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Quantum-Mechanical Studies of Model Peroxidase Compound I Complexes

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Peroxidases are oxidative metabolizing heme proteins that are converted to their biologically active iron–oxo (ferryl, compound I, Fe=O) form, two oxidizing equivalents above the resting state, by reaction with peroxides. The deprotonation of the imidazole of the proximal histidine, a common endogenous ligand in these proteins, by a nearby Asp residue which is present in both CCP and HRP has been suggested to be important in forming and stabilizing this ferryl intermediate of peroxidases. In the study presented here, we have used the INDO semiempirical quantum chemical method to investigate the possible effects of this deprotonation on the electronic structure and reactivity of the ferryl compound (compound I) form of the active site of peroxidases. The striking result is obtained that, in the deprotonated form only, in addition to the classical a_{1u} and a_{2u} porphyrin π-cation-radical states, a new low-lying imidazole π-cation-radical electronic state is obtained. In this imidazolate π-radical state, there is one unpaired spin on the imidazole ring, and the negative charge is almost entirely on the porphyrin ring, which has little unpaired spin density. These results suggest that deprotonation is a plausible mechanism for enhancement of the radical character of the imidazole ligand in compound I of peroxidases. Such enhanced radical character provides a coherent explanation for some unusual experimental observations in these systems. In particular, the presence of radical character on the imidazole ring helps explain enigmatic aspects of recent NOE–NMR and resonance Raman spectra reported for the compound I intermediate of horseradish peroxidase (HRP). Both of these results inferred some radical character on the imidazole ligand. Enhanced radical character on the imidazole of the proximal histidine ligand in CCP-I could also account for the rapid transfer of an electron to it from the Trp 191 stacked above it. Since the residue equivalent to Trp 191 is absent in the active site of HRP, there is no possibility of an electron transfer from it, thus accounting for a more stable compound I in HRP even with enhanced imidazole radical character.

Introduction

Peroxidases are oxidative metabolizing heme proteins that are converted to their biologically active iron–oxo (ferryl, compound I, Fe=O) form, two oxidizing equivalents above the resting state, by reaction with hydroperoxides.[1-3] An imidazole of a proximal histidine residue is the endogenous ligand common to these proteins. The deprotonation of the labile $H_{\delta 1}$ proton of this imidazole ring has long been suggested to be important in forming and stabilizing this ferryl intermediate of peroxidases.[4-7]

There are a number of recent studies that lend further support to this hypothesis. Crucial among these is evidence provided by the first high-resolution X-ray structure determination of a peroxidase.[8] The 1.7-Å resolution crystal structure of cytochrome C peroxidase (CCP) reveals that there is an asparate residue near the $N_{\delta 1}$ of the proximal imidazole that could serve as a proton acceptor of the $H_{\delta 1}$ imidazole proton.[1,2] Our recent calculations of models for the proton relay system in this enzyme indicate favorable energetics for this proton transfer and imply that complete deprotonation of the distal histidine could occur.[9] Sequence alignments performed between horseradish peroxidase (HRP) and CCP[10] reveal that this Asp residue is conserved in HRP. Moreover, such a favorable proton acceptor is absent in the X-ray structure of Met Mb and could help explain why this system, with a heme unit identical with that in CCP including a proximal histidine ligand, is not an active peroxidase.[1] Additional evidence for the importance of this proton transfer provided by a recent experimental study of a model heme system that mimics peroxidase activity. Results of this study led to the conclusion that hydrogen bonding to the imidazole $H_{\delta 1}$ increases the rate of compound I formation.[11] NMR studies of peroxidases consistently reveal the presence of such a proton in the resting state but provide evidence for imidazole $H_{\delta 1}$ deprotonation[12,13] in six-coordinated complexes, such as CyanoHRP. These observations, taken together, suggest that, in the resting state of peroxidases, the imidazole is protonated but that deprotonation is a beneficial component of compound I formation.

