

EPR and ENDOR Detection of Compound I from *Micrococcus lysodeikticus* Catalase†

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ABSTRACT: We present the first EPR and ENDOR examination of a catalase compound I (Cat I), the one formed by peracetic acid treatment of *Micrococcus lysodeikticus* catalase. The Cat I rapid-passage EPR signal ($g_{\perp}^{\text{eff}} = 3.32$; $g_{\parallel}^{\text{eff}} \sim 2$) appears quite different from those reported previously for the compounds I from horseradish peroxidase (HRP I) and chloroperoxidase. Nonetheless, all three signals can be explained by the same model for exchange coupling between an $S = 1$ oxoferryl $[\text{Fe}=\text{O}]^{2+}$ moiety and a porphyrin π -cation radical ($S' = 1/2$) (Schulz, C. E., *et al.* (1979) *FEBS Lett.* 103, 102–105). The signal for Cat I is unlike those for the two peroxidases in that it reflects a *ferromagnetic* rather than *antiferromagnetic* exchange. Preliminary ^1H ENDOR spectra for Cat I appear to differ from the proton (^1H) ENDOR spectra of HRP I; the latter, along with the ^{14}N ENDOR spectra, indicate that the porphyrin radical in HRP I exhibits a predominantly A_{2u} -like state having large spin densities on porphyrin N and C(β). The proton ENDOR spectrum of Cat I is insensitive to H/D exchange, which indicates that the $[\text{Fe}=\text{O}]^{2+}$ moiety is not protonated. Consideration of the EPR results for a series of compounds I suggests that the sign and magnitude of the exchange parameter (J) is correlated with the nature of the proximal axial ligand.

Catalase is a hemoprotein that catalyzes the decomposition of hydrogen peroxide via an intermediate, termed compound I, that is two equivalents more oxidized than the native ferric enzyme (Schonbaum & Chance, 1976). This enzyme, and the closely related peroxidases, such as horseradish peroxidase (HRP) (Dunford, 1991) and chloroperoxidase (CPO) (Dawson & Sono, 1987; Griffin, 1991), exhibit a compound I in which one of the two oxidizing equivalents is stored as a low spin oxoferryl moiety ($(\text{Fe}^{\text{IV}}\text{O})^{2+}$; $S = 1$) and the other is stored as a porphyrin-centered π -cation radical ($S' = 1/2$), with the green color of compound I being characteristic of the porphyrin π -cation radical. HRP I (Schulz *et al.*, 1979) and CPO I (Rutter *et al.*, 1984) give unusual EPR signals that quantitate to approximately one unpaired electron per heme group. Schulz *et al.* (1979) have shown that such signals are a manifestation of an exchange interaction,

$$\hat{H}_{\text{ex}} = J\hat{S}\cdot\hat{S}' \quad (1)$$

between the ferryl iron ($S = 1$) and the porphyrin radical ($S' = 1/2$), where J is the exchange parameter. ENDOR experiments confirmed that the broad HRP I EPR signal is associated with the porphyrin π -cation radical (Roberts *et al.*, 1981b). In addition, ^{17}O ENDOR showed that an oxygen atom remains bound to the ferryl iron of HRP I (Roberts *et al.*, 1981a), as confirmed in resonance Raman (Paeng & Kincaid, 1988; Chuang & Van Wart, 1992) and EXAFS experiments (Penner-Hahn *et al.*, 1986).

No EPR or ENDOR observations of catalase compound I have been reported. However, Jones and Middlemiss (1972)

have shown that an exceptionally stable compound I is formed in the reaction of *Micrococcus lysodeikticus*¹ catalase with peracetic acid. Catalase compound I (Cat I) accumulates as a result of peracetic acid's slow catalytic turnover (Jones & Middlemiss, 1972). Use of this "pseudosubstrate" provides opportunities for examining the physical nature of this catalytic intermediate, and we now present the first EPR and ENDOR examination of a catalase compound I, that formed by peracetic acid treatment of *M. lysodeikticus* catalase. The three-dimensional structure of this enzyme has recently been reported at 1.5 Å (Murshudov *et al.*, 1992).

