Density Functional Study on the Electronic Structures of Model Peroxidase Compounds I and II

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Abstract: The electronic structures of $[Fe(Por)(Im)O]^{1+}$ and [Fe(Por)(Im)O] (model compounds I and II, respectively) have been studied on the basis of density functional theory or DFT (Por = porphine, Im = imidazole). The $a_{2\mu}$ π -cation radical state (⁴A_{2u}) was determined to be the ground state of compound I with total spin equal to $\frac{3}{2}$, while the $a_{1u} \pi$ -cation state (⁴A_{1u}) was found to be 0.15 eV higher in energy than the ⁴A_{2u} state. Since, in both states, the spins were localized to the porphyrin ring ($S = \frac{1}{2}$) and the Fe–O center (S = 1), the magnetic coupling interaction between the two spin sites was examined by using a broken symmetry method. The calculated J value revealed very weak magnetic coupling for the A_{2u} state, which corresponded to the experimental data. The calculated J value revealed strong antiferromagnetic coupling for the A_{1u} state. The calculated Mössbauer spectrum parameters (quadrupole splitting and asymmetry) were similar for both the A_{1u} and A_{2u} states, and both agreed well with experimental values. On the other hand, the calculated hyperfine coupling constants for the nitrogen and the proton of the porphyrin ring were different in the two states. Although the experimental coupling constant values of the pyrrole nitrogen atoms were intermediate between the calculated values for the A_{2u} and A_{1u} states, the experimental values for the meso protons were closer to the values calculated for the A_{2u} state. These results suggest that the electronic structure of compound I is closer to the A_{2u} state than to the A_{1u} state. However, these results also suggest that there is the possibility that the electronic structure of compound I is an admixture of the A_{1u} state and the A_{2u} state. The electronic structure of compound II was calculated and compared with the electronic structure of compound I. The energetics of the redox reaction between the two compounds is discussed.

Introduction

Peroxidases, such as horseradish peroxidase (HRP), are hemeprotein enzymes that catalyze substrate oxidation by hydrogen or alkyl peroxides. Two intermediates, compounds I (HRP-I) and II (HRP-II), have been shown to be involved in the HRP reaction process¹ as shown in the following scheme:

$$HRP + H_2O_2 \rightarrow HRP-I + H_2O \tag{1}$$

$$HRP-I + AH_2 \rightarrow HRP-II + AH \bullet$$
 (2)

$$HRP-II + AH_2 \rightarrow HRP + AH_{\bullet} + H_2O$$
(3)

HRP-I is two oxidizing equivalents above the ferric resting state of the enzyme and is considered to have a Fe–O bond with a porphyrin π -cation radical. HRP-II, produced by one-electron reduction of HRP-I, is also considered to have a Fe–O bond, but the π -cation hole on the porphyrin disappears following recombination with an electron.

The electronic and magnetic properties of HRP-I, HRP-II, and their related compounds have been extensively investigated by magnetic susceptibility,^{2,3} Mössbauer,^{4–9} ESR,^{9,10} electronic absorption,^{11–13} ENDOR,^{14,15} NMR,^{3,13,18–21} resonance

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Raman,^{22–27} EXAFS,^{16,17} and X-ray crystallography.²⁸ Magnetic susceptibility measurements have indicated that there are

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three unpaired electrons ($S = \frac{3}{2}$) for HRP-I and two unpaired electrons (S = 1) for HRP-II.^{2,3} Mössbauer spectra, as well as ESR spectra, suggest that both HRP-I and -II have an Fe(IV) configuration (S = 1).⁴⁻¹⁰ The observed quadrupole splitting was determined to be approximately 1.3 mm/s; the isomer shift was roughly equal to 0.1 mm/s, and the asymmetric parameter was 0.0 for both compounds. These values are different from ferrous and ferric Fe porphyrin values, but they are similar to values for model compounds considered to be ferryl Fe porphyrins such as FeTPP(py)O. In addition, electronic spectrum studies have suggested that the extra spin ($S = \frac{1}{2}$) in HRP-I is distributed on a porphyrin ring as a π -cation radical, yielding a net spin of 3/2 when the S = 1/2 porphyrin is coupled to the Fe(IV) center with $S = 1.^{11,12}$ ENDOR study for HRP-I by Roberts et al.¹⁵ provided direct evidence for a π -cation radical center. Their observations of hyperfine structures for ${}^{14}N$, C_a, and C_{β} protons and their comparisons with the theoretical studies led them to conclude that the structures came from the porphyrin radical formed when one electron was removed from the $a_{2\mu} \pi$ molecular orbital.

That one O atom is bound to the Fe(IV) was suggested by earlier ¹⁸O isotopic substitution studies on chloroperoxidase.²⁹ Structural information on the Fe–O bond in HRP-I and HRP-II was provided by EXAFS studies,^{16,17} and structural data on Fe(IV)-oxo-"picket-fence" porphyrin were collected from an X-ray crystallographic study.²⁸ The Fe–O distances were reported to be 1.64 and 1.604 Å, respectively. The ¹⁷O ENDOR study on HRP-I suggested that about 25% of the spin on the Fe–O center was located at the oxygen site with axial symmetry.¹⁴ Similarity between the Fe–O electronic structures in HRP-I and HRP-II was further revealed by Mössbauer spectrum parameters^{4–9} and EXAFS spectroscopy.^{16,17}

Theoretical studies for HRP-I and HRP-II were performed by using several quantum chemical methods. Charge iterative extended Hückel calculations^{30,31} for model compounds of the ground state of HRP-I ($S = \frac{3}{2}$) indicated that two unpaired electrons were located over the Fe-O center in nearly degenerate and extremely delocalized antibonding $(d\pi + p\pi)$ orbitals. These studies also indicated that the remaining unpaired electron was located in the highest energy, half-filled $a_{2\mu}$ porphyrin π -orbital. UHF³² and RHF^{33,34} INDO methods were also used to investigate the model compounds. Mössbauer quadrupole splittings and the NMR chemical shifts of HRP-I correlated with the electronic configuration corresponding to Fe(IV) (S = 1) and porphyrin π -cation radical ($S = \frac{1}{2}$). Approximately 20% of the unpaired spin density in the Fe-O center was calculated to be on the O atom; this value was in agreement with the experimentally determined value of approximately 25%. However, the a_{2u} π -cation radical (the ${}^{4}A_{2u}$ state) was found to be several kilocalories per mole higher in energy than the a_{1u} π -cation radical (the ⁴A_{1u} state). Ab initio UHF study^{35,36} also insisted that the ground state of the HRP-I model compound is the ${}^{4}A_{1u}$ state rather than the ${}^{4}A_{2u}$ state. It is important to note that the structure of the model compound used in those calculations lacked an axial ligand corresponding to the imi-

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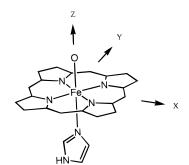


Figure 1. Geometry used for the calculations of model compounds I and II. The imidazole molecule lies in the xz plane.

dazole of HRP. Ab initio RHF and CASSCF calculations37 on model HRP-I and HRP-II that incorporated pyridine as an axial ligand revealed that configuration interaction effects are essential for the description of the Fe-O bond in both HRP-I and HRP-II. Although the character of the Fe-O center and of the porphyrin π -cation radical are essentially the same, X α multiple scattering calculations³⁸ yielded an electronic structure that differed from that obtained with other types of calculations. The two $d\pi - p\pi$ orbitals of the Fe–O center were calculated to be lower in energy than the a_{1u} and a_{2u} porphyrin π -orbitals and to admix more with occupied e_g porphyrin π -orbitals. These alternative descriptions are significant because they point out slight differences concerning the spin distribution of the porphyrin ring that are important for the interpretation of hyperfine structures. Local density functional (LDF) calculations³⁹ using the local von Barth-Hedin functional also exhibited almost the same spin distribution as the $X\alpha$ calculations, although a sixth, axial ligand was not included in the model compounds.

