J. Am. Chem. Soc. **1981**, *103*, 663-670

Electron Pathways in Catalase and Peroxidase Enzymic Catalysis. Metal and Macrocycle Oxidations of Iron Porphyrins and Chlorins

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Abstract: Charge iterative extended Hückel calculations are presented for compound II, the one-electron oxidation intermediate of horseradish peroxidase (HRP), and for compounds I, the two-electron oxidation transients of HRP and catalase (CAT) observed in the catalytic cycles of the hydroperoxidase enzymes. Compound II is described in terms of a ferryl configuration ($O{=}Fe^{IV}$), and compounds I are described as ferrylporphyrin π-cation radicals. The validity of the iron π-cation calculations is supported by favorable comparison of parallel computations for porphyrin π cations of diamagnetic metals with new and previously reported ESR results for radicals of zinc tetrabenz-, *meso*-tetramethyl-, (^{14}N and ^{15}N) tetraphenyl-, and magnesium (^1H and ^2H) octaethylporphyrins. The calculated electronic configurations and unpaired spin density profiles for the ferryl π cations satisfactorily account for the physical properties reported for compounds I of HRP (in the native protoporphyrin IX form or reconstituted with deuteroporphyrin), chloroperoxidase, and CAT. The ground states of the π cations, "a_{1u}" or "a_{2u}", are determined by peripheral substitution and axial ligation, and the axial ligand of CAT I is predicted to differ from that of HRP I. The combination of model studies and calculations suggests that ^2H, ^{13}C, and ^{15}N NMR studies of isotopically substituted proto and deutero HRP I would confirm the electronic profiles predicted. ^{15}N NMR in particular would clearly discriminate between a_{1u} and a_{2u} configurations. As an additional test of the ferryl π-cation hypothesis, calculations are presented for a proposed ferrylchlorin π cation of *Neurospora crassa* catalase, which contains an iron chlorin prosthetic group. Compound I of this unusual heme is predicted to occupy an "a_2" ground state with the spin distribution and optical spectra reported here for synthetic chlorin radicals.

Introduction

An extensive body of evidence has established[2-4] the crucial roles played by π radicals (i.e., electrons have been added to or abstracted from the π system) of porphyrin derivatives in the electron-transport chain of photosynthetic organisms. Although such radicals have also been invoked[5-9] in the catalytic cycles of heme-containing enzymes, their existence and function remain subject to considerable controversy.[10-13] We consider here a role for π cations in enzymes which contain prosthetic groups comprised of iron porphyrins (horseradish peroxidase [HRP] and catalase [CAT]) and iron chlorins (*Neurospora crassa* catalase).

Catalases decompose hydrogen peroxide to oxygen and water in two steps, eq 1 and 2, whereas peroxidases react with peroxides

$$\text{CAT} + H_2O_2 \rightarrow \text{compound I} + H_2O \qquad (1)$$

$$\text{compound I} + H_2O_2 \rightarrow \text{CAT} + H_2O + O_2 \qquad (2)$$

and other oxidants to form an intermediate compound I which then reacts with an array of oxidizable organic and inorganic substrates in consecutive one-electron steps (eq 3-5).

$$\text{HRP} + H_2O_2 \rightarrow \text{compound I} + H_2O \qquad (3)$$

$$\text{compound I} + AH_2 \rightarrow \text{compound II} + AH\cdot \qquad (4)$$

$$\text{compound II} + AH\cdot \rightarrow \text{HRP} + A + H_2O \qquad (5)$$

The catalytic cycles of HRP and CAT have been extensively reviewed.[12,13] Salient features are the appearance, on treatment with peroxides, of green species (compounds I, or HRP I and CAT I), which are two electron oxidation products of the ferriheme proteins.[14,15] Magnetic susceptibility measurements of the transients indicate[16] three unpaired electrons, $S = {}^3/_2$, for compounds I and two electrons, $S = 1$, for compound II of HRP, which is one oxidation level above the resting enzyme. The Mössbauer

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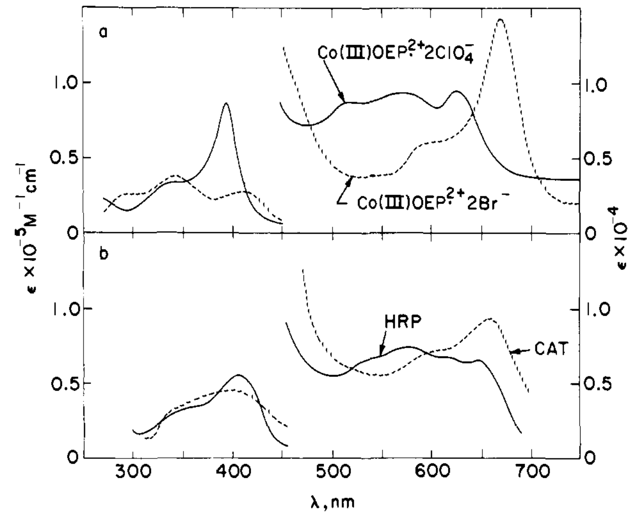

Figure 1. (a) Optical absorption of the radicals of cobaltic octaethylporphyrin in CH_2Cl_2 with bromide counterions (=$^2A_{1u}$ spectrum (---)) and with perchlorate counterions (=$^2A_{2u}$ spectrum (—)). (b) Compound I of HRP (—) and compound I of CAT (---) (from ref 5a).

spectra of compounds I and II exhibit[7,17,18] similar isomer shifts which differ from those of the parent heme and which are consistent with an Fe^{IV} oxidation state. ^{18}O experiments on compound I of chloroperoxidase, a chloride specific peroxidase, suggest[19] one oxygen as an iron ligand, resulting in $O{=}Fe^{IV}$ (ferryl) or HO—Fe^{IV} configurations for the iron in compounds I and II.

A number of electronic configurations have been proposed[12,13] to account for the above properties of compounds I and II. However, the recent report[7] of an ESR signal, centered at $g = 2$ and associated with HRP I, narrows these down to two reasonable alternatives. Dolphin, Fajer, Felton, and co-workers proposed[5] that compounds I consist of oxygen or hydroxide-bound Fe^{IV} coupled to π cations of the porphyrin because the optical spectra of compounds I resemble those of a series of synthetic porphyrin π cations. These exhibit two distinct types of optical spectra in the visible region attributable to two different ground states of the porphyrin radicals, $^2A_{1u}$ or $^2A_{2u}$ (D_{4h} symmetry). In addition, in cobaltic octaethylporphyrin radicals, the two ground states were determined by the axial ligand of the metal, and comparison of the optical spectra of HRP I and CAT I with those of the cobalt radicals revealed a close analogy between HRP I and species typical of a $^2A_{2u}$ state whereas CAT I exhibited spectra characteristic of a $^2A_{1u}$ state (Figure 1).

Morishima and Ogawa challenged[11] this interpretation after noting that NMR spectra of the heme peripheral substituents in HRP I shifted 35–60 ppm relative to HRP II and showed no significant line broadening. They concluded that the spectral changes were inconsistent with an unpaired electron localized on the porphyrin and suggested instead that the second electron lost by the resting enzyme was abstracted from a nearby protein residue, as is indeed observed by ESR[20] and ENDOR[21] in the two-electron oxidation intermediate of cytochrome *c* peroxidase. The optical spectrum of the latter differs,[20] however, from those of HRP I and CAT I and resembles compound II instead.