The catalase I rapid-passage EPR signal ($g_{\perp}^{\text{eff}} = 3.32$, $g_{\parallel}^{\text{eff}} \sim 2$) appears quite different from those reported previously for HRP I (Schulz *et al.*, 1979) and CPO I (Rutter *et al.*, 1984). However, it can be explained by the same spin-coupling model (Schulz *et al.*, 1979), but we find that, unlike the cases of two peroxidases, it reflects a *ferromagnetic* exchange interaction between the oxoferryl iron ($S = 1$) and radical ($S' = 1/2$) moieties. Preliminary ^1H ENDOR spectra are consistent with an oxoferryl porphyrin π -cation radical formulation for catalase compound I. However, they appear to differ from the proton (^1H) ENDOR spectra of HRP I which, along with the ^{14}N ENDOR spectra, indicate a predominantly A_{2u} -like state having large spin densities on porphyrin N and C(β) (Roberts *et al.*, 1981b). The proton ENDOR spectrum of catalase I is insensitive to H/D exchange, which indicates that the $[\text{Fe}=\text{O}]^{2+}$ moiety is not protonated. Consideration of the EPR results for a series of compounds I suggests that the sign and magnitude of the exchange parameter J can be correlated with the nature of the bonding between the ferryl iron and the proximal axial ligand.

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¹ The current name for *M. lysodeikticus* is *Micrococcus luteus*, but we have retained the former name to facilitate reference between this study and others, such as the recent crystal structure determination (Murshudov *et al.*, 1992).

EXPERIMENTAL PROCEDURES

Procedures for the isolation and purification of catalase from *M. lysodeikticus* have been reported previously (Jones *et al.*, 1970). Catalase in 10 mM phosphate buffer, pH 7, was concentrated for EPR and ENDOR analysis by ultrafiltration (Amicon YM-10 membrane). EPR and ENDOR samples were 0.50 mM in protein-bound heme. Concentration was determined from heme optical density at 406 nm ($\epsilon = 103 \text{ mM}^{-1} \text{ cm}^{-1}$) (Brill & Williams, 1961).

Catalase compound I was generated by the method of Jones and Middlemiss (1972). This procedure uses the pseudosubstrate peracetic acid to trap the compound I form of the enzyme (Jones & Middlemiss, 1972). Typically, 25 μL of a 20 mM peracetic acid stock solution was added to 300 μL of enzyme in a quartz EPR tube at ambient temperature. Final peracetic acid concentration was 1.5 mM. The sample was frozen 30 s after mixing by immersion in liquid nitrogen. The peracetic acid stock solution (20 mM) was prepared by dilution of a 35% reagent solution (Aldrich) with pH 7 phosphate buffer. Residual hydrogen peroxide was removed from the stock solution by catalase pretreatment as previously described (Jones & Middlemiss, 1972). D_2O exchange was performed by 100-fold dilution of the enzyme with D_2O buffer followed by reconcentration by ultrafiltration.

EPR spectra were recorded at 2 K with a Varian E-109 X-band spectrometer as described in detail elsewhere (Venters, 1985). They were obtained with 100-kHz field modulation as the absorption-mode EPR signal, or as the dispersion-mode EPR signal under rapid-passage conditions. The former is the conventional display and ideally gives the derivative of the EPR absorption envelope; the latter display gives to a first approximation the EPR absorption envelope itself. ENDOR peak positions were determined from scans of increasing frequency; peak positions did not shift appreciably when the scan direction was reversed. EPR and ENDOR spectral conditions are given in the appropriate figure captions.

RESULTS

EPR. Figure 1 compares the 2 K absorption-derivative EPR spectra of native *M. lysodeikticus* catalase (A) and its peracetic acid oxidation product (B). The EPR spectrum of the native enzyme exhibits features from the catalase high-spin ferric heme at $g_y = 6.58$, $g_x = 5.56$. A poorly resolved pattern trailing about 400 G to low field from $g \sim 2.02$ arises from adventitious Cu(II) introduced into the sample by a corroded syringe needle (the initial enzyme preparation contained no Cu detectable by atomic absorption). In addition, there is a weak impurity signal near $g = 2.02$. Slappendel *et al.* (1981) have calculated the g -values for a high-spin ferric iron for large zero-field splitting as a function of E/D , the ratio of rhombic to axial zero-field splitting parameters. The experimentally observed values of g_y and g_x for catalase agree well with theory for $E/D = 0.021$. The Cu(II) signals near $g = 2$ (Figure 1A) obscure the feature associated with g_z , which is predicted to equal 1.98.

