ENDOR Study of Oxoiron(IV) Porphyrin π-Cation Radical Complexes as Models for Compound I of Heme Enzymes

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Electron nuclear double resonance (ENDOR) spectroscopy of oxoiron(IV) porphyrin π-cation radical complexes with pyrrole β-substituted porphyrins is reported. Hyperfine coupling constants (A^{H}) of the pyrrole β-methyl groups were determined. Comparison of the A^{H} values with those of compound I of peroxidase and catalase indicated the a_{1u} porphyrin π-cation radical states of these compound I.

Oxoiron(IV) porphyrin π-cation radical species is a reaction intermediate in the catalytic cycles of heme-containing peroxidases and catalases.[1] This intermediate, formally two oxidation equivalents above the ferric porphyrin resting state, is commonly known as compound I.[1] A similar compound I intermediate has been proposed as an active oxygen atom donor in catalytic cycles of cytochromes P-450.[2] For over a decade, because of the importance in biological functions, the electronic states of compound I have been studied by several physicochemical methods, such as electronic absorption, NMR, resonance Raman, EPR, MCD, Mössbauer, and EXAFS spectroscopy. However, our understanding of the relationship between electronic structure and reactivity of compound I remains incomplete.

Electron nuclear double resonance (ENDOR) spectroscopy is a powerful tool to determine the electronic state of compound I. Roberts et al. first reported ^{1}H and ^{14}N ENDOR spectra of compound I of horseradish peroxidase (HRP), which suggested the a_{2u} porphyrin π-cation radical state of HRP compound I.[3] Moreover, the ^{17}O ENDOR signals of HRP compound I allow estimation of spin density on the iron-bound oxo ligand.[4] Benecky et al. showed EPR and ^{1}H ENDOR spectra of compound I of *Micrococcus lysodeikticus* Catalase (*Ml*-CAT),[5] which differ from those of HRP, suggesting a different porphyrin π-cation radical state. Recently, ENDOR spectra of compound I of chloroperoxidase (CPO) were reported.[6] Analysis of hyperfine coupling constants indicated that spin delocalization to the axial thiolate ligand is not so large; $\rho_S < 0.23$. While these ENDOR studies clearly demonstrate that ENDOR spectroscopy provides considerable insight into the relationship between the electronic structure and reactivity, application of ENDOR spectroscopy to compound I has been limited. This is due to absence of ENDOR spectral data of compound I model complexes, which are expected to provide a direct probe for the ENDOR assignment of compound I. Here we report the first ENDOR spectroscopy of compound I model complexes, oxoiron(IV) porphyrin π-cation radical complexes.

Because of similarity in the porphyrin structure to those of naturally occurring compounds, we utilized oxoiron(IV) porphyrin π-cation radical complexes with the pyrrole β-substituted porphyrins, **1**–**4**, shown in Figure 1. The electronic structures of **1**–**4** were characterized by absorption, ^{1}H NMR, and EPR spectroscopy, which showed the a_{1u} porphyrin π-cation radical state with weak ferromagnetic coupling with the ferryl iron spins.[7] The EPR spectrum of **1** in dichloromethane–toluene–methanol shows perpendicular and parallel components of the ESR signal at $g = 3.6$ and 2.0 (Figure S1),[8] which was identical to our previous result in dichloromethane–methanol (5:1).[7] Moreover, the EPR spectrum of **1** was close to those of compound I of ascorbate peroxidase, lignin peroxidase, and *Ml*-CAT.[5,9] Figure 2a shows the ENDOR spectrum of **1** with magnetic field set at 324.4 mT, where the peak of the parallel component ESR signal is. The signals, located in the frequency range of 12–16 MHz, result from ^{1}H ENDOR signals, which exhibit a pair of ENDOR lines separated by a hyperfine coupling constant, A^{H}, and mirrored about the proton Larmor frequency, ν_H (13.9 MHz). We can identify at least two sets of magnetically distinguishable ^{1}H ENDOR resonances in Figure 2b. To assign these ENDOR signals, we measured the ENDOR spectrum of meso-d_4-labeled **1** (Figure 2c). The ENDOR signals for the meso proton were assigned at 13.7 and 14.1 MHz from a difference spectrum between Figures 2b and 2c (see Figure S2).[8] A pair of signals around 12.5 and 15.3 MHz was assigned to the pyrrole β-methyl proton and the other pyrrole β-mesityl signals were in the peaks from 13.5 to 14.3 MHz. Therefore, hyperfine-coupling constants (A^{H}) of the meso proton and the pyrrole β-methyl protons were estimated to be 0.4 and 2.7 MHz, respectively, which are consistent with the a_{1u} radical state as previously assigned.[7]

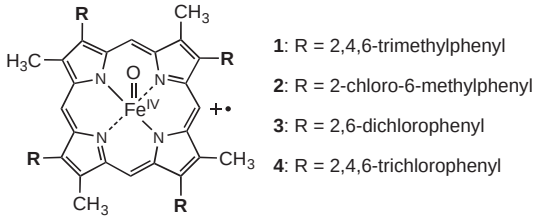

Figure 1. Structures of oxoiron(IV) porphyrin π-cation radical complexes used in this study.

