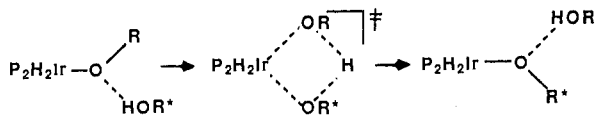
To gauge the effect of increased coordination number on bond length, note that the Ir-P distance increases only 0.04 Å upon addition of CO.[13,14]

2. The reactivity of $IrH_2(OR)(PCy_3)_2$ is high, consistent with it being unsaturated either in the ground state or in a thermally accessible excited state (eq 1). Thus, it adds CO under mild conditions (within time of mixing at <1 atm, 25 °C) to give *cis,trans*-$IrH_2(OR)CO(PCy_3)_2$.[15]

3. Addition of stoichiometric water to $IrH_2(OR)(PCy_3)_2$ also gives an adduct. On the basis of the minimal change in the hydride chemical shift (0.55 ppm), we conclude that this adduct involves only hydrogen bonding of water to the alkoxide oxygen.[16,17] Consistent with weak bonding of this type, exposure of $IrH_2(OR)(PCy_3)_2 \cdot H_2O$ to vacuum (1 h) during and after removal of solvent furnishes $IrH_2(OR)(PCy_3)_2$ again. It is noteworthy that there is no evidence for conversion to $IrH_2(OH)(PCy_3)_2$. Proton NMR monitoring of a titration of $IrH_2(OR)(PCy_3)_2$ with the *alcohol* ROH shows rapid exchange (i.e., a single coalesced OCH_2 signal) at 25 °C.[15] Although this process may be initiated by formation of a hydrogen bond to uncoordinated alcohol (A), we suggest that formation of an Ir-O bond to the incoming alcohol (B) is the next step. This is why $IrH_2(OR)CO(P^iPr_3)_2$ does not



A B

exchange rapidly with free ROH.[8] Intermediate or transition-state B requires conversion of the original π-donor alkoxide in A to a simple σ-donor mode of bonding, which simultaneously makes that oxygen a better proton acceptor.

4. Goldman and Halpern demonstrated[8] the rapid conversion shown in eq 3. We suggest that this heterolytic splitting of the

$$IrH_2(OR)(P^iPr_3)_2 + 2H_2 \rightarrow IrH_5(P^iPr_3)_2 + ROH \quad (3)$$

first mole of H_2 consumed in this reaction proceeds via an H_2 adduct $Ir(H)_2(H_2)(OR)(P^iPr_3)_2$, in which the alkoxide oxygen is more electron rich (i.e., minimal π-donation to Ir) and thus well suited to accept H^+ from the Bronsted acidic η^2-H_2 ligand. This Ir-O hydrogenolysis also occurs within the time of mixing for $IrH_2(OR)(PCy_3)_2$, and we are studying this mechanistic proposal with further experiments.[18]

It is generally true that chloro compounds and hydride/chloro compounds are frequently isolable as unsaturated species, while unsaturated polyhydrides devoid of π-donor ligands are almost never isolable. For example, compare[1] the formulas $ReCl_4P_2$ with ReH_7P_2; $ReCl_3P_3$ with ReH_5P_3; $OsCl_4P_2$ with OsH_6P_2; $OsCl_3P_3$ with OsH_4P_3; $RuH_3Cl(PCy_3)_2$[20] with $RuH_6(PCy_3)_2$; $IrHCl_2P_2$,[19] $IrH_2Cl_2P_2$,[21] and IrH_2ClP_2[19] with IrH_5P_2. On the basis of our structural results with alkoxide as the coligand, we propose that this trend exists because any unsaturation is moderated by the presence of the (weak) π-donor chloride ligands.[22] More importantly however, this hypothesis seems to translate into a valuable method for enhancing the reactivity of transition-metal polyhydrides. While π-donation by alkoxides is well established toward early transition metals,[23] the same is not true for the late transition elements.[24] Indeed, it has been concluded[24] that "...there are no significant π interactions between...alkoxide...ligands and late-transition-metal centers...." While this may be true for four-coordinate Rh^I and Pd^{II} or Pt^{II}, the evidence we present here indicates significant alkoxide π-donation to Ir^{III}. We propose that the difference originates in the higher electrophilicity of the higher valent metal, as well as the presence of a more suitable acceptor orbital on a five-coordinate d^6 complex than on a four-coordinate d^8 species.

