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Can the Replacement of a Single Atom in the Enzyme Horseradish Peroxidase Convert It into a Monoxygenase? A Density Functional Study

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Density functional calculations on horseradish peroxidase mutants are presented, whereby one or two of the nitrogen atoms of the axial histidine ligand have been replaced by phosphorus atoms. Our calculations show that phosphorus entices a push effect on the oxoiron group, whereas a histidine side chain withdraws electrons. As a result, we predict that a phosphorus-substituted histidine ligand will convert the active form of a peroxidase into a monoxygenase. This subsitution may be useful for the bioengineering of commercially exploitable enzymes.

Peroxidases are heme enzymes that catalyze the detoxification of hydrogen peroxide to water and molecular oxygen.[1] The active species (Compound I, CpdI) of heme peroxidases, such as horseradish peroxidase (HRP) or cytochrome *c* peroxidase (C*c*P), contains an oxoiron group embedded in a heme.[2] The peroxidases differ from other heme enzymes such as the cytochrome P450 enzymes in the nature of the axial ligand bound to iron, namely the active species in P450 enzymes is bound to the protein backbone via a thiolate linkage of a cysteinate residue, whereas peroxidases generally bind to a histidine side chain.[3] The differences in axial ligand result in different chemical properties of the active species, i.e., a cysteinate ligand exerts a push effect on the iron, while histidine withdraws electron density.[4] As a result, these two ligands give differences in electronic as well as catalytic properties to the enzyme active site.[5,6] Attempts to mutate the axial ligand of C*c*P into cysteinate were unsuccessful and resulted in oxidation of the ligand into cysteic acid.[7] In the past we extensively studied the axial ligand effect on the catalytic properties of oxoiron porphyrin models using theoretical methods.[4c,6] In line with this, in this work we present the effect of replacing one or more nitrogen atoms of the histidine axial ligand by phosphorus atoms and the changes this exerts on the catalyst.

Recent studies on α-iminophospholide ligands in coordination chemistry showed extensive charge delocalization from the phosphorus atom.[8] Moreover, biphosphines were shown to have strong π-accepting properties and as a result stabilize transition metals in low oxidation states.[9] To find out what effects the replacement of a nitrogen atom by a phosphorus atom has on the electronic and catalytic properties of HRP CpdI, we have investigated models of CpdI with only the axial bound nitrogen atom replaced by phosphorus (CpdI(1P)) and a model in which both nitrogen atoms of the imidazole ring are replaced by phosphorus (CpdI(2P)).

The model of the active species of peroxidase CpdI is an oxoiron porphyrin (without side chains) that is bound to an axial imidazole group, and the overall charge of the model is +1. All geometries were fully optimized with Jaguar 5.5 using the UB3LYP hybrid density functional method.[10,11] We employed an LACVP basis set for the geometry optimizations and ran single point calculations with an LACV3P+* basis set to confirm the energetics.[12] All energetics reported in this work are calculated at the LACV3P+* level of theory with ZPE corrections at the LACVP level. The charge distributions were calculated with the Natural Bond Analysis (NBO) program as implemented in Gaussian-03.[13,14]

Figure 1 shows the optimized geometries and group spin densities (ρ) of CpdI(1P) and CpdI(2P) in the lowest lying quartet and doublet spin states. Similarly to CpdI of peroxidase and P450 models, CpdI(1P) and CpdI(2P) are also described as a triradicaloid system: two unpaired electrons located in π^* orbitals along the FeO bond and the third one in a nonbonding heme orbital with a_{2u} symmetry.[5,15] These three electrons are either ferromagnetically coupled into an overall quartet spin state or antiferromagnetically coupled into a doublet spin state. As the coupling between the π^*_{FeO} and a_{2u} electrons is weak, the doublet-quartet energy gap is small: ΔE+ZPE = −0.2 kcal mol^{-1} in CpdI(1P) and +0.1 in CpdI(2P), where a minus sign implies a doublet spin ground state. These energy differences are virtually identical to those obtained for HRP CpdI, where an energy gap of +0.1 kcal mol^{-1} was obtained using the same methods and basis sets. The group spin densities match those obtained for HRP CpdI closely, the only minor difference being a slightly higher absolute value on the axial ligand here.[5b] In P450 models also a significant axial ligand spin density was obtained due to mixing of the a_{2u} orbital with a σ_S orbital on the ligand.[4c] It appears that the phosphorus substituted structures have similar mixing patterns but to a somewhat lesser degree than thiolate ligated systems.