We examined ^{1}H ENDOR measurements of **1** at different magnetic field sets, but the ENDOR signal was not detected. Moreover, we could not observe ^{14}N ENDOR for **1**. To examine ^{17}O ENDOR spectroscopy, the oxo ligand of **1** was labeled with 40% ^{17}O-enriched *m*-chloroperoxybenzoic acid. However, we have not been able to observe ^{17}O ENDOR. These may be due to insufficient saturation of the EPR signal of **1** at 4 K (Figure S1).[8]

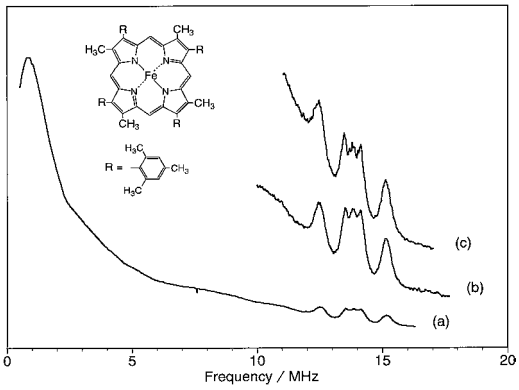

Figure 2. (a) ENDOR spectra of **1** at 4 K. (b) Expansion of ^{1}H ENDOR region of (a). (c) ^{1}H ENDOR region of meso-d_4-labled **1**.

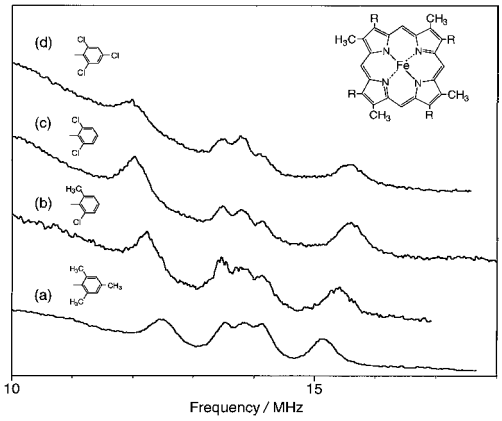

Figure 3. ^{1}H ENDOR spectra of (a) **1**, (b) **2**, (c) **3**, and (d) **4** at 4 K.

To investigate the electron-withdrawing effect on the spin density, we measured ENDOR spectra of **2**–**4** at 4 K with magnetic field set at 324.4 mT (Figure 3) and the hyperfine-coupling constants for **1**–**4** are summarized in Table 1. Interestingly, the A^H value for the pyrrole β-methyl proton increases with increasing electron-withdrawing effect of the pyrrole β-substituent, **1** < **2** < **3** < **4**. This is consistent with a previous ^{1}H NMR study, in which paramagnetic down field shift of the pyrrole β-methyl proton signal became larger as the electron-withdrawing effect of the pyrrole β-substituent was stronger.[7]

The hyperfine coupling constant (A^H) of the pyrrole β-methyl proton can be estimated by an equation, $A^H = A_{aniso} + \rho(B_1 + B_2 \cos^2\theta)$, where A_{aniso} is an anisotropic hyperfine coupling constant, ρ is a π-spin density on the pyrrole β-position, θ is the angle between the $C_{py\text{-}\beta}$–C_{methyl}–H plane and the pyrrole β-carbon $2p_z$ axis, and $B_1 = 0$–10 MHz and $B_2 = 140$ MHz for the methyl proton. Since rotation of the pyrrole β-methyl group is frozen at 4 K, the observed A^H value would result from the methyl proton(s) with one orientation. As expected from the above equation, the observed A^H values for **1**–**4** linearly correlated to the a_{iso} values calculated from their ^{1}H NMR shifts (Table 1),[7] indicating $A^{\parallel}_{aniso} \approx -2$ MHz and $\theta \approx 30^\circ$.[10] This is reasonable orientation to avoid steric interaction with either the meso-proton or the pyrrole β-phenyl group.

Table 1. Hyperfine coupling constants for the pyrrole β-methyl groups of **1**–**4** and compound I of heme proteins

Compound	A^H /MHz	$a_{iso}{}^c$ /MHz	ρ^c
1	2.67	3.45	0.049
2	3.16	3.78	0.054
3	3.58	4.11	0.059
4	3.67	4.16	0.059
HRP[a]	≈4.4		
Ml-CAT[b]	≈6		

a) Ref 3. b) Ref 5. c) Calculated from ^{1}H NMR shifts (ref 7).

From the present ^{1}H ENDOR results, we expect that the A^H value of the pyrrole β-methyl proton in ^{1}H ENDOR of frozen solution is less than 10 MHz for compound I with the a_{1u} porphyrin π-cation radical state.[11] On the other hand, ^{1}H NMR shift of the pyrrole proton for the meso-sunstituted porphyrin complex suggested $\rho = 0.013$ on the pyrrole β-position for the a_{2u} porphyrin π-cation radical state.[7] Thus, the A^H value of the pyrrole β-methyl proton is expected to be less than 4 MHz for compound I with the a_{2u} porphyrin π-cation radical state. The hyperfine coupling constants of the pyrrole β-methyl groups for compound I of HRP and *Ml*-CAT are in the range of the a_{1u} porphyrin π-cation radical states, but larger than that of the a_{2u} porphyrin π-cation radical state. Therefore, this study clearly indicates that the compound I of both HRP and *Ml*-CAT are consistent with the a_{1u} porphyrin π-cation radical state, but not the a_{2u} porphyrin π-cation radical state.

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10 $A^H = A^{\parallel}_{aniso} + a_{iso}(B_1 + B_2 \cos^2\theta)/(B_1 + B_2 \cos^2 45)$. When $B_1 = 0$, $A^H = A^{\parallel}_{aniso} + a_{iso}(2\cos^2\theta)$. The correlation was shown in Figure S3.[8] From the slope (1.39) and y intercept (−2.1) of the correlation line, the θ and A_{aniso} were found to be 33.5° and −2.1 MHz, respectively.
11 The maximum A_{aniso} component was assumed to be +2 MHz. The maximum a_{iso} for the a_{1u} and a_{2u} radicals were found to be 8.3 and 1.8 MHz, respectively, from $a_{iso} = 140 \times \rho$ for $\theta = 0$.

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