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Article

What External Perturbations Influence the Electronic Properties of Catalase Compound I?

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We have performed density functional theory calculations on an active-site model of catalase compound I and studied the responses of the catalytic center to external perturbations. Thus, in the gas phase, compound I has close-lying doublet and quartet spin states with three unpaired electrons: two residing in π^*_{FeO} orbitals and the third on the heme. The addition of a dielectric constant to the model changes the doublet–quartet energy ordering but keeps the same electronic configuration. By contrast, the addition of an external electric field along one of the principal axes of the system can change the doublet–quartet energy splitting by as much as 6 kcal mol^{-1} in favor of either the quartet or the doublet spin state. This sensitivity is much stronger than the effect obtained for iron heme models with thiolate or imidazole axial ligands. Moreover, an external electric field is able to change the electronic system from a heme-based radical [Fe═O(Por$^{\bullet+}$)OTyr$^-$] to a tyrosinate radical [Fe═O(Por)OTyr$^\bullet$]. This again shows that oxo–iron heme systems are chameleonic species that are influenced by external perturbations and change their character and catalytic properties depending on the local environment.

Introduction

Heme enzymes have many different functions in biosystems ranging from oxygen transport by hemoglobins to detoxification mechanisms and drug metabolism by the cytochromes P450.[1] Although all of these enzymes contain an iron heme active center, there are small differences in the active-site regions of these enzymes that establish the nature and catalytic properties of these enzymes. Catalases are important heme-type enzymes that detoxify hydrogen peroxide into water and molecular oxygen.[2] To this end, hydrogen peroxide binds to the resting state of the enzyme that contains an Fe$^{\text{III}}$ atom inside a heme group (porphyrin, Por). A protonation machinery subsequently converts the hydrogen peroxide−iron complex into an oxo−iron species (compound I, CpdI) and water (reaction 1). CpdI was characterized by electron paramagnetic resonance (EPR) and electron nuclear double resonance (ENDOR) techniques as a triradical system with the metal in oxidation state Fe$^{\text{IV}}$ and two unpaired electrons on the FeO moiety, which are ferromagnetically coupled to a third unpaired electron located on the heme (Por$^{\bullet+}$).[3] Moreover, these studies indicated that the axial ligand of iron heme systems fine-tunes the intrinsic properties of the catalyst and thereby influences the nature and catalytic properties of the enzyme.

$$\text{Por}-\text{Fe}^{\text{III}} + \text{H}_2\text{O}_2 \rightarrow \text{Por}-\text{Fe}^{\text{III}}-\text{H}_2\text{O}_2 \rightarrow \text{Por}^{\bullet+}\text{Fe}^{\text{IV}}{=}\text{O (CpdI)} + \text{H}_2\text{O} \quad (1)$$

$$\text{Por}^{\bullet+}\text{Fe}^{\text{IV}}{=}\text{O (CpdI)} + \text{H}_2\text{O}_2 \rightarrow \text{Por}-\text{Fe}^{\text{III}} + \text{H}_2\text{O} + \text{O}_2 \quad (2)$$

Catalase CpdI reacts with a second molecule of hydrogen peroxide to form another water molecule and molecular oxygen, whereby the system returns to the resting state and the catalytic cycle is completed (reaction 2). Catalase is one of the most thoroughly studied heme enzymes, and as a result, there are many crystal structures available with

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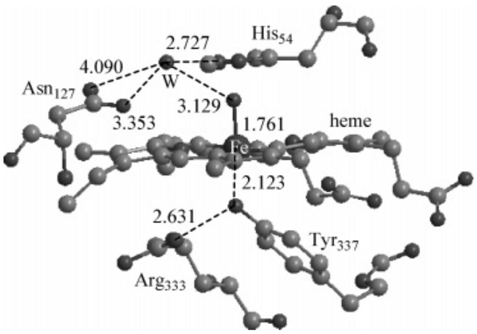
Chart 1. Structure of the Active Site of Catalase CpdI with Labels Taken from the 1MQF pdb[a]



[a] W is a water molecule. All bond lengths are in angstroms.[4]

characteristics as shown in Chart 1.[4] The active center contains an Fe atom embedded in a heme group that is bound to the protein backbone via a tyrosinate axial ligand (Tyr_{337}). The phenolate oxygen atom of Tyr_{337} forms H-bonding interactions with the two amine groups of a nearby arginine residue (Arg_{333}). On the distal side, several polar amino acid groups, such as His_{54} and Asn_{127}, and crystal water molecules (W) are involved in proton relay mechanisms as well as substrate and product binding processes.