Electron correlation is an important part of the description of the electronic structures of HRP-I and HRP-II. Previous disparities, including the results of X α calculations, prompt thorough analysis. Nonlocal density functional theory (NL-DFT) incorporates electron correlation and is more sophisticated than X α calculations. The DFT calculations presented in this study will provide new insight into the electronic structures of compounds I and II, including the ground state of compound I and the spin coupling between the Fe–O center and the π -cation radical. The DFT calculations treat the energetics of the compound I \rightarrow compound II redox process, and the nature of the orbitals involved.

Details of the Calculations

A. Molecular Geometry. Fe-porphyrin complexes with O and imidazole axial ligands were used as model compounds of HRP-I and HRP-II. The geometries of the Fe-porphyrin complexes were based on the X-ray structures of previous model compounds published by Collman et al.⁴⁰ In the model compounds, the distance between the Fe atom and the axial imidazole was set to 1.98 Å. The distance between the Fe atom and the axial O atom was set to 1.70 Å. In the HRP-II model, the Fe-O distance was varied in order to examine the potential energy curve. The porphyrin X-ray structure was idealized to D_{4h} symmetry. The coordinate axes were arranged so that the nitrogen atoms were on the bisector of the *x* and *y* axes. The imidazole was placed in the *xz* plane so that the molecular symmetry was Cs (Figure 1). Following this convention, the notation of the D_{4h} symmetry group

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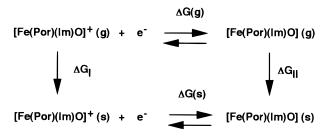
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Scheme 1



was employed in labeling the molecular orbitals (MO's) of the porphyrin ring, although the symmetry was not perfectly maintained.

B. Density Functional Calculations. All calculations were carried out with the Density Functional LCAO computer program of Baerends, Ros, and co-workers (AMOL codes)⁴¹ on a Cray-YMP supercomputer at the Scripps Research Institute. Vosko–Wilk–Nusair (VWN) LSD⁴² potential was used with nonlocal Becke exchange correction⁴³ and nonlocal Perdew correlation correction.⁴⁴ The nonlocal corrections were incorporated into the potential during the SCF procedure (except as noted). The Slater-type basis set was applied with a frozen core approximation. For Fe, the 1s, 2s, and 2p atomic orbitals (AO) were frozen, and the 1s AO were also frozen for C, N, and O. The calculations were performed with a triple- ζ basis set with polarization functions (4p AO for Fe, 3d AO for C, N, and O, and 2p AO for H). A spin-unrestricted open-shell method was used for all calculations.

Calculations including all electrons were performed in order to estimate hyperfine coupling constants. For the study of the total bonding energy dependence on the Fe–O distance in the HRP-II model, the double- ζ basis set and VWN potential without nonlocal correction were employed in the SCF calculation. This change was neccesary to keep CPU working periods within reasonable limits and to reduce disc space. Following the SCF calculation, nonlocal corrections were added to the total bonding energy as the perturbation energy.

The hyperfine coupling constants were evaluated on the basis of the SCF wave functions derived from calculation where core orbitals were unfrozen (described above). The equations were:

$$a = (4\pi/3S_{\rm i})g_{\rm e}\beta_{\rm e}g_{\rm N}\beta_{\rm N}[\rho^{\alpha}(0) - \rho^{\beta}(0)]$$
(4)

$$A_{\rm pq} = (g_{\rm e}\beta_{\rm e}g_{\rm N}\beta_{\rm N}/2S_{\rm i})\sum_{r}\sum_{s}\rho_{r\rm s}^{\rm spin}\int\chi{\rm s}\frac{3r_{\rm p}r_{\rm q}-\delta_{\rm pq}r^{2}}{r^{5}}\chi_{r}\,{\rm d}\upsilon$$
 (5)

where *a* and A_{pq} are isotropic and anisotropic hyperfine coupling constants, and where $S_i = S_{Fe-O} = 1$ or $S_{por} = \frac{1}{2}$ for intrinsic hyperfine parameters on the Fe–O or porphyrin radical centers, respectively. In the A_{pq} calculation for atom A, the contribution from the AO of A (on-center contribution) was analytically obtained. The contribution of overlap portions and of other atoms (off-center contribution) was estimated by numerical integration.

The Mössbauer spectrum parameters for ⁵⁷Fe, the asymmetry parameter (η) and quadrupole splitting (ΔE_Q), were calculated by using the following equations:

$$\eta = \frac{|V_{xx} - V_{yy}|}{|V_{zz}|} \tag{6}$$

$$\Delta E_{\rm Q} = \frac{1}{2} e Q |V_{zz}| \left(1 + \frac{\eta^2}{3}\right)^{1/2} \tag{7}$$

The V values are the principal components of the electronic field gradient (EFG) at the Fe nucleus. Q is the quadrupole moment of the Fe nucleus (equal to 0.15 barn⁴⁵), and e is the electron charge. The EFG tensors were approximated by using the Sternheimer factors for Fe. The EFG tensors represent the contribution of both valence electrons in orbitals centered on the Fe (V^{ral}) and ligand charges surrounding it (V^{lat}).

$$V_{\rm pq} = V_{\rm pq}^{\rm val} + V_{\rm pq}^{\rm lat} \tag{8}$$

$$V_{\rm pq}^{\rm val} = -e(1-R_s) \sum_{r} \sum_{s} \rho_{rs} \int \chi_s \frac{3r_{\rm p}r_{\rm q} - \delta_{\rm pq}r^2}{r^5} \chi_r \,\mathrm{d}\nu \qquad (9)$$

$$V_{\rm pq}^{\rm lat} = e(1 - \gamma_{\infty}) \sum_{j} q_{j} \frac{3R_{\rm p}R_{\rm q} - \delta_{\rm pq}R^{2}}{R^{5}}$$
(10)

The factors $(1 - R_s)$ and $(1 - \gamma_{\infty})$ are the Sternheimer corrections 0.92 and 10.5, respectively. *R* is the vector to a ligand with a Mulliken charge of q_j . The Mössbauer calculations used a small frozen core through Fe(2p), but with properties evaluated over valence Fe(3d,4s,4p) orbitals and all ligand orbitals. This both gave the most stable Mössbauer results and is an appropriate basis set for using the Sternheimer factors above to account for nonspherical core deformation.

C. Redox Potential Calculations. A thermodynamic cycle (Scheme 1) was used to calculate the redox potential between compound **I** and compound **II** in aqueous solution. Electrostatic calculations were used to obtain the free energies of solvation ΔG_{II} and ΔG_{II} . DFT calculations yielded the enthalpy difference $\Delta H(g)$ for the gas phase for the redox reaction. The entropy difference $\Delta S(g)$ was ignored since it is expected to be insignificant compared to $\Delta H(g)$. In solution, the free energy difference is given by the following equation:

$$\Delta G(s) = \Delta G(g) + \Delta G_{\rm I} - \Delta G_{\rm II} \tag{11}$$

The redox potential can be calculated with this equation. An experimental value for the standard hydrogen potential (-4.5 V) was used for calculation of the standard redox potential. Electrostatic solvation energies were calculated by using the MEAD (Macroscopic Electrostatics with Atomic Detail) suite of programs developed by Bashford.⁴⁶ This approach is based on the Poisson equation for solvation free energies. The solute was treated as a set of irregularly shaped objects with point charges at positions corresponding to the atomic nuclei, and the solvent as a continuous dielectric medium. The ESP charges obtained from the DFT calculations were used as a set of atomic charges. The free energy difference for charging the solute in a gas phase and in solution was calculated by solving the macroscopic Poisson equation with a finite difference method. More details of this approach are described in the references.⁴⁶