We present here charge iterative extended Hückel (IEH) molecular orbital calculations and magnetic and optical results for porphyrin and chlorin radicals which lead to the following conclusions. (1) The magnetic susceptibilities and optical, Mössbauer, ESR, and NMR spectra of compounds I of HRP and CAT are consonant with a ferryl configuration ($O{=}Fe^{IV}$, $S = 1$) weakly coupled to a π-cation radical of the porphyrin macrocycle. (2) The ground state[22] of the porphyrin π cation, "a_{2u}" or "a_{1u}", is determined by peripheral substitution and axial ligation, and the axial ligand of CAT probably differs from that of HRP: it is either weakly bound, nonnitrogenous, or absent. (3) NMR examination of HRP I and CAT I reconstituted with hemes substituted with ^{15}N pyrroles or with ^{13}C or ^2H at the methine positions would provide further evidence for the π-cation hypothesis. (4) A similar ferryl π-cation configuration is predicted for the catalase found in *Neurospora crassa* which contains[23] an iron chlorin prosthetic group. The chlorin should exhibit an "a_2" ground state (C_{2v} symmetry) with the optical characteristics of model chlorin cations presented herein.

Experimental Section

The program and parameters[24] used for the IEH calculations have been described.[25] Zinc tetrabenzporphyrin (ZnTBP) was synthesized by the method of Vogler et al.[26] and oxidized by electron transfer to the oxidized salt of zinc tetraphenylporphyrin[27] ($ZnTPP^+{\cdot}ClO_4^-$). Methyloctaethylchlorin (MeOEC, 2-hydro-2′-methyl-3,3′,7,8,12,13,17,18-octaethylporphyrin) was prepared according to Chang.[28] H_2TPP enriched in ^{15}N was prepared[27] by condensation of benzaldehyde with pyrrole comprised of 95% ^{15}N (Merck and Co.). ZnTPP-^{15}N, ZnMeOEC, and CoMeOEC were obtained by reaction of the free bases with zinc and cobalt acetates by standard methods.[27] The cation radicals were generated by controlled potential electrolyses at a platinum electrode in dichloromethane or butyronitrile with tetrabutylammonium perchlorate (TBAP) as electrolyte.[3,4,27] The techniques for ESR and ENDOR measurements have been reported.[4a]

Results and Discussion

Porphyrins, Horseradish Peroxidase and Catalase. Mössbauer results for compound II of HRP differ[7,17,18] from those of the resting ferric enzyme, which suggests that the metal rather than the porphyrin has undergone electron abstraction to a formal oxidation state of Fe^{IV}. ^{18}O studies on chloroperoxidase further indicate[19] that oxygen is complexed during the enzymatic cycles. An intermediate ferryl species, $O{=}Fe^{IV}$, would therefore stabilize the tetravalent state of the iron by sharing the charge density with the oxygen.[6] ESR spectra of Fe^{II}HRP nitrosyl complexes indicate[29] that one axial ligand of HRP is a nitrogenous residue, probably the imidazole group of a histidine. Compound II may then be described as a ferryl imidazole complex, $O{=}Fe^{IV}$–imidazole. The magnetic susceptibility[16] of HRP II dictates an intermediate spin state for the Fe^{IV} with two unpaired electrons. A number of model ferric porphyrins undergo[5d,30-33] one-electron oxidation to both high- and low-spin Fe^{IV}, and a synthetic $O{=}Fe^{IV}$TPP–imidazole exhibits[32] $S = 1$ and resembles compound II optically.

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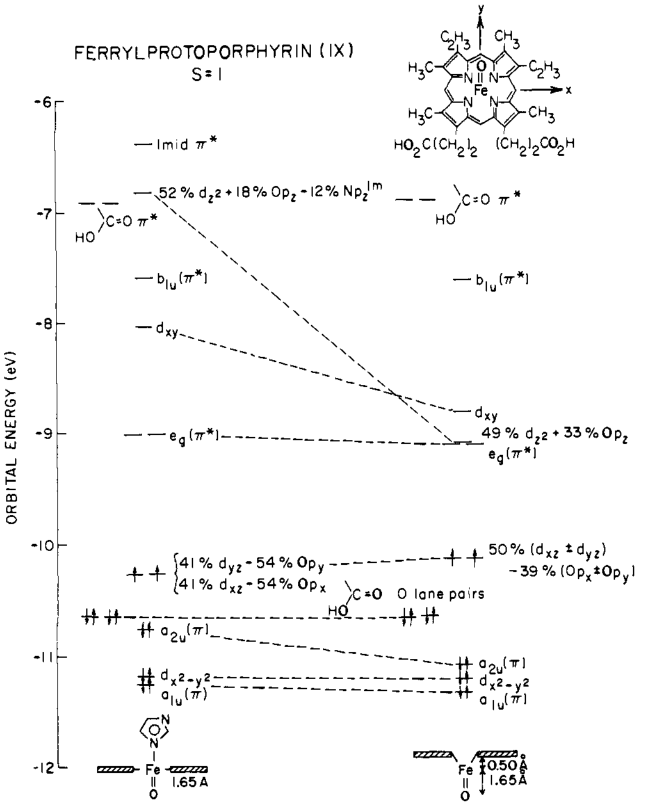

Figure 2. Energy level diagrams for ferrylprotoporphyrin IX with spin state $S = 1$, with and without an imidazole axial ligand. The porphyrin core and imidazole coordinates are given in ref 25b. The ring substituents were placed in calculated positions using standard distances and angles. Fe–N(imid) = 1.957 Å, with the imidazole staggered relative to the pyrrole nitrogens (from the crystal structure of $(imid)_2Fe^{III}TPP{\cdot}Cl$: Collins, D. M.; Countryman, R.; Hoard, J. L. *J. Am. Chem. Soc.* **1972**, *94*, 2066–72). The ferryl Fe=O distance of 1.65 Å is based on the metal–oxygen distances found for vanadyl (Pettersen, R. C. *Acta Crystallogr., Sect. B* **1969**, *B25*, 2527–2539) and titanyl porphyrins (Guilard, R.; Latour, J.-M.; Lecomte, C.; Marchon, J.-C.; Protas, J.; Ripoll, D. *Inorg. Chem.* **1978**, *17*, 1228–1237).

Energy level diagrams calculated for ferrylprotoporphyrin IX compounds II in $S = 1$ spin states, with two extremes of axial ligation, a tightly bound imidazole and no sixth ligand, are shown in Figure 2. As had been noted in previous IEH calculations for imidazole ferrylporphine,[6a] the iron d_{xz} and d_{yz} orbitals mix extensively with the oxygen p_x and p_y orbitals, thereby stabilizing the formal Fe^{IV} oxidation state. Indeed, the charge densities computed for the iron (0.23) fall within the range of values typically obtained for iron(III) porphyrins (0.21–0.26).[24c]

Oxidation of the $S = 1$ imidazole compound II to compound I would result in abstraction of an electron from the a_{2u} porphyrin orbital (Figure 2) yielding an $S = {}^3/_2$ species with the optical characteristics of an "a_{2u}" radical,[22] as observed for HRP I.[34] Unpaired spin densities for such a radical species are shown in Figure 3. Because of the small mixing between the iron and porphyrin orbitals,[35] the electron density on the metal remains relatively unperturbed (Figure 3) and thus accounts for the similar Mössbauer parameters observed[7] in compounds I and II of HRP. The weak coupling between the $S = 1$ of the iron and the $S = {}^1/_2$ of the porphyrin radical also provides[36] a rationale for the broad ESR spectrum of HRP I.

