An ab Initio Model System Investigation of the Proposed Mechanism for Activation of Peroxidases: Cooperative Catalytic Contributions from the Ion and Microsolvent Water

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In the enzymatic cycle of the peroxidase family of heme proteins, hydrogen peroxide is transformed into the catalytically active species, an Fe═O heme species known as compound **I**. The postulated mechanism involves the formation of a transient HOOH−Fe heme complex that is transformed via proton transfer to the oxywater isomer H_2OO−Fe heme species, which is followed by facile cleavage of the O−O bond to yield compound **I** and water. The proton transfer process is thought to be aided by a highly conserved distal histidine that serves as a catalyst. The plausibility of this step has been assessed in this work by characterizing the isomerization of HOOH to H_2OO in complexes with Fe^+, Fe^{2+}, Fe^{3+}, Na^+, Mg^{2+}, and Al^{3+} using second-order perturbation theory and the coupled-cluster method in conjunction with various basis sets. The putative catalytic role of a proton acceptor was investigated by determining the influence of one microsolvent water on the Mg^{2+} system. The results demonstrate that although the gas-phase isomerization is highly endothermic and possesses a large activation energy, the metal ion significantly stabilizes the oxywater isomer. The $Na^+ - Mg^{2+} - Al^{3+}$ sequence of complexes reveals that the stabilization effect increases sharply with the charge on the ion. Fe^+ and Fe^{2+} calculations found a small amount of additional stabilization with respect to the corresponding Na^+ and Mg^{2+} systems. While the barrier to isomerization was not significantly reduced by binding peroxide to a cation alone, it was reduced dramatically when a single water was added to the Mg^{2+} system. The ion contributes to this effect by increasing the protonicity of the H that is being transferred, allowing it to interact more strongly with the water. The proton transfer is thus strongly enhanced by cooperative contributions by the metal ion and microsolvent water.

Introduction

Peroxidases are ubiquitous metabolizing oxidative heme proteins that are widely distributed in plants, fungi, and bacteria, and also play a role in mammalian physiology.[1] The defining characteristic of all peroxidase oxidations[2] is that stoichiometric amounts of hydrogen peroxide (HOOH) are transformed into the catalytically active Fe═O ferric heme species known as compound **I**. The postulated mechanism for this process consists of several steps as shown in Figure 1: (i) HOOH displaces the H_2O ligand from the initial resting state, (ii) a proton is transferred from one oxygen to the other, and (iii) the O−O bond cleaves, yielding compound **I** and a departing H_2O. The proton transfer is thought to be aided by a highly conserved distal histidine that serves as a catalyst. The resting state and compound **I** have been characterized spectroscopically,[3] but the intermediate peroxide species are apparently too transient to be observed directly with the experimental methods presently in use.

Theoretical methods can often probe mechanistic issues that are currently inaccessible to experiment and thus complement laboratory studies. For example, two recent computational studies have addressed the nature of transient peroxide intermediates using different models and levels of theory. Harris and Loew[4] utilized semiempirical INDO/ROHF/CI calculations to predict the structures and electronic spectra of two candidate peroxide intermediates. Loew and Dupuis[5] employed the ROHF method to study the binding of HOOH to an iron−porphyrin complex with an imidazole distal axial ligand. These and other studies have provided strong support for the existence of a transient peroxide−peroxidase intermediate. They have also provided a detailed description of the electronic structure and spectra of the resting state and compound **I** species. However, due to the complexity of the iron−heme system, no computational study to date has characterized the detailed pathway of the key proton-transfer step in order to probe its postulated role in the overall pathway between the resting state and compound **I**.

The present work uses ab initio quantum chemical methods to study simple model systems in order to assess the plausibility of the crucial initial proton transfer, which corresponds to the gas-phase isomerization of hydrogen peroxide to oxywater, HOOH to H_2OO. Four components of the protein system can modify the energetics with respect to the gas-phase behavior: (i) the metal ion; (ii) moieties present in the binding pocket that may serve as catalysts, such as water and amino acids; (iii) the porphyrin macrocycle; and (iv) a distal ligand on the ion. In this work, the impact of the first two components has been investigated in small systems where high-level correlated molecular orbital methods could be employed. It is intended to serve as a foundation for future studies on full heme model systems using methods suited to larger systems. While it is intended to provide insight into peroxidases, the core chemistry—an ion-assisted isomerization further aided by solvent or other moieties—may well be present in other systems. With the stated approach, we were able to ascertain the individual and cumulative effects of the different components that are present.