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Supplementary Material Available: Listing of fractional coordinates and spectroscopic data for $IrH_2(OCH_2CF_3)(PCy_3)_2$ and $IrH_2(OCH_2CF_3)CO(PCy_3)_2$ (3 pages). Ordering information is given on any current masthead page.

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Evidence for a Weak Mn=O Bond and a Non-Porphyrin Radical in Manganese-Substituted Horseradish Peroxidase Compound I

Robert J. Nick, Gigi B. Ray, Kenneth M. Fish, Thomas G. Spiro,* and John T. Groves*

Department of Chemistry, Princeton University Princeton, New Jersey 08544

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We have recently described the isolation and characterization of several oxomanganese(IV) porphyrin complexes.[1] Interest in these complexes derives from the activity of manganese porphyrins as oxidation catalysts[2] and as comparators for the biologically significant iron heme derivatives.[3] These synthetic manganese(IV) species exhibit the characteristic EPR spectra of an $S = {}^3/_2$ Mn(IV) spin system, and surprisingly, the resonance Raman (RR) and IR spectra revealed an unusually weak Mn=O stretching frequency in the range of 711-757 cm^{-1} depending on the trans-axial ligand.[1b,c] We have ascribed this feature to the unique high-spin d^3 electronic state of these complexes. Oxidized forms of manganese-substituted horseradish peroxidase[4,5] (MnHRP) and

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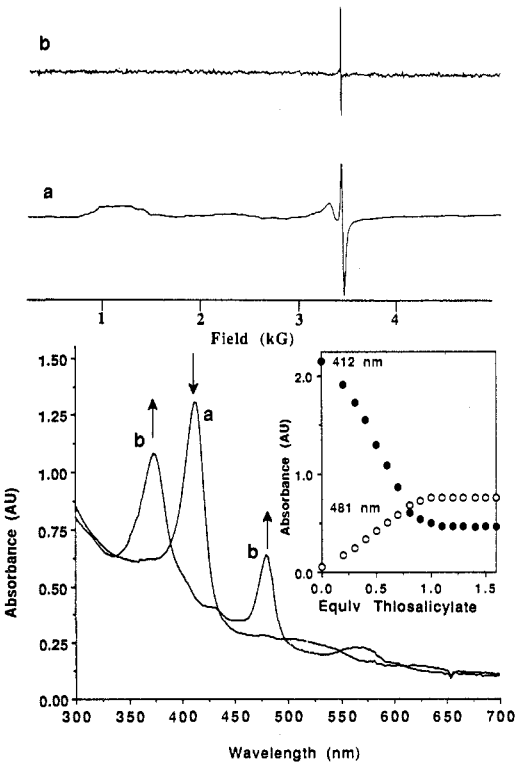

Figure 1. Upper panel: (a) X-band EPR of 800 μM MnHRP compound I in phosphate buffer pH 7.2 generated by the reaction of resting MnHRP with 1 equiv of mCPBA. (b) X-band EPR of MnHRP compound I which has been reduced with 1 equiv of sodium thiosalicylate. Lower panel: (a) Visible spectrum of 6.5 μM MnHRP compound I generated by oxidation of resting MnHRP with 1 equiv of mCPBA. (b) Visible spectrum of 6.5 μM MnHRP compound I which has been reduced with 1 equiv of sodium thiosalicylate, yielding an Mn(III) species. Visible spectra both taken in 0.1 M phosphate buffer at pH 7.2. Inset: Visible titration of 1 mL of 10.3 μM MnHRP compound I with thiosalicylate.

manganese-substituted cytochrome *c* peroxidase[5] have also been reported. However, the axial ligand in oxidized MnHRP has been suggested[6] to have a hydroxo rather than an oxo formulation. We describe here the generation, isolation, and characterization of compound I of MnHRP. RR and EPR data support an oxomanganese(IV)–protein free radical configuration for this species.