One of the important factors that influence the catalytic properties of CpdI of heme enzymes is the nature of the axial ligand. Thus, it was realized that the axial ligand can induce a push or a pull effect on the oxo−iron moiety and thereby subtly change the electronic properties of the enzyme.[5] Further studies into this phenomenon on synthetic oxo−iron porphyrin systems indeed confirmed that the axial ligand can regulate the oxidative power of the catalyst because of electron-donating or -withdrawing properties.[6] In particular, the thiolate ligand in cytochrome P450 and chloroperoxidase (CPO) enzymes induces a push effect on the oxo−iron group that enhances the oxygenation properties of the catalyst.[5,7]

Oxo−iron heme complexes have been amply studied with theoretical methods.[8] In particular, Ryde and co-workers[9] showed the effect of the axial ligand on the catalytic cycle geometries of several heme enzymes. In the case of catalase CpdI, Green[10] identified the importance of the H-bonding interactions of the Arg_{333} side chain with the phenolate oxygen atom of the tyrosinate axial ligand because it moved charge and spin density away from the axial ligand onto the porphyrin group in agreement with the EPR and ENDOR studies mentioned above.[3] Yet other theoretical studies on the activity of catalase focused on formate binding to the active site.[11]

In previous work, we showed that the electronic properties of heme enzymes such as the doublet−quartet energy splitting and the ordering of the electronic states can be influenced by external perturbations such as H bonding or an applied electric field.[12] These types of effects can have a considerable impact on the activity and catalytic properties of the enzyme. For instance, benzene hydroxylation by a model of P450 CpdI was shown to proceed via a C−O bond activation step, leading to a cationic intermediate with low barriers on the doublet spin state surface but much higher barriers on the quartet spin state surface.[13] By contrast, the sulfoxidation reaction as catalyzed by the same system proceeds on a dominant quartet spin state surface.[14] Yet, alkenes, such as propene or styrene, react on competing quartet and doublet spin state surfaces with close-lying barriers and similar reaction mechanisms.[15] In a model for cytochrome *c* peroxidase (C*c*P) CpdI, an applied electric field shifted the charge and spin densities away from the tryptophan (Trp) onto the heme and the system essentially changed from $Fe{=}O(Por){-}Trp^{\bullet+}$ to $Fe{=}O(Por^{\bullet+}){-}Trp$.[12d] Generally, oxo−iron heme systems have close-lying quartet and doublet spin states and react with substrates via two-state reactivity patterns.[16] Therefore, understanding the mechanisms that influence the quartet−doublet energy splitting is important because a change in this splitting may lead to changes in the catalytic properties of the enzyme.

Recent experimental work showed that induced electric fields by proteins shift the vibrational frequencies (Stark shifts) and thereby influence protein function.[17] Thus, the polar and charged groups in proteins create electric fields that perturb the rest of the protein, thereby changing its inherent properties and function. These electrostatic effects

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were found to vary strongly between various proteins.[18] It should be noted that not only can electric fields influence protein and enzyme function but also magnetic fields can do this although there is debate going on about the magnitude of this effect.[19] Nevertheless, it is important to understand the way in which an active site of an enzyme reacts to local perturbations, such as a dielectric constant or an external electric field. To this end, we have investigated the electronic properties of catalase CpdI and its behavior under extreme conditions.

Thus, although many heme enzymes are very much alike, there are small differences that distinguish them from each other and that give each heme enzyme its own distinctive qualification. In particular, the active center of heme enzymes has been shown to be affected considerably to small external perturbations that consequently also affect the catalytic activity of the enzyme. As such, it is important to fully understand the environmental effects that influence the low-lying electronic states and the quartet−doublet energy splitting. In the past, we extensively studied these effects for models of cytochrome P450, horseradish peroxidase (HRP), and C*c*P enzyme models.[8c,12] A detailed analysis of this kind has never been performed on catalase CpdI, however; therefore, we believe it is timely to present the results of a thorough analysis of the ordering of the low-lying electronic states as a function of external perturbations in order to fully understand and appreciate the factors that stabilize the active site of catalase CpdI. As we will show here, similarly to other heme enzymes the quartet−doublet energy gap is indeed influenced by external perturbations as well as the charge and spin distributions. There are, however, critical differences between the heme enzymes that make catalase CpdI unique.

Methods

We use generally accepted methods, which we will briefly summarize here.[12d,20] As before, we use the unrestricted hybrid B3LYP density functional theory (DFT) method in combination with a double-ζ-quality LACVP basis set on the Fe atom and a 6-31G basis set on the rest of the atoms.[21] All structures were fully optimized (without constraints) using the *Jaguar 5.5* program package,[22] and an analytical frequency in *Gaussian-98* verified that the structures are indeed local minima.[23] To improve the energetics, we ran single-point calculations using the LACV3P+* basis set in *Jaguar*.[21c]

As a model for catalase CpdI, we used the 1MQF pdb[4] as a starting point, of which we selected the oxo−iron heme group and the phenol group of Tyr_{337} and replaced Arg_{333} by a methylguanidinium group. The side chains of the heme were replaced by H atoms because we showed before that these have little effect on the electronic properties of the active site.[24] Subsequently, we added H atoms to the system to get a model with stoichiometry $[\mathrm{C}_{28}\mathrm{H}_{25}\mathrm{FeN}_7\mathrm{O}_2]^+$. Previous work of Green and Ryde et al. showed that this is an appropriate model of catalase CpdI.[9,10] To identify the electronic ground state, we tested the system in the doublet, quartet, and sextet spin states.