Results

1. Effect of Basis Sets and Nonlocal Corrections on the Calculations for Compounds I and II. The calculations for compounds I ($^{4}A_{2u}$ state) and II were carried out using double- ζ or triple- ζ basis sets. VWN potential was applied as exchange and correlation potentials with and without Becke and Perdew (BP) nonlocal corrections. The double- ζ and triple- ζ basis sets yielded significantly different MO energies for the two compounds, although the character of the MO's and the value of the energy difference between them were similar in both basis sets. In both compounds, the MO energies of the triple- ζ basis

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Table 1. Effect of Basis Sets and Nonlocal Corrections on AOPopulation and Spin Population in Model Compound I (${}^{4}A_{2u}$ state)

	A	AO population (spin population	n)					
	dout	ole-ζ	trip	le-ζ					
	VWN ^a	VBP ^b	VWN	VBP					
Fe d_{xy}	0.84 (0.06)	0.77 (0.06)	0.81 (0.05)	0.75 (0.06)					
d_{vz}	1.50 (0.45)	1.49 (0.47)	1.50 (0.47)	1.48 (0.49)					
d_{xz}	1.52 (0.45)	1.51 (0.46)	1.51 (0.47)	1.49 (0.48)					
$d_{x^2-y^2}$	1.77 (0.21)	1.93 (0.05)	1.82 (0.15)	1.91 (0.06)					
d_{z^2}	0.96 (0.03)	0.93 (0.04)	0.92 (0.02)	0.89 (0.03)					
$O p_x$	1.43 (0.53)	1.44 (0.53)	1.44 (0.51)	1.45 (0.50)					
p _y	1.44 (0.52)	1.45 (0.51)	1.45 (0.50)	1.46 (0.49)					
\mathbf{p}_z	1.43 (0.02)	1.44 (0.00)	1.50 (0.02)	1.50 (0.00)					
Fe	0.48 (1.22)	0.63 (1.17)	0.53 (1.13)	0.66 (1.24)					
0	-0.37(1.06)	-0.40(1.04)	-0.38(1.04)	-0.43(0.99)					
Por	0.45 (0.71)	0.36 (0.80)	0.49 (0.83)	0.45 (0.78)					
Im	0.44 (0.01)	0.41 (0.00)	0.36 (0.01)	0.33 (0.01)					

^{*a*} Vosko–Wilk–Nusair potential. ^{*b*} VWN potential with Becke exchange and Perdew correlation corrections.

set were approximately 0.5-0.7 eV higher than the values from the double- ζ basis set. However, the effect of nonlocal corrections on MO energies was less than the choice of basis set. In the ${}^{4}A_{2\mu}$ state of compound I, HOMO and LUMO were meaningfully, although slightly changed by nonlocal corrections. The HOMO of a β -spin consists primarily of the $d_{x^2-v^2}$ orbital of Fe, while the LUMO has the a2u orbital character of porphyrin ring orbitals. In the calculation with nonlocal corrections, the HOMO and LUMO were almost completely separated into orbitals with the expected characteristics. In the calculation without nonlocal corrections, the HOMO and LUMO characters were partially admixed, although the two orbitals remained distinct. This is reflected in the mulliken charges and spin populations, especially of the $d_{x^2-y^2}$ orbital of Fe and the porphyrin ring (see Table 1). This change in the charge distributions leads to the difference observed in the Mössbauer spectrum parameters: quadrupole splitting (ΔE_0) and asymmetric parameters (η). With nonlocal corrections, the ΔE_0 increased from 0.61 to 0.86 and the η decreased from 0.15 to 0.02. Both parameters approached the experimental values (Table 5). This indicated that the nonlocal corrections were significant in the calculations for compound I and that the nonlocal correction should yield superior results. Therefore, we performed the SCF calculations for both compounds with the nonlocal corrections with the triple- ζ basis set. In compound II, however, the effect of the corrections on molecular orbitals was relatively small.

2. Quantum Chemical Calculations of Model Compounds I and II. (a) State 1: Compound I, [Fe(Por)(Im)O]⁺. The $a_{2u} \pi$ -cation radical state (⁴A_{2u}) was determined to be the ground state of compound I with a total spin of ³/₂, and the a_{1u} cation state (⁴A_{1u}) was found to be 0.15 eV higher in energy than the ⁴A_{2u} state. In both states, the spins were localized to the porphyrin ring (S = 1/2) and the Fe–O center (S = 1). The states ⁴A_{2u} and ⁴A_{1u} result from parallel spin coupling between the two spin sites. In order to assess the magnetic coupling interaction, the antiferromagnetic states ($M_s = 1/2$) were calculated and the J values, which were defined as the Heisenberg parameter in a Hamiltonian of the form $\neq J$ **S**₁·**S**₂,⁴⁷ were estimated on the basis of a broken symmetry

Table 2. AO Population and Spin Population in ModelCompounds I and \mathbf{H}^a

	com	pd I	
	⁴ A _{2u} state	⁴ A _{1u} state	compd II
$Fe^b d_{xy}$	0.75 (0.06)	0.73 (0.06)	0.74 (0.06)
d_{vz}	1.48 (0.49)	1.47 (0.48)	1.46 (0.49)
\mathbf{d}_{xz}	1.49 (0.48)	1.49 (0.48)	1.48 (0.50)
$d_{x^2-y^2}$	1.91 (0.06)	1.96 (0.01)	1.96 (0.01)
d_{z^2}	0.89 (0.03)	0.88 (0.03)	0.88 (0.03)
$O^b p_x$	1.45 (0.50)	1.45 (0.50)	1.46 (0.48)
\mathbf{p}_{v}	1.46 (0.49)	1.46 (0.49)	1.47 (0.48)
\mathbf{p}_z	1.50 (0.00)	1.51 (-0.01)	1.52 (-0.01)
Fe ^c	0.66 (1.24)	0.66 (1.15)	0.66 (1.19)
O ^c	-0.43(0.99)	-0.44(0.98)	-0.46(0.96)
Por ^c	0.45 (0.78)	0.48 (0.89)	-0.46(-0.12)
Im ^c	0.33 (-0.01)	0.31 (-0.02)	0.27 (-0.02)

^{*a*} VBP, triple- ζ basis set. ^{*b*} Obital populations and spin populations (in parentheses). ^{*c*} Net charges on groups and spin populations (in parentheses).

(BS) method.⁴⁸ Both $S = \frac{3}{2}$ and $\frac{1}{2}$ contribute to the BS state estimate of mixed spin under the condition of $M_s = \frac{1}{2}$. The energy for the mixed-spin BS state is related to those of the two pure spin states as follows.

$$E(BS) = \frac{1}{3}E(S = \frac{3}{2}) + \frac{2}{3}E(S = \frac{1}{2})$$
(12)

Since both E(BS) and $E(S = \frac{3}{2})$ were estimated directly from DFT calculations involving a single configuration wave function, the energy of the doublet ($S = \frac{1}{2}$), which can only be expressed by a multiconfiguration wave function, could also be obtained by the above equation. Although the Mulliken charges of the antiferromagnetic state represented those of the mixed BS state (Table 3), the total bonding energies of the antiferromagnetic state and the ferromagnetic state of compound **I** were calculated as pure spin states.

The J values were given by the equation:

$$E(S = \frac{3}{2}) - E(BS) = 2J(S1)(S2) = J$$
 (13)

The *J* values obtained were -1.1 cm^{-1} for the ${}^{4}\text{A}_{2u}$ state and 696 cm⁻¹ for the ${}^{4}\text{A}_{1u}$ state (see Table 3). These results are not strictly quantitative, since they were derived from very small energy difference between the ferromagnetic state ($S = {}^{3}/_{2}$, $M_{\rm s} = {}^{3}/_{2}$) and the BS state ($M_{\rm s} = {}^{1}/_{2}$). Nevertheless, these results predict that the magnetic coupling between the two spin sites is very weak for the A_{2u} state and strongly antiferromagnetic for the A_{1u} state. In addition, it is important to note that the A_{1u} antiferromagnetic state (${}^{2}\text{A}_{1u}$) and both the A_{2u} states (${}^{2}\text{A}_{2u}$, ${}^{4}\text{A}_{2u}$) are energetically very similar.