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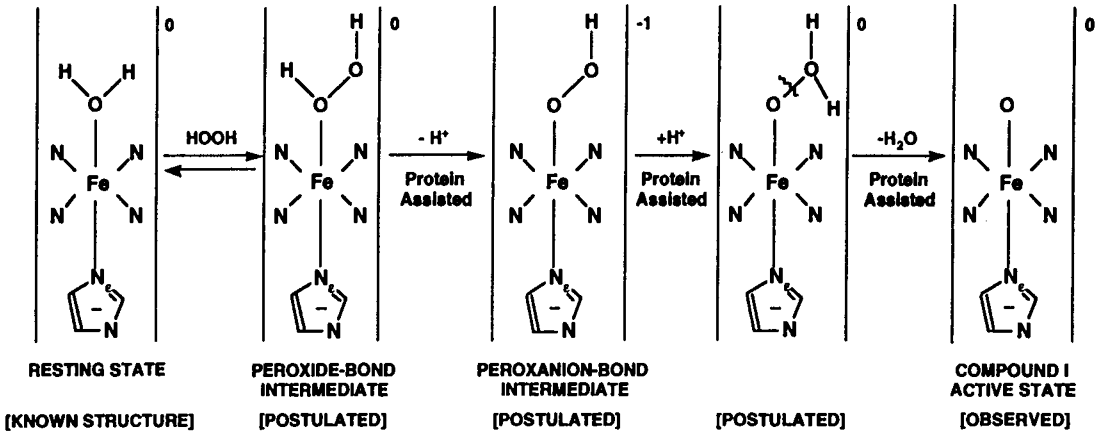

Figure 1. Proposed pathway for transformation of peroxidases from the ferric resting state to the catalytically active oxidative species, compound **I**.

The model system calculations reported here begin with the bare gas-phase isomerization process and characterize the manner in which additional components alter the barrier height and isomerization energy as well as the O−O bond length. The varying impact of adding Fe^+, Fe^{2+}, Fe^{3+}, Na^+, Mg^{2+}, and Al^{3+} ions alone was first investigated. We then examined the extent and nature of the further mitigating influence of a single catalytic water molecule on the Mg^{2+}−peroxide/oxywater system. Although in the real system this role is thought to be played by an amino acid present in the binding pocket, water is a suitable substitute in a model system. The dramatic changes obtained in both the isomerization energy and barrier to isomerization demonstrate how effectively environmental factors can alter gas-phase behavior. They also provide additional support for the proposed mechanism of compound **I** formation in peroxidases.

The respective catalytic effects of ions and of water and other proton acceptors have been reported in earlier studies. For example, Wakatsuki et al.[6] describe the manner in which Rh(I) modifies the isomerization energetics of acetylene and vinylidene, which is quite analogous to the present problem. Likewise, water has been shown[7] to significantly alter the energetics of the HOOH to H_2OO isomerization. Other proton acceptors can function in a similar manner. For example, the presence of H_2 in the reaction

$$HOC^+ + H_2 \rightarrow HCO^+ + H_2 \qquad (1)$$

reduces a large gas-phase barrier for HOC^+ to HCO^+ isomerization to about 1 kcal/mol.[9] Although the influence of ions and microsolvent have been characterized separately, the present work demonstrates that they may function cooperatively as well, each playing very specific roles to enhance the critical proton transfer in peroxidases.

Methodology

The MOLPRO[9] and GAUSSIAN[10] suites of ab initio electronic structure codes were employed in this study. Correlation contributions are important here for two reasons: to provide a more balanced treatment of the isomers and associated transition state and to properly describe the intermolecular interactions of the various species. Each of the species were characterized with second-order Møller−Plesset (MP2) or Rayleigh−Schrodinger (RS2) perturbation theory, with the latter used for open-shell species. Structures of the smaller closed-shell species were also optimized using the coupled cluster method CCSD(T).[11] A basis set constructed by Wachters[12] was used for Fe, while correlation consistent basis sets[13] were employed for the remaining elements: sets of cc-pVDZ quality were used for the metal ions, while aug-cc-pVDZ sets were employed for H and O. The diffuse functions present in the augmented correlation consistent basis sets are needed to describe the intermolecular character of the metal−peroxide and peroxide−water interactions. The d functions were spherically contracted, and only valence electrons were correlated. Analytical gradient methods were used for finding MP2 stationary points. Numerical methods were used for the CCSD(T) and RS2 optimizations. Zero-point energies (ZPE) were computed analytically for some of the species in order to correct relative energies.