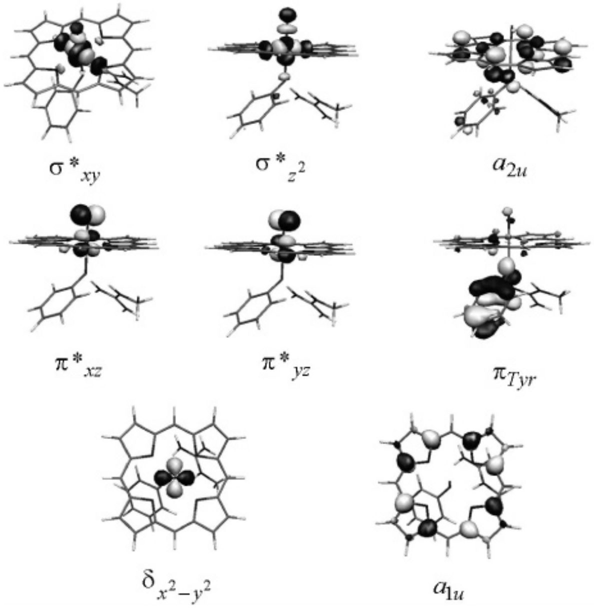

Figure 1. High-lying occupied and low-lying virtual orbitals of catalase CpdI.

We used several procedures to test the effect on the doublet−quartet energy splitting such as the addition of a dielectric constant to the system using the self-consistent reaction field model as implemented in *Jaguar 5.5*.[22] We used a large range of dielectric constants (ϵ) representing chlorobenzene ($\epsilon = 5.7$, $r_{\mathrm{P}} = 2.72$), 1,2-dichloroethane ($\epsilon = 10.65$, $r_{\mathrm{P}} = 2.51$), nitrobenzene ($\epsilon = 35.74$, $r_{\mathrm{P}} = 2.73$), and water ($\epsilon = 80.37$, $r_{\mathrm{P}} = 1.40$), whereby r_{P} is the probe radius. In addition, we investigated the effect of an applied external electric field on the energetics and spin densities of the lowest-lying electronic states using standard procedures in *Gaussian*.[23] A typical electric field induced by a dipole moment in a protein is on the order of 10 MV cm^{-1} and may lower the barriers of transition states by 2.3 kcal mol^{-1}.[17] In this work, we use a series of applied electric fields in the range of ±0.015 au (±77 MV cm^{-1}), which are well higher than typical protein values but should give the maximum boundaries of response of the enzyme to local electric fields.

Results

The oxo−iron species of heme enzymes has many close-lying molecular orbitals, and as a result, CpdI has many close-lying electronic states.[8c,25] Figure 1 shows the high-lying occupied and low-lying virtual orbitals of catalase CpdI. On the left-hand side, we show the five metal 3d orbitals, which split into the usual $\mathrm{t}_{2g}-\mathrm{e}_g$ set of orbitals. The lower

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Table 1. Low-Lying Electronic States with Relative Energies and Group Spin Densities (ρ) for Catalase CpdI[a,b]

label	orbital occupation	ΔE^c	ρ_{Fe}	ρ_O	ρ_{Por}	ρ_{Tyr}	ρ_{Arg}
$^4A_{2u}$	$\delta^2\pi^*{}_{xz}{}^1\pi^*{}_{yz}{}^1a_{1u}{}^2a_{2u}{}^1$	0.0	1.01	1.02	0.74	0.23	0.00
$^2A_{2u}$	$\delta^2\pi^*{}_{xz}{}^1\pi^*{}_{yz}{}^1a_{1u}{}^2a_{2u}{}^1$	−0.3	1.10	0.99	−0.85	−0.24	0.00
$^4A_{1u}$	$\delta^2\pi^*{}_{xz}{}^1\pi^*{}_{yz}{}^1a_{1u}{}^1a_{2u}{}^2$	3.1	1.06	1.01	0.93	0.00	0.00
$^2A_{1u}$	$\delta^2\pi^*{}_{xz}{}^1\pi^*{}_{yz}{}^1a_{1u}{}^1a_{2u}{}^2$	4.1	1.05	1.02	−1.03	−0.04	0.00
$^6A_{2u}$	$\delta^1\pi^*{}_{xz}{}^1\pi^*{}_{yz}{}^1\sigma^*{}_{xy}{}^1a_{1u}{}^2a_{2u}{}^1$	11.5	3.02	0.72	1.19	0.07	0.00

[a] Relative energies in kcal mol^{-1} with respect to the $^4A_{2u}$ state. [b] Energies obtained from UB3LYP/LACV3P+* single points on UB3LYP/LACVP-optimized structures. [c] The reference energy is −1739.406 46 au.