In the work reported here, we have used the semiempirical quantum-chemical INDO/RHF method to investigate the effects of imidazole $H_{\delta 1}$ deprotonation on the electronic structure and reactivity of the ferryl (compound I) form of the active site of peroxidases. In previous theoretical studies using the extended Huckel method, a small amount of unpaired spin on the imidazole in a model for HRP-I was inferred even in the protonated state.[14] In another study using the same method, an imidazolate form of HRP-I was suggested, but not investigated, as a possible source of rhombicity in this system.[15] In particular, in this studies, we have addressed the question of whether deprotonation could lead to increased radical character on the histidine. The presence of such radical character could help explain puzzling aspects of recent (NMR)[16] and resonance Raman spectra[17] reported for the compound I intermediate of HRP. Detailed NOE studies of compound I of HRP[16] revealed one peak at 21 ppm that was found not to originate from the heme unit and was assigned to the proximal His 170 β-CH_2. This assignment implies that there is significant radical character on the axial histidine of HRP-I. In further support of this possibility, recent resonance Raman spectra of HRP-I exhibited features of unexplained origin that were not characteristic of typical metalloporphyrin π cation radicals.[17]

Significant radical character on the proximal histidine could also help explain the rapid electron-transfer process from the protein to the heme unit that occurs in CCP-I. The cation radical in CCP-I has at various times been considered to reside on Trp 51,[18] Met 230 and 231,[19] and Met 172.[20] However, several site

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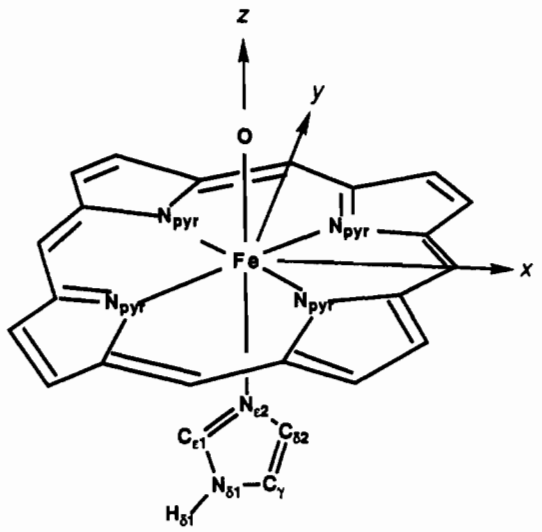

Figure 1. Model peroxidase compound I used in studies. r(Fe–O) = 1.65 Å; r(Fe–$N_{\epsilon 2}$) = 2.05 Å. The β-CH_2 group of the imidazole side chain is connected to C_γ. Calculations are performed with the rotational angle (N_{pyr}–Fe–$N_{\epsilon 2}$–$C_{\delta 2}$) τ_{Im} = 23°.

directed mutagenesis studies[21-23] have eliminated these from consideration, and Trp 191 has now been identified[23,24] as the most likely radical center that transfers an electron to the heme unit. In CCP, Trp 191 is within an interaction distance (3.4 Å) of the proximal histidine. Thus, a proximal histidine that has significant radical character in CCP-I would be an ideal electron acceptor from Trp 191 forming the "ES" complex via a π–π charge-transfer complex with one oxidizing equivalent on the Trp 191 radical and the other remaining on the heme unit. The Trp 191 residue is not conserved in HRP, which could account for the lack of electron transfer to the heme, even if the imidazole of the proximal His were to have appreciable radical character.

Our previous calculations of the protonated HRP-I electronic structure using the INDO method[25] revealed that, in the classical [Fe^{IV}=O; S = 1] a_{1u} or a_{2u} π-cation quartet states of this complex, there is no significant spin delocalization on the imidazole. In other model system calculations, only these a_{1u} or a_{2u} cation states were considered.[26] In the light of the recent experimental evidence implicating possible deprotonation of the imidazole in HRP-I and CCP-I, in the studies reported here, we have broadened the original calculations to include characterization of the active site for a model peroxidase compound I with both a neutral and a deprotonated proximal imidazole ligand. The surprising result is obtained that in the deprotonated state, there is a low-energy form of a peroxidase compound I intermediate with significant radical character on the imidazole. This result provides a coherent explanation for some of the novel experimental observations of these systems.