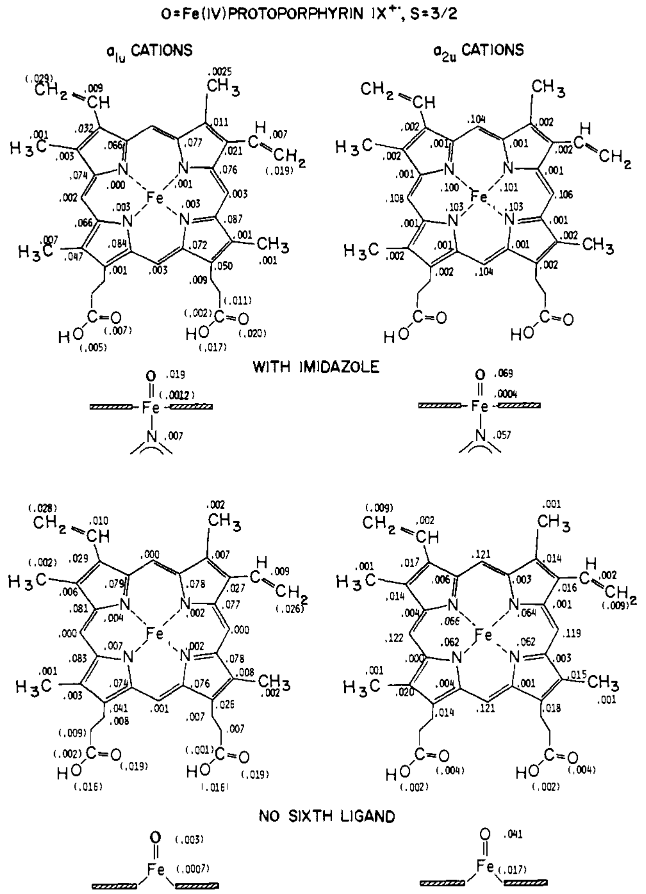

Figure 3. Calculated unpaired spin densities for $S = {}^3/_2$ ferryl protoporphyrin IX π-cation radicals, with and without imidazole ligands, with an electron abstracted from the a_{1u} (left) and a_{2u} (right) orbitals. The spin densities shown are those of the half-filled porphyrin π orbitals only. The numbers in parentheses include unpaired spin density contributions from orbitals other than p_z for C, N, and O or d_{z^2} for Fe, if they are significant. The spin densities of the a_{1u} (but not the a_{2u}) radicals are sensitive to the orientation of the 2,4 vinyl groups relative to the porphyrin plane. The values shown were obtained with vinyls 30° out of the macrocycle plane (Caughey, W. S.; Ibers, J. A. *J. Am. Chem. Soc.* **1977**, 99, 6639–45). If the vinyl groups are rotated perpendicular to the porphyrin, the ρ values are redistributed around the periphery of the molecule (to more closely resemble deuteroporphyrin, Figure 6): the 1, 2, 3, 4, 5, 6, 7, and 8 β-carbons carry ρ's of 0.021, 0.012, 0.027, 0.009, 0.000, 0.048, 0.038, and 0.002 without ligand, and 0.015, 0.020, 0.037, 0.006, 0.013, 0.024, 0.021, and 0.014 with imidazole.

We consider now the NMR spectra reported for native HRP, HRP II, and HRP I. The positions monitored are the methyl substituents on the β-pyrrole carbons, and these are observed at 55–85, ~14, and 50-80 ppm downfield from DSS for the three species, respectively.[11] A calculation for the resting enzyme ($S = {}^5/_2$, imidazole–iron(III) protoporphrin IX) places spin densities, ρ, of 0.028–0.035 at the β-carbons adjacent to the methyl substituents[37] whereas computations for compound II (ferrylprotoporphyrin) reduce these values to 0.003–0.004, in reasonable agreement with the trend observed experimentally (55–85 ppm → 14 ppm in compound II), if the shifts are assumed to arise solely from the Fermi contact terms.[38] (Pseudocontact shifts are considerably smaller in hemes.[38b]) On oxidation of compound II

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[34] Because of the similarity in the heme methyl shifts of compound I to native HRP and compound II to low-spin ferric systems, Morishima and Ogawa[11] raised the possibility of an $S = 2$ spin state for compound I. Our calculations suggest that such a configuration is highly disfavored for $O{=}Fe^{IV}$ because of the large energy gap between the $d_{x^2-y^2}$ and d_{z^2} orbitals. This gap is even larger for the $S = 2$ form relative to the orbitals depicted in Figure 2 for $S = 1$.

[35] Removal of an electron from *either* the a_{2u} or a_{1u} π orbitals does not alter the orbital patterns of Figure 2 and leaves the Fe=O bonding unaffected: the maximum change in the Fe, O coefficients is ~1%. As shown in Figure 3, the total contribution of the iron to the porphyrin a_{1u} and a_{2u} orbitals is very small.

[36] See ref 7 for a detailed discussion of this point.

[37] The proton NMR shifts observed reflect the unpaired spin densities at the carbon atoms in the π system to which the protons or methyl groups are attached.

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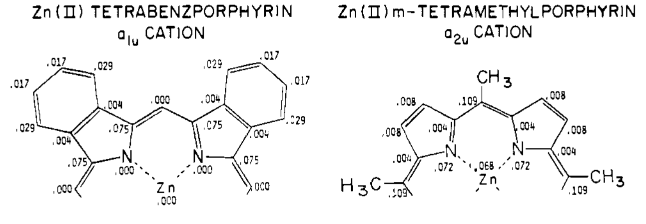

Figure 4. Unpaired spin densities for the cation radicals of zinc *meso*-tetramethylporphyrin ($^2A_{2u}$) and zinc tetrabenzporphyrin ($^2A_{1u}$). The zinc was placed 0.33 Å out-of-plane with Zn–N = 2.08 Å.[54c]

to compound I, the methyl resonances again migrate downfield to 50–80 ppm. Such a positive shift simply requires an increase in ρ of the order of 0.01–0.02 at the β-carbons since calculations for the imidazole ferryl π cations indicate that the iron orbitals contribute ρ values between 0.003 and 0.004. (For weak exchange interactions between the iron and porphyrin, the total NMR shifts observed in HRP I can be approximated[38a] as the sum of the contributions made by the unpaired spin densities due to the iron and to the radical:[39] $\Delta H \approx k_1\rho_{Fe} + k_2\rho_{\pi\cdot}$.)

Examination of Figure 3 reveals that the salient features of the imidazole-bound a_{2u} cation are the localization of spin at the meso carbons and nitrogens whereas the α- and β-pyrrole carbons are practically devoid of unpaired spin density.[40] Since only an additional 0.01 in spin density at the β-carbons is required to satisfactorily account for the methyl shifts observed in HRP I relative to HRP II, the a_{2u} cation calculations are reasonably consistent with the experimental NMR data. (It would be naive to expect absolute agreement for ρ values of the order of 0.01.) Obviously, the protoporphyrin substituents monitored by NMR in compound I are positioned at the sites of least unpaired spin whereas the meso protons, which should exhibit significantly larger contact shifts and line broadening, are either masked by the protein resonances or undetected. The electronic profile calculated for HRP I (Figure 3) suggests considerably more sensitive tests of the π-cation formulation: reconstitution of the enzyme with protoporphyrin enriched in ^2H or ^{13}C at the meso positions or with ^{15}N at the pyrrole nitrogens should lead to major changes in the ^2H, ^{13}C, or ^{15}N spectra of compound I relative to compound II.