Calculations were performed with two groups of ions: the closed-shell sequence Na^+, Mg^{2+}, and Al^{3+} and the open-shell iron ions Fe^+, Fe^{2+}, and Fe^{3+}. In each case, structures for HOOH−M^{p+} and H_2OO−M^{p+} were sought. For the closed-shell ions, the intervening transition states were also sought. There is an inherent difficulty treating divalent and trivalent gas-phase ions: they dissociate to $HOOH^+$ (or H_2OO^+) and $M^{(p-1)+}$, the surface of which crosses the desired HOOH (or H_2OO) + M^{p+} surface. If the crossing occurs at a small enough Fe−O distance, there may be no minimum on the computed ground-state surface. In previous work on the Fe^{3+}−H_2O system, Curtiss et al.[14] located a minimum, but the crossing to the Fe^{2+}−H_2O^+ surface occurred very close to it. We encountered no difficulty with either of the Fe^{2+} species or with Fe^{3+}−HOOH, but no structure for Fe^{3+}−H_2OO was found. As the Fe−O distance was increased, the surface only exhibited a shoulder where the charge distribution changed to repulsive H_2OO^+ + Fe^{2+}. However, the Al^{3+} calculations did not exhibit this instability and provide insight into the environmental influence of a trivalent ion.

In the characterization of intermolecular complexes, it is often advisable to correct binding energies for basis set superposition error (BSSE) using an approximation such as the counterpoise (CP) method of Boys and Bernardi.[15] The focus here is not on the binding energies, however, but on on the relative energies of the isomers. Also, BSSE tends to be a very small fraction of the binding energy in ionic interactions.[16] As a test of the need to apply a correction in the present study, we used the CP method to determine the BSSE in the Mg^{2+}−HOOH and Mg^{2+}−H_2OO species. It was found to be the usual 1−2 kcal/mol in each case. The change in the relative energy, however, was less than 1 kcal/mol due to cancelation.

Results

Selected structural parameters for the various species studied here are given in Table 1. The corresponding total energies,

TABLE 1: Selected Geometrical Parameters for Peroxide and Oxywater Isomers and Transition States in Complexes with Various Metal Ions (M) Computed at the MP2/RS2 Level

	r(OO) (Å)			*r*(MO) (Å)		
species	X = HOOH	X = TS	X = H_2OO	X = HOOH	X = TS	X = H_2OO
X	1.470	1.570	1.506			
Na^+·X	1.481	1.563	1.487	2.450	2.283	2.260
Mg^{2+}·X	1.496	1.580	1.455	2.048	1.958	1.913
Al^{3+}·X	1.571	1.568	1.472	1.870	1.855	1.830
Fe^+·X	1.480		1.475	2.316		2.039
Fe^{2+}·X	1.498		1.458	2.097		1.914
Fe^{3+}·X	1.495			1.976		
Mg^{2+}·X·H_2O	1.524	1.513	1.485	1.921	1.877	1.876

isomerization energies, and barriers to isomerization are presented in Table 2. High-spin states were most stable for the Fe^+/Fe^{3+} (sextet) and Fe^{2+} (quintet) systems.[17] Figure 2 displays the structures and relative energetics for the isomerization of HOOH to H_2OO for three different systems: (i) the gas phase, (ii) interacting with Mg^{2+}, and (iii) interacting with both Mg^{2+} and H_2O. In the ion−oxywater complexes, only the terminal O is coordinated closely to the ion. In the ion−peroxide complexes, however, the structures are symmetric, with equal MO bond lengths. By contrast, in previous calculations[4,5] on heme models in which the porphyrin macrocycle is present, peroxide was found to bind asymmetrically to Fe in an end-on manner. This difference can be attributed to the additional interaction of HOOH with the anionic N atoms in the porphyrin ring and the low energy of the bending mode associated with breaking symmetry.