The optimized geometries of CpdI(1P) and CpdI(2P) are similar to those obtained for either HRP or P450 CpdI.[5,15] The Fe−O distances are closer to those obtained for P450 CpdI than those for HRP CpdI, where values of 1.651 (1.648) Å and 1.621

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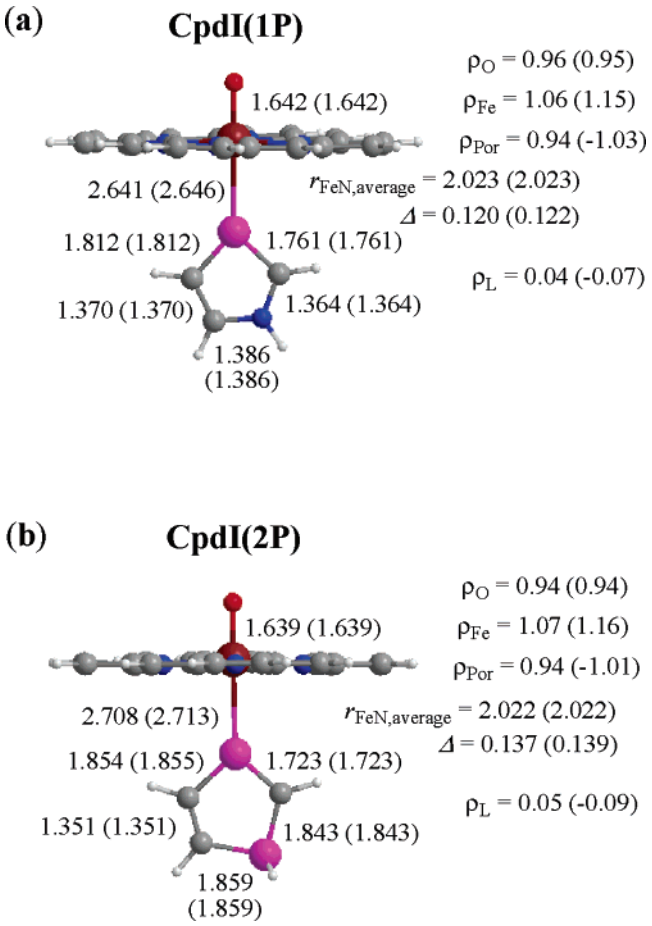

Figure 1. Optimized geometries and group spin densities (ρ) of (a) CpdI(1P) and (b) CpdI(2P). All bond lengths are in Ångstroms. Δ is the displacement of the iron atom with respect to the plane of the porphyrin.

(1.620) Å, respectively, for the quartet (doublet) spin states were found.[5b,16] Due to differences in hybridization between a nitrogen and phosphorus atom, the proton attached to the second phosphorus atom of the imidazole ring of CpdI(2P) is not in the plane of the imidazole ring.

The big difference between HRP CpdI and the phosphorus substituted CpdI species is the large displacement (Δ) of the iron atom with respect to the plane through the four nitrogen atoms. In HRP CpdI, the imidazole axial ligand interacts only weakly with the iron atom, hence the atom is almost in the plane of the porphyrin ring (Δ = 0.077−0.079 Å). However, the stronger push effect of the phosphorus atom lifts the iron atom much more out of the plane of the four nitrogen atoms by 0.137 (0.139) Å in the quartet (doublet) spin states of CpdI(2P). The displacement of the iron atom from the plane of the heme influences the molecular orbitals strongly. Thus, the singly occupied π^*_{xz} and π^*_{yz} orbitals in CpdI(1P) and CpdI(2P) can interact with a virtual e^*_g orbital on the nitrogen atoms of the heme as shown in Figure 2. This will lower the energy levels of the singly occupied π^* orbitals.