Because of the negligible mixing between the porphyrin and iron orbitals, the validity of the calculations for the iron π cations can be assessed by comparing calculations for metalloporphyrins containing diamagnetic metals (Figure 4) against the resolved ESR spectra obtained[2a,5c,e,27] for a series of zinc and magnesium radicals which exhibit $^2A_{2u}$ optical signatures. The unpaired spin density distributions in these radicals were mapped by a combination of ^{15}N and ^2H isotopic substitutions and computer simulations of the resolved ESR spectra (Figure 5a,b). The spectra could only be accounted for in terms of large unpaired spin densities at the meso positions and at the nitrogens, with undetectable amounts at the β-pyrrole positions, in agreement with the MO calculations (Figure 4). Because of the relatively small energy gap between the a_{2u} and a_{1u} orbitals, model studies have shown[2a,5,27] that changes of the porphyrin substituents or of the axial ligands can invert the radical ground state from $^2A_{2u}$ to $^2A_{1u}$, with concomitant changes in unpaired spin densities, ESR, and optical spectra. In contrast to the $^2A_{2u}$ states observed in zinc and magnesium *meso*-tetramethyl- or tetraphenylporphyrins, magnesium octaethylporphyrin, $MgOEP^{+\cdot}$, displays[2a,5c,27] little unpaired spin density at the β-pyrrole atoms and at the nitrogens, with $a_H \approx$ 1.5 G at the meso positions (Figure 5c). Additional support for this profile is found in the cation of zinc tetrabenzporphyrin (ZnTBP) which is predicted[24a,41] to occupy a $^2A_{1u}$ state and for which calculated unpaired spin densities are shown in Figure 4. The experimental ESR spectrum, Figure 5d, can be fitted with three different proton splittings determined by ENDOR and assigned, on the basis of the IEH calculations, to four protons, a_H = 1.2 G (meso positions), and to two sets of eight protons, a_H = 0.55 and 0.32 G (peripheral protons), in reasonable agreement with the values found for $MgOEP^{+\cdot}$. In Zn- and $MgOEP^{+\cdot}$, no evidence of metal or counterion hyperfine interaction is observed.[5c] The sum of the unpaired spin densities at the metal, counterion, nitrogen, meso, and β positions amounts[5c] to ~40% of the unpaired electron, leaving, by default, the α-carbons of the pyrroles to carry ~60% of the spin, as expected from the MO calculations. (Both the IEH results cited here and self-consistent field Pariser Parr Pople calculations[27] appear to underestimate the spin density at the meso positions in $^2A_{1u}$ radicals. This may be due to a small mixing of the a_{1u} and a_{2u} orbitals.) Clearly, the MO calculations for the zinc and magnesium porphyrin cations provide a reasonable description of the experimental ESR spectra and thus lend confidence to the profile predicted for the Fe^{IV} π cations.

The effect of the porphyrin substituents in influencing the ground-state occupancy observed in the model systems has also been noted in HRP. Dinello and Dolphin found[5g] that HRP I reconstituted with deuteroporphyrin exhibited an absorption spectrum at −42 °C characteristic of an a_{1u} radical, instead of the a_{2u} spectrum observed in the native protoporphyrin enzyme.

Because ESR data for deutero Fe^{II}(HRP)NO complexes still show[29] the presence of a nitrogenous axial ligand, the switch from a_{2u} to a_{1u} in deutero HRP I at low temperatures cannot be attributed to differences in axial ligation, if the imidazole is retained during the catalytic cycle. (The calculations actually favor an a_{2u} configuration for a tightly bound sixth ligand.) The question then arises as to why the relatively small electrophilic and structural changes caused by exchange of the vinyl groups of protoporphyrin for the protons of deuteroporphyrin induce a ground-state inversion. The answer may lie in NMR[42] and X-ray[43] results for aquo metmyoglobin which show interactions between the vinyl groups of protoporphyrin and the threonine C4 and phenylalanine H15 protein residues. The observation[44] that deuteroporphyrin can flip over by 180° on insertion into myoglobin and HRP apoproteins suggests that the porphyrin–protein coupling is altered (with possible concomitant modifications of the imidazole binding and orientation) on reconstitution with deuteroporphyrin, thereby further enhancing the structural substituent effects on the ground-state occupancy of HRP I. Additional support for this idea derives from the fact that reconstitution of HRP with 2,4-substituted acetyl or formyldeuteroporphyrins leads[5g] to the normal a_{2u} optical spectra of HRP I.

Unpaired spin densities calculated for ferryldeuteroporphyrins IX a_{1u} and a_{2u} π cations with and without imidazole ligands are presented in Figure 6. The a_{2u} deutero radical profiles parallel those obtained for protoporphyrin: no appreciable spin density resides at either the α- or β-carbons of the pyrroles when the iron is bound by imidazole. Removal of the ligand and displacement of the iron 0.5 Å out-of-plane redistributes spin density away from the pyrrole nitrogens and onto the meso and β-pyrrole carbons at the ring periphery. In contrast to the a_{2u}, the a_{1u} profiles exhibit significant sensitivity to ring substituents at the C_β: in the deutero radicals, either three (with imidazole) or two (without imidazole) methyl groups are adjacent to atoms with sizable unpaired spin densities.

NMR spectra of deutero HRP I at ambient temperatures show[9] more line broadening and larger average contact shifts (50–100

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(39) The unpaired spin density contributions by both the ferryl and π-cation moieties to the β-pyrrole positions are positive, as shown by INDO calculations[6b] on ferrylporphines and their π-cation radicals, and SCF-PPP calculations[27] on porphyrin π cations. Indeed, the NMR spectrum of deuteroporphyrin-substituted HRP I[9] reveals that the 2,4-protons (which replace the vinyl groups of protoporphyrin) shift upfield, in the opposite direction of the methyl groups. These results are consistent with positive spin densities at the C_β's because Q, the proportionality constant which relates unpaired spin density to the contact shift, is positive for a methyl proton and negative for an aromatic proton.

(40) Similar profiles are predicted for radicals of iron(IV) porphine by IEH and INDO calculations.[6]

(41) Schaffer, A. M.; Gouterman, M. *Theor. Chim. Acta* **1972**, *25*, 62–82.

(42) La Mar, G. N.; Budd, D. L.; Smith, K. M.; Langry, K. C. *J. Am. Chem. Soc.* **1980**, *102*, 1822–7.

(43) Takano, T. *J. Mol. Biol.* **1977**, *110*, 569–84.