three of those split further into a one-below-two (δ, π^*) subset because the $\delta_{x^2-y^2}$ orbital is a nonbonding orbital that cannot form overlap with the orbitals on the heme ligand. The π^* orbitals (π^*_{xz}, π^*_{yz}) reflect the antibonding interactions of the $3d_{xz,yz}$ orbitals on Fe with the $2p_{y,z}$ orbitals on O along the Fe−O bond. The e_g set of orbitals are σ^*_{xy} for the antibonding interactions between the metal and the pyrrole N atoms and $\sigma^*_{z^2}$ for the antibonding interactions along the O−Fe−O axis. These five orbitals are essentially similar in shape to those obtained before with other heme enzymes with either thiolate, chloride, or imidazole as axial ligands.[8,10,12b,20a] The system also has two high-lying heme orbitals, which in D_{4h} symmetry have the labels a_{1u} and a_{2u}, and a high-lying π orbital on the tyrosinate ligand (π_{Tyr}).

The complete set of orbitals shown in Figure 1 is occupied by nine electrons: we computed the lowest-lying electronic states in various overall spin states (see Table 1). All states are labeled according to the heme orbital that is singly occupied, and the superscript next to the label identifies the overall spin state. In all states, the metal is in oxidation state Fe^{IV} and the π_{Tyr} orbital is doubly occupied. All attempts to create an electronic state with π_{Tyr} singly occupied failed, and the calculation converged to one of the lower lying states in Table 1. Analogously to other heme enzymes such as P450 and HRP,[12b,25,26] the lowest-lying electronic state has $\delta^2\pi^*{}_{xz}{}^1\pi^*{}_{yz}{}^1a_{1u}{}^2a_{2u}{}^1$ occupation. The a_{2u} orbital can be ferromagnetically or antiferromagnetically coupled to the two π^* orbitals in either an overall quartet or doublet spin state ($^{4,2}A_{2u}$). These two states are virtually degenerate in the gas phase, with a small preference of $^2A_{2u}$ over $^4A_{2u}$. Because of single occupation of the π^*_{xz}, π^*_{yz}, and a_{2u} molecular orbitals, there is a spin density of around 2 on the oxo−iron group. The rest is distributed over the Por (0.74 in $^4A_{2u}$ and −0.85 in $^2A_{2u}$) and the tyrosinate ligand (0.23 in $^4A_{2u}$ and −0.24 in $^2A_{2u}$). Our calculated spin densities are in good agreement with the ones obtained by Green,[10] who found spin densities of $\rho_{Por} = 0.83$ and $\rho_{Tyr} = 0.21$, although Ryde et al.[9] found somewhat more spin density on the axial ligand. Nevertheless, our results are in line with resonance Raman spectroscopy on catalase CpdI, which also indicated spin delocalization toward the axial ligand.[27]

In addition, we checked the possibilities of having the a_{1u} orbital singly occupied rather than a_{2u} but found these states to be at least 3.1 kcal mol^{-1} higher in energy. This matches the state ordering obtained for HRP CpdI, where we found similar excitation energies.[12b] The a_{2u} orbital is known to mix with orbitals located on the axial ligand.[8c] Thus, the lone-pair orbital on the thiolate ligand in P450 CpdI mixes strongly with the a_{2u} orbital and raises it in energy. Because, in the P450s, the a_{1u} orbital does not mix with other orbitals, the energy gap between the $^4A_{2u}$ and $^4A_{1u}$ states is quite large (21.3 kcal mol^{-1}),[25] whereas a_{2u} and a_{1u} are degenerate in an isolated Por group.[28] In catalase CpdI (Figure 1), there appears to be a little bit of mixing of the a_{2u} orbital with the π_{Tyr} orbital, and as a result, a significant amount of spin density has accumulated on the axial ligand. However, this mixing is not large enough to destabilize the a_{2u} orbital over the a_{1u} orbital considerably; hence, the $^4A_{2u}/^4A_{1u}$ energy gap is virtually the same as the one obtained for HRP CpdI.[12b] We also tested the sextet spin state, but because of the excitation of an electron from the nonbonding (δ) orbital to an antibonding (σ^*_{xy}) orbital, this state is considerably higher in energy than the quartet and doublet spin states.

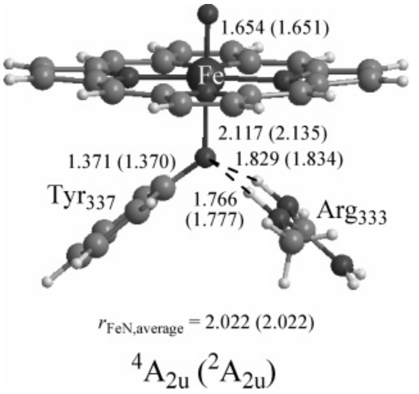

Figure 2. Optimized geometries of catalase CpdI in the $^4A_{2u}$ ($^2A_{2u}$) states as obtained with UB3LYP/LACVP in *Jaguar 5.5*. All bond lengths are in angstroms.

