmuch as the perchlorate ligand is quite labile, it is easily displaced by titration with a cold solution ($-80\text{ }^\circ\text{C}$) of the nitrogenous base (Im or 2-MeIm), at which point the solution remains green and was checked by UV-vis electronic absorption spectrophotometry.⁹ While not reported previously, the NMR spectrum of this complex (pyrrole-H, -14.7 ppm; O-Me, 26.0 and 24.6 ppm; m-H, 71.5 and 70.3 ppm; p-Me, 11.1 ppm at -80°) is also consistent with formation of a ${}^2A_{2u}$ radical.⁹

The observed RR spectra are shown in Figure 1. The top two traces are those corresponding to the known compounds, $\text{Fe(III)TMP}(\text{ClO}_4)$ and $\text{O}=\text{Fe(IV)(TMP}^+)(\text{ClO}_4)$, in dichloromethane.^{5,6b,c} Upon oxidation, the heme core mode labeled ν_4 shifts to lower frequency and loses intensity in a manner consistent with previous observations of the general behavior of metalloporphyrin π -cation radicals.^{2,11} The mode designated as ν_2 is observed to shift to lower frequency, appearing as a weak feature at 1514 cm^{-1} , consistent with the formation of a ferryl porphyrin π -cation radical having a largely ${}^2A_{2u}$ ground state.^{2,11} In the low-frequency region, a relatively strong band appears at 835 cm^{-1} , its assignment to the $\nu(\text{Fe}=\text{O})$ stretching mode being confirmed by its shift to 799 cm^{-1} when the oxidation is carried out with ${}^{18}\text{O}_3$, as had been reported previously.^{6b,c}

As is seen from inspection of the bottom two traces in Figure 1, in the region above 1000 cm^{-1} , the general appearances of the RR spectra of the two π -cation radical complexes with nitrogenous bases are entirely consistent with the spectral behavior expected for a ferrylporphyrin π -cation radical, the ν_4 shifting from 1371 cm^{-1} in the top trace to 1354 and 1356 cm^{-1} in the bottom two traces, respectively. Similarly, it is seen in the bottom two traces that the ν_2 mode shifts down by about 40 cm^{-1} (to 1528 or 1529 cm^{-1}), compared to the value observed for the neutral macrocycle (top trace), behavior indicative of formation of a ${}^2A_{2u}$ ferrylporphyrin π -cation radical.^{2,5–8} It is also interesting to note that, for the two model compounds bearing a nitrogenous base trans-axial ligand, the ν_2 occurs at a slightly higher frequency than that observed for the ferrylporphyrin π -cation radical which contains the very weakly coordinating trans-axial ligand, ClO_4^- , that is, 1528 and 1529 cm^{-1} for the former two vs 1514 cm^{-1} for the latter. This behavior is consistent with previously documented RR spectral behavior of these ferrylporphyrin π -cation radicals, where it was noted that the ν_2 increases with the donor strength of the trans-axial base.^{6b}

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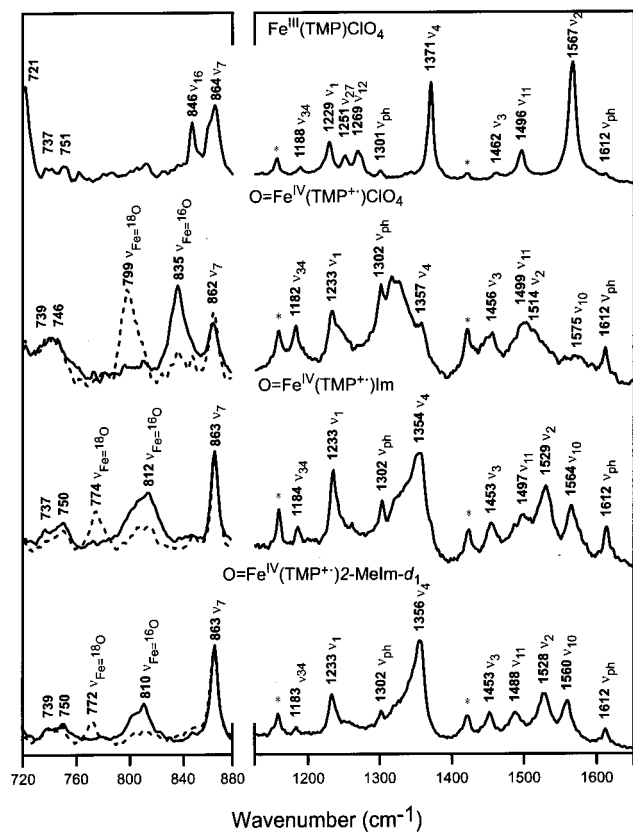


Figure 1. Resonance Raman spectra of Fe(III)TMP(ClO₄) and oxo-Fe(IV)(TMP) π -cation radical and its complexes with imidazole and 2-Melm-d1 at -80 °C in dichloromethane. Spectra were acquired with excitation by the 406.7 nm line of a Kr⁺ laser (~ 5 mW at sample) using equipment and methods described previously (8b). The concentration of iron porphyrin is 1 mM. In each panel, the top trace is the spectrum of the ferric porphyrin, the second trace is its oxo-iron(IV) π -cation radical, the third trace is the imidazole complex (1 molar equiv of imidazole) of the oxo-iron(IV) π -cation radical, and the fourth trace is the 2-Melm-d1 complex (1 molar equiv of 2-Melm-d1) of the oxo-iron(IV) π -cation radical. Dotted traces in the left panel represent the spectra of the ¹⁸O-isotopomers.