Figure 1B shows the 2 K EPR spectrum of *M. lysodeikticus* catalase subsequent to addition of peracetic acid (1.5 mM) to the native enzyme (0.5 mM). The ferriheme signal of the native enzyme has almost totally disappeared, and there is a new pattern extending from $g = 3.44$ to $g \sim 2$. Although this new signal appears weak, its shape indicates that it is strongly saturated, even at the low microwave power employed (45 dB), and that the 100-kHz field modulation is inducing the rapid-passage condition. As will become clear, the new

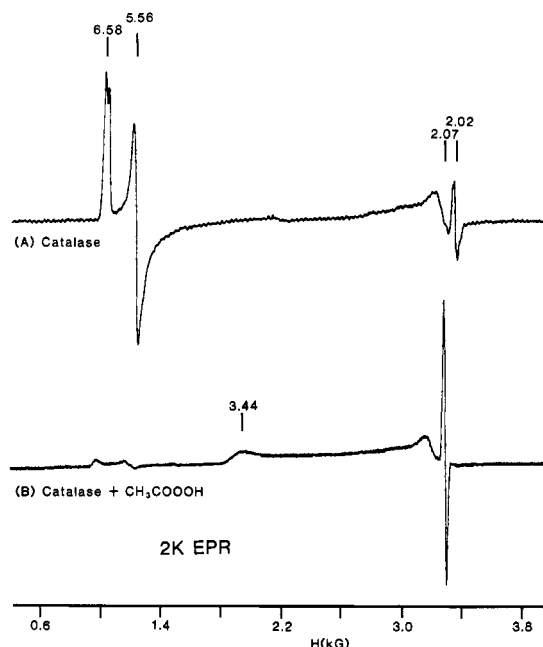


FIGURE 1: Low-temperature absorption-derivative EPR of catalase (A) and its peracetic acid oxidation product (B). (A) Conditions: enzyme concentration, 0.50 mM in 10 mM potassium phosphate, pH 7; absorption-mode derivative EPR; temperature, 2 K; microwave frequency, 9.525 GHz; microwave power, 45 dB; modulation amplitude, 5 G; modulation frequency, 100 kHz; field sweep rate, 16.67 G/s; time constant, 0.128 s; gain, 5×10^2 . (B) Conditions were as in (A) except enzyme and peracetic acid concentrations were 1.5 mM; and the gain was 2.5×10^2 .

resonance is associated with catalase compound I (Cat I). In addition, a sharp, $g = 2$, free radical signal of negligible integrated intensity is observed along with the Cu^{2+} .

Observation of an EPR signal under rapid-passage conditions is optimized through detection of the dispersion-mode derivative (Mailier & Taylor, 1973) and leads to a resonance pattern that approximates the undifferentiated EPR absorption envelope. Figure 2 shows the rapid-passage dispersion-mode derivative EPR spectra of the native enzyme (A) and its peracetic acid oxidation product (B). The dispersion spectrum of the native enzyme (Figure 2A), like its absorption counterpart (Figure 1A), consists of two EPR signals. The absorption envelope of the ferriheme signal extends from 1.0 to 3.4 kG ($g = 6.8$ to 2) with a maximum at $g_x = 5.2$ (1.3 kG). The Cu(II) impurity signal superimposed upon the ferric heme signal now is clearly seen to exhibit a maximum at ca. 3.4 kG and to extend to ca. 2.65 kG.

Figure 2B shows the dispersion EPR spectrum of the peracetic acid oxidation product of bacterial catalase. The resting-state ferriheme signal is strongly diminished and has been replaced by a signal of comparable integrated intensity that can be assigned to catalase compound I. The EPR spectra of both HRP I (Schulz *et al.*, 1979) and CPO I (Rutter *et al.*, 1984) likewise are seen clearly only in the dispersion mode. The Cat I resonance extends from 1.8 to ca. 3.4 kG and represents the absorption envelope of a center having an axial g -tensor with $g_{\perp} \sim 3.32 > g_{\parallel} \sim 2$. Superimposed upon the Cat I signal is the absorption envelope (3.35 kG, 400-G width) of the adventitious Cu(II) present in the enzyme preparation. Computer subtraction of the residual ferriheme EPR signal from Figure 2B (the result is shown in Figure 2C) reveals that the preparation contains less than 20% residual unoxidized enzyme.