The optimized geometries of catalase CpdI in the $^4A_{2u}$ and $^2A_{2u}$ states are shown in Figure 2. The Fe−O bond is short (1.654 Å in $^4A_{2u}$ and 1.651 Å in $^2A_{2u}$), typical for a double bond. These bonds are somewhat shorter than the experimentally obtained value[4] of 1.761 Å but match the experimental data of 1.62−1.7 Å for HRP CpdI excellently.[29] The long Fe−O distance found in the pdb of catalase CpdI presumably indicates that a proton is bound to the oxo group because usually distances of this order of magnitude are obtained for Fe−OH bonds rather than for Fe═O bonds.[30] Models on catalase CpdI predicted Fe−O distances of 1.622 (1.620) Å and Fe−O_{Tyr} distances of 2.175 (2.300) Å for the quartet (doublet) spin states.[9] These data are in reasonable agreement with our optimized geometry. Earlier calculations

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Table 2. Relative Energies of the $^{4,2}A_{2u}$ and $^{4,2}A_{1u}$ States under the Influence of a Dielectric Constant (ϵ) of Various Strengths[a]

label	$\Delta E_{\epsilon=1}$[b]	$\Delta E_{\epsilon=5.7}$[c]	$\Delta E_{\epsilon=10.65}$[c]	$\Delta E_{\epsilon=35.74}$[c]	$\Delta E_{\epsilon=80.37}$[c]
$^4A_{2u}$	0.0	0.0	0.0	0.0	0.0
$^2A_{2u}$	−0.3	0.2	0.1	−0.1	−0.2
$^4A_{1u}$	3.1	6.1	6.0	5.7	5.3
$^2A_{1u}$	4.1	7.0	6.9	7.3	7.1

[a] Relative energies in kcal mol^{-1} with respect to the $^4A_{2u}$ state. [b] Energies obtained from UB3LYP/LACV3P+* single points on UB3LYP/LACVP-optimized structures. [c] Calculated at the UB3LYP/LACVP level of theory.

of us and others on oxo−iron Por systems gave similar Fe−O distances of around 1.64−1.65 Å for P450,[24,25,31] 1.62−1.64 Å for HRP models,[12b,26c] and 1.657−1.663 Å for C*c*P models.[12d,32] Our calculated Fe−O_{Tyr} and O_{Tyr}−N_{Arg} distances of 2.117 (2.135) and 2.719 (2.728) Å, respectively, for the quartet (doublet)-optimized geometries match the 1MQF pdb distances of Chart 1 perfectly. Therefore, our model correctly reproduces electronic as well as geometric characteristics of catalase CpdI.

Although catalase CpdI has a nonplanar heme (Chart 1), a full optimization of the model converted to an almost planar structure. In oxo−iron heme models of peroxidases ruffling of the Por ring was shown to stabilize the system by about 2 kcal mol^{-1}.[33] To test the effect of a nonplanar heme on the quartet−doublet energy gap (ΔE_{QD}), we ran single-point calculations with the distorted heme group as taken from the 1MQF pdb inserted into the optimized geometry of the $^4A_{2u}$ structure described above. In contrast to peroxidase models,[33] ruffling of the heme raises the energy strongly, whereas the group spin densities and quartet−doublet energy gap, on the other hand, undergo only minor changes. Therefore, the nonplanarity of catalase CpdI is not an intrinsic property of the oxo−iron heme system but more likely the result of electrostatic interactions of the heme with neighboring groups in the enzyme active site.

Because the proximal site of catalase CpdI contains many polar residues, we also ran a series of test calculations on the effects originating from this site. Thus, we added a H_3O^+ molecule hydrogen bonded toward the oxo group at a distance of 2.0 Å and ran single-point calculations. As expected, a hydrogen-bonded H_3O^+ molecule polarizes the group spin densities on the oxo−iron group toward the Fe atom strongly ($\rho_{Fe} = 1.32$; $\rho_O = 0.74$). At the same time, the tyrosinate ligand gains radical character ($\rho_{Tyr} = 0.33$), while it is reduced on the Por ring ($\rho_{Por} = 0.60$). Nevertheless, the doublet−quartet energy gap is similar to the one obtained in the gas phase without H_3O^+ ($\Delta E_{QD} = -0.6$ kcal mol^{-1} for ^{4,2}CpdI·H_3O^+ using the LACV3P+* basis set).

