Calculated Spin Densities and Quadrupole Splitting for Model Horseradish Peroxidase Compound I: Evidence for Iron(IV) Porphyrin (S = 1) π Cation Radical Electronic Structure

Sir:

In the enzymatic reaction of horseradish peroxidase (HRP) with peroxide, two intermediate complexes have been identified, the green primary compound HRP-I, which is 2 oxidizing equiv above the ferric resting state of the enzyme, and the red secondary compound HRP-II, which is formed by one-electron reduction of HRP-I.[1] Neither the resting state nor the reactive forms of the detailed electronic structure and the nature of the axial ligands have been completely resolved despite extensive research in the past few years. Experimental evidence points to an imidazole ligand and, possibly, a H_2O attached to the iron in the resting state[2] while in the intermediates the iron is thought to bind a single oxygen atom of the substrate in both HRP-I and HRP-II[3,4a] (see Figure 1).[4b] There is evidence for both high-spin and low-spin ferric ion in the resting state,[5] and the detailed electronic states of both HRP-I and HRP-II are also in doubt, due mainly to apparently conflicting evidence from different spectroscopic measurements. Susceptibility data[6] as well as recent ESR,[7] Mössbauer,[8-10] and electronic spectra studies[3] support an (S = 1) Fe(IV) configuration for both HRP-I and HRP-II, with the porphyrin ring forming an a_{2u} π cation radical in HRP-I.

Very recently, Morishima and Ogawa[11] have proposed an alternative structure involving iron(IV) in a high-spin (S = 2) configuration and a single electron distributed on the protein rather than on the porphyrin ring and spin coupled to the high-spin iron(IV), yielding a net spin of $^3/_2$. This proposal was made on the basis of similar heme methyl protein shifts in the ^1H NMR spectra of HRP and HRP-I. The apparent similarity of the shifts was interpreted to exclude a porphyrin π cation radical. However, it has not been established that a π cation radical would necessarily significantly alter the ^1H NMR spectra of the methyl groups. In fact, La Mar and de Ropp[12] have recently presented evidence against the interpretation of Morishima and Ogawa. They claim that the NMR data are inconsistent with a high-spin iron(IV) but can be interpreted in terms of a porphyrin π cation radical.

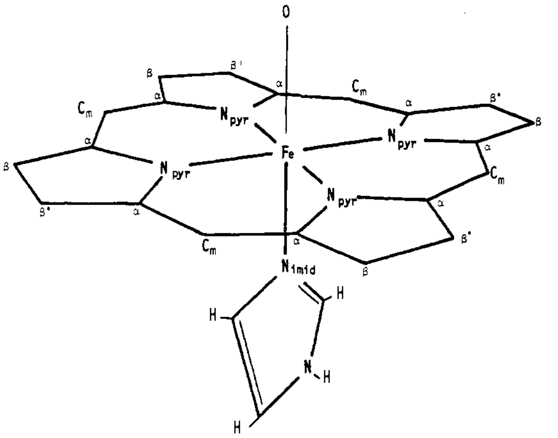

Figure 1. Porphine–ferryl model used for HRP-I and HRP-II. $C_{\beta'}$ atoms indicate positions of methyl substituents in protoporphyrin IX, with C_m levels bridging methine carbon atoms. In HRP-I and HRP-II, the Fe–O distance is 1.75 Å. In the resting-state model, the oxygen atom is replaced by a H_2O molecule with the Fe–O distance 2.00 Å. The remaining geometry is based on the X-ray structure of model oxyheme.[4b]

Table I. Calculated Spin Density Distributions in Model HRP Fe(III) (d^5) Resting State, HRP-I in Fe(IV) (d^4, $S = 1$) por a_{1u} π ($S = {}^1/_2$) and Fe(IV) (d^4, $S = 1$) por a_{2u} π ($S = {}^1/_2$) Electronic States, and HRP-II in Fe(IV) (d^4, $S = 1$) por π ($S = 0$) Electronic State

	HRP resting state		HRP-I		
			a_{2u} cation	a_{1u} cation	HRP-II
	$S = {}^5/_2$	$S = {}^1/_2$	$S = {}^3/_2$	$S = {}^3/_2$	$S = 1$
Fe d_{z^2}	0.72	0.01	0.04	0.05	0.04
Fe $d_{x^2-y^2}$	0.98	0.98	0.00	0.00	0.00
Fe d_{xy}	0.63	0.01	0.04	0.05	0.04
Fe d_{xz}	0.95	0.00	0.81	0.82	0.83
Fe d_{yz}	0.95	0.00	0.81	0.82	0.83
O p_x	0.00	0.00	0.17	0.15	0.15
O p_y	0.01	0.00	0.17	0.15	0.15
O p_z	0.02	0.00	−0.03	−0.04	−0.04
N_{pyr} p_z	0.05	0.02	0.12	−0.05	0.00
C_m p_z	0.10	0.05	0.28	−0.14	0.00
C_α p_z	−0.07	−0.04	−0.08	0.19	0.01
C_β p_z	0.01	0.00	0.00	0.03	0.00
N_{pyr} σ	0.11	0.00	0.00	−0.03	−0.01
C_m σ	0.01	0.01	0.02	−0.02	0.00
C_α σ	0.00	0.00	−0.02	0.02	0.00
C_β σ	0.00	0.00	0.00	0.00	0.00
N_{imid}	0.11	0.00	0.00	−0.01	−0.01