Methods and Procedures

A model Compound I Peroxidase complex (Figure 1) was used, consisting of a regularized Fe–porphyrin complex with the same geometry as in previous studies[25] and an oxygen atom and imidazole as axial ligands. An Fe=O distance of 1.65 Å was chosen in accordance with several experimental determinations,[27-29] and an Fe—$N_{\epsilon 2}$ imidazole distance of 2.05 Å was selected consistent with the X-ray structure of CCP.[8] Following convention, the porphyrin ring was placed in the xy plane, and the D_{4h} symmetry group, although not perfectly maintained, was assumed in labeling the oribtals and states considered. Although the effect of the rotations of imidazole ring on the calculated energies and electronic structures of the different spin states has been shown to be small,[30] the effect on the relative energies of three different quartet states was examined here by calculations with three different values of the rotation angle τ_{Im} = 0, 23, and 45°. The rotational angle τ_{Im} is defined as the dihedral angle of N_{pyr}–Fe–$N_{\epsilon 2}$–$C_{\delta 2}$ (Figure 1). As shown in Table I, the effect of imidazole rotation on the relative energies of these states is very small. In the following calculations, the geometry with τ_{Im} = 23° was used. To investigate the effect of deprotonation, both imidazole and imidazolate models of the axial ligand were included in this study.

Table I. Effect of Imidazole Rotations on the Relative State Energies

cation radical	ΔE, kcal/mol		
	τ_{Im} = 0°	τ_{Im} = 23°	τ_{Im} = 45°
a_{1u}	0	0	0
a_{2u}	5.5	5.7	6.3
d_{xy}	23.0	23.7	24.4

Table II. Calculated Relative Energies and Quadrupole Splittings for the Manifold of Quartet States in Model Peroxidase Compound I

compound I model	radical cation[a]	ΔE,[b] kcal/mol	ΔE_Q,[c] mm/s	η[d]
imidazole	d_{xy}	23.7	1.34	0.08
	a_{2u}	5.7	1.49	0.02
	a_{1u}	0.0	1.47	0.03
imidazolate	d_{xy}	16.1	1.69	0.27
	Im π	6.2	1.61	0.45
	a_{2u}	2.8	1.33	0.32
	a_{1u}	0.0	1.32	0.35

[a] This column indicates MO occupancy of the third unpaired electron of the quartet state. The other two unpaired electrons occupy the Fe–O π^* orbitals that are symmetric relative to the Fe–O axis. [b] Calculated relative state energies. [c] Calculated values of quadrupole splitting. Experimental value for HRP compound I ΔE_Q = 1.38 mm/s.[34] [d] Asymmetry in calculated electric field gradient at Fe nucleus. Experimental η = 0.

In previous calculations, we have definitely established that the quartet states, rather than doublet or sextet states, are the lowest lying states of the peroxidase compound I. In the studies reported here, we have calculated the relative energies and electron and spin distributions of a manifold of low-lying quartet spin states using a spin-restricted open-shell INDO–RHFSCF method, which is described in detail elsewhere[31] and which we have applied to a model P450[32] system and to a series of ferric heme complexes.[33] These results have demonstrated the reliability of the method in predicting the correct order of states of different spin multiplicities, in agreement with experimental observations. The nature and accessibility of the quartet states in compound I complexes of different heme proteins is a long-standing question. In this study, we have examined states with different occupancies of the third unpaired electron, specifically states in which it is assigned to the a_{1u}, a_{2u} porphyrin π orbitals, the imidazole π orbital, or the Fe d_{xy} orbital, respectively. For all of these possibilities, each of the other two unpaired electrons are assigned to one of the two Fe–O π^* orbitals.