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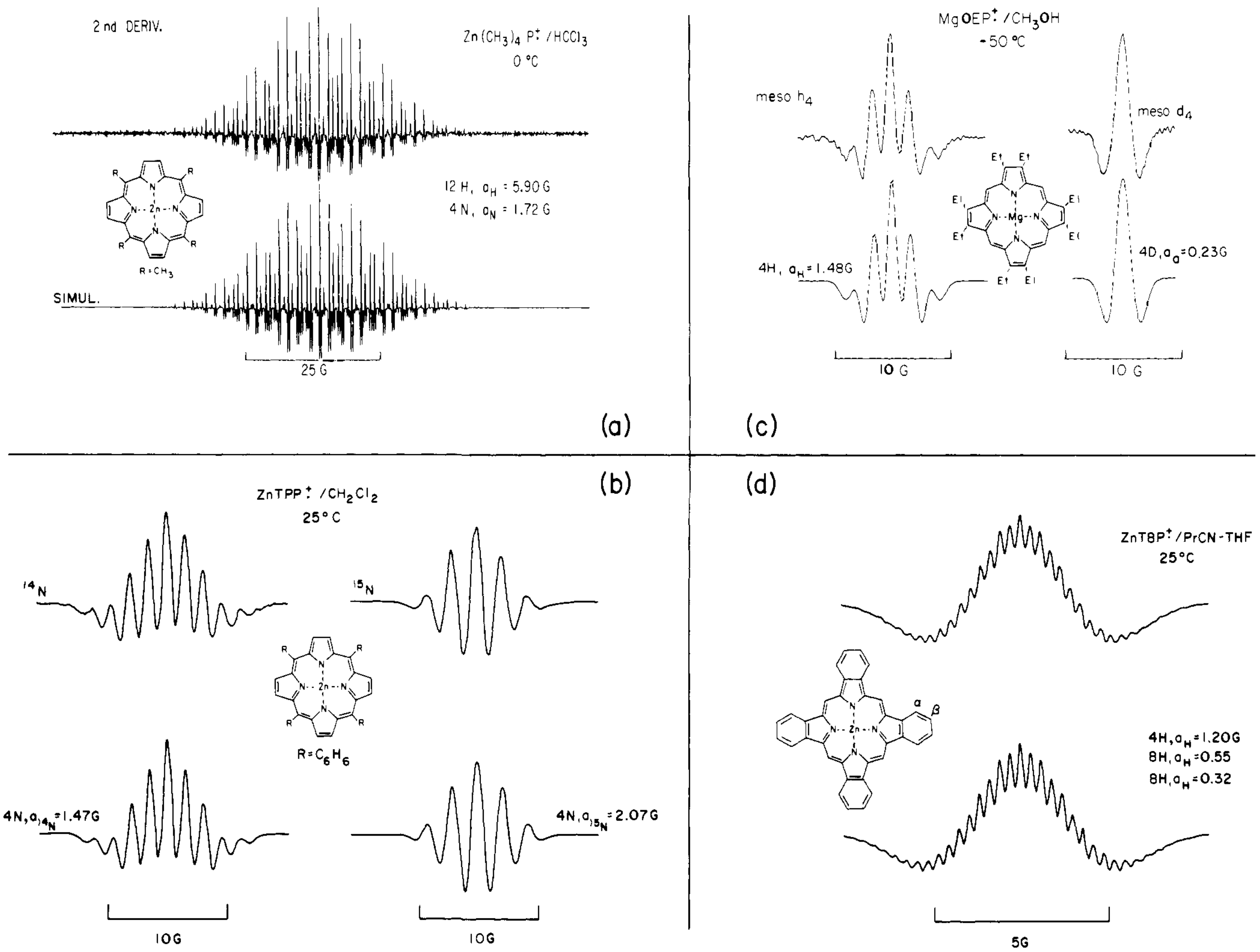

Figure 5. Second derivative ESR spectra: (a) $Zn(CH_3)_4P\cdot^+ClO_4^-$ ($^2A_{2u}$). The simulation assumes 12 protons (4 methyl groups), $a_H = 5.90$ G, and 4 nitrogens, $a_N = 1.72$ G. The narrow line widths and close agreement between the observed and simulated ESR spectra preclude any signficant unpaired spin density at any other nuclei (from ref 5e). (b) $ZnTPP\cdot^+ClO_4^-$ ($^2A_{2u}$). ^{14}N: The 9-line spectrum is due to four nitrogens, $a_{^{14}N} = 1.47$ G. The large unpaired spin density at the meso positions is not evident because the phenyl groups are oriented out of the plane of the porphyrin (from ref 27). ^{15}N: The 5-line spectrum arises from the four nitrogens with $a_{^{15}N} = 2.07$ G. (The nuclear spins of ^{14}N and ^{15}N are $I = 1$ and $^1/_2$. $a_{^{15}N}/a_{^{14}N} = 1.40$ is fixed by the gyromagnetic ratios of the isotopes.) (c) $MgOEP^+\cdot ClO_4^-$ ($^2A_{1u}$). h$_4$: The simulation assumes four protons, $a_H = 1.48$ G. Small splittings for the nitrogens, $a_N \approx 0.3$ G, or the methylene groups, $a_H^{CH_2} \approx 0.3$ G, can be accommodated within the simulated line widths. d$_4$: The spectrum obtained on exchanging the four meso protons by deuterons. The simulation includes four deuterons, $a_D = 0.227$ G. (The nuclear spins of ^1H and ^2H are $I = {}^1/_2$ and 1, and $a_D/a_H = 0.1535$; from ref 5c and 27). (d) $ZnTBP^+\cdot ClO_4^-$ ($^2A_{1u}$). The simulation is based on three proton splittings determined by ENDOR and assigned on the basis of the MO calculations to the four meso protons, $a_H = 1.2$ G, the eight α protons of the benzene rings, $a_{H_\alpha} = 0.55$ G, and the eight β protons, $a_{H_\beta} = 0.32$ G.

ppm) for the methyl substituents than protoheme. Although qualitatively consistent with the shift patterns observed,[45] calculated spin densities for the a_{1u} imidazole radical are too large by approximately a factor of 2. The calculation may therefore simply overestimate the ρ values, or the room-temperature NMR spectra may reflect some mixing of the a_{1u} and a_{2u} states. (Such a combination becomes allowed because of the lack of symmetry in the system.) Optical spectra of deutero HRP I at room temperature do in fact deviate[5g] from the characteristic a_{1u} features observed at −42 °C. More importantly however, the NMR shifts of deutero HRP I certainly lie well within the range of unpaired spin densities predicted by the calculations for a_{1u} and a_{2u} ferryl π cations.

Since catalase contains protoheme as the prosthetic group, the a_{1u} optical spectrum of its compound I (Figure 1) may be attributable to a ligation different from that of HRP. Indeed, whereas Fe^{II}(HRP)NO complexes clearly show hyperfine splittings due to a nitrogenous sixth ligand, the corresponding (CAT)NO complex does *not*.[29] Examination of the energy level diagrams for ferryl compounds (Figure 2) shows that the a_{2u} radical is clearly preferred when the heme is strongly bound to imidazole but that the gap between the a_{1u} and a_{2u} orbitals narrows in the absence of a sixth ligand. Calculations[47a] for model ferrylporphines free of ligand or bound to imidazole, imidazolate, mercaptan, and mercaptide predict that the difference between the a_{2u} and a_{1u} orbitals increases in the order: no ligand < mercaptide ≪ imidazole < mercaptan < imidazolate.[47b] In agreement with these considerations, compound I of chloroperoxidase which is a protoheme believed[48] to be complexed by a sulfydryl anion, exhibits[49]

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(45) The contact shifts exhibited by substituents at the β-pyrrole positions are influenced not only by the unpaired spin densities at nearby carbon atoms but also by the protein environment[44] and axial ligand orientation.[46] Because protoheme and deuteroheme have different orientations in the HRP pocket,[44] the contact shifts of the methyl groups cannot be wholly attributed to differences in spin density.

(46) Traylor, T. G.; Chang, C. K.; Geibel, J.; Berzinis, A.; Mincey, T.; Cannon, J. *J. Am. Chem. Soc.* **1979**, *101*, 6716–31. Goff, H. *Ibid.* **1980**, *102*, 3252–4. Walker, F. A. *Ibid.* **1980**, *102*, 3254–6. Traylor, T. G.; Berzinis, A. P. *Ibid.* **1980**, *102*, 2844–6.

