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Resonance Raman Study of a High-valent Fe=O Porphyrin Complex as a Model for Peroxidase Compound II

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Resonance Raman spectroscopy is applied to a Fe^{IV} oxo porphyrin with imidazolate as the axial ligand. The $\nu_{Fe=O}$ mode is observed at 792 cm^{-1}, which is 23 cm^{-1} lower than that of the analogous 1–methylimidazole complex and similar to that of horseradish peroxidase compound II (787 cm^{-1}) at alkaline pH, for which presence of an anionic histidine was previously postulated. This study thus provides a useful model compound of horseradish peroxidase compound II.

Horseradish peroxidase (HRP) catalyzes the oxidation of organic substrates using H_2O_2 as a specific oxidant. Upon reaction with H_2O_2, HRP sequentially forms two reaction intermediates known as compound I and compound II, before returning to the original ferric state. Compound I and compound II are respectively 2 and 1 oxidative equivalents higher than the Fe^{III} state,[1] and respectively correspond to the Fe^{V} and Fe^{IV} formal oxidation states. Compound II of HRP exhibits a $\nu_{Fe=O}$ resonance Raman (RR) band at 775 and 787 cm^{-1} at pH 7 and 11, respectively.[2] The 12 cm^{-1} upshift of the $\nu_{Fe=O}$ mode of HRP compound II upon alkalization is caused by elimination of the hydrogen bond between the oxygen atom and the distal His and is regarded as a "distal effect." The $\nu_{Fe=O}$ mode of the Fe^{IV} state of myoglobin (Mb)[3] at pH 8.5 is located at ca. 800 cm^{-1} (sperm whale Mb at 797 cm^{-1} and horse heart Mb at 804 cm^{-1}), where no hydrogen bond exists between the oxygen atom and the distal His. There is a 13 cm^{-1} frequency difference in the $\nu_{Fe=O}$ mode between HRP at 787 cm^{-1} and Mb at 800 cm^{-1}. This has been interpreted as a result of a "proximal effect." Both HRP and Mb have a proximal His residue.[4,5] However, the proximal His of HRP has the anionic character of imidazolate (Im^-), while the proximal His of Mb is considered to be neutral.

Many Fe=O porphyrin model complexes with 1-methylimidazole (1MeIm) or a solvent molecule acting as the axial ligand have been prepared and characterized by RR spectroscopy,[6] in order to obtain insights into the electronic structures and reactivities of hemoproteins. However, there have been no reports of model complexes with Im^- as a *trans* axial ligand. In the present study, we have prepared an Fe=O porphyrin model complex with Im^- as the axial ligand and identified the $\nu_{Fe=O}$ mode at 792 cm^{-1}, which is significantly lower than that of an analogous complex with 1MeIm as the axial ligand (815 cm^{-1}). Thus, the imidazolate complex could be regarded as a model for compound II of HRP. The experimental details are described in Supporting Information.[7]

Figure 1 depicts absorption spectra of (TMP)[8](Fe^{IV}=O) (A), +1MeIm (B), and +Im^- (C). (TMP)(Fe^{IV}=O) gives absorption maxima at 416 and 543 nm. (TMP)(Fe^{IV}=O)(1MeIm) has absorption maxima at 425, 556, 569, and 604 nm. (TMP)(Fe^{IV}=O)(Im^-) has absorption maxima at 423, 558, 566, and 604 nm. The absorption spectra of the 6-coordinate complexes (Figures 1B and 1C) are similar to each other and distinct from that of the 5-coordinate complex (Figure 1A).