Since the a_{2u} orbital in the phosphorus substituted CpdI systems is not stabilized, the one-electron reduction of CpdI to form CpdII is similar in CpdI(1P) and HRP CpdI, i.e., 6.55 eV vs 6.44 eV. In principle, reduction of CpdI in all cases results in the creation of a triplet spin state with configuration $\pi^*_{xz}{}^1$ $\pi^*_{yz}{}^1$ $a_{2u}{}^2$. One-electron reduction of CpdI(2P), however, leads to dissociation of the axial ligand from the oxoiron heme system. The vertical electron affinity, however, is similar to the one obtained for CpdI(1P) and HRP CpdI.

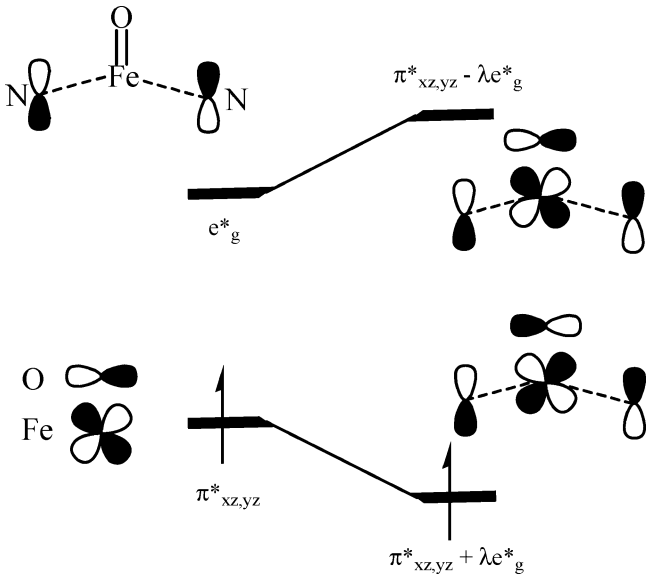

Figure 2. Orbital mixing between the singly occupied $\pi^*_{xz,yz}$ orbitals and the virtual e^*_g orbitals in CpdI(1P) and CpdI(2P).

Subsequently, we calculated the NBO populations for HRP CpdI, CpdI(1P) and CpdI(2P). The group charges on the Fe, O, porphyrin, and ligand groups are virtually identical for all systems studied (Supporting Information). However, close inspection of the charge on the atoms in the axial ligand position gives some dramatic differences. In particular, the axial nitrogen atom in HRP CpdI is negatively charged (−0.52), whereas the phosphorus atom in CpdI(1P) is positively charged (+0.48). This is a full charge reversal in the axial ligand position. Therefore, the electronic repulsion between the phosphorus and iron atoms will lengthen the Fe−P distance and lift the iron atom out of the plane through the heme nitrogen atoms. Therefore, the phosphorus substituted histidine ligand will entice a push effect on the oxoiron group, whereas a histidine group pulls electrons. This may also have strong consequences for the catalytic properties of the enzyme and may give a phosphorus substituted peroxidase mutant P450 type qualities.

In summary, bioengineered phosphorus substituted histidine axial ligands of oxoiron heme systems change the active center considerably with respect to peroxidases. Thus, the phosphorus atom induces a push effect on the oxoiron group, whereby the iron is lifted above the plane of the heme and the Fe−O bond is weakened. This bond weakening will make monoxygenase processes easier, and therefore this single-atom mutation may convert a peroxidase into a monoxygenase enzyme, but this requires further experimental as well as theoretical testing.

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Supporting Information Available: Group spin densities and charges of all structures described in this work (9 Tables) as well as detailed methodology and ref 14 in full. This material is available free of charge via the Internet at http://pubs.acs.org.

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