(47) (a) The differences between the a_{2u} and a_{1u} orbital energies for ferrylporphines as a function of axial ligand are no ligand, 0.475 eV, < methylmercaptide, 0.541 eV, < imidazole, 0.683 eV, < methylmercaptan, 0.734 eV, < imidazolate, 0.768 eV. The methylmercaptide and mercaptan calculations had the iron in-plane and Fe–S = 2.36 Å. (b) An imidazolate ligand for HRP is appealing because it causes only minimal changes in the ρ's of the a_{2u} radical, when compared to imidazole, but splits the d_{xz} and d_{yz} orbitals of the resting enzyme and may thus explain the rhombicity of the ferriheme.[13]

(48) Cramer, S. P.; Dawson, J. H.; Hodgson, K. O.; Hager, L. P. *J. Am. Chem. Soc.* **1978**, *100*, 7282–90.

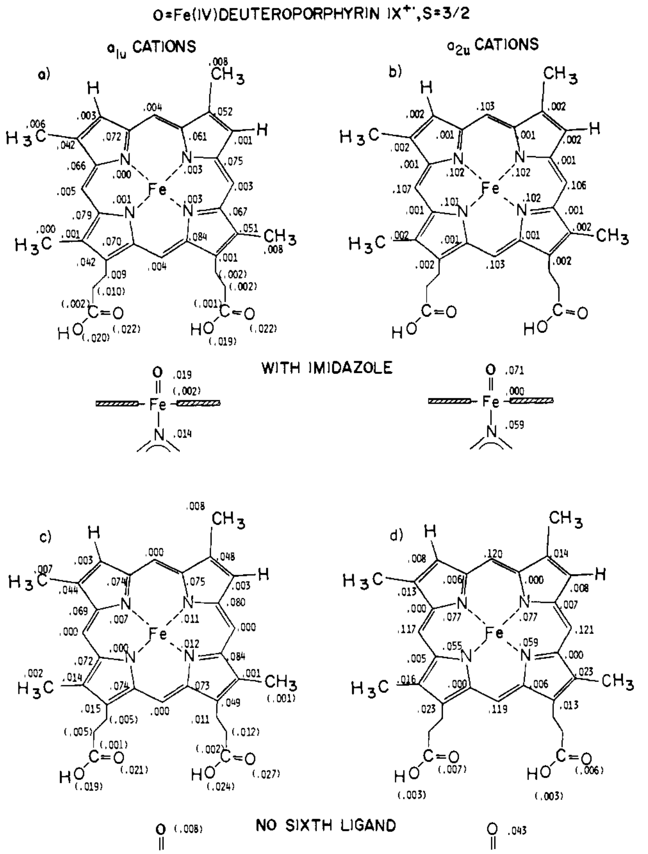

Figure 6. Calculated unpaired spin densities for $S = {}^3/_2$ ferryldeuteroporphyrin radicals: (a) a_{1u} with Fe in-plane, Fe–N(imid) = 2.107 Å, (b) a_{1u} with Fe 0.5 Å out-of-plane and no sixth ligand, (c) a_{2u} with Fe in-plane, Fe–N(imid) = 1.957 Å, and (d) a_{2u} with Fe 0.5 Å out-of-plane and no sixth ligand. The imidazole–Fe distance had to be lengthened in (a) in order for the calculation to converge.

catalatic activity and an optical spectrum similar to CAT I, i.e., an a_{1u} radical. These results suggest therefore that the sixth ligand of CAT I is either less tightly bound than that of HRP, nonnitrogenous, or absent. Unpaired spin densities for a_{1u} ferryl proto- and deuteroporphyrin radicals without ligands are shown in Figures 3 and 6.

Chlorins and *Neurospora crassa* Catalase. Jacob and Orme-Johnson recently isolated[23] a catalase from *Neurospora crassa* which contains an iron chlorin as the prosthetic group. The enzyme decomposes H_2O_2 efficiently, in common with catalases that contain protoporphyrins. This unusual heme offers an opportunity to further test the Fe^{IV} π cation hypothesis by spectroscopic techniques because π cations of chlorins exhibit unpaired spin density profiles and optical characteristics which differ significantly from those of porphyrins (vide infra). The oxidation potentials required to remove two electrons from synthetic iron(III) chlorins lie[5d,50,51] within 200 mV of those of iron porphyrins (+1.2 V (vs. SCE) for chlorins vs. 1.4 V for porphyrins). Therefore, *N. crassa* catalase is likely to undergo oxidation by the same intermediates as normal catalases. Energy level diagrams for $S = 1$ ferrylchlorins with and without axial ligands are shown in Figure 7. A configuration for compound I of *N. crassa* catalase similar to other catalases is evidently reasonable, except that a_2 and b_2 orbitals (for an assumed C_{2v} symmetry) replace the corresponding a_{1u} and a_{2u} orbitals of the porphyrins and are nearly degenerate. Unpaired spin densities for $O{=}Fe^{IV}$ cations in these states are shown in Figure 8. They are characterized by high-spin

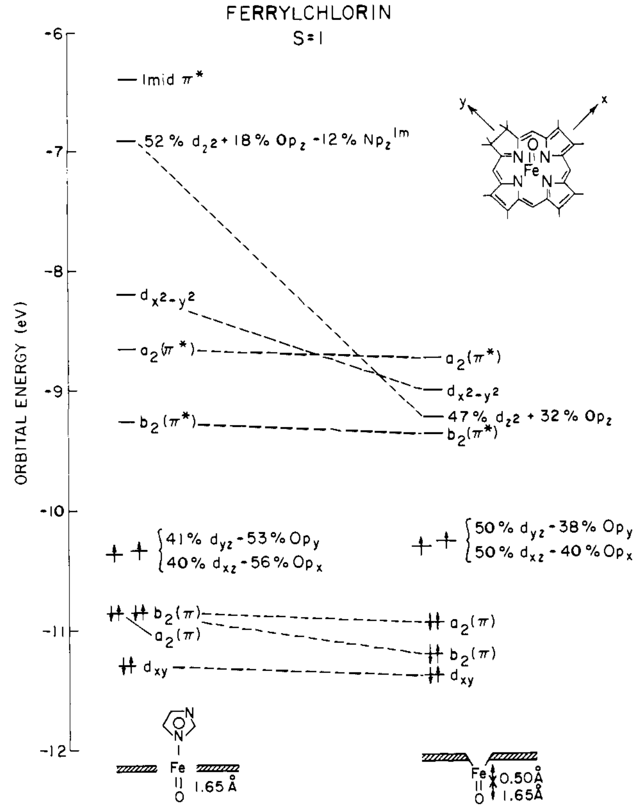

Figure 7. Energy level diagrams for ferrylchlorins in the $S = 1$ spin state, with and without an imidazole ligand. The chlorin ring coordinates are described in ref 54c, with Fe–N(unsaturated pyrroles) = 2.01 Å and Fe–N(saturated pyrrole) = 2.03 Å. Fe–N(imid) = 1.957 Å.