Comparison of Optimized Structures for Oxywater and Peroxide species. The optimized structures in Table 1 can be used to compare the geometries of the peroxide and oxywater isomers in the gas phase and as modified by the presence of the five ions. The calculated M^{p+}−HOOH binding energies of −22.5, −83.9, and −215.7 kcal/mol for the Na^+, Mg^{2+}, and Al^{3+} complexes reflect the dependence of the interaction energies on the magnitude of the ionic charge. Likewise, the cation−oxygen ligand distances r(MO) in the peroxide isomers steadily contract as the charge increases, at 2.450, 2.048, 1.870 Å for the three closed-shell ions, respectively. The respective values of r(MO) exhibit a similar trend for the oxywater isomers, at 2.260, 1.913, and 1.830 Å for the Na^+, Mg^{2+}, and Al^{3+} complexes. Similar trends are exhibited by the Fe^+ and Fe^{2+} complexes.

The presence of an ion significantly distorts the geometries of HOOH and H_2OO. The results indicate that binding peroxide to a cation increases O−O bond length from the gas-phase value of 1.470 Å to 1.481, 1.496, and 1.571 Å for the Na^+, Mg^{2+}, and Al^{3+} complexes, respectively. The Fe^+ and Fe^{2+} values are 1.481 and 1.498 Å, nearly identical to their closed-shell analogues. The calculated O−O bond lengths in the corresponding oxywater species are shorter than in the gas-phase species: 1.506 Å in isolated H_2OO vs 1.487, 1.455, and 1.472 Å for the Na^+, Mg^{2+}, and Al^{3+} complexes, respectively. Again the results for Fe^+ and Fe^{2+} are comparable to Na^+ and Mg^{2+}. For each of the divalent and trivalent ions, the O−O distances are actually shorter in the oxywater isomers than in the peroxide forms. These trends are observed at the CCSD(T) level as well, but the values, 1.532, 1.487, and 1.484 Å for Na^+, Mg^{2+}, and Al^{3+}, respectively, exhibit more consistent dependence upon the charge, which reflects the improved treatment of the dative O−O bond using this method. Each of the ions induce contraction of the O−O bond in the oxywater species, which implies a slight strengthening of the bond. This is a small effect, however, compared to the relative ease with which O can be extracted from H_2OO instead of from HOOH; bond cleavage leading to compound **I** formation cannot proceed without the proton transfer. As discussed below, the key effect of the metal ion is to improve the energetics for forming the oxywater species.

Isomerization of Peroxide to Oxywater in the Gas Phase. At the MP2/(cc-pVDZ, aug-cc-pVDZ) level, gas-phase oxywater is 49.7 kcal/mol less stable than hydrogen peroxide (50.0 kcal/mol with the ZPE correction), and the barrier to isomerization is very high, 57.1 kcal/mol (54.9 kcal/mol with the ZPE corection). At the CCSD(T) level, the isomerization energy and barrier height drop slightly, to 45.0 and 51.3 kcal/mol, respectively. These values are commensurate with those reported in the more detailed study of Meredith et al.[18]

The large difference in the relative stabilities of the isomers is due to the nature of the O−O bond in H_2OO. Unlike the usual σ bond in HO−OH, the bond in oxywater is dative, arising from the interaction of one of the lone pairs on water and the axial 2p orbital on the terminal O· H_2O−O dissociates to ground-state H_2O and O in the ^{1}D excited state. It is a weaker bond, e.g., 30.6 kcal/mol for H_2O−O vs 46.6 kcal/mol for HO−OH at the MRCI/aug-cc-pVDZ//CCSD(T)/aug-cc-pVDZ level.

Isomerization of M^{p+}·HOOH complexes for M^{p+} = Na^+, Mg^{2+}, Al^{3+}, Fe^+, and Fe^{2+}. The addition of a cation significantly alters the energetics of the peroxide−oxywater isomerization as indicated by the results presented for the Na^+, Mg^{2+}, Al^{3+}, Fe^+, and Fe^{2+} ion complexes in Table 2. As seen in this table, the ion preferentially stabilizes the oxywater isomer, and the effect varies markedly with the magnitude of the ionic charge. Values of the isomerization energy are 36.8, 16.8, and −9.3 kcal/mol from the MP2 calculations for Na^+, Mg^{2+}, and Al^{3+} cases, respectively. The RS2 results for Fe^+ and Fe^{2+} of 26.4 and 12.1 kcal/mol are roughly comparable to the behavior of the Na^+ and Mg^{2+} complexes, but do exhibit a greater stabilization effect. As in the previous discussion on ion-induced distortion of the O−O bond length, the ionic charge is the primary factor that determines the extent to which the oxywater is stabilized with respect to peroxide. If Fe^{3+}−H_2OO had been stable, the trends for the other five ions suggest that it would have been more stable than Fe^{3+}−HOOH, by 10 kcal/mol or more.