Results and Discussion

Table II gives the calculated relative energy of each of the quartet states for the neutral and deprotonated imidazole system

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Table III. Iron and Oxygen Atomic Orbital and Spin Populations in Model Peroxidase Compound I Complexes

protonation state	state	orbital population (spin population)						
		$Fe_{d_{z^2}}$	$Fe_{d_{x^2-y^2}}$	$Fe_{d_{xy}}$	$Fe_{d_{xz}}$	$Fe_{d_{yz}}$	O_{p_x}	O_{p_y}
imidazole	d_{xy}	0.91 (0.00)	0.91 (0.00)	0.02 (0.99)	0.90 (0.55)	0.87 (0.56)	1.14 (0.43)	1.17 (0.41)
	a_{2u}	0.88 (0.01)	0.52 (0.00)	2.00 (0.00)	0.59 (0.70)	0.58 (0.70)	1.42 (0.28)	1.43 (0.28)
	a_{1u}	0.89 (0.00)	0.52 (0.00)	2.00 (0.00)	0.59 (0.70)	0.58 (0.70)	1.42 (0.28)	1.43 (0.27)
imidazolate	d_{xy}	0.92 (0.00)	0.86 (0.00)	0.02 (0.99)	0.93 (0.53)	0.80 (0.60)	1.09 (0.45)	1.24 (0.38)
	Im π	0.88 (0.00)	0.53 (0.00)	2.00 (0.00)	0.71 (0.64)	0.49 (0.75)	1.30 (0.34)	1.52 (0.23)
	a_{2u}	0.90 (0.01)	0.50 (0.00)	2.00 (0.00)	0.62 (0.69)	0.48 (0.75)	1.39 (0.30)	1.53 (0.22)
	a_{1u}	0.90 (0.00)	0.50 (0.00)	1.99 (0.00)	0.62 (0.68)	0.48 (0.76)	1.38 (0.30)	1.54 (0.22)

Table IV. Net Charges and Spin Density on Iron, Oxygen, Porphyrin, and Imidazole of Model Peroxidase Compound I Complexes

protonation state	state	net charge (spin density)			
		Fe	O	Im	Por
imidazole	d_{xy}	1.59 (2.10)	−0.56 (0.83)	0.23 (0.01)	−0.26 (0.06)
	a_{2u}	1.47 (1.41)	−0.74 (0.56)	0.19 (0.02)	0.08 (1.01)
	a_{1u}	1.47 (1.40)	−0.74 (0.56)	0.17 (0.01)	0.10 (1.03)
imidazolate	d_{xy}	1.57 (2.11)	−0.62 (0.82)	−0.59 (0.01)	−0.37 (0.06)
	Im π	1.43 (1.39)	−0.74 (0.57)	0.15 (1.00)	−0.84 (0.04)
	a_{2u}	1.46 (1.44)	−0.80 (0.53)	−0.64 (0.05)	−0.02 (0.98)
	a_{1u}	1.46 (1.44)	−0.81 (0.52)	−0.68 (0.01)	0.02 (1.03)

together with the calculated values of the quadrupole splitting and their anisotropy. For the neutral imidazole system, the imidazole π cation radical could not be found. An initial configuration of this type converged to the a_{1u} cation radical. In contrast, for the deprotonated model system, in addition to the porphyrin π radical and the Fe d_{xy} type of states, the imidazolate π radical was obtained. The a_{1u} cation radical was found to be the ground state for both imidazole and imidazolate complexes. The calculated values of ΔE_Q and η for this ground state are in excellent agreement with experiment.[34] The a_{2u} and imidazolate cation radicals are a few kcal/mol higher in energy and have similar values of ΔE_Q and η. The energy of the d_{xy} cation radical is substantially higher.

