Saddle Distortions of Ferryl-Porphyrin Models for Peroxidase Compound I: A Density Functional Study

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Peroxidases catalyze a wide range of substrate oxidations.[1] In heme-based peroxidases such as lactoperoxidase (LPO)[2] and horseradish peroxidase (HRP),[3] the active site iron reacts with hydrogen peroxide to generate an intermediate, compound **I**, which can directly oxidize certain substrates to generate species with important biological activity. The latter process requires two electrons. One-electron oxidations can also be carried out by another intermediate, compound **II**, which is produced by a one-electron reduction of **I** (Scheme 1).

The physical properties of compounds **I** and **II** have been studied intensively.[1] Although there are structural and chemical differences between the various peroxidases, the basic features of compounds **I** and **II** are similar. Theoretical studies have explored the electronic structures of model Fe porphyrin systems including recent density functional theory (DFT) calculations with imidazole[4,5] or CH_3S^- [6,7] as the proximal ligand. The latter work focuses on Cytochrome P450, but many of the computational features are relevant.

The peroxidase model used previously is shown schematically in Figure 1. Experiment suggests that **I** possesses three unpaired electrons ($S = 3/2$) with two associated with the Fe=O unit and one localized on the porphyrin, thus generating a π-cation radical. The authors assumed a fixed geometry and calculated the electronic structures and relative energies of two different porphyrin radical states. Antony et al.[5] also varied the proximal ligand. Both groups concluded that the a_{2u} π-cation radical state is the ground state although the a_{1u} state is quite close to this.

A potential shortcoming of the calculations on models for compound **I** in peroxidase is the assumption of a fixed geometry. Pronounced distortions of the porphyrin plane can be induced by substituents on the ring carbons and/or coordination to transition metals.[8−11] Given the small energy difference between the a_{2u} and a_{1u} states in the planar model system, it is important to verify the earlier conclusions using a fully optimized geometry. Moreover, the active-site structure ultimately affects the enzyme's mode of action. Accordingly, this communication reports geometry-optimized structures for model systems based on the proposed structure of compound **I**.[12]

DFT has rapidly established itself as the method of choice for theoretical treatments of transition metal complexes.[13−15] Our
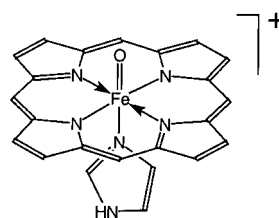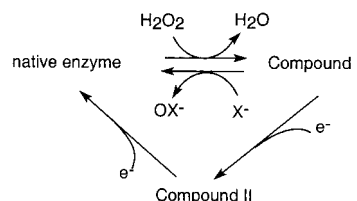

Figure 1. Schematic representation of compound **I** model [FeO-(porphyrin)(HIm)]$^+$.

Scheme 1

experience with the Amsterdam Density Functional (ADF) code shows that for high-oxidation metal-oxo species, the local density approximation (LDA)[16] (with the correlation functional of Vosko, Wilk, and Nusair[17]) gives better M−L distances than gradient-corrected functionals, while the reverse holds for energies.[18−23] The present Fe porphyrin systems are quite large with the added complication of closely spaced energy levels requiring a careful evaluation of the ADF convergence criteria.[24]

Preliminary calculations on C_{4v} model species with no proximal imidazole encountered severe SCF convergence problems and those calculations which completed successfully often displayed non-Aufbau states. A non-Aufbau state was therefore imposed on [FeO(porphyrin)(HIm)]$^+$. The computed geometry showed a planar porphyrin system, **A**, (Figure 2a) which is also found for the P450 model systems.[6,7] Compared with the previous DFT results on peroxidase models,[4,5] the present 'a_{2u}' and '$d_{x^2-y^2}$' β MOs depicted in Figure 2a of ref[4] (46a′ and 47a′) are reversed, and the 'a_{2u}' level for the fully-optimized structure is marginally lower than the metal-based MO. Given that the geometries in each case are slightly different and that the two MOs are separated by only 0.02 eV, all three sets of calculated results are very similar, and the previously reported electronic structure and spin