ENDOR. Figure 3 shows the ENDOR spectrum obtained by setting the magnetic field to g_{\perp} for catalase I, the maximum

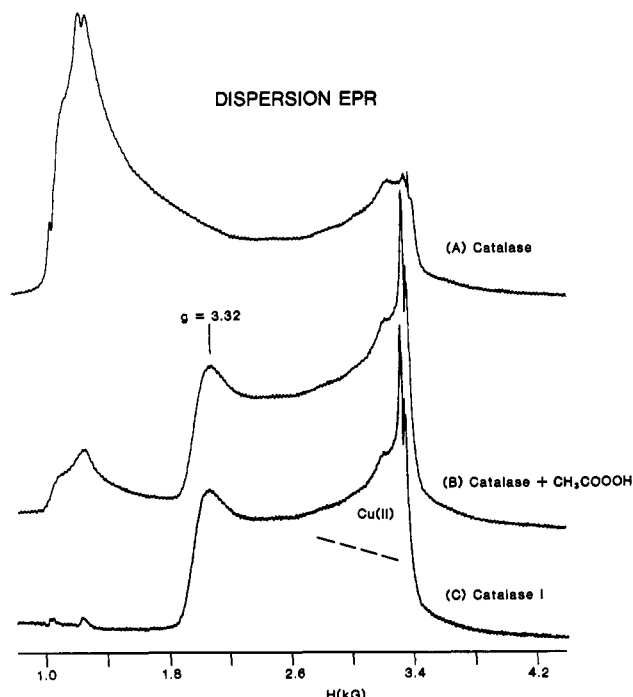


FIGURE 2: Dispersion EPR of catalase (A) and its peracetic acid oxidation product (B). These traces, taken under rapid-passage conditions, approximate the EPR absorption envelope itself. (A) Conditions were as in Figure 1A except dispersion-mode EPR was employed, microwave power was 40 dB, and the gain was 2.5×10^2 . (B) Conditions were as in Figure 1B except the gain was 8×10 . (C) Computer-generated difference spectrum: [peracetic acid oxidation product (B)] - [(native enzyme (A)) $\times 0.0634$].

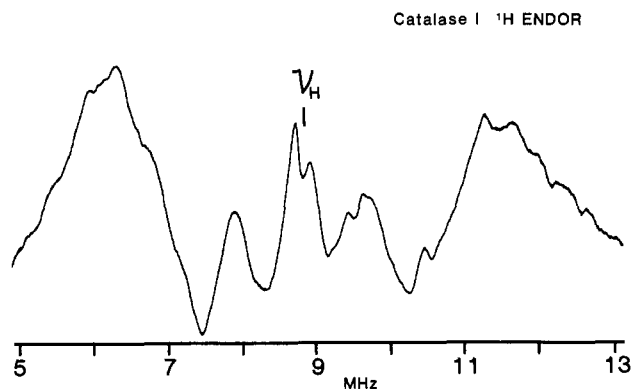


FIGURE 3: ENDOR spectrum of catalase compound I. Conditions: sample, prepared as described in Figure 1B; dispersion-mode EPR; temperature, 2 K; ENDOR scan rate, 1 MHz/s; time constant, 0.064 s; microwave frequency, 9.525 GHz; microwave power, 40 dB; magnetic field, 2050 G ($g = 3.34$); modulation amplitude, 5 G; modulation frequency, 100 kHz. The spectrum shown is a sum of 750 scans. A sloping baseline has been corrected by computer subtraction of a curve generated by a least-squares fit of the experimental background to a third-order polynomial.

of its dispersion EPR signal envelope (2.05 kG, $g = 3.32$). Each set of magnetically distinguishable protons is expected to exhibit a pair of ENDOR lines with separation A^H and mirrored about the proton Larmor frequency, ν_H (8.7 MHz for Figure 3). Examination of Figure 3 shows at least five sets of magnetically distinguishable protons, with all observed proton hyperfine couplings $A^H < \sim 6$ MHz. These signals are assigned to an oxoferryl porphyrin π -cation radical in analogy to the case of HRP, where Roberts *et al.* (1981b) showed by deuterium labeling that the proton ENDOR of HRP I arises from structural protons on the porphyrin macrocycle. None of the resonances in the catalase I 1H