Subsequently, we investigated the effect of a dielectric constant on the ordering and relative energies of the $^{4,2}A_{2u}$ and $^{4,2}A_{1u}$ states (Table 2). A dielectric constant of either $\epsilon = 5.7$ or 10.65 reverses the ordering of $^4A_{2u}$ and $^2A_{2u}$ and makes the quartet spin state the ground state, although by a small margin. EPR and ENDOR studies determined ferromagnetic coupling of the two π^* orbitals with a_{2u}, which supports a $^4A_{2u}$ ground state. A larger dielectric constant ($\epsilon = 35.74$ or 80.37) only has a minor effect on the relative energies of these four states and makes the doublet spin state the ground state again, although by a very small margin. The separation between the A_{2u} and A_{1u} states stays significant and is well higher in a dielectric constant; therefore it is not expected that the A_{1u} states will play a role of importance in catalase CpdI. To illustrate the effect of a dielectric constant on the group spin densities, we show in Figure 3 the group spin densities in $\epsilon = 5.7$ and 10.65. As can be seen, the spin densities on the oxo−iron group are slightly more polarized toward the Fe in a dielectric constant, while in the gas phase, the spin density is equally distributed over the oxo−iron group. No changes in the spin densities on the heme and tyrosinate ligands are obtained in a dielectric constant with respect to the gas-phase results. With larger values of a dielectric constant, virtually the same group spin densities and charges are obtained; therefore, it seems that the magnitude of the dielectric constant does not have much effect on the charge and spin densities.

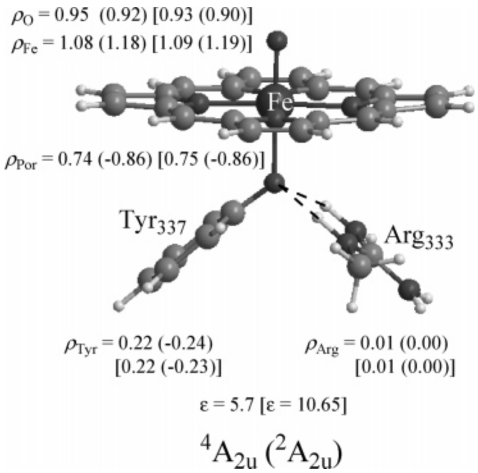

Figure 3. Group spin densities of the $^4A_{2u}$ ($^2A_{2u}$) states in $\epsilon = 5.7$ and 10.65. Values in square brackets refer to $\epsilon = 10.65$.

Thereafter, we investigated the effect of an applied electric field on the doublet−quartet energy gap and the charge and spin distributions of the system. In previous work, we showed that with an oxo−iron heme model with a thiolate axial ligand, an external electric field can change the system from a charge-separated $Por^{\bullet+}S^-$ system into a $PorS^\bullet$ situation.[12c] Similarly, in C*c*P CpdI, an applied electric field changes the system from a Trp radical to a Por radical or vice versa.[12d] Figure 4 shows the energy gap between the $^4A_{2u}$ and $^2A_{2u}$ states of catalase CpdI as a function of an applied electric field. As can be seen, changes in the $^4A_{2u}$/$^2A_{2u}$ gap of up to almost 6 kcal mol^{-1} are obtained with an applied electric field along the *y* direction. Moreover, it does change not only the relative energy between the quartet and doublet spin states but also the spin state ordering. Therefore, the active species of catalase CpdI is extremely sensitive to external perturbations, and an induced electric field of the enzyme environment should be able to modulate the quartet−doublet energy gap. In C*c*P, an applied electric field kept the quartet−doublet

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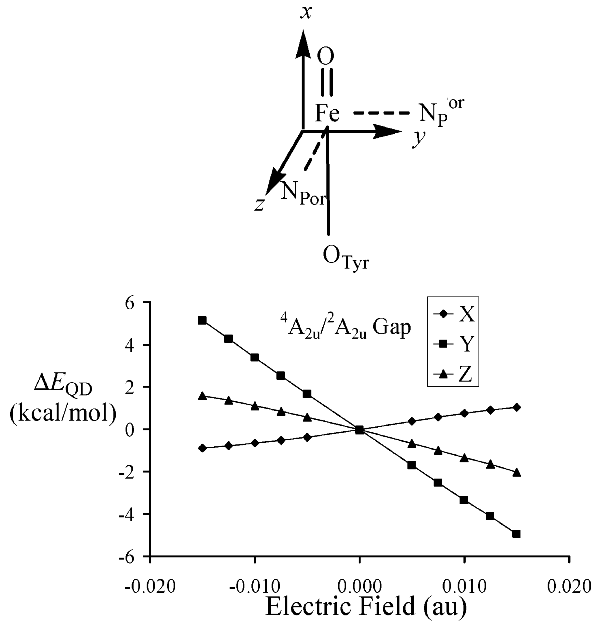


Figure 4. Quartet−doublet energy gap (ΔE_{QD} in kcal mol^{-1}) between the $^4A_{2u}$ and $^2A_{2u}$ states as a function of an applied electric field along one of the principal axes of the system as defined on the top. A negative value means a $^4A_{2u}$ ground state.