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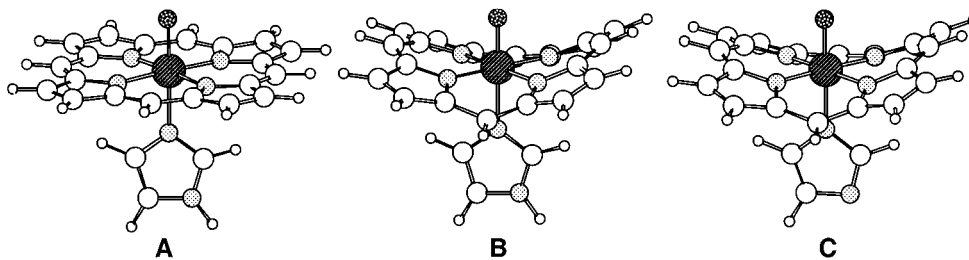(10) Duval, H.; Bulach, V.; Fischer, J.; Renner, M. W.; Fajer, J.; Weiss, R. *J. Biol. Inorg. Chem.* **1997,** *2,* 662−666.

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(24) The geometry optimizations employed the LDA functional (with the Vosko-Wilk-Nusair correlation) as implemented in ADF version 2.3.0. Basis sets were uncontracted triple-ζ + polarization STO expansions (ADF sets IV) and were used in conjunction with the frozen core approximation (1s−2p on Fe; 1s on C, O, and N) and the standard fitting sets. The integration precision was set to 5 (default 4) with an SCF convergence criterion of 10^{-6}. Geometry optimization convergence levels were 0.0001 hartrees for the energy, 0.001 for gradients, 0.005 Å for bond lengths, and 0.2° for angles. Total binding energies were computed post-SCF using the Becke's 1988 exchange and Perdew's 1986 correlation functionals.

Figure 2. DFT-optimized structures for compound **I** models. (**A**) [FeO(porphyrin)(HIm)]⁺, non-Aufbau state; (**B**) [FeO(porphyrin)(HIm)]⁺, Aufbau state; (**C**) [FeO(porphyrin)(Im)], Aufbau state. Legend: small dots, N; small open circles, H; large open circles, C; diagonal hatching, Fe; coarse speckle, O.

Table 1. Selected Calculated Charge and Spin Densities (in Italics) Derived from Mulliken Population Analyses of the Structures Depicted in Figure 2

	[FeO(porphyrin)-(HIm)]⁺ planar (**A**)	[FeO(porphyrin)-(HIm)]⁺ saddle (**B**)	[FeO(porphyrin)-(Im)] saddle (**C**)
Fe	0.62 *1.05*	0.67 *1.45*	0.66 *1.43*
O	−0.41 *0.98*	−0.39 *1.00*	−0.44 *0.99*
C_m	0.11 *0.20*	0.13 *0.12*	0.11 *0.08*
N	−0.33 *0.09*	−0.34 *0.06*	−0.33 *0.05*
porphyrin	0.41 *0.94*	0.34 *0.54*	0.03 *0.37*
imidazole	0.38 *0.03*	0.38 *0.01*	−0.25 *0.21*

distributions for compound **I** with a planar porphyrin system remain relevant. The optimized Fe=O, Fe−N$_{porph}$, and Fe−N$_{Im}$ (1.64, 1.98, and 2.03 Å, respectively) are in excellent agreement with values derived from EXAFS studies of compound **I** from HRP (Fe=O, 1.64 ± 0.03 Å; Fe−N, 2.00 ± 0.02 Å).[25]