Table III shows the electron and spin populations on the iron d and oxygen p_π orbitals in each of the states for each model. Table IV lists the net charge and spin densities on the iron, oxygen atoms and on the imidazole and porphyrin rings in each of these states. The calculated spin densities on the O atom due to delocalization in the Fe–O π^* orbitals are similar for the a_{1u}, a_{2u}, and Im π-radical states and in good agreement with the experimental observations[35] (0.28 on O_{p_x} and 0.27 on O_{p_y}). These results indicate that the position of the third unpaired electron has very little effect on the radical character of the oxygen atom and that the description obtained for these low-lying quartet states is reliable. Finally, Table V gives the unpaired spin densities on the imidazole ring atoms in the imidazole radical state of the deprotonated model for peroxidase compound I. The presence of this low-lying imidazole radical quartet state with very different charge and spin distributions is dramatic evidence for the ease of electron and spin transfer in this labile biologically active oxidizing state.

The main new insight obtained from these calculations is the effect of deprotonation on the electronic structure and possible type of accessible states for labile peroxidase compound I intermediates. Deprotonation does not significantly affect the charge or spin distribution on the iron, on the oxygen atom, or on the porphyrin ring in the electronic states common to both forms of compound I (Tables III and IV). Instead, in the imidazolate form of the a_{1u}, a_{2u}, and d_{xy} states, the imidazole ring retains a large part of the negative charge resulted from its deprotonation, and it has very little spin density. The striking effect of deprotonation is the appearance of a low-lying state corresponding to an imidazole radical. In this imidazole π radical state, there is nearly one unpaired spin on the imidazole ring, and the negative charge

Table V. Spin Distribution on Imidazole Ring in the Imidazolate π-Cation-Radical State of Peroxidase Compound I

atom[a]	spin density
$N_{\epsilon 2}$(ligand)	0.020
$C_{\delta 2}$	0.395
C_γ	0.243
$N_{\delta 1}$	0.058
$C_{\epsilon 1}$	0.286
tot.	1.002

[a] See Figure 1 for the atomic assignment.

is centered almost entirely on the porphyrin ring, which has no significant unpaired spin density.

The results obtained clearly indicate that deprotonation is a mechanism by which radical character can be transferred from the porphyrin ring to the imidazole in the compound I state of peroxidases. Thus, it provides a coherent explanation for the experimental evidence for unpaired spin density on the imidazole ring from NOE NMR studies of HRP-I.[16] We see from Table V, that the ring carbon C_γ, which is the atom attached to the β-CH_2 group, has substantial unpaired spin density, consistent with the assignment of an NOE peak in HRP-I to the β-CH_2 of the histidine.[14] While an Im π state is of higher energy than the porphyrin π-cation states, it is only 6 kcal/mol above the ground state. Given the approximations in the methods used, these results suggest that such an Im π state is energetically accessible and hence likely to contribute to the observable properties. The calculated quadrupole splittings of the imidazole π-radical state is similar to that for the porphyrin π radicals, and the presence of such a state is consistent with the observed value for HRP-I.

These results can also be used to help explain the anomalous results found in the resonance Raman spectra of HRP-I,[17] which were not characteristic of that observed for classical metalloporphyrin π cation radicals. One of the interpretations of the data considered by the authors was extensive delocalization of the porphyrin π cation radicals of HRP-I onto the axial ligands.

Finally, these results can also explain the facile transfer of an electron to the heme unit from a Trp 191 known to occur in CCP-I.[24] In CCP, Trp 191 and the proximal histidine are arranged in a stacked orientation, close enough (~3 Å) for charge transfer. Thus electron transfer by Trp 191 would be facilitated by enhanced radical character on the proximal imidazole in CCP. By contrast, sequence alignment of HRP with CCP[10] reveals that the Trp 191 residue is not present at the active site of HRP. Hence, such charge transfer would not occur even if the imidazole has radical character.

The studies reported here suggest that enhanced radical character is imparted to the imidazole in the deprotonated state

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of both CCP and HRP. Since proton transfer to a nearby Asp residue is possible in both proteins, it could be important in formation of an initial compound I state in both species. Since Trp 191 is absent in HRP, this state is more stable in HRP but is easily transformed to the ES complex in CCP.

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Registry No. CCP, 9029-53-2; His, 71-00-1; Trp, 73-22-3; peroxidase, 9003-99-0.