Mn^{III}HRP was prepared from apoHRP by reconstitution with 1 equiv of Mn^{III}PPIX.[4] Oxidation of Mn^{III}HRP with *m*-chloroperoxybenzoic acid (mCPBA) was evidenced by isosbestic changes in the visible spectrum and the appearance of an oxidized intermediate, MnHRP-I (λ_{max} = 412 nm), after the addition of 1.0 molar equiv of the oxidant. Samples of MnHRP-I prepared in this way were stable enough to be isolated by Sephadex chromatography at 4 °C. The EPR spectrum of isolated MnHRP-I or samples prepared in situ upon addition of 1.2 equiv of mCPBA displayed signals at g 5.25 and 2.08 characteristic of manganese(IV) and a sharp free-radical feature at g 2.00 (Figure 1a, top). Titration of MnHRP-I to Mn^{III}HRP required 2 equiv of thiosalicylate. The first equivalent eliminated the Mn(IV) signals in the EPR spectrum (Figure 1b, top) and restored the characteristic split Soret visible spectrum of the manganese(III) enzyme (Figure 1b, bottom). However, a second equivalent of thiosalicylate added in several aliquots gradually titrated the g 2.00 EPR signal. Thus, the two oxidation equivalents of the peroxy-acid converted Mn^{III}HRP to Mn(IV) *and* produced a protein free radical. By contrast, we corroborate the finding of Yonetani et al.[4] that only a weak g 2 signal is observed when

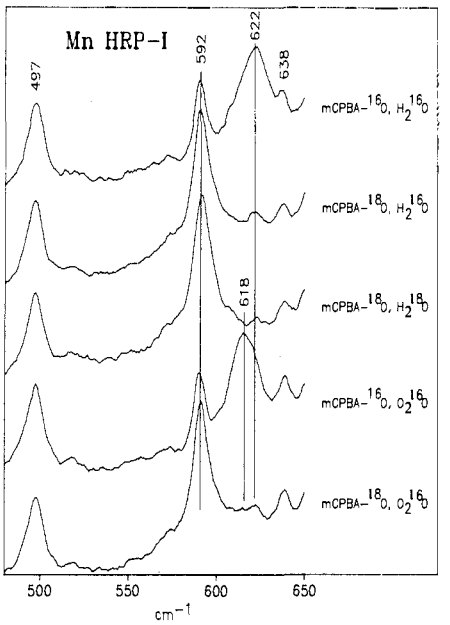

Figure 2. Resonance Raman spectra of Mn-substituted horseradish peroxidase compound I, obtained by oxidation of Mn^{III}HRP with 1.2 equiv of mCPBA. Isotopic substitution of the oxidant (^{16}O- or ^{18}O-containing mCPBA) and the solvent (0.1 M phosphate buffer, pH 7.2 in $H_2{}^{16}O$, $H_2{}^{18}O$, or $D_2{}^{16}O$) is indicated in the figure. Spectra were obtained with 413.1-nm excitation (Coherent Innova 100-K3) by using <10-mW power incident on a stirred sample of 800 μM protein, kept at 5 °C. Total accumulation time was 160 s/spectrum, collected on a SPEX 1877 triplemate monochromator with intensified diode array detection.

Mn^{III}HRP is oxidized with hydrogen peroxide.