The spin (S = 1) on the Fe–O center was equally distributed on the Fe and the O atoms in both the ${}^{4}A_{2u}$ and the ${}^{4}A_{1u}$ states (see Table 2). The MO analysis of the ${}^{4}A_{2u}$ state, expressed as the ferromagnetic alignment between the Fe–O center and the a_{2u} radical, is summarized in Table 4. The principal AO of spin distribution for the Fe–O center were d_{xz} and d_{yz} for the Fe atom and p_x and p_y for the O atom. These construct two nearly degenerate, antibonding $\pi(d\pi-p\pi)$ MO's (48a' and 33a'' in α -spin). There are strong π -bonding interactions between the Fe atom and the O ligand which were principally exhibited by 39a', 25a'', and 41a' MO's in α -spin and 43a', 28a'', 31a'', and 45a' MO's in β -spin. The σ -interaction is relatively weak as evidenced by the 36a' MO in α -spin and the 37a' MO in β -spin. On the other hand, the bonding between the Fe atom

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Table 3. Comparison of Ferromagnetic Coupling with Antiferromagnetic Coupling in Model Compound I

			total bonding energy ^b		
electronic state	Fe	0	porphyrin	imidazol	(eV)
a _{2u} cation, ferromagnetic	0.66 (1.24)	-0.43 (0.99)	0.45 (0.78)	0.33 (-0.01)	-316.427
a _{2u} cation, antiferromagnetic	0.65 (1.14)	-0.43(0.90)	0.45 (-1.01)	0.33 (-0.03)	-316.427
a _{1u} cation, ferromagnetic	0.66 (1.15)	-0.44(0.98)	0.48 (0.89)	0.31 (-0.02)	-316.281
a _{1u} cation, antiferromagnetic	0.66 (1.03)	-0.40 (0.90)	0.41 (-0.90)	0.33 (-0.02)	-316.411

^a The charges and spin populations reported for each antiferomagnetic state are the values for the corresponding broken symmetry (BS) state. ^b Total bonding energies for pure spin states, ferromagnetic, $S = \frac{3}{2}$, and antiferromagnetic, $S = \frac{1}{2}$, $\frac{4}{4}$, $\frac{2}{4}$, $\frac{4}{4}$, $\frac{2}{4}$, $\frac{4}{10}$, $\frac{2}{4}$, $\frac{2}{10}$, $\frac{2}{4}$, $\frac{1}{10}$, $\frac{2}{4}$, $\frac{2}{10}$, $\frac{$

Table 4.	Composition of the Princi	pal Molecular Orbitals for Model	Compound I (${}^{4}A_{2u}$ State, α - and β	3-spin)
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	energy			grou	ıp popı	ulation	(%)	overlap population (%)			
MO	(EV)	occu.	primary contributors (%)	Fe	0	Por	Im	Fe-O	Fe-Por	Fe-Im	
			(a) α-Spin								
21a''	-13.172	1.0	Fe d_{xy} (17.7), N(Por) $2p_x$ (12.4), N(Por) $2p_y$ (12.4), $C_\beta 2p_x$ (15.0), $C_\beta 2py$ (14.8), H_β 1s (18.8)	17.7	0.0	82.3	0.0	0.0	7.7	0.0	
36a'	-13.097	1.0	Fe d_{z^2} (29.3), O $2p_z$ (6.5), N(Por) $2p_x$ (5.3), N(Por) $2p_y$ (9.0), $C_{\beta} 2p_x$ (5.5), Im $2p_z$ (19.9)	30.7	7.3	36.0	25.5	1.1	5.4	3.9	
24a‴	-12.235	1.0	Fe d_{yy} (15.0), $C_{\alpha} 2p_x$ (11.3), $C_{\alpha} 2p_y$ (9.1), $C_{\beta} 2p_x$ (21.2), $C_{\beta} 2p_y$ (18.5)	15.1	0.0	84.9	0.0	0.0	4.6	0.0	
39a'	-12.003	1.0	Fe d_{1x} (14.6), O 2p _x (26.0), N(Por) 2p _x (9.3), C _{α} 2p _x (10.6), C _{β} 2p _x (8.5)	19.3	26.2	54.4	0.2	7.3	1.5	-0.1	
25a‴	-12.003	1.0	Fe d_{yz} (20.9), O 2p _y (28.2), N(Por) 2p _y (6.7), C _{α} 2p _y (7.2), C _{β} 2p _z (5.1), C _{β} 2p _y (5.7)	24.0	28.5	44.5	3.1	7.9	1.1	0.4	
26a‴	-11.914	1.0	Fe d_{yz} (8.0), N(Por) $2p_y$ (8.4), $C_{\alpha} 2p_y$ (7.8), $C_{\beta} 2p_y$ (5.6), Im $2p_y$ (43.6)	11.9	0.2	43.3	45.6	0.0	1.6	4.6	
41a'	-11.381	1.0	Fe d_{xz} (23.1), O $2p_x$ (10.3), N(Por) $2p_x$ (13.5), N(Por) $2p_y$ (9.3), $C_{\alpha} 2p_x$ (6.4)	25.7	12.2	60.3	1.4	2.3	5.1	-1.2	
46a′	-9.387	1.0	Fe $d_{x^2-y^2}(93.4)$	93.4	0.0	6.5	0.0	0.1	-2.8	0.0	
47a′	-8.985	1.0	N(Por) $2p_z$ (26.2), $C_\beta 2p_z$ (5.6), $C_m 2p_z$ (56.5)	0.4	0.9	96.5	1.5	0.0	1.5	-1.8	
32a″	-8.709	1.0	$C_{\alpha} 2p_{z}$ (72.8), $C_{\beta} 2p_{z}$ (21.4)	0.0	0.0	99.8	0.1	0.0	0.0	0.0	
48a′	-8.606	1.0	Fe d_{yz} (52.3), O $2p_x$ (38.7)	52.8	38.7	7.9	0.6	-9.2	-5.3	0.0	
33a″	-8.562	1.0	Fe d_{yz} (51.9), O $2p_y$ (38.4)	52.4	38.4	6.2	3.0	-9.2	-4.6	-1.5	
			(b) β -Spin								
21a''	-13.066	1.0	Fe d _{xy} (12.4), N(Por) 2p _x (11.6), N(Por) 2p _y (11.8), C _{β} 2p _x (17.0), C _{β} 2p _y (17.1), H _{β} 1s (19.4)	12.4	0.0	87.6	0.0	0.0	6.5	0.0	
37a′	-12.881	1.0	Fe d_{z^2} (24.5), N(Por) $2p_x$ (7.2), N(Por) $2p_y$ (5.3), Im $2p_z$ (31.8)	26.1	4.8	27.9	40.6	0.7	5.0	5.0	
24a‴	-12.116	1.0	Fe d_{xy} (15.8), N(Por) $2p_x$ (7.1), N(Por) $2p_y$ (7.3), $C_{\alpha} 2p_x$ (10.5), $C_{\alpha} 2p_y$ (9.3), $C_{\beta} 2p_x$ (18.2), $C_{\beta} 2p_y$ (17.0)	15.8	0.0	84.1	0.0	0.0	6.3	0.0	
43a′	-10.467	1.0	Fe d_{xz} (23.8), O $2p_x$ (15.7), N(Por) $2p_z$ (9.3), C _{β} $2p_z$ (38.0)	23.8	16.1	59.0	1.0	4.9	3.7	-0.5	
28a‴	-10.299	1.0	Fe d _{yz} (18.6), O 2p _y (14.8), N(Por) 2p _z (11.8), C _β 2p _z (24.8), Im 2p _y (20.2)	18.6	15.0	44.7	21.6	4.5	3.0	-2.0	
31a″	-9.010	1.0	Fe d_{yz} (18.0), O $2p_y$ (24.5), N(Por) $2p_z$ (17.3), C _{β} $2p_z$ (29.8)	18.2	24.6	56.7	0.5	6.7	-6.0	-0.1	
45a′	-9.002	1.0	Fe d_{xz} (18.2), O $2p_x$ (24.0), N(Por) $2p_z$ (18.0), C _{α} $2p_z$ (5.1), C _{β} $2p_z$ (29.8)	18.3	24.0	57.5	0.2	6.6	-6.0	0.0	
32a″	-8.752	1.0	$C_{\alpha} 2p_{z}$ (73.2), $C_{\beta} 2p_{z}$ (21.2)	0.0	0.0	99.8	0.2	0.0	0.0	0.0	
46a′	-8.598	1.0	Fe $d_{x^2-y^2}$ (88.2)	88.3	0.1	11.5	0.1	0.1	-1.8	-0.2	
47a′	-8.574	0.0	Fe $d_{x^2-y^2}$ (5.4), N(Por) $2p_z$ (26.1), $C_m 2p_z$ (51.9)	5.9	0.9	91.4	1.1	0.0	1.3	-1.7	
48a′	-6.883	0.0	Fe d_{xz} (38.1), O $2p_x$ (47.0)	39.2	47.0	13.2	0.9	-8.4	-2.7	0.2	
33a″	-6.878	0.0	Fe d_{yz} (37.1), O $2p_y$ (45.6)	38.3	45.5	12.6	3.8	-8.1	-2.3	-0.5	