ENDOR spectrum (Figure 3) are deuterium exchangeable; this indicates that the oxoferryl moiety is not protonated. However, the maximum values of the 1H hyperfine couplings observed at $g = 3.34$ are roughly one-half those observed for HRP I at $g \approx 2$ ($A^H < \sim 12$ –13 MHz) (Roberts *et al.*, 1981b). We take this to mean that the π radical on the porphyrin of Cat I differs significantly from that of HRP I, where 1H and ^{14}N ENDOR studies showed that the porphyrin π -cation radical is predominantly of A_{2u} symmetry.

We have not been able to observe ^{14}N ENDOR from catalase compound I. This observation is consistent with the reduced 1H couplings in suggesting that the porphyrin π -cation radical has increased A_{1u} character. However, in the analogous HRP I, ^{14}N ENDOR is relatively weak and was readily observable only at high protein concentrations (~ 4 mM), whereas the sample of catalase I is ~ 0.5 mM. Therefore, the failure to date to observe ^{14}N ENDOR in catalase I is not strong evidence regarding the symmetry of the radical state. It is hoped that the results from further ENDOR studies, in conjunction with those from resonance Raman spectroscopy (Paeng & Kincaid, 1988; Palaniappan & Turner, 1989; Chuang & Van Wart, 1992), will resolve this issue.

Spin-Coupling Analysis. The presence of a broad rapid-passage EPR signal for catalase I is qualitatively similar to the case of HRP I (Schulz *et al.*, 1979) and CPO I (Rutter *et al.*, 1984). Note however that $g_{\parallel} \sim 2$ and $g_{\perp} < 2$ for CPO I (Rutter *et al.*, 1984) but $g_{\perp} > 2$ for catalase I. Nonetheless, the spin-coupling model of Schulz *et al.* accounts quantitatively for the new EPR data for Cat I, as well as for the green compound I intermediates of HRP (Schulz *et al.*, 1979) and CPO (Rutter *et al.*, 1984). In this model the $S = 1$ oxoferryl moiety and the $S' = 1/2$ porphyrin π -cation radical are weakly exchange coupled (eq 1); in combination with a positive zero-field splitting at the $[Fe=O]^{2+}$ moiety (splitting parameter D), this causes the $(2S + 1)(2S' + 1) = (3)(2) = 6$ energy levels of the oxoferryl porphyrin π -cation radical spin system to form three Kramers' doublets. The lowest doublet is well separated in energy from the other two and gives rise to the observed EPR spectra, which can be represented in terms of an effective spin $S^{eff} = 1/2$. The effective g values, g_{\parallel}^{eff} and g_{\perp}^{eff} , are determined by the ratio J/D (Schulz *et al.*, 1979; Rutter *et al.*, 1984). To a first approximation, $g_{\parallel}^{eff} \approx g_e$, and $g_{\perp}^{eff} \approx g_e - 2g_{\perp}^{Fe}J/D$, where g_{\perp}^{Fe} is associated with the isolated $[Fe=O]^{2+}$ unit, but more exact expressions are readily derived; a plot of the latter is presented by Rutter *et al.* (1984). Using the exact equations, $g_{\perp}^{eff} \sim 3.32$, and parameters for $[Fe=O]^{2+}$ similar to those for CPO I, the catalase I EPR data can be explained by this model in terms of ferromagnetic exchange: $J < 0$, with $|J|/D = 0.40$. It is useful to note that exchange couplings characterized by this ratio (or smaller) cause a negligible ($\leq 3\%$) change in the hyperfine couplings to protons on the porphyrin and thus do not modify the discussion of such couplings in terms of molecular orbitals.