Axial ligand sensitivities for other macrocycle modes are not as firmly established from previous work.^{6b} Thus, ν_{10} shifted only slightly (about 5 cm⁻¹) for the chloride complex, relative to the perchlorate derivative, but by 10–15 cm⁻¹ for the nitrogenous bases studied here. As was noted previously,^{6b} the ν_3 mode is insensitive, as confirmed here. While the earlier studies involving the ligands, fluoride, chloride, perchlorate, and triflate^{6b} had not indicated a trend in the frequency of ν_{11} , the present study, employing the much stronger ligands, imidazole and 2-methyl imidazole, provides tentative evidence for sensitivity. Thus, this mode is observed at 1488 cm⁻¹ for O=Fe(TMP⁺)(2-Melm), 11 cm⁻¹ lower than for the ferric perchlorate complex. The apparent frequency (1497 cm⁻¹) of this mode for the O=Fe(TMP⁺)(Im) complex would seem to contradict this trend. However, we note that the ν_{11} mode is much more weakly enhanced in the spectra of the cation radicals than for the ferric complexes or for the neutral ferrylhemes.² Thus, even slight contamination by either of these two impurities, whose ν_{11} modes occur near 1500 cm⁻¹, could account for an apparently high frequency for the imidazole complex of the ferryl π -cation. High-frequency shoulders on the ν_4 and ν_{10} bands in the spectrum of this complex (corresponding to strong bands in the spectrum of the ferric complex in the top trace in Figure 1) also suggest a slight amount of this impurity. Thus, while there is some indication that ν_{11} may be sensitive to axial ligation in these ferryl cation radicals, further work will be needed to clarify this point. In any case, other regions of the

spectrum are less susceptible to spectral contamination by small amounts of these impurities (i.e., the ν_4 and ν_2 bands of these impurities are exceptionally strong).

In the lower-frequency region of the RR spectra of the two Compound I models (bottom two traces), the $\nu(\text{Fe}=\text{O})$ stretching mode is observed at 812 or 810 cm⁻¹ as a somewhat broadened, moderately strong band, which is apparently overlapped by a low-frequency mode of the ferrylporphyrin π -cation macrocycle. When ¹⁸O₃ is employed as the oxidant (dashed lines), a new band appears at 774 and 772 cm⁻¹, assigned as the $\nu(\text{Fe}=\text{O})$, while the stronger components of the broadened bands at 812 and 810 cm⁻¹ disappear, revealing the underlying macrocycle modes. While exact frequencies cannot be precisely determined for the $\nu(\text{Fe}=\text{O})$ modes, owing to this overlap, it is noted that the estimated ($\Delta^{16}\text{O}/^{18}\text{O}$) shifts of 38 cm⁻¹ are in quite reasonable agreement with that expected (36 cm⁻¹).

The results obtained here provide, for the first time, the RR spectrum of a ferrylporphyrin π -cation radical, wherein the coordinated ligand trans to the ferryl fragment is physiologically relevant.

The $\nu(\text{Fe}=\text{O})$ frequencies observed here are substantially higher than those observed for the Compound I and II intermediates of peroxidases and other heme proteins; for example, for horseradish peroxidase compound II this mode appears between 776 and 789 cm⁻¹, depending on pH.^{2–4} Two factors can account for this difference between the models and the enzyme intermediates. Here the trans-axial imidazole bases are not hydrogen bonded, whereas in the peroxidases the proximal histidyl imidazole is strongly H-bonded to an aspartate or other proton-accepting residue, an interaction which imparts a high degree of imidazolate character, increasing the donor strength and lowering the $\nu(\text{Fe}=\text{O})$ frequency.^{1,2} Furthermore, it is well established^{1–4} that the ferryl fragment in the enzyme intermediates can interact with distal pocket H-bond donors to lower the $\nu(\text{Fe}=\text{O})$ frequency, whereas no such H-bond donors are present in the model systems being studied here.

One of the essential issues which has not been possible to address until now is the isolated effect of macrocycle oxidation upon the frequency of the $\nu(\text{Fe}=\text{O})$ stretching mode. In the past, attempts to address this question have been frustrated by the inability to maintain constant ligation upon oxidation, because of the tendency for the neutral ferrylporphyrins to remain five coordinate or add a neutral ligand in the trans position and the propensity for the ferrylporphyrin π -cation radicals to add an anionic trans-axial ligand to neutralize the excess positive charge. Thus, the present study provides the first opportunity to compare the RR spectrum of a neutral parent ferrylporphyrin with the oxidized π -cation radical bearing a similar trans-axial ligand. The previously reported $\nu(\text{Fe}=\text{O})$ frequencies for the Compound II models, bearing *N*-methyl-imidazole, occur near 820 cm⁻¹ in toluene.^{2b} While the present study was conducted in CH₂Cl₂, this difference in solvent is expected to account for only about 4 cm⁻¹; that is, the $\nu(\text{Fe}=\text{O})$ frequency of the Compound II models would be expected to occur near 816 cm⁻¹ in CH₂Cl₂.² Such considerations would suggest that the isolated effect of macrocycle oxidation on the Fe=O fragment of these models is small, the observed shift in $\nu(\text{Fe}=\text{O})$ being only about 4–6 cm⁻¹ to lower frequency. Further studies, employing other trans-axial bases and solvents of differing polarity and H-bonding capabilities, will soon be undertaken in order to properly calibrate the sensitivity of this key $\nu(\text{Fe}=\text{O})$ stretching frequency.

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