and the imidazole ligand consisted not only of π -interaction (26a" MO in α -spin) but also of σ -interaction (36a' and 37a' MO's in α - and β -spin, respectively). The relatively large population of d_{z^2} AO reflected electron donation from the imidazole through the σ -interaction. The covalency between the Fe and the porphyrin ring was also shown by 21a" and 24a" MO's (in α - and β -spin) and a significant population of d_{xy} AO. The d_{xz} and d_{yz} orbitals were overlapped with the porphyrin ring orbitals in several MO's. For example, the 43a' and 28a" MO's in β -spin revealed π -bonding interaction between the $d\pi$ -p π orbitals of the Fe–O center and the p_z orbital of the porphyrin ring with 31a" and 45a' forming antibonding MO's between them. These results indicated that there was a relatively strong interaction between the Fe atom and the porphyrin ring which would influence the magnetic coupling between these two spin sites. Furthermore, it was evident that the electronic structure of the Fe atom in this system could not be adequately described by a simple electronic configuration, such as: $(d_{x^2-y^2})^2(d_{xz})(d_{yz})$, as shown in Tables 1 and 2.

The Mössbauer spectrum parameters obtained in the calculations, the quadrupole splitting (ΔE_0) and the asymmetric parameter (η) , were in excellent accord with the experimental results^{49,53–56} in magnitude, sign, and direction. The ΔE_{0} value of the ⁴A_{1u} state was slightly closer to the experimental

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Table 5. Mössbauer Parameters of Model Compounds I and II

				ΔE_{Ω}		
	V_{xx}	V_{yy}	V_{zz}	(mm/s)	η	direction ^a
compd I						
${}^{4}\text{\AA}_{2u}$ state	-0.42	-0.44	0.86	0.86	0.02	z
${}^{4}A_{1u}$ state	-0.55	-0.48	1.03	1.03	0.06	z
compd II	-0.62	-0.55	1.18	1.18	0.06	z
]	Experimental Res	sults			
compd I		1				
\hat{HRP} -I ^b (4.2 K)				1.25	0.0	
JRP-I ^c (77 K)				1.33		z
compd II						
\hat{HRP} -II ^b (4.2 K)				1.51	0.0	
JRP-II ^c (77 K)				1.46	0.03	z
$Fe(TPP)(1-MeIm)O^d$ (4.2 K)				1.26		z
$Fe(TP_{piv}P)(1-MeIm)O^{e}$ (4.2 K)				1.37		
$Fe(TPP(2,6-Cl))(1-MeIm)^{f}(4.2 \text{ K})$				1.35		

^{*a*} Direction of the principal axis of the electric field gradient tensor. ^{*b*} Reference 47. ^{*c*} Compound **I** of Japanese radish peroxidase. Reference 51. ^{*d*} Reference 52. ^{*e*} Reference 53. ^{*f*} Reference 54.

 Table 6.
 Hyperfine Coupling Constants of Model Compound I (MHz)

	$A_{ m iso}$	A_{zz}	$A_{\rm iso} + A_{\rm zz}$	expt ^a
⁴ A _{2u} state				
N1(Por)	2.35	8.45	10.8	7.2
N2(Por)	2.35	8.29	10.6	
⁴ A _{1u} state				
N1(Por)	-2.25	-2.06	-4.31	
N2(Por)	-2.17	-2.00	-4.17	
⁴ A _{2u} state				
H _{meso1}	-13.5	-2.00	-15.5	9.26-11.90
H _{meso2}	-13.2	-1.99	-15.1	
H _{meso3}	-13.4	-2.00	-15.4	
⁴ A _{1u} state				
H _{meso1}	2.17	-2.99	-0.82	
H _{meso2}	1.99	-2.97	-0.98	
H _{meso3}	2.13	-3.01	-0.88	

^a ENDOR data for HRP-I. Reference 15.

magnitude than the corresponding value of the ${}^{4}A_{2u}$ state (Table 5). Partial electron outflow from the $d_{x^2-y^2}$ orbital to the a_{2u} hole, indicated by the decrease in both the Mulliken population of the $d_{x^2-y^2}$ orbital and the positive charge on the porphyrin ring compared with the ${}^{4}A_{1u}$ state, may have been responsible for the smaller value of ΔE_Q for the ${}^{4}A_{2u}$ state. In contrast, no orbital mixing was observed between the $d_{x^2-y^2}$ and the a_{1u} hole due to symmetry differences.

The hyperfine coupling constants for the nitrogen atoms and the protons on the porphyrin ring and the O atom in the Fe-O center in compound I were evaluated on the basis of the SCF wave functions. The coupling constants of pyrrole nitrogen atoms calculated by assigning them to A_z were 10.6 to 10.8 MH_z in the ${}^{4}A_{2u}$ state and -4.2 to -4.3 MHz in the ${}^{4}A_{1u}$ state. The absolute values were slightly higher and lower, respectively, compared to the 7.2 MHz ENDOR observation (Table 6). There was significant β -spin population on the nitrogen in the ${}^{4}A_{1u}$ state due to the spin polarization effects induced by the α -spin in the $d\pi$ -p π orbitals of the Fe–O center and the p π orbitals of neighboring C_{α} 's. These polarizations also would be explained by the significant overlap between the $d\pi - p\pi$ orbitals and the $p\pi$ orbitals of nitrogen in β -spin MO's. The significant difference between the ⁴A_{2u} state and ⁴A_{1u} state was illustrated by the coupling constants of the meso proton which were equal to -15.1 to -15.5 MHz and -0.82 to -0.98 MHz, respectively. Although the calculated values for the ⁴A_{2u} state were slightly higher than the experimental results of 9.26 to 11.90 MHz, the ENDOR results for the meso protons were more consistent with