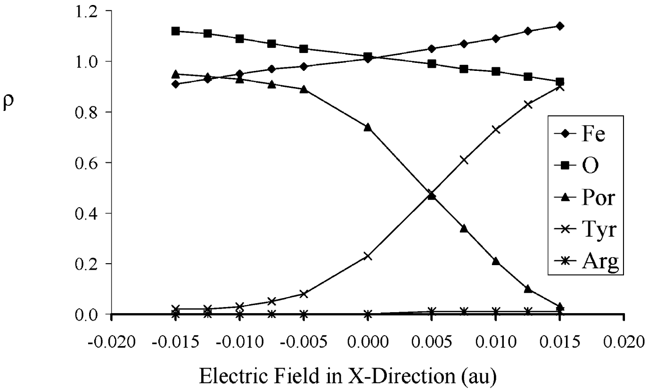


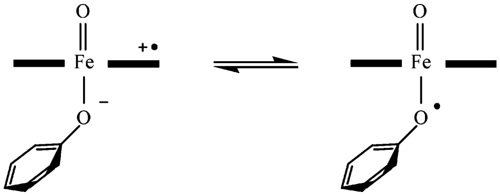
Figure 5. Group spin densities in the $^4A_{2u}$ state under the influence of an applied external electric field along the *x* axis.

energy gap within ±0.5 kcal mol^{-1} even when the strength and direction of the field was changed.

In Figure 5, we present the effect of an applied external electric field along the *x* axis on the group spin densities of the $^4A_{2u}$ state of catalase CpdI. Because of single occupation of the π^*_{xz} and π^*_{yz} orbitals, there is a spin density of 2 on the FeO moiety. In the gas phase, the spin density is equally distributed over both atoms of the FeO moiety ($\rho_{Fe} = 1.01$; $\rho_{O} = 1.02$), but an external electric field effect changes the charge and spin polarization of the oxo−iron group. Thus, with an applied electric field of 0.015 au along the Fe−O bond, the spin densities change to $\rho_{Fe} = 1.14$ and $\rho_{O} = 0.92$, whereas with a field in the opposite direction, the values are $\rho_{Fe} = 0.91$ and $\rho_{O} = 1.12$. Nevertheless, the sum of the spin densities on the oxo−iron group stays virtually the same regardless of the direction and magnitude of the applied electric field.

By contrast, the spin densities on the Por and tyrosinate residues change considerably. In the gas phase, most of the spin density for single occupation of the a_{2u} orbital is on the heme ligand ($\rho_{Por} = 0.74$), with a small contribution on the tyrosinate ligand ($\rho_{Tyr} = 0.23$). However, with an electric field in the positive *x* direction, the system changes to a tyrosinate radical, whereas in the opposite direction, the system retains the heme radical situation (Scheme 1). Thus, the system can exist in a charge-separated situation with a cation radical on the heme and an anionic axial ligand or have a neutral heme and a radical axial ligand. These two valence bond (VB) states mix, and external perturbations determine the percentage of each of these VB forms in the system. In particular, the mixing depends on the ionization energy of the axial ligand, the electron affinity (EA) of CpdI, and the difference in the electrostatic interaction energy.[12b] Furthermore, a spin density of $\rho = 1$ on the Por ligand suggests a dominant charge-separated species, i.e., Fe=O-(Por$^{\bullet+}$)OTyr$^-$, whereas a spin density of $\rho = 1$ on the tyrosinate ligand implies an Fe=O(Por)OTyr$^\bullet$ situation. Thus, in the gas phase, the dominant form is the charge-transfer structure Fe=O(Por$^{\bullet+}$)OTyr$^-$ due to $\rho_{Por} = 0.74$ and $\rho_{Tyr} = 0.23$. However, applied electric fields can completely reverse the spin densities and create systems that are either purely Fe=O(Por$^{\bullet+}$)OTyr$^-$ or Fe=O(Por)OTyr$^\bullet$.

Scheme 1. Mixing of Two VB Structures of Catalase CpdI



Our attempts to create an electronic state ($^4\Pi_{Tyr}$) in the gas phase with $\delta^2\pi^*_{xz}{}^1\pi^*_{yz}{}^1a_{1u}{}^2a_{2u}{}^2\pi_{Tyr}{}^1$ occupation failed and always converged back to the more stable $\delta^2\pi^*_{xz}{}^1\pi^*_{yz}{}^1a_{1u}{}^2a_{2u}{}^1$ solution. Therefore, the energy gap between the $^4A_{2u}$ and $^4\Pi_{Tyr}$ states should be considerable and at least 20 kcal mol^{-1} in energy. Note as well that the spin densities on the Arg residue are minimal and stay negligible during all experiments. The spin densities with an applied electric field along the *y* and *z* axes follow trends similar to the one depicted in Figure 5 (see the Supporting Information). Although the Por is in the *yz* plane of symmetry, the tyrosinate ligand, by contrast, is not located in either *xy*, *xz*, or *yz* planes but under an angle of each of these. Therefore, a perturbation along one of the three principal axes will influence the charge and spin densities on the tyrosinate ligand and consequently also on the Por group.