Significantly, there was no evidence in the visible spectrum or high-frequency RR spectrum of MnHRP-I of a porphyrin cation radical. Accordingly, the second oxidation equivalent in MnHRP-I has been shifted from the porphyrin ring, as in FeHRP-I,[7] to an amino acid residue reminiscent of compound I of FeCCP.[8] The peroxy-acid oxidation of Mn(III) porphyrins has been shown to produce a fleeting intermediate which has been ascribed an oxomanganese(V) formulation rather than oxomanganese(IV) porphyrin cation radical.[9] We suggest that manganese(IV) has stabilized the porphyrin HOMO relative to that of the iron(IV) complex both in the model system and in MnHRP. We attribute this change to porphyrin → manganese d_π electron donation for which one finds evidence in the large upfield shift of the pyrrole proton resonances in the ^2H NMR spectrum of oxomanganese(IV) porphyrins.[1b,c]

Figure 2 shows a segment of the 413.1-nm-excited RR spectrum of MnHRP, to which 1.2 equiv of mCPBA has been added. A band at 622 cm^{-1} in this spectrum disappears when mCPBA-^{18}O was used, and the missing intensity appears under the nearby porphyrin mode at 592 cm^{-1}. The observed downshift of ca. 30 cm^{-1} is consistent with the 28-cm^{-1} downshift, calculated for an Mn–O diatomic oscillator. Oxidation with mCPBA-^{18}O yields the same shifts in $H_2{}^{16}O$ and $H_2{}^{18}O$, showing that the Mn-bound oxygen does not exchange with solvent. Model O=Mn^{IV} porphyrin complexes likewise show slow oxo exchange with H_2O.[1a] An ^{18}O-sensitive band near 630 cm^{-1} has also been observed by Makino et al.[6] in the RR spectrum of MnHRP upon treatment with H_2O_2. They reported an 18-cm^{-1} shift to lower frequency in D_2O and assigned the band to the Mn–OH stretch of bound hydroxide. This result was obtained from a rather noisy difference spectrum, however, and our spectrum in D_2O, which is stronger and better resolved, clearly shows that the D_2O downshift is only 4 cm^{-1}. This shift is insufficient to support assignment to bound

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hydroxide, which would require greater than 10 cm^{-1} D_2O sensitivity. *We assign the band instead to an Mn=O manganyl stretch, with the small D_2O downshift reflecting involvement of H-bonding from a distal protein residue.*

The dramatic 135-cm^{-1} lowering of this frequency relative to the typical value in 5-coordinate Mn^{IV}=O porphyrins[1b] is attributed to the imidazolate character of the proximal histidine ligand in HRP[10] and to strong distal H-bonding, possibly from Arg 38, which is indicated by NMR analysis to lie above pyrrole ring III.[11] For compound II of FeHRP, the H-bonding group is believed to be a distal histidine because the Fe^{IV}=O stretching RR band shifts to a higher frequency as the pH is raised through 6.9 (isoenzyme A) or 8.8 (isoenzyme C).[10] In FeCCP compound I, which is insensitive to pH (4-8), strong H-bonding to distal Arg 48 is most likely responsible for its 22-cm^{-1}-lower Fe^{IV}=O stretching frequency than that of neutral pH FeHRP-II.[12] We have found no change in the RR spectrum of MnHRP-I (isoenzyme C) between pH 6.4 and 10.5. Perhaps the enhanced basicity of Mn^{IV}=O, a consequence of its weak bond, induces a switch in the H-bond donor group in MnHRP-I.

Thus, compound I of MnHRP is shown to be an oxomanganese(IV) protein radical species with an unusually weak but exchange-resistant Mn=O bond.

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First Observation of Alkene Radical Anions by Electron Spin Resonance Spectroscopy: Electron and Hole Trapping in Hexene/*n*-Hexane Mixed Crystals Irradiated at 4.2 K

Hachizo Muto,* Keichi Nunome, and Kaoru Matsuura

Government Industrial Research Institute, Nagoya Hirate-cho, Kita-ku, Nagoya 462, Japan
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In the present work, we report the first observation of alkene radical anions and their geometrical and electronic structures clarified by electron spin resonance (ESR) spectroscopy. Recently, considerable interest has been taken in the electronic structures of ethylene and alkene radical cations.[1-7] Alkene radical anions may play an important role in the radiolysis of organic compounds as well as the cations. However, the anions have never been observed by ESR and optical spectroscopies, because they have very large negative electron affinities (EA ≈ -2.3 eV[8]) and are unstable. Previously, we found that the radiation-induced electrons were trapped in the defects of the mixed crystals of binary *n*-alkanes with different molecular chains.[9] This observation predicted the possibility of formation of the alkene anions by scavenging the electrons, if the mixed crystals of alkene and alkane were irradiated at a very low temperature. Irradiation of the mixed crystals at 4.2 K has led to the successful ESR detection of the radical anions together with the corresponding cations.