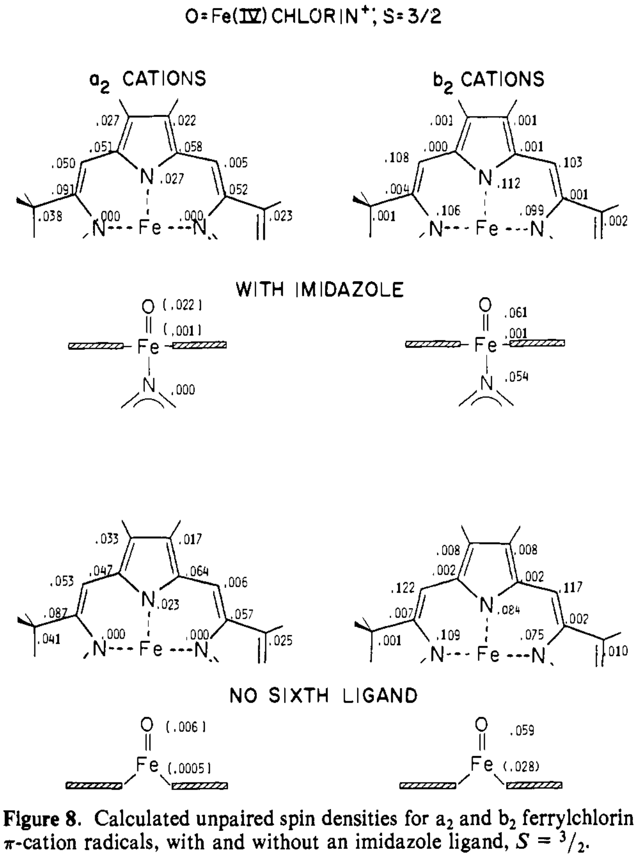

Figure 8. Calculated unpaired spin densities for a_2 and b_2 ferrylchlorin π-cation radicals, with and without an imidazole ligand, $S = {}^3/_2$.

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(49) Palcic, M. M.; Rutter, R.; Araiso, T.; Hager, L. P.; Dunford, H. B. *Biochem. Biophys. Res. Commun.* **1980**, *94*, 1123–7.

(50) $E_{1/2}$ = 1.22 V for the two-electron oxidation of $ClFe^{III}MeOEC$ in CH_2Cl_2. E_0's for compound I/compound II of HRP A_2 and C have been reported as ~0.7 V at pH ~6. Surprisingly, E_0 for compound II/Fe^{III}HRP is nearly the same. (Hayashi, Y.; Yamazaki, I. *J. Biol. Chem.* **1979**, *254*, 9101–6.)

(51) Stolzenberg, A. M.; Spreer, L. O.; Holm, R. H. *J. Chem. Soc., Chem. Commun.* **1979**, *1077*–8.

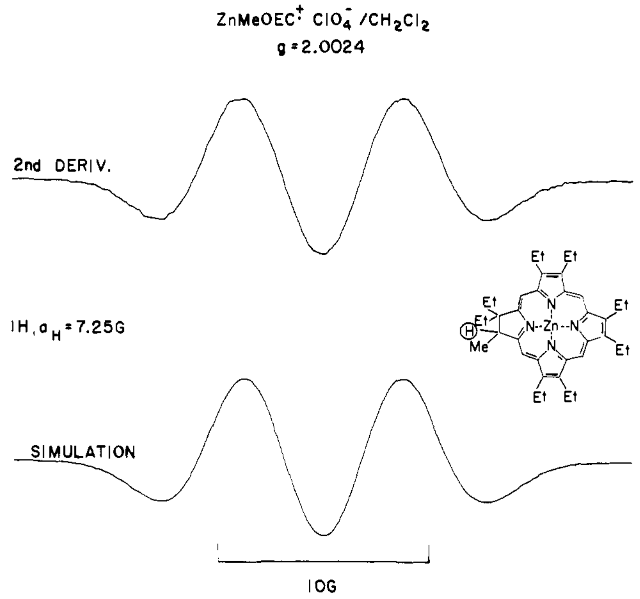
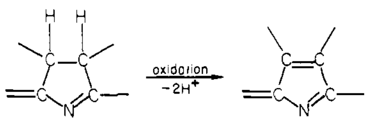


Figure 9. Second-derivative ESR spectrum of ZnMeOEC$^{+}\cdot$ClO$_4^-$ in CH$_2$Cl$_2$ at 25 °C. The simulation demonstrates that one proton (a_H = 7.25 G) determines the pattern observed. This splitting is assigned to the proton on the reduced pyrrole ring on the basis of the MO calculations and by analogy with selectively deuterated chlorins.[2a,4b] The smaller splittings expected from the calculated spin densities[2a,4b] at the nitrogens, meso protons, methylene, and methyl groups are not resolved but can readily be accommodated within the spectral envelope.

densities at the α-carbons in the a$_2$ radical to be contrasted to the significant densities at the meso and nitrogen positions in b$_2$ (these are respectively analogous to the a$_{1u}$ and a$_{2u}$ porphyrin radicals). ESR studies of model zinc and magnesium chlorins indicate[2a,4b] that the ^{2}A$_2$ (electron abstracted from the a$_2$ orbital) is the normal ground state. (Significant variations in hyperfine splittings have, however, been observed in the chlorin cations as a function of axial ligand and temperature and have been attributed[2a,4b] to mixing between the ^{2}A$_2$ and ^{2}B$_2$ states.) By analogy with the model compounds, we assume that the profile of the iron(IV) chlorin π cations will effectively be that of an a$_2$ radical. The unpaired spin densities for this state (Figure 8) suggest therefore that NMR spectra of such radicals should exhibit significant line broadening and contact shifts for the substituents on all the rings (especially the reduced one) and two meso positions.

Jacob and Orme–Johnson noted[23] that the chlorin isolated from *N. crassa* did not undergo the oxidative dehydrogenation to porphyrin typical of chlorins in which the reduced ring bears vicinal protons



We have therefore chosen methyloctaethylchlorin (Figure 9) as a synthetic model of the *N. crassa* prosthetic group. Because MeOEC does not contain vicinal dihydro substituents on the reduced ring, it too is immune to dehydrogenation to porphyrin. In dichloromethane, ClFe^{III}MeOEC displays absorption bands at 378 nm ($\epsilon = 9.1 \times 10^4$ M^{-1} cm^{-1}) and 607 nm ($\epsilon = 2.2 \times 10^4$) whereas the native enzyme absorbs[23] at 400 and 590 nm with $A_{400}/A_{590} = 4.89$.

The cation radical of ZnMeOEC exhibits a doublet ESR signal, centered at $g = 2.0024$, with a splitting constant of 7.25 G in CH$_2$Cl$_2$ at 25 °C (Figure 9). This splitting is comparable to those observed[2a,4b] in several chlorin radicals (5–7G), and which have been assigned to the protons on the reduced rings by selective deuteration. The Zn cation thus also conforms to the unpaired spin density profile predicted for a ^{2}A$_2$ state.

As a guide to the optical transitions that may be expected for compound I of *N. crassa* catalase, Figure 10 displays spectra of the cation radicals of Zn- and Co^{III}MeOEC. Oxidation results in small shifts of the Soret bands,[52] a loss of the 617 nm bands characteristic of chlorins, and the appearance of weak, broad bands stretching into the near infrared. By analogy with compounds I of HRP and CAT, for which Co^{III} π cations have proven[5] to be successful models, spectra similar to those observed for the oxidized zinc and cobalt chlorins may be expected to characterize compound I of *N. crassa* catalase.