The MP2 energy differences for the closed-shell set of ions are closer to the more accurate CCSD(T) results than in the gas phase isomerization. As in the isolated system, the relative stability of oxywater is increased slightly at the higher level of theory. Oxywater possesses a large dipole moment component along the O−O due to charge transfer within its dative bond. This property accounts for both the preferential stabilization of the oxywater isomer when bound to an ion as well as the increased stabilization as the charge increases. The increasing influence of the cation is great enough that Al^{3+}·H_2OO is more stable than Al^{3+}·HOOH, a change of almost 60 kcal/mol with respect to the gas-phase value.

This cation-assisted formation of the oxywater complex provides convincing support for the proposed formation of the corresponding oxywater species as a ligand for the heme iron in peroxidases. However, while the activation energy barrier is also lowered (to 50.9, 44.7, and 44.6 kcal/mol for Na^+, Mg^{2+}, and Al^{3+}, respectively), the changes are not as pronounced as in the increasing stability of the oxywater isomer. In all cases, the barrier continues to be a substantial impediment to isomerization. However, the increasing cationic charge effects another key property in the isomerization, the effective charge of the

TABLE 2: Total Energies and Energy Differences for Hydrogen Peroxide−Oxywater Isomerization in the Gas Phase and in Complexes with Various Species[a]

species	method	X = HOOH	X = TS	X = H_2OO	$\Delta E_{barrier}$	ΔE_{isom}
X	MP2	−151.218 030	−151.126 965	−151.138 844	57.1 (54.9)	49.7 (50.0)
	CCSD(T)	−151.243 341	−151.161 612	−151.171 641	51.3	45.0
$Na^+\cdot X$	MP2	−312.924 919	−312.843 850	−312.866 261	50.9	36.8
	CCSD(T)	−312.949 959		−312.896 254		33.7
$Mg^{2+}\cdot X$	MP2	−350.175 898	−350.104 705	−350.149 109	44.7 (41.2)	16.8 (17.3)
	CCSD(T)	−350.200 809		−350.175 960		15.6
$Al^{3+}\cdot X$	MP2	−391.556 108	−391.484 981	−391.570 967	44.6	−9.3
	CCSD(T)	−391.581 123		−391.598 064		−10.6
$Fe^+\cdot X$[b]	RS2	−1413.460 888		−1413.418 795		26.4
$Fe^{2+}\cdot X$[b]	RS2	−1412.992 922		−1412.973 654		12.1
$Mg^{2+}\cdot X\cdot H_2O$	MP2	−426.502 696	−426.483 543	−426.484 020	12.0 (12.7)	11.7 (12.3)

[a] Total energies in hartrees (E_h); energy differences in kcal/mol (with zero-point energy corrected values in parentheses). [b] H basis set: cc-pVDZ.

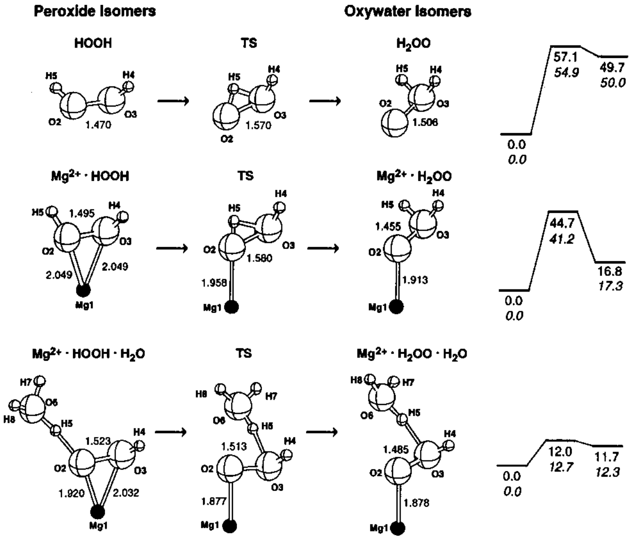

Figure 2. Structures of hydrogen peroxide (HOOH), oxywater (H_2OO), and the associated transition state (TS) in the gas phase, bound to Mg^{2+}, and bound to Mg^{2+} and one water molecule. Selected bond lengths (in angstroms) are shown. The isomerization diagrams (with energy differences in kcal/mol relative to the peroxide isomer and zero-point energy corrected values in italics) indicate that the presence of the ion alone greatly stabilizes the oxywater isomer, but the barrier remains high until the water stabilizes the transition state.