Discussion

In this work, we showed the results on environmental (external) effects that influence the doublet−quartet energy splitting of a model of catalase CpdI. Although in the gas phase, there is a small preference of the $^2A_{2u}$ state over the $^4A_{2u}$ state, in a dielectric constant of either $\epsilon = 5.7$ or 10.65 the quartet spin state is lower. This is in agreement with EPR and ENDOR studies, which found a quartet spin ground state for catalase CpdI.[3]

To verify the effect of an oxygen bound axial ligand on the quartet−doublet energy splitting, we present in Table 3

Table 3. Doublet−Quartet Energy Splitting (ΔE_{QD}) and the EA of Cpd I for Several Fe═O(Por)L Heme Systems with Various Ligands L

L	ΔE_{QD} [a,b] (kcal mol^{-1})	EA(CpdI)[b] (eV)
OH$^-$	0.98	3.30
OSer$^-$	0.19	3.40
ImH[c]	0.01	6.41[e]
SH$^-$	−0.19	3.06[f]
OTyr$^-$ [d]	−0.30	6.12

[a] A positive value denotes a quartet spin ground state. [b] Energies obtained at the LACV3P+* level of theory with zero-point-energy corrections at the LACVP level. [c] Model of C*c*P Cpd I from ref 12d. [d] Model of catalase Cpd I from this work. [e] From ref 12b. [f] From ref 7b.

the quartet−doublet energy splitting (ΔE_{QD}) of a series of oxo−iron Por systems Fe═O(Por)L with oxygen-based axial ligands L (L = OH$^-$, OSer$^-$, and OTyr$^-$) and non-oxygen-based axial ligands L (L = SH$^-$, ImH). These ligands mimic the active sites of CpdI of P450 (SH$^-$), HRP (ImH), and catalase (OTyr$^-$), whereby ImH represents an imidazole group. In addition, we also calculated other hypothetical oxygen-bound axial ligands, such as the minimal system OH$^-$ and a deprotonated serine amino acid (OSer$^-$). As can be seen from Table 3, the axial ligand distinctly influences the quartet−doublet energy splitting in such a way that a strong electron-donating ligand like OH$^-$ stabilizes the quartet over the doublet spin state by almost 1 kcal mol^{-1}, while a tyrosinate ligand as in catalase seems to stabilize the low spin stronger. Thus, it was shown that the quartet−doublet energy gap correlates to the spin-coupling constant *J*, which has a positive value for catalase CpdI, is neutral in HRP and negative in P450.[10,26] These trends were assigned to differences in the axial ligands.[10] However, here we find no correlation between ΔE_{QD} and the EA of CpdI (Table 3). In principle, the quartet−doublet energy gap is dependent on the amount of mixing and therefore exchange stabilization of the a_{2u} molecular orbital with the two π^* orbitals. With the Fe atom in the plane of the Por ring, this mixing will be negligible and the doublet and quartet spin states are degenerate. If the mixing is favorable, it will increase the exchange stabilization and lower the quartet over the doublet spin state considerably.

Also shown in Table 3 are the EAs of the various CpdI species, which is the energy difference between CpdI and CpdII, the one-electron-reduced form of CpdI. Generally, the electron addition to CpdI leads to a triplet spin state with orbital occupation $\delta_{x^2-y^2}{}^2\pi^*_{xz}{}^1\pi^*_{yz}{}^1a_{2u}{}^2$; i.e., the a_{2u} molecular orbital accepts one electron. Because the a_{2u} orbital in Fe═O(Por)SH mixes strongly with a lone-pair orbital on the thiolate ligand, this results in a small EA of 3.06 eV because of the push effect of the thiolate ligand.[7b] Axial ligands, such as ImH and tyrosinate, are much weaker ligands that show much lesser mixing of the a_{2u} orbital with other high-lying orbitals. As a result, the energy to accept an electron into the a_{2u} orbital is much higher and, hence, the EA is larger. Table 3 shows also the EA values for systems with an OH$^-$ or serinate axial ligand. These ligands apparently also induce a push effect on the metal and give low EAs of 3.40−3.44 eV. Nevertheless, the EA of the system with thiolate as the axial ligand is by far the lowest, so it is not surprising that nature has chosen a thiolate ligand for a catalyst that can oxygenate substrates. The reason that no enzymes with a serinate axial ligand exist is probably because it requires too much energy to deprotonate serine, while this is much more thermodynamically favorable for a cysteine residue.

Conclusions

DFT calculations on a model of catalase CpdI show that the active center is extremely sensitive to external perturbations. In particular, a dielectric constant or an applied external electric field can change the ordering and energy differences of the quartet and doublet spin states. If the reactions catalyzed by CpdI in the quartet spin state differ distinctly from the ones in the doublet spin state, this effect can have serious consequences on the activity of the enzyme.

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Supporting Information Available: Twelve tables with relative energies, group spin densities, and charges of various CpdI structures under different environmental conditions. Also provided are nine figures with effects of an electric field on energies and group spin densities. This material is available free of charge via the Internet at http://pubs.acs.org.

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