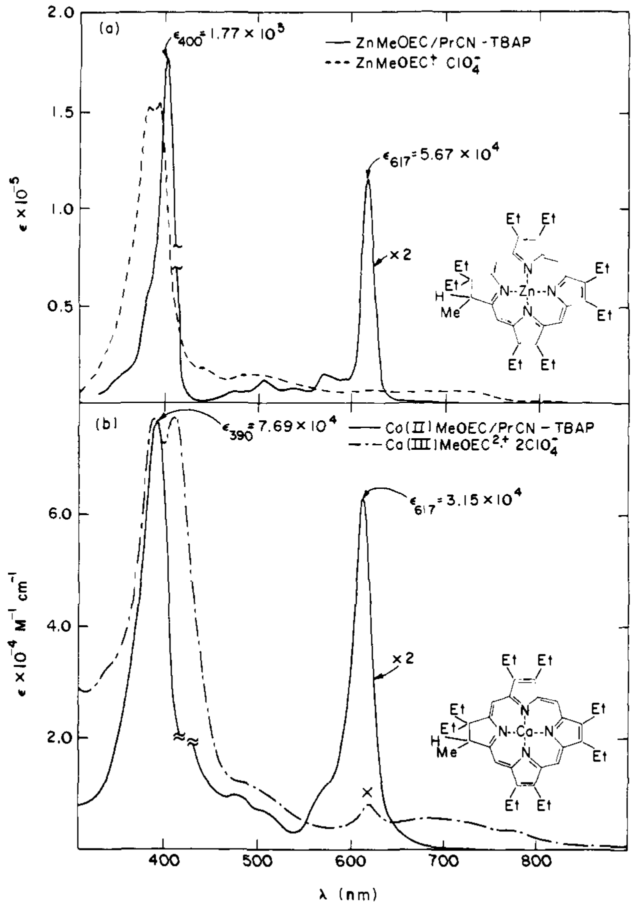


Figure 10. Optical absorption spectra, in butyronitrile: (a) ZnMeOEC (—) and its cation radical (---). (b) Co^{II}MeOEC (—) and its *two-electron* oxidation product Co^{III}MeOEC$^{\cdot 2+}$2ClO$_4^-$. (---; x may represent a small amount of unoxidized Co^{III}MeOEC$^+$ClO$_4^-$.)

In conclusion, our results and other recent theoretical[6] and experimental studies[7–9] suggest that ferryl π cation formulations for compounds I of HRP and CAT are eminently reasonable and satisfactorily account for their reported physical properties.

The calculations suggest that ^{2}H, ^{13}C, and ^{15}N NMR studies of isotopically substituted proto and deutero HRP would confirm the electronic profiles predicted. ^{15}N NMR in particular would clearly distinguish between a$_{1u}$ and a$_{2u}$ radical configurations. The different spin density distribution of the two ground states may also couple differently with the $S = 1$ iron and therefore yield different ESR parameters for the signals associated[7] with compounds I. Compounds I and II should be amenable to EXAFS[53] investigations: edge shifts and the near edge structure could establish the ferryl configuration, and analysis of the absorption fine structure may distinguish the different ligation suggested for HRP and CAT. The optical spectra and unpaired spin densities presented above should also help verify the postulated radical nature of compound I of *N. crassa* catalase and further test the Fe^{IV} π cation hypothesis.

Finally, we note that iron π cations may also mediate electron transport in other heme proteins such as nitrite and sulfite reductases, which utilize[51,54] iron isobacteriochlorins (sirohemes)

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(52) Note the unusual split Soret bands in the radicals.

(53) Stern, E. A. *Contemp. Phys.* **1978**, *19*, 289–310.

to catalyze the six-electron reductions of nitrite and sulfite to ammonia and hydrogen sulfide, respectively. A combination of experiment and theory suggests[54] that, because of the ease of oxidation of the isobacteriochlorin skeleton, π radicals play a role in the enzymatic cycles of nitrite and sulfite reductases. ESR results confirm[54b,c] the theoretical prediction[54c] that oxidation of pyridine or imidazole CO complexes of iron(II) isobacteriochlorins proceeds via the macrocycle and unambiguously establish the existence of iron π cation radicals.

Note Added in Proof: Hoffman and co-workers[55] have obtained ^{1}H and ^{14}N ENDOR hyperfine splittings which further support the assignment of HRP I to a π cation. The isotopic substitution experiments suggested above for NMR are obviously equally applicable for ENDOR.

Acknowledgment. We thank the authors of ref 6, 7, 9, 44, 49, and 55 for providing us with their results prior to publication. This work was supported by the Division of Chemical Sciences, U.S. Department of Energy, Washington, D.C., under Contract No. DE-AC02-76CH00016 at Brookhaven National Laboratory and by NSF Grant CHE 7815285 at Michigan State University.

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(54) (a) Richardson, P. F.; Chang, C. K.; Spaulding, L. D.; Fajer, J. *J. Am. Chem. Soc.* **1979**, *101*, 7736–8. (b) Chang, C. K.; Fajer, J. *Ibid.* **1980**, *102*, 848–51. (c) Richardson, P. F.; Chang, C. K.; Hanson, L. K.; Spaulding, L. D.; Fajer, J. *J. Phys. Chem.* **1979**, *83*, 3420–4.

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Communications to the Editor

Cycloreversion Reactions of Phenylated Cage Compounds Induced by Electron Transfer[1]

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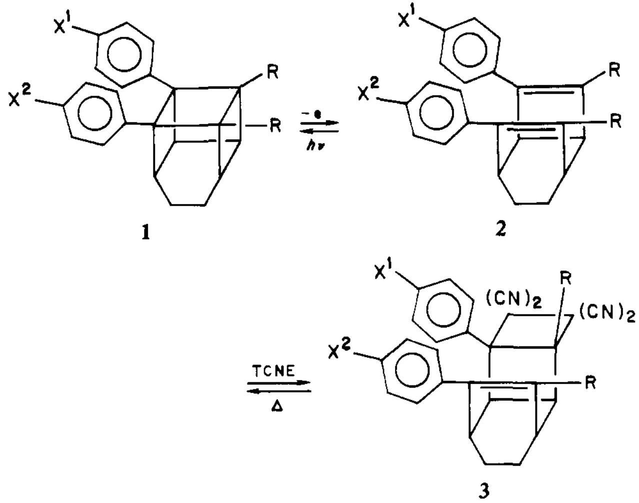
Received June 30, 1980

In addition to the ring-opening reactions of cage compounds catalyzed with acid[2] and metal ions,[3] photochemically and thermally induced valence isomerizations between cyclic dienes and cage compounds are well documented.[4,5] We have found that electron transfer both in the ground and excited states also can induce cycloreversion of certain cage compounds possessing phenyl groups. In this connection there are reported examples of ring-opening reactions of monocyclic phenylated cyclobutanes[6] and cyclopropanes[7] which proceed via electron-transfer reactions. However, our findings can provide a new procedure for the cycloreversion reactions of cage compounds which occur with extraordinary high efficiency.

The observed reactions are divided into three types: (1) ring opening reactions induced by electron transfer or charge transfer in the dark; (2) reactions induced by irradiation of the charge-transfer complexes; (3) reactions which occur by electron transfer between the ground state of the cage compounds and the excited state of the electron acceptors. We describe here the relationship between the types of reaction and the electron-donating or -accepting properties of substrates or electron acceptors. The mechanism of type 3 is mainly described here.

Table I. Charge-Transfer Band Maxima of **1** and **2** with TCNE in CH_2Cl_2



	R	X¹	X²	1, nm	2, nm
a	Me	OMe	OMe	700 (596)[a]	740 (627)[a]
b	Me	OME	H	630	680
c	Me	H	OMe	590	640
d	Me	H	H	538 (end)[a,b]	627 (547)[a]
e	Me	Cl	Cl		601
f	CO_2Me	H	H	420 sh	end[b]

[a] Chloranil instead of TCNE. [b] End absorption, 500–600 nm.

The charge-transfer absorption bands resulting from the interaction of bis(homocubane) derivatives **1a–f**[8] with tetracyanoethylene (TCNE) are shown in Table I, together with those of dienes **2a–f** with TCNE. Although the charge-transfer absorption band of 4-phenylhomocubane with TCNE has been reported,[9] Table I reveals that the substitution of *p*-anisyl for phenyl, especially in **1a** and **1b**, results in large red shifts of the absorption maxima. This is ascribed to a through–space[10] or through–bond

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