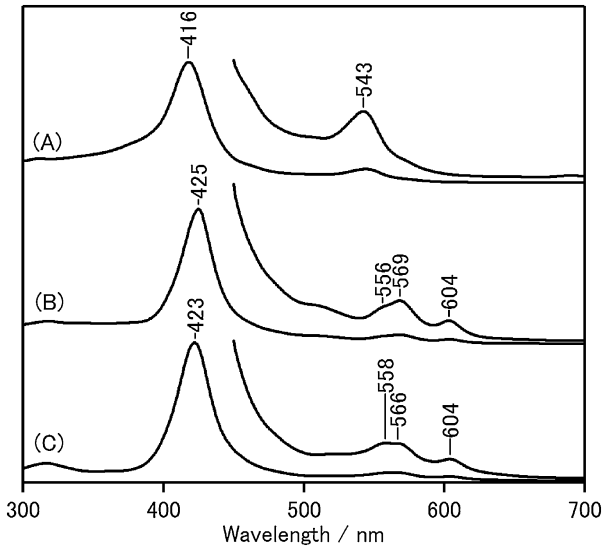

Figure 1. Absorption spectra of (TMP)(Fe^{IV}=O) in the absence and presence of trans ligand at −40 °C in chlorobenzene. (A) (TMP)(Fe^{IV}=O), (B) (TMP)(Fe^{IV}=O)(1MeIm), and (C) (TMP)(Fe^{IV}=O)(Im^-). The concentration of TMP was 0.2 mM in the presence of 0, 0.2, and 0.4 mM ligand for (A), (B), and (C). Expanded spectra in the visible region are for 1 mM TMP in the presence of 0, 1, and 2 mM ligand for (A), (B), and (C).

Figure 2 depicts RR spectra of (TMP)(Fe^{IV}=^{16}O) (A), (TMP)(Fe^{IV}=^{16}O)(1MeIm) (B), and (TMP)(Fe^{IV}=^{16}O)(Im^-) (C). Figures 2A′, 2B′, and 2C′ are obtained for the corresponding ^{18}O-complexes and Figures 2A″, 2B″, and 2C″ are the isotopic difference spectra (see the legend). RR difference spectra designated by A″, B″, and C″ exhibit the $\nu_{Fe=O}$ mode at 841/809, 815/778, and 792/760 cm^{-1}, respectively, for the ^{16}O/^{18}O complex. Upon addition of 1MeIm, the $\nu_{Fe=O}$ mode at 841 cm^{-1} for the 5-coordinate species (Figure 2A) becomes downshifted to 815 cm^{-1} (Figure 2B). This downshift is 5 cm^{-1} lower than the downshift observed for the same complex in toluene.[6] Upon addition of Im^-, instead of 1MeIm, the $\nu_{Fe=O}$ mode downshifts further to 792 cm^{-1} by 23 cm^{-1} (Figure 2C). Notably the pattern and the frequency of the porphyrin vibra-