DISCUSSION

This study reports the first EPR and ENDOR observations of a catalase compound I, that from the enzyme isolated from *M. lysodeikticus*. The magnetic resonance ENDOR data presented here is consistent with the formulation of catalase I as containing an oxoferryl porphyrin π -cation radical. Although the EPR spectra of Catalase I and the analogous compounds I of horseradish peroxidase and chloroperoxidase appear quite different, nevertheless, all three can be analyzed in terms of spin coupling, as described by eq 1, between the zero-field-split ferryl iron ($S = 1$) and the porphyrin π -cation

radical ($S' = 1/2$). Likewise there exist several oxoferryl porphyrin (P) π -cation radical model systems, $[(P)Fe=O]^+$, that can be analyzed similarly (Groves *et al.*, 1981; Groves & McMurry, 1985; Groves & Watanabe, 1988; Hashimoto *et al.*, 1991; Mandon *et al.*, 1992).

For all these compounds I the spin coupling can be characterized by the sign of J and the relative magnitude of the exchange interaction and zero-field splitting parameter, the ratio $|J|/D$. The well-characterized model complexes, $[(P)Fe=O]^+$, represent an example of strong ferromagnetic exchange ($J < 0$) where $|J|/D > \sim 1$. Our results show that catalase I is the first protein-bound system to exhibit ferromagnetic exchange ($J < 0$), with an intermediate strength of $|J|/D = 0.4$. HRP I exhibits extremely weak exchange ($|J|/D < 0.10$); in fact, simulation of the broad HRP I EPR signal centered at $g = 2$ requires that J/D be assigned a distribution in J/D that includes positive (antiferromagnetic) and negative (ferromagnetic) values (Schulz *et al.*, 1979). Finally, CPO I exhibits appreciable antiferromagnetic exchange ($J > 0$), with $|J|/D = 1.02$ (Rutter *et al.*, 1984). Thus, J/D goes from being large and negative (ferromagnetic), to moderately negative, through near-zero, to moderately positive (antiferromagnetic) in the series $[(P)Fe=O]^+$, Cat I, HRP I, CPO I. The parameter D is uniformly positive and has been found to vary only over a modest range; for example, $D = 20$ – 25 cm^{-1} for the $[(P)Fe=O]^+$ model compounds, $D = 22\text{ cm}^{-1}$ for HRP I (Schulz *et al.*, 1979), and $D \sim 35\text{ cm}^{-1}$ for CPO I (Rutter *et al.*, 1984). Thus, the changes in $|J|/D$ within this series of compounds I primarily result from changes in the exchange coupling, J .

The variation within this series from ferromagnetic to antiferromagnetic coupling between $[Fe=O]^{2+}$ and the porphyrin π -cation radical (P^+) must reflect an increasing departure from the idealized 4-fold symmetry provided by the porphyrin N_4 coordination of iron (Gans *et al.*, 1986). The two odd electrons in an oxoferryl, $[Fe=O]^{2+}$, $S = 1$ moiety reside one each in the two antibonding Fe–O molecular orbitals that have π -symmetry with respect to the diatomic axis; they have the form $\psi_x = (1 - c^2)^{1/2} d_{xz}^{Fe} - cp_x^O$, and correspondingly for ψ_y (Roberts *et al.*, 1981a). In a 4-fold-symmetric metalloporphyrin the half-filled orbitals of the $[Fe=O]^{2+}$ center and the π -molecular orbitals of the porphyrin radical moiety are orthogonal by symmetry. In such a case correlation effects tend to force the spins of the two magnetic subsystems to align parallel, and the result can be described in terms of a ferromagnetic exchange, $J_F < 0$. If the symmetry is reduced to such a degree that the magnetic orbitals of the metal and the macrocycle no longer are orthogonal, this introduces an additional bonding interaction that tends to align the subsystem spins antiparallel and that can be described in terms of an antiferromagnetic contribution to the exchange, $J_{AF} > 0$. In general, the observed exchange coupling, J , is the result of a competition between the two opposing tendencies and can be written as $J = J_F + J_{AF}$.

It is reasonable to infer that a major element in this reduction of symmetry for the proteins is π -bonding between Fe^{IV} and the proximal endogenous axial ligand. However, as emphasized by Reed and co-workers (Gans *et al.*, 1986), a simple reduction from 4- to 2-fold symmetry would not be sufficient to allow a net overlap between the d_{xz}^{Fe} , d_{yz}^{Fe} orbitals of $[Fe=O]^{2+}$ and the odd-electron π -orbital of the ring; the distortion must include a further lowering in symmetry, for example, a buckling of the ring. This presumably explains why no compound I yet observed has strong AF coupling, such that the total molecular spin is a good quantum number,

with $S = 1/2$. Nonetheless, small deviations from strict orbital orthogonality can lead to dominant AF interactions, and as we now discuss, the anticipated order of increasing π -interactions by the axial ligands does match the increases in AF contribution to J .