an electron hole present in the a_{2u} orbital rather than in the a_{1u} orbital. The ¹⁷O hyperfine constants we have calculated in this study display axial symmetry in accordance with the ENDOR observations. As shown in Table 7, the predicted O π spin population is 50% of the total Fe-O spin, which is larger than the ENDOR empirical estimate (approximately 25%) based on comparison with nitroxide radicals. The 50% value for the O π spin population does agree well, however, with previous first principles calculations. Ghosh³⁹ obtained 43% for compound I and 41% for compound II with local DFT calculations on an iron-oxo-porphyrin model (no axial ligand), while Yamamoto et al.^{37a} using CASSCF, calculated 43% for a compound II model (with pyridine as the axial ligand) (see Table 7 for results from other calculations). To examine this more closely, we directly computed the ¹⁷O hyperfine tensor using the hyperfine equations cited previously, and using $S_i = S_{Fe-O} = 1$ for the Fe-O center site spin. Then the A tensor for the triplet Fe-O center was calculated with NL-DFT (no frozen core) from the sum of the isotropic and anisotropic parts as $A_{xx}^{tot} = -28.6$ MHz, $A_{yy}^{\text{tot}} = -27.4$ MHz, and $A_{zz}^{\text{tot}} = +26.7$ MHz. These values compare fairly well with the measured ENDOR ¹⁷O of Roberts, Hoffman, and co-workers¹⁴– $A_x^{OT} = 35$ MHz, and $A_y^{OT} = 36$ MHz. The A_z^{OT} value cannot be measured, and the signs of A_x^{OT} and A_y^{OT} cannot be measured in these ENDOR experiments. We suspect the significant quantitative discrepancy between the empirical ENDOR estimate of the oxygen spin (25%) and that predicted by first principles calculations (40-50%) is due at least in part to the extrapolation from nitroxide systems (where $S = \frac{1}{2}$) to Fe–O systems (where the site spin $S_{Fe-O} = 1$), and the factor $(1/S_i)$ required in the A tensor equations. Further, nitroxide systems and Fe-O complexes may differ significantly in oxygen covalency, and the isotropic spin polarization induced by (p_x, p_y) spin densities versus (p_z) densities will also affect the isotropic tensor.

(b) State 2: Compound II, [Fe(Por)(Im)O]. One-electron reduction of compound I generates neutral compound II with the disappearance of a porphyrin π -cation radical. The electron affinities of compound I were calculated to be 6.11 eV for the ${}^{4}A_{2u}$ state and 6.26 eV for the ${}^{4}A_{1u}$ states. The electrostatic solvation energies of compounds I and II in aqueous solution were -1.99 and -0.94 eV, respectively, reflecting the positive charge of compound I. The redox potential (versus NHE) for compound I was evaluated to be 1.61 V for the gas phase and 0.56 V for the aqueous solution. The experimentally determined redox potential for HRP-I/HRP-II was reported to be approximately 0.95 V.⁵⁷ The experimental redox potential for HRP is higher than our calculated result in aqueous solution

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Table 7. Comparison of π -Electron Spin Densities and Energies in HRP-I and Its Model Compound I [Fe(Por)(O)(L)]⁺

method axial ligand (L)	experiment HRP-I	PPP- non	· .	IE Ir			D-UHF ^c Im		<i>io</i> UHF ^d one		io RHF ^e Py	Xα ^f Py	LDF ^g none		FT ^h Im
cation type		a _{2u}	a_{1u}	a _{2u}	a _{1u}	a _{2u}	a _{1u}	a _{2u}	a _{1u}	a _{2u}	a_{1u}	a_{2u}	a _{2u}	a _{2u}	a _{1u}
N _{pyr}	$\pm (0.043 - 0.060)^{j}$	0.05	0.00	0.10	0.00	0.12	-0.05	0.18	-0.10			0.07	0.05	0.08	-0.02
C_m	$\pm (0.17 - 0.04)^{j}$	0.19	0.00	0.11	0.00	0.28	-0.14	0.36	-0.18			0.14	0.18	0.18	-0.03
C_{α}		-0.01	0.10	0.00	0.08	-0.08	0.19	-0.14	0.24				-0.01	-0.03	0.12
C_{β}	$\pm (0.036)^{j}$	0.01	0.03	0.00	0.02	0.00	0.03	0.00	0.03			0.03	0.02	0.00	0.03
Ó	0.50^{k}			1.15	1.10	0.34	0.30			0.88	0.85	0.84	0.85	0.99	0.99
$\Delta E ({ m eV})$				0.00	0.683	0.00	-0.354	0.00	-0.412	0.00	-0.054			0.00	0.146

^{*a*} Reference 60a. ^{*b*} Reference 31. ^{*c*} Reference 32. ^{*d*} Reference 36. ^{*e*} Reference 37b. ^{*f*} Reference 38. ^{*g*} Reference 39. ^{*h*} This work. ^{*i*} [Por]⁺. ^{*j*} ENDOR estimation from ref 15. ^{*k*} ENDOR estimation from ref 14.

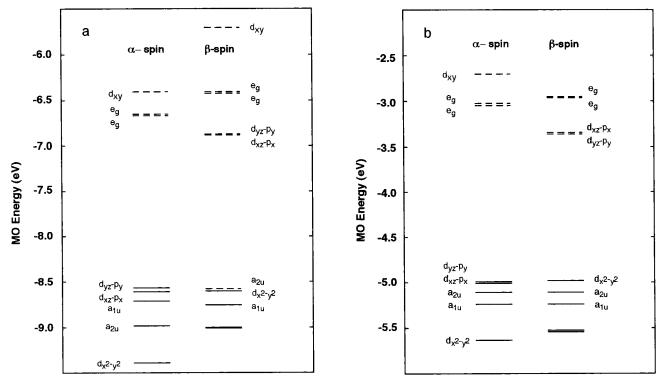


Figure 2. Energy levels for occupied and unoccupied orbitals of (a) compound I, $S = \frac{3}{2}, \frac{4}{2}$ state, and (b) compound II, S = 1.

(lying between this and the predicted gas phase), probably as a consequence of the lower dielectric protein environment (compared to water) which surrounds the Fe-porphyrin prosthetic group.

The addition of an electron had little effect on the electronic structure of the Fe–O center. This is demonstrated by the similar occupation of the d-orbital of the Fe atom and the p-orbital of the O atom (Table 2) and by the similar quadrupole splitting (Table 5) in compounds I and II. The slight difference in quadrupole splitting between the two compounds (0.86 vs 1.18, the same trend was observed in the experimental data) may be related to the subtle change of $d_{x^2-y^2}$ orbital population and the disappearance of a cation hole on the porphyrin ring. The cause of the population change was considered to be the movement of an electron out of the $d_{x^2-y^2}$ orbital into the a_{2u} hole in the ${}^4A_{2u}$ state of compound I.

The character of the MO's and the order of their energy levels are similar in compounds **I** and **II**, although the MO energies of compound **II** shift to levels which are approximately 3.5 eV higher than those of compound I (Figure 2). The β -spin HOMO of compound **II** was determined to be the $d_{x^2-y^2}$ orbital of Fe rather than the $a_{2u} \pi$ -cation radical orbital of the porphyrin ring, the β -spin LUMO of the ⁴A_{2u} state of compound **I**. This transition of the $d_{x^2-y^2}$ orbital to HOMO level can be explained both by the increase of the ligand field for the $d_{x^2-y^2}$ orbital that occurred with the addition of an electron to the π -orbital and by the large intraatomic exchange splitting observed between the α - and β -spin orbitals (also seen in compound I). On the other hand, the β -spin LUMO of compound II was found to be the $d\pi$ -p π orbital formed between the Fe and the O atoms, as was expected.