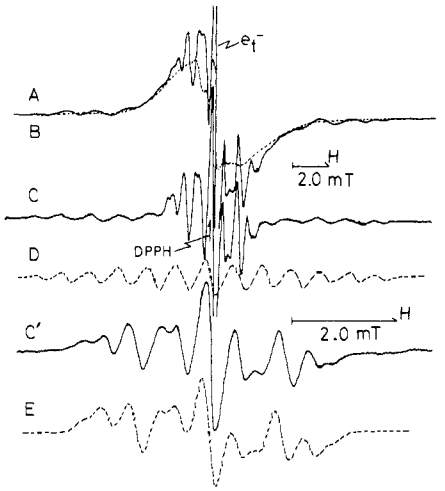

Figure 1. The first-derivative absorption ESR spectra observed for *trans*-3-hexene (4.0 mol %)/*n*-hexane-d_{14} mixed crystals (A) irradiated at 4.2 K and (B) after illuminated by visible light. (C) The difference spectrum obtained by subtracting spectrum B from spectrum A. (C') The expanded spectrum of the difference spectrum observed for the *trans*-3-hexene (8.0 mol %)/*n*-hexane-d_{14} mixed crystals. The ESR spectral simulations for the *trans*-3-hexene (D) cation and (E) anion radicals.

Figure 1A shows the first-derivative absorption ESR spectrum observed for *trans*-3-hexene (4.0 mol %)/*n*-hexane-d_{14} mixed crystals irradiated at 4.2 K by X-rays (45 kV, 40 mA, for 15 min). Figure 1B is the remaining ESR spectrum after photobleaching with visible light. It was the same spectrum as that observed for the irradiated pure *n*-hexane-d_{14}. The difference spectrum obtained by subtracting the spectrum in Figure 1B from that in Figure 1A is shown in Figure 1C, indicating that two kinds of photobleachable radicals were formed from the solute 3-hexene molecules in addition to a small amount of trapped electrons. The trapped electrons were no longer produced by increasing the solute concentration to 8.0 mol % (Figure 1C'). A similar difference ESR spectrum was obtained in the protiated hexane matrix except for slightly broader line widths. One species gives a wide resonant magnetic field spread over $\sim$18 mT, and the other has a narrow spread of $\sim$5 mT. The double integration of the difference spectrum indicated that the amounts of the two species were nearly equivalent. The former species was assigned to the 3-hexene radical cation by the spectral simulation, which is shown in Figure 1D. The ESR parameters obtained are listed in Table I. Two pairs of C-H β-proton hyperfine couplings are 4.6 and 2.9 mT, and a pair of C-H α-proton couplings is 1.3 mT. The cations are known to be stabilized by hyperconjugation and to have large β-proton hyperfine couplings compared with neutral delocalized radicals.[1-7] The large size of the proton hyperfine couplings observed in the present study agrees well with those reported for alkene cations[1-5] and supports the assignment.

The other species gives a strong seven-line ESR spectrum at the center in Figures 1C and 1C'. This species would be assigned to the 3-hexene radical anion, since it was formed as the counter radical to the 3-hexene cation with an equivalent yield and since both radicals were simultaneously photobleached. This assignment was confirmed by a competitive reaction of electron capture between 3-hexene and 1-chloro-*n*-hexane-h_{13} molecules in the C_6D_{14} matrices. Instead of the 3-hexene anion, the chain-end alkyl radical (CH_2CH_2-C_4H_9) was selectively formed by dissociative electron attachment to the 1-chlorohexane molecules along with the 3-hexene cation.[10] The ESR spectral simulation of the anion is shown in Figure 1E, and the ESR parameters obtained are listed in Table I. The shoulders of the outermost lines of the spectrum

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