The model compounds $[(P)FeO]^+$ clearly represent a case where the half-occupied π -molecular orbital of the porphyrin cation radical has negligible overlap with the magnetic orbitals of the $[Fe=O]^{2+}$ moiety. For $[(P)Fe=O]^+$ it may well be that the anion is not bound to iron (Bill *et al.*, 1990), and in any case the 4-fold symmetry of the $[(P)FeO]^+$ models would not be appreciably perturbed by weak coordination of triflate ion or methanol (Mandon *et al.*, 1992). Thus orbital orthogonality is maintained, $J_{AF} \rightarrow 0$, and $J \approx J_F < 0$. In contrast, for CPO I, where the axial ligand is a strongly π -bonding cysteinyl thiolate (Dawson & Sono, 1987), the effective symmetry is lowered and orbital overlap is increased to the point that antiferromagnetic coupling dominates: $J_{AF} > |J_F|$, and $J > 0$. HRP I, where the axial ligand is imidazole or imidazolate (LaMar *et al.*, 1981; Thanabal *et al.*, 1988), represents a presumably fortuitous balance where $|J_{AF}| \sim |J_F|$ and $J \sim 0$. As described here, Cat I, where the axial ligand is phenolate (Hu & Kincaid, 1992; Murshudov *et al.*, 1992), lies between the model and HRP I; J_{AF} for Cat I is the smallest of the values of the three enzymes studied, and the net interaction is ferromagnetic. Note that the suggested shift of the π -radical state in Cat I toward being more A_{1u} -like would lower the spin density on the porphyrin N; although this should decrease the magnitude of both J_F and J_{AF} , it is plausible that the effect on J_{AF} would be greater.

Interpretation of the trend in J in terms of increasingly unsymmetric oxoferryl heme environment is in part supported by considering the g -tensors of the parent high-spin ferriheme states ($S = 5/2$). A high-spin ferriheme of 4-fold effective symmetry would have an axial g -tensor of $\delta g \propto g_y - g_x = 0$ (this corresponds to a ratio of the zero-field splitting parameters for the $S = 5/2$ state of $\lambda = E/D = 0$). Reduction of symmetry through π -bonding between the ferriheme and the axial ligand and/or by deformations of the porphyrin will progressively increase $\delta g(\lambda)$. Consistent with expectations, the $[(P)Fe^{3+}A]^+$ compounds are high spin, with $g_x \approx g_y \approx 6$ and $\delta g \approx 0$, whereas the g -tensor for ferri-CPO shows a large rhombic splitting, $\delta g = 3.10$ (Dawson & Sono, 1987); HRP and catalase have intermediate values of δg (1.2 for catalase; 0.9 for HRP), in parallel with their intermediate position in the scale of J/D . Although catalase and HRP exchange places in the series organized by J/D and δg , π -bonding between the proximal ligand and a heme ferric ion can be expected to differ strongly from that of the more electron deficient $[Fe=O]^{2+}$ moiety, and thus it would be valuable to have a direct measure of λ for the different compounds I. This is possible for the $[(P)Fe=O]^+$ compounds, whose EPR spectra show rhombic splittings that have been analyzed to yield small values of λ (Mandon *et al.*, 1992). However, a distribution in J eliminates all resolution in the EPR spectrum of HRP I and may well mask any rhombic splittings in Cat I and CPO I.

In summary, the EPR and preliminary ENDOR data for Cat I show that it differs in quite significant ways from both HRP I and CPO I and more specifically show that the oxoferryl and porphyrin π -cation radical spin subsystems of $[(P)Fe=O]^+$ embedded in a protein can exhibit net exchange couplings that are ferromagnetic as well as antiferromagnetic. It may well be that this fine tuning correlates with the bonding properties of the axial ligand and, thus, with chemical and enzymatic activity as well.

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