The equilibrium Fe-O distance and the bond strength in compound **II** were examined by using a double- ζ basis set and VWN functional with perturbative nonlocal corrections. Figure 3 shows the potential energy curve where a fourth-order polynominal was fitted to the calculated energy values. The minimum point of 1.68 Å was similar to the 1.64 Å bond length obtained by EXAFS studies of HRP-II.¹⁷ This value was 0.07 Å longer than the bond length of 1.604 Å obtained by the X-ray analysis of Fe(T_{piv} PP)O.⁸ The force constant of the Fe-O bond was obtained as the second derivative of the polynominal at the equilibrium distance. Using the force constant and reduced masses for Fe and O atoms, the bond stretching frequency was evaluated to be 842 cm⁻¹, in accordance with experimental values. The observed values are 790 and 776 cm^{-1} for HRP-II (at high and low pH, respectively)⁵⁸ and 820 cm⁻¹ for Fe(TPP)-(1-MeIm)O,⁵⁹ 807 cm⁻¹ for Fe(TP_{piv}P)(1-MeIm)O,⁶⁰ and 818 cm⁻¹ for Fe(TPP(2,6-Cl))(1-MeIm)O.61

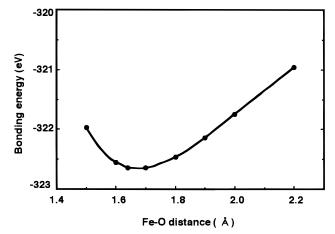


Figure 3. Potential energy curve as a function of the Fe–O distance of model compound **II**.

Discussion

The total $S = \frac{3}{2}$ spin structure of compound **I**, constructed from both the Fe–O center with S = 1 and the porphyrin π -cation radical with S = 1/2 (both weakly ferromagnetically spin coupled), has been supported by various experimental studies including electronic absorption, ENDOR, Mössbauer spectroscopy, and ESR. The nature of the π -cation radical, however, still remains controversial. The radical can be formed by removing an electron from either one of the nearly degenerate a_{1u} or a_{2u} orbitals of the porphyrin ring to yield either the A_{1u} or A_{2u} ground state configuration. The relative energies of these two orbitals are sensitive to the axial ligands and to peripheral substitutions of the porphyrin ring.^{31,62} Previous work had assigned an A_{2u} radical ground state to HRP-I, because the optical spectrum was similar to the π -cation radical of Co^{III}-(OEP)(ClO₄)₂ thought to be characteristic of the A_{2u} radical.¹¹ Several independent ESR,63 NMR,64 and resonance Raman65 studies of porphyrin π -cation radicals, however, have shown that their optical spectra are not reliable indicators of their ground state configurations.

On the other hand, Roberts et al.¹⁵ detected the hyperfine structure of ¹H and ¹⁴N with HRP-I by ENDOR measurements and estimated the π -electron spin densities of the porphyrin ring based on the hyperfine coupling constants with empirical equations. They compared their results with theoretical calculations. In PPP-CI calculation,^{62a} the π -spin density of pyrrole nitrogen (N_{pyr}) is evaluated to be 0.05 in the ⁴A_{1u} state and 0.00 in the ⁴A_{1u} state (see Table 7). Since the former value is in

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H.; Yoshimura, T.; Kamada, H. *Inorg. Chem.* **1996**, *35*, 2373. (63) Rutter, R.; Valentine, M.; Hendrich, M. P.; Hager, L. P.; Debrunner, excellent agreement with ENDOR data, it was concluded that HRP-I is in the ${}^{4}A_{2u}$ ground state. The DFT calculations in the present study as well as two other calculations, INDO-UHF³² and *ab initio* UHF,³⁶ however, indicated substantial negative spin density on N_{pyr} in the ${}^{4}A_{1u}$ state that was induced by spin polarization effects. Therefore, these calculations did not rule out the possibility that ${}^{4}A_{1u}$ was the ground state for HRP-I.

The spin density of meso carbons (C_m) calculated by DFT methods cannot exclude either radical state, although the two UHF methods are consistent with the ${}^4A_{1u}$ state rather than the ${}^4A_{2u}$ state. Since the estimation of spin densities from the ENDOR spectra was based on several approximations, the hyperfine coupling constants were calculated directly from the molecular orbitals obtained by DFT calculations. The calculated constants for meso protons of the ${}^4A_{2u}$ state are comparable with the experimental data. The values calculated for the ${}^4A_{1u}$ state, on the other hand, were significantly different (see Table 6). In addition, the calculated constants of N_{pyr} in the ${}^4A_{2u}$ state were also consistent with the experimental data. These results suggested that the electronic structure of HRP-I was more similar to the ${}^4A_{2u}$ state than to the ${}^4A_{1u}$ state.

DFT calculations for the energy difference between the two radical states supported the contention that the ground state was the ${}^{4}A_{2u}$ radical state. This, however, contrasted with INDO and *ab initio* calculations. The energy difference between the ${}^{4}A_{2u}$ and the ${}^{4}A_{1u}$ ferromagnetic states was very small (0.15 eV). In fact, there was evidence for the mixing of the A_{2u} and A_{1u} ground states in some π -cation radical species. This indicated that these states did constitute the "predominant character" of these species.^{65bc,66-68} Several spectroscopic studies, including ESR,⁶³ NMR,⁶⁹ MCD,^{66b} resonance Raman spectra,⁶⁷ and ENDOR, have determined that the formation of HRP-I is predominantly like the $A_{2u} \pi$ -cation radical.

The ground state of model compounds for the porphyrin π -cation radical is sensitive to the axial ligands and the peripheral substituents. Strong donor axial ligands like imidazole stabilize the A_{2u} state relative to the A_{1u} state. The calculations performed in the present study have indicated that the oxidation of compound II to the ${}^{4}A_{2u}$ state of compound I decreases the electron density of pyrrole nitrogen due to the removal of an electron from the a_{2u} orbital which largely populates the nitrogen atom. The ⁴A_{1u} state has almost the same density as compound II. The Mulliken charge on the nitrogen is -0.391 for ${}^{4}A_{2u}$, -0.405 for ${}^{4}A_{1u}$, and -0.407 for compound **II**. Therefore, the electrostatic repulsion between the pyrrole nitrogen ligands and the d-orbitals of the Fe atom enhanced by electron donation from the imidazole axial ligand is less in the ${}^{4}A_{2u}$ state than in the ${}^{4}A_{1u}$ state. A related argument is based on Mulliken population analyses and total charges. In the ${}^{4}A_{2u}$ state, the $d_{x^2-v^2}\beta$ -spin orbital, which is the HOMO in β -spin, admixes with the a_{2u} hole orbital. This causes the electron flow from the imidazole to the porphyrin ring via the Fe orbital. In the ⁴A_{1u} state, such orbital mixing does not occur because of the different symmetry. These effects may cause the ${}^{4}A_{2u}$ state to be slightly lower in energy than the ${}^{4}A_{1u}$ state.

Spin coupling between the Fe–O center (S = 1) and the porphyrin π -cation radical ($S = \frac{1}{2}$) was observed in compound **I**. DFT calculations indicated that in the A_{2u} configuration, this

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coupling interaction was relatively weak (-1.1 cm^{-1}) . In the A_{1u} configuration, however, there was strong antiferromagnetic interaction (696 cm^{-1}). The former is compatible with the experimental |J| value of 4 cm⁻¹ from Mössbauer and ESR spectra of HRP-I performed by Schulz et al.⁴⁹ Schulz et al. presumed that different heme ligand conformations had different exchange interactions. This resulted in a distribution of J values that introduced apparent anisotropy. In other compound I complexes, the large variation in the magnitude and sign of Jhas been reported. These include values <-80 and -43 cm⁻¹ for [(TMP)Fe=O]⁺,^{50,51} ±1.5 and -38 cm⁻¹ for [TPP(2,6-Cl)- $Fe=O]^{+,50,51} - 22 \text{ cm}^{-1}$ for [(TTMPP)Fe=O]^{+,50} and 76 \text{ cm}^{-1} for chloroperoxidase compound I^{52} (Here J > 0 is defined to be antiferromagnetic, consistent with the spin Hamiltonian H $= JS_1 \cdot S_2$.) Significantly, synthetic compound I complexes displayed strong ferromagnetic couplings, while couplings in the less symmetrical enzymes were found to be weak or antiferromagnetic.