With no constraints on the MO occupations, a converged Aufbau state was located. In contrast to planar **A**, a highly distorted 'saddle' porphyrin geometry resulted, **B**, (Figure 2b) which is actually about 10 kJ mol⁻¹ (∼0.1 eV) *lower* in energy. The ruffling does not produce a significant change in the Fe=O or Fe−N$_{Im}$ distances (1.64 and 2.02 Å, respectively) nor in the overall dimensions of the porphyrin ring. For example, opposing H atoms are 10.4 Å apart in **A** and 10.3 Å in **B**. Moreover, the metal and the porphyrin N donors remain essentially coplanar in the saddle structure. The principal 'mechanical' effect of the distortion is to displace the pyrrole-bridging C$_m$ atoms about 0.6 Å and the pyrrole carbons about 0.25 Å from the porphyrin N$_4$ plane. The main electronic consequence of the saddle distortion is that about half of the 0.8 spins originally located on the C$_m$ atoms of **A** move on to the iron center (see Table 1) and appears in orbitals which are perpendicular to the Fe=O bond. The saddle distortion thus tends to deactivate the porphyrin, at least with respect to free-radical processes, while the total spin on the oxygen remains virtually unchanged. However, the increase in the *ratio* of oxygen to porphyrin spins makes the oxygen center a relatively more attractive target for reaction. The spin distribution in **B** is about halfway between the S = 1 on Fe=O, S = 1/2 on porphyrin for **A**, and S = 3/2 all located in the ferryl moiety.

There are no structural precedents for Fe=O porphyrin complexes in the Cambridge Structural Database. However, analysis of other metal-oxo systems suggests a planar porphyrin ring structure for [FeO(porphyrin)(HIm)]⁺. The bond lengths derived from EXAFS measurements for compound **I** in HRP[25] are certainly in good agreement with the planar structure **A**. Moreover, a planar structure for **I** in HRP appears consistent with resonance Raman spectroscopy.[26] We await further experimental data from other peroxidases for evidence of a saddle geometry. Meanwhile, the calculations indicate that the redistribution of spin density—the atomic charges change very little—leads to a smaller metal and hence shorter Fe−N$_{porph}$ distances.

The important feature, then, is that the two rather different structures have comparable energies. Unfortunately it is not practical to confirm whether all the computed structures correspond to local minima. The saddle geometry is more likely to be a minimum since it has the lower energy but the planar structure may not be at a stationary point at all. Presumably, though, substituents on the porphyrin and/or changes in the proximal ligand may remedy this. For example, the calculations on the P450 Compound I model systems consistently give a planar ring structure.[6,7]

Structural flexibility is a recurring theme in heme chemistry where small variations can induce marked changes in the overall active-site structure.[27] Heme-based peroxidases share many similarities, but there are also significant differences. For example, chloroperoxidase and myeloperoxidase can oxidize Cl⁻, Br⁻, and I⁻, while LPO and HRP are unable to oxidize chloride.[1] One might speculate, then, that if enzymes switch from a planar heme to a saddle geometry, the concomitant changes in both the electronic and geometrical structure may be at least partly responsible for the observed differences in peroxidase reactivity.

It is also known that some protonation event, possibly on the proximal histidine, is associated with the reaction of LPO compound **I** with oxidizable substrates.[28] If correct, then the compound **I** model systems may already have a deprotonated imidazole ligand. The optimized geometry for [FeO(porphyrin)-(Im)] (**C**) is shown in Figure 2c. As expected, the Fe−N$_{Im}$ distance shortens, albeit by only 0.04 to 1.99 Å which still places it in the range derived from EXAFS measurements on HRP compound **I**. The rest of the structure is not significantly affected. However, the decrease by one unit of charge does alter the charge- and spin-density distributions. As expected, the change in total charge is mainly associated with the imidazole group ($\Delta\rho = -0.63$), but the porphyrin also makes a significant contribution ($\Delta\rho = -0.31$). This is further reflected in a reduction of the total spin on the porphyrin with about 0.2 spins being transferred to the imidazole moiety. Deprotonation of the proximal ligand is thus predicted to further reduce the reactivity of the porphyrin system to radical attack.

Finally, the geometric and electronic structure changes accompanying any switching between planar and saddle conformations may also be the key to the observed reactivity variations for heme-based systems with proximal ligands other than histidine.

Supporting Information Available: Cartesian coordinates and MO energies for A, B, and C.

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