Thus, in order to further elucidate the electronic structure of HRP-I, we have calculated the spin densities of a model HRP-I complex with an unsubstituted porphyrin ring in two electronic configurations corresponding to an (S = 1) Fe(IV) por π (S = $^1/_2$) cation radical, with the porphyrin electron in either an a_{1u} or an a_{2u} π orbital, and compared these with the spin densities calculated for the resting-state model with both high-spin and low-spin ferric iron, and for a model HRP-II complex with an (S = 1) Fe(IV) configuration. These calculations were performed with a new all-valence-electron INDO-type SCF method developed specifically to include transition metals and configuration interaction.[13] This method has been successfully employed to investigate the ground-state properties of a number of model heme compounds.[14-16] In the present case, the calculated spin densities were obtained from an unrestricted Hartree–Fock self-consistent-field calculation for each model and each electron configuration chosen and are corrected for spin contamination by a spin annihilation procedure.[13]

The calculated spin densities for both high-spin Fe(III) (d^5, $S = {}^5/_2$) and low-spin (d^5, $S = {}^1/_2$) Fe(III) resting states and (d^4, S = 1) Fe(IV), por a_{1u} π ($S = {}^1/_2$) and por a_{2u} π ($S = {}^1/_2$), HRP-I states are shown in Table I. The calculated spin-densities for the Fe(IV) (d^4, S = 1) por π (S = 0) state of HRP-II, formed by one-electron reduction of the HRP-I configuration, are shown as well. We see from this table that, in addition to the iron and oxygen ligands, the spin density in the model HRP-I a_{2u} π cation resides primarily on the pyrrole nitrogen and on the methine carbon atoms whereas in the model HRP-I a_{1u} π cation the spin

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Table II. Calculated Relative Energies and Quadrupole Splittings for Model HRP-I and HRP-II Intermediates

	ΔE, eV	ΔE_Q, mm/s	η	$V_{ii}^{\max}$
HRP-I ($S = {}^{3}/_{2}$)				
Fe(V) ($S = {}^{3}/_{2}$) por π ($S = 0$)	1.100	1.64	0.27	V_{yy}
Fe(IV) ($S = 2$) por π ($S = {}^{1}/_{2}$)	1.358	−0.89	0.12	V_{zz}
Fe(IV) ($S = 1$) por a_{1u} π ($S = {}^{1}/_{2}$)	0.000	1.43	0.02	V_{zz}
Fe(IV) ($S = 1$) por a_{2u} π ($S = {}^{1}/_{2}$)	0.354	1.43	0.01	V_{zz}
Fe(III) ($S = {}^{5}/_{2}$) por π ($S = -1$)	0.849	0.34	0.24	V_{zz}
Fe(III) ($S = {}^{1}/_{2}$) por π ($S = 1$)	0.649	−2.65	0.20	V_{zz}
HRP-II ($S = 1$)				
Fe(IV) ($S = 1$) por π ($S = 0$)	−5.825	1.51	0.02	V_{zz}
Expt[a]				
HRP-I		1.38	0	V_{zz}
HRP-II		1.44	0	V_{zz}

[a] See ref 10.

density resides primarily on the α and methine carbon atoms. In neither configuration is there a significant amount of spin density of any C_β atom, including the four $C_{\beta'}$ atoms which have the methyl substituents in protoporphyrin heme units. Addition of substituents to the porphyrin ring does not appreciably change the spin density in these π orbitals.[17] Neither the resting states nor the model HRP-II has a significant amount of spin density on these β carbon atoms. However, both the resting state and HRP-I have significant π spin density on the C_α and C_m carbon atoms, in contrast to HRP-II which has no spin density on these atoms. These results are consistent with the fact that the observed CH_3 proton chemical shift in NMR spectra is similar for the resting state and HRP-I and different for HRP-I and HRP-II.