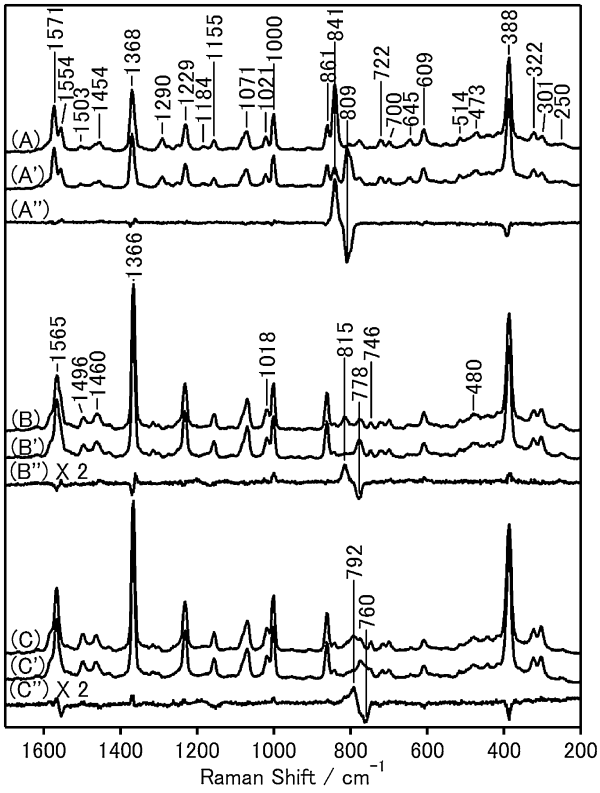

Figure 2. RR spectra of (TMP)(Fe^{IV}=O) in the absence and presence of trans ligand at −40 °C in chlorobenzene. (A) (TMP)(Fe^{IV}=O), (B) (TMP)(Fe^{IV}=O)(1MeIm), and (C) (TMP)(Fe^{IV}=O)(Im^-). The concentration of TMP was 1 mM. The concentration of ligand was 0, 1, and 2 mM for (A), (B), and (C). Spectra (A), (B), and (C) were obtained for ^{16}O-complexes. Spectra (A′), (B′), and (C′) for ^{18}O-complexes. Spectra (A″), (B″), and (C″) are isotopic difference spectra (^{16}O–^{18}O: A″ = A–A′, B″ = B–B′, and C″ = C–C′).

tional modes obtained for (TMP)(Fe^{IV}=O)(1MeIm) and (TMP)(Fe^{IV}=O)(Im^-) are virtually identical (Figures 2B and 2C), indicating that structural differences in the porphyrin core are essentially negligible. On the other hand, the difference of these two complexes is prominent in the $\nu_{Fe=O}$ mode. Since each of the $\nu_{Fe=O}$ frequencies in Figures 2B and 2C were unchanged for the ligand concentration between 0.25 and 10 mM (data not shown), it is not likely that a direct interaction of the added ligand with the oxygen atom causes the downshift in the $\nu_{Fe=O}$ mode. The $\nu_{Fe=O}$ mode of (TMP)(Fe^{IV}=O)(Im)[8] is at 792 cm^{-1}, and addition of tetrabutylammonium hydroxide, which is expected to remove the NH proton to generate Im^-, does not change the frequency (data not shown).

Downshift of the $\nu_{Fe=O}$ mode upon transformation from the 5- to 6-coordinate complex by ligation of 1MeIm is caused by electron donation from the nitrogen atom of 1MeIm to Fe, which reduces donation from the oxo ligand, resulting in the weakening of the Fe=O bond.[6] The lower $\nu_{Fe=O}$ frequency of HRP compound II (787 cm^{-1}) relative to the Mb ferryl state (800 cm^{-1}) has been interpreted as a result of larger electron donation from His, whereas the proximal His of HRP has Im^- character. The ν_{Fe-His} modes of HRP[9] and Mb[10] in the Fe^{II} state are located at 244 and 220 cm^{-1}, respectively, and the higher frequency for HRP is also due to the Im^- character of its proximal His.[9] The X-ray structure of HRP[4] revealed the presence of a hydrogen bond between the NH of the distal His170 and the main chain C=O of Asp247, which gives anionic character to His170. For the model compounds, the ν_{Fe-His} modes[9,11] of Fe^{II}(OEP)(2MeIm$^-$)[8] and Fe^{II}(OEP)(2MeIm)[8] are located at 233 and 208 cm^{-1}, consistent with the trend identified in the proteins. An inverse relationship between the $\nu_{Fe=O}$ and the ν_{Fe-His} frequencies, has been pointed out.[6a] This indicates that the anionic character of the proximal His is common for the Fe^{II} and Fe^{IV} states.

In conclusion, the present results demonstrate that the Im^- ligand actually lowers the $\nu_{Fe=O}$ frequency relative to that of neutral 1MeIm ligand and that the (TMP)(Fe^{IV}=O)(Im^-) could serve as a model complex for compound II of HRP. Since the $\nu_{Fe=O}$ frequency is identical for complexes of (TMP)(Fe^{IV}=O) + Im^- and (TMP)(Fe^{IV}=O) + Im systems, it is deduced that the NH proton of Im is removed upon ligation to Fe^{IV}. The high basicity of the oxygen atom may contribute to the deprotonation. It is also likely that the high valent state of Fe significantly lowers the pK_a of the NH proton of Im. These characteristics are expected to be important for proper functioning of certain biological systems, since formation of high-valent iron complexes such as compound II in the protein during enzyme catalysis reaction could be communicated to the proximal side via this mechanism.

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7 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.
8 Abbreviations: Im; imidazole, OEP; octaethylporphyrin dianion, TMP; tetramesitylporphyrin dianion, 2MeIm; 2-methylimidazole, 2MeIm$^-$; 2-methylimidazolate.
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