Since the a_{1u} radical forms an antiferromagnetic coupling with the spin of the Fe-O center, it is likely that there is significant overlap of the radical orbital and the unpaired $d\pi - p\pi$ orbitals of the Fe-O center. With the C_s symmetry of the model compound, it is possible that the a_{1u} orbital of the porphyrin ring admixes with the $d_{yz}-p_y$ unpaired orbitals of the Fe–O center. This is to be distinguished from the orthogonal interaction between the two spin orbitals in the synthetic complexes with C_{2v} symmetry (mentioned above). The antiferromagnetic exchange coupling formed between the π -cation radical and the Fe-O center unpaired orbital occurs when these orbitals are non-orthogonal and when they overlap significantly. In the unpaired orbital of the a_{1u} antiferromagnetic cation (BS) state, the mixing of the d_{yz} - p_y of the Fe-O center with the p_y of the C_{α} and C_{β} pyrrole atoms was clearly observed: d_{yz}-p_y (73%), $p_v(C_{\alpha})$ (16%), and $p_v(C_{\beta})$ (6%) in the β -spin 32a" MO of the BS state.

In contrast, the analysis of spin population in the a_{2u} antiferromagnetic cation state exhibited less orbital mixing compared to the a_{1u} antiferromagnetic state (data not shown) despite the fact that similar orbital mixing is also predicted for the a_{2u} radical state. In addition to differences in net overlap of unpaired electrons in the A_{2u} and A_{1u} states, there should be a major difference in the size of the ferromagnetic term based on the following considerations. The a_{2u} orbital has overall symmetry z in the ideal D_{4h} point group, which means that all $N(pyrrole) p_z$ orbitals add symmetrically. These are orthogonal by symmetry to $Fe(d_{xz})$ and $Fe(d_{yz})$ within D_{4h} . Then the unpaired electron in the a_{2u} orbital and the unpaired electrons in $Fe(d_{xz})$ and $Fe(d_{yz})$ have a large differential overlap, but very small net overlap, which is just the condition for a substantial ferromagnetic coupling term. With other antiferromagnetic interactions from overlap terms, the net coupling for A_{2u} is expected to be small, as observed from our calculations. As a result, in the A_{2u} state of the HRP-I model compound, there was weak coupling-the result of approximate cancellation of the ferromagnetic and antiferromagnetic contributions to J. Further, the closer the situation is to the D_{4h} symmetry limit, the stronger one would expect the ferromagnetic terms to become compared to the antiferromagnetic terms (given an A_{2u} ground state), as seen with the symmetrical synthetic compound I analogues. By contrast, for the A_{1u} radical state, there is no $N(pyrrole) p_z$ contribution in the radical orbital, giving a very small differential overlap, and a very small ferromagnetic contribution to the coupling. With other antiferromagnetic terms, as discussed above, the net coupling is substantial and antiferromagnetic. Despite the difficulty in obtaining small energy differences (in quantum chemical terms) with high accuracy, the DFT calculations do predict a small coupling for the A_{2u} radical state, in agreement with experimental trends. As the mixing of A_{2u} with A_{1u} increases induced by lowered symmetry, one expects enhanced antiferromagnetic coupling, as seen, for example, in chloroperoxidase.

Our DFT calculations revealed that the three electronic configurations of compound **I**, the ${}^{4}A_{2u}$, the ${}^{2}A_{2u}$, and the ${}^{2}A_{1u}$ states, are very similar energetically. This indicated the possibility that mutual conversions or mixings of states may readily occur, especially when environmental conditions (such as proximal ligands, porphyrin substituents, or protein interactions) distort the symmetry of the Fe-porphyrin electronic structure. In this way, the specificity and the efficiency of the compound I reaction could be regulated by subtle structure modifications. The electrostatic properties of regions within the protein are important to the compound I reaction. This is evidenced by the sensitivity of the redox potential between compounds I and II to the electrostatic properties of the environment. The calculated aqueous solution potential (0.56 V) was significantly lower than the corresponding gas phase potential of 1.61 V.

Comparison of the electronic structures of compounds I and **II** revealed the similarity of the Fe–O centers, and this was reflected in similar Mössbauer parameters for the two compounds. The reactivities of compounds I and II with respect to one-electron reduction clearly differ (Figure 2). In compound II, the two lowest empty orbitals (which are minority β spin) are nearly degenerate $d\pi - p\pi$ orbitals of the Fe–O center where the electron density on the O atom was 43%. In contrast, the LUMO (minority β spin) of compound **I** is the π -cation radical orbital of the porphyrin ring, which lies 1.5 eV lower in energy than the $d\pi - p\pi$ orbitals of the Fe–O center. During the redox reaction with substrates, first compound I and then compound II receives one electron from the substrate. Only after reduction of compound II is the Fe-O oxygen activated to labile water (or hydroxyl). Since in the compound II reduction the spin state changes from S = 1 to $\frac{1}{2}$, and the electron adds to the Fe–O center, while the resting enzyme has a ferric site with total $S = \frac{5}{2}$, clearly the $S = \frac{1}{2}$ to $\frac{5}{2}$ transition (along with increased electron density in $d\pi - O\pi$) provides part of the driving force for the reaction. Very recently, theoretical studies of the transition peroxide intermediate from an earlier step of the catalytic cycle were reported with use of *ab initio* and INDO methods.70

Conclusions

In this study, the electronic structures of model compounds I and II were well characterized by sophisticated DFT calculations with nonlocal corrections and triple- ζ basis sets including polarization functions. The calculated Mössbauer spectrum parameters for both compounds and the magnetic couplings between the porphyrin π -cation radical and the Fe–O center of compound I were in good agreement with the experimental data. The nonlocal corrections were found to be important in the description of compound I.

Our DFT calculations assigned the ${}^{4}A_{2u}$ state as a ground state of compound I on the basis of the total bonding energy, weak ferromagnetic spin coupling (both calculated and observed), and the hyperfine coupling constants, particularly of meso protons, which were evaluated directly from the SCF wave functions. This study, however, also indicated that the energy difference between the A_{2u} and A_{1u} states was very small and

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would be significantly affected by the subtle change of the electron donation from the axial ligand and the slight distortion of molecular symmetry leading to the orbital mixing: The electron donation from the imidazole and the orbital mixing between the a_{2u} hole orbital of the porphyrin ring and the $d_{x^2-y^2}$ orbital of the Fe, which have the same symmetry, cause the A_{2u} state to be slightly lower in energy than the A_{1u} state. Several spectroscopic studies have presented the possibility for the mixing of the A_{2u} and A_{1u} ground states in some π -cation radical species. DFT calculations performed here provide further support for the proposition that the admixture or the dynamic interchange between the A2u and A1u states of compound I would be subtly controlled with the structural alteration of the axial ligand and the peripheral substituents and the fluctuation of the environmental conditions such as the protein region.

This study also indicated that the magnetic coupling interaction between the Fe–O center and the porphyrin π -cation radical was different between the A_{2u} and A_{1u} states, i.e., very weak ferromagnetic for the former state and antiferromagnetic for the latter state, which was attributed to the significant covalency between the Fe and the porphyrin ring. Our approach to the magnetic coupling may adequately account for the large variation in the magnitude and sign of the J value observed in several types of compound I complexes.

Refined NL-DFT calculations for the electronic structure and the magnetic interaction of compounds I and II, thus, appears to provide very useful information on the factors that dominate the chemical properties of the two compounds.

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Supporting Information Available: Basis Sets for the calculations, and Mulliken charges, α - and β -spin populations, net spin populations, and ESP charges on all atoms, and molecular orbital compositions for compound I (${}^{4}A_{2u}$ and ${}^{4}A_{1u}$) and for compound II (19 pages). See any current masthead page for ordering and Internet access instructions.

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