migrating H in the transition state. The net atomic charge (Mulliken population) on this atom is 0.48 with no ion and 0.59, 0.68, and 0.77 for the Na^+, Mg^{2+}, and Al^{3+} complexes, respectively. As the ionic charge increases, the migrating H becomes more protonic and is thus able to interact more effectively with proton acceptors such as water or nearby amino acids with large proton affinities. In the final study described below, we have explicitly investigated this possible catalytic effect.

Isomerization of $Mg^{2+}\cdot HOOH\cdot H_2O$. In the final study, a single water molecule was added to each of the optimized structures for the three critical points on the isomerization reaction pathway for the Mg^{2+} complex from above, and the geometries were reoptimized. As shown in Figure 2 and Table 2, the presence of one water alters the energetics of the process in a remarkable manner. Not only does it provide an additional 5 kcal/mol stabilization for oxywater (16.8 vs 11.7 kcal/mol), it drastically reduces the barrier to isomerization from 44.7 to 12.0 kcal/mol, a change of over 30 kcal/mol, which drops the barrier to just 0.3 kcal/mol above the oxywater isomer (0.4 kcal/mol with the ZPE correction). Adding water also lengthens the O−O bond in both minima but decreases it in the transition state.

In systems such as this, good proton acceptors such as water can stabilize transition states significantly by altering their character to become more intermolecular in nature. In this case, and particularly in the transition state, the migrating H atom is shared as a proton between the catalytic water and the moiety undergoing isomerization. The structures very much resemble a distorted H_3O^+ interacting with $Mg^+\cdot OOH$. The binding energy of the water to the initial $Mg^{2+}\cdot HOOH$ complex is 41.3 kcal/mol with respect to the separated $H_2O + Mg^{2+}\cdot HOOH$ species. This is much larger than the $H_2O - HOOH$ interaction energy of 9.7 kcal/mol reported by Bach et al.[7] at the MP4/6-31G*//MP2/6-31G* level. It is thus evident that the presence of the cation greatly enhances the interaction of water with the peroxide moiety in the complex by increasing the protonicity of the migrating H.

Conclusions

In this study we have investigated the isomerization of hydrogen peroxide to oxywater when bound to an isolated metal ion in order to gain understanding into the analogous process in peroxidases where it is a crucial step in the proposed formation of compound **I**. The results of these calculations demonstrate that although the isomerization of HOOH to H_2OO in the gas phase is a highly endothermic process with a large activation energy, the presence of an ion greatly stabilizes the oxywater isomer. Furthermore, examining the sequence of complexes with Na^+, Mg^{2+}, and Al^{3+} demonstrates that the stabilization effect increases with the magnitude of the ionic charge. Analogous complexes with sextet Fe^+ and quintet Fe^{2+} behaved comparably. While the barrier to isomerization remains very high in all of these complexes, the migrating hydrogen becomes more protonic as the ionic charge increases, which improves its ability interact with a proton acceptor such as water. Adding a single water molecule to the Mg^{2+}−peroxide/oxywater system dramatically reduces the barrier by over 30 kcal/mol, to a value of 12 kcal/mol. Thus the ion and the solvent work in concert to make the overall isomerization process much more favorable. As an effective proton acceptor, water catalytically reduces the barrier, which is precisely the role widely assumed for the conserved distal imidazole in most peroxidases and for the glutamate variant discovered in the structure of chloroperoxidase.

The results of these calculations provide additional direct support for the widely accepted mechanism of compound **I** formation from the peroxide intermediate of peroxidases and for the proposed role of the imidazole in the conserved distal histidine. The conclusions drawn from this study will be further tested in future work by corresponding computational investigations using more complex iron-porphyrin model systems. The present study provides initial geometries that will be very useful for locating transition states in those systems. Although the conclusions of this work need to be tested in more complex models, they strongly suggest that compound **I** formation requires active assistance from appropriately positioned amino acid residues or trapped solvent molecules.

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