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Metal Phthalocyanine Ground States: Covalence and ab Initio Calculation of Spin and Charge Densities

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Ab initio UHFS DV-Xα calculations of the low-lying states of manganese, iron, and cobalt phthalocyanines have been carried out with basis sets corresponding to about "double-ζ" quality. Comparison with experimental spin and charge densities on β-polymorph crystals shows the ground states to be ^4E_g, ^3E_g (or less likely $^3B_{2g}$), and $^2A_{1g}$, respectively. The molecules are highly covalent, with σ donation into $3d_{x^2-y^2}$ of ca. 0.7 e and π back-donation into the macrocycle of ca. 1 e from $d_{xz,yz}$, with the other 3d orbitals fairly ionic in character. The π back-donation differs strongly between "up" and "down" spin, the up spin being mostly ionically localized in metal $3d_{xz,yz}$ orbitals whereas the down spin is strongly covalently delocalized onto the macrocycle. This large differential covalence appears in experiment as well as the calculation. Such covalence is compatible with ESR, magnetic and Mossbauer data on the crystals. Consequently, the assignment of ground state with ionic ligand field models can be misleading. The 3d dominated orbitals lie in the order $3d_{x^2-y^2} \gg 3d_{z^2} > 3d_{xy} \sim 3d_{xz,yz}$, with up-spin members lower in energy than down-spin ones, giving the observed ground states. The closeness of the $3d_{xy}$ and $3d_{xz,yz}$ down-spin orbitals in the calculation for FePc suggests that the contribution of $^3B_{2g}$ and ^3E_g in the spin-orbit mixed ground state may vary greatly depending on small changes in axial ligation. This means small changes in such ligation can produce changes in both spin and charge populations on metal and Pc of about 1 e.

Introduction

Metal complexes of tetrapyrrole ligands are widespread in biological systems and, probably concomitantly, their properties are often very sensitive to their chemical environment, both intra- and intermolecular. Metal phthalocyanines and simple porphyrins have often been used as model compounds to try to understand the details of the metal-ligand bonding in important biological molecules. In addition, metal phthalocyanines have an extensive redox chemistry with applications in electro- and photocatalytic and semiconduction processes.[1]

The compounds have a rich array of properties with complex spectra and electrochemistry, highly variable from one metal to another. The magnetic and ESR properties also cover a wide range. The first mentioned properties are broadly understood in terms of molecular orbitals models, such as iterated extended Hückel, which emphasize the covalent mixing of metal and macrocycle orbitals and the importance of charge transfer as well as intra-ring transitions.[2] On the other hand, historically, the magnetic, ESR, and related properties have been interpreted in terms of a ligand field model, with its inherently ionic basis. While such modeling has been successful in many respects, such as the explanation of the occurrence of unusual intermediate spin states, if pressed too far, inconsistent interpretation can result.[3]

Aside from the question of covalence, a further possible source of confusion is the sensitivity of ground states to intermolecular effects. For example, the ESR properties of CoPc are very different between its two polymorphs.[4] This has been recognized in the electronic spectra,[5] where attempts at a detailed understanding are restricted to weakly axially ligated solution or vapor data. In our case, we are primarily interested in the β-polymorph, so solution or vapor data bear on this only to a limited extent.

Attempts at a more fundamental understanding of the electronic structure of metal phthalocyanine free molecules by use of ab initio calculations have been made most notably by Hartree-Fock (HF),[6] Xα,[7,8] and INDO[9] methods for states of ferrous porphine, and by Xα methods for copper porphine,[7] as well as other systems.[10-15] The HF calculation shows little covalence in the metal-macrocycle bonding and takes account of electron-electron correlation by only limited configuration interaction. In contrast, the Unrestricted Xα and INDO calculations give a markedly covalent description. A beginning has been made in the proper inclusion of spin-orbit coupling for copper porphine. In these UHFS cases, and in the earlier calculation on iron and copper tetraazaporphyrin,[10] good agreement is obtained with spectroscopic, Mossbauer, and resonance data. The message to be derived from the Xα calculations is, first, covalence is important and, second, because of the energy

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