Thus, the observed NMR results are completely consistent with a description of HRP-I as a Fe(IV) ($S = 1$) por π ($S = {}^{1}/_{2}$) radical, with either a_{1u}- or a_{2u}-type spin density on the porphyrin π ring and with the a_{2u} π cation structure for normal and the a_{1u} π cation structure for reconstituted deuteroheme HRP-I. Furthermore, the spin densities calculated for the a_{2u} π and a_{1u} π cation radical structures are consistent with the characteristic ESR parameters observed for the two types of radicals, i.e., ESR spectra in keeping with high spin density at the methine carbon positions and nitrogen splittings in the former and small spin density at the methine carbon positions and no resolved nitrogen splittings in the latter.[18]

To further investigate the plausibility of these electronic structures for HRP-I, we have calculated the relative energies, electric-field gradients at the iron nucleus, and quadrupole splittings for them as well as for a number of other previously proposed electronic structures for HRP-I: Fe(V) (d^3, $S = {}^{3}/_{2}$), with all oxidizing equivalents and unpaired spin density localized on the iron; Fe(IV) (d^4, $S = 2$), a high-spin variation of the iron(IV) state as suggested by Morishima and Ogawa,[11] but with the spin-coupled electron on the porphyrin ring; and high-spin Fe(III) (d^5, $S = {}^{5}/_{2}$) and low-spin (d^5, $S = {}^{1}/_{2}$) Fe(III) states of model HRP-I, with both oxidizing equivalents and unpaired electrons on the porphyrin ring rather than on the iron.

The spin densities calculated for all of these states are compatible with the observed ^1H NMR of HRP and HRP-I, i.e., little or no spin density on $C_{\beta'}$ atoms. Table II gives the relative energies and quadrupole splittings calculated for each of these electronic configurations. We see from this table that the Fe(IV) (d^4, $S = 1$) por a_{2u} π ($S = {}^{1}/_{2}$) and Fe(IV) (d^4, $S = 1$) por a_{1u} π ($S = 1$) states both have very similar quadrupole splittings and are in best agreement with experiment. Furthermore, both the a_{1u} and a_{2u} cation radicals have similar energies, with the a_{1u} cation radical predicted to be the lowest energy state. This behavior may certainly be correct for the unsubstituted porphyrin ring used in these calculations. Recent electronic spectra[19] and ^1H NMR studies[11] of both normal HRP-I and HRP-I reconstituted with deuterohemin demonstrate the sensitivity of an a_{1u} vs. an a_{2u} π cation to ring substituents. The protoporphyrin HRP-I appears to be an a_{2u}-type radical[3] and the deutero HRP-I an a_{1u}-type radical.[19] Our results indicate how close these two states are in energy and suggest that if reconstituted HRP-I were prepared with simple porphine heme an a_{1u} cation radical would be obtained.

Of the other possible configurations, only the (d^3, $S = {}^{3}/_{2}$) Fe(V) state of the model HRP-I yields a value of ΔE_Q consistent with the observed value. However, for this state, the principal value of the electronic-field gradient at the iron nucleus is predicted to be in the heme plane rather than perpendicular to it. Mössbauer resonance experiments on oriented samples are suggested to determine the principal direction of the field gradient to help distinguish between an Fe(IV) and an Fe(V) structure.

We have also calculated the electronic structure and quadrupole splittings for a model of HRP-II. The most favorable HRP-II has a (d^4, $S = 1$) Fe(IV) electronic structure with no porphyrin π cation character. As seen in Table I, the calculated value of ΔE_Q for this state is in good agreement with experiment,[8-10] and the lower energy of HRP-II compared to HRP-I is consistent with the facile reduction observed.

The results presented here, together with other experimental and theoretical evidence, now point to an Fe(IV) (d^4, $S = 1$) por a_{2u} π ($S = {}^{1}/_{2}$) cation as the most plausible electronic state of HRP-I. The ^{13}C and ^{15}N NMR studies recently suggested by investigators[11] should be very useful in verifying the π cation nature of HRP-I and the sensitivity of an a_{1u} or a_{2u} ground state to porphyrin ring substituents, which is strongly suggested by ours and other existing results. Such studies would directly link observed peak positions and line widths to calculated values of spin densities for a_{1u}- and a_{2u}-type cation radicals and, hence, further aid in distinguishing between such radicals. These experiments would also help determine which theoretical method, if any, gives the more reliable quantitative correlations to observed frequency shifts and line broadening.

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Gas-Phase Complexes of Cu^+ and Ag^+ via Thermionic Emission Sources

Sir:

The gas-phase clustering of molecules about ions can provide important information about both solvation phenomena and the chemistry of gas-phase complexes. Although the coordination complexes of transition-metal ions have been extensively studied in solution, relatively few studies involving transition-metal-ion complexes have been carried out in the gas phase. These ICR studies have been mostly limited to complexes that can be generated via electron-impact fragmentation of volatile inorganic compounds.[1] The most recent development in ICR work involving