The Structure and Spin Coupling of Catalase Compound I: A Study of Noncovalent Effects

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Catalase compound I contains an iron(lV)-oxo porphyrin-radical; in this species, an $S = 1$ iron-oxo unit and an $S = {}^1/_2$ radical, located on the porphyrin ring, couple to give doublet and quartet states that are mixed by the zero-field splitting of the ferryl moiety. The result is a Kramer's doublet ground state with a distinctive EPR signature.[1]

In bovine liver catalase (BLC). this signal decays with time, and a new signal corresponding to a tyrosine radical appears.[2] The formation of this tyrosine radical may be linked to the propensity of some catalases to bind NADPH.[3] Bound NADPH prevents the accumulation of compound II**,** an inactive ferryl form of the enzyme.[4] Experiments suggest that NADPH does this not by reducing compound I or compound II directly but by reacting with a species termed compound II*. This intermediate, a precursor to compound II, is formed when the porphyrin radical of compound I moves to a protein residue, presumably a tyrosine. Although it is not known which of the 20 possible BLC tyrosine residues is oxidized during the formation of compound II*,[5,6] our previous work would seem to indicate the proximal ligand.[7]

We have suggested that donating axial-ligands (thiolate or imidazolate) can alter the electronic structure of compound I. Density functional calculations reveal that in these systems it is the axial ligand, not the porphyrin, that is preferentially oxidized to generate the radical species. In light of these results, one might expect that it is the donating tyrosinate ligand of BLC that is oxidized during the formation of the BLC Tyr• species. EPR investigations, however, reveal only weak dipolar interactions between the BLC ferryl moiety and tyrosine radical, indicating the proximal ligand is not oxidized.[8]

Why might the tyrosinate ligand of catalase be different from the other donating ligands we have examined? Perhaps the answer lies in the proximal charge-relay system, which has recently been proposed to tune the metal site and stabilize compound I.[6] This charge-relay network contains an arginine that forms two hydrogen bonds to the proximal ligand. Our work on imidazole- and imidazolate-ligated intermediates suggests hydrogen bonds can modify the active-site electronic structure, changing the location of the radical species. Given these results, it seems that the catalase charge-relay system could increase the potential of the proximal tyrosinate ligand, forcing oxidation at the porphyrin ring in compound I and distant tyrosine residues in compound II*.

To gain insight into the radical species of BLC and to examine the function of the proximal charge-relay system, we have performed density functional calculations on active-site models of perferryl catalase. Our results are striking: the charge-relay system not only determines the location of the radical species, it also determines the structure of the compound I intermediate. Using GAUSSIAN94,[9] unrestricted calculations were initially performed on two different active-site models of catalase compound I. In the first model, the tyrosinate axial ligand was replaced with a phenolate, and a porphine was substituted for the protoporphyrin unit, yielding the $Fe(N_4C_{20}H_{12})(OC_6H_5)O$ 50-atom species. In the second model, the proximal arginine was included in the form of a methylguanidinium ($C_2N_3H_8^+$), resulting in a 63-atom complex. The geometry of each spin state was optimized at the B3LYP/6-311G level,[9,10] and exchange couplings *(J* values) were obtained from a Heisenberg Hamiltonian ($\mathscr{H} = -JS_1 \cdot S_2$) using the broken symmetry method of Noodleman.[11]

Our results are summarized in Table 1. In agreement with our previous work, we find the donating phenolate ligand is oxidized during compound I formation. Over 90% of the radical's spin density resides on the proximal ligand. Oxidation of the phenolate ligand results in a rotation of the phenyl ring about the C−O bond. This rotation, which is not observed in catalase,[12] increases the π interaction between the ferryl unit and the ligand radical. As a result, the phenolate-ligated intermediate displays strong antiferromagnetic coupling *(*$J = -90$ cm^{-1}), in contrast to the ferromagnetic coupling observed for catalase compound I *(*$J \approx 10$ cm^{-1})[13] and the weak dipolar coupling found for the BLC Tyr• species.[8]

The natural magnetic orbitals (NMOs)[14] for the phenolate-ligated complex are shown in Figure 1. One electron resides in each of the FeO π* orbitals (center and right). These electrons couple ferromagnetically, giving the well-characterized $S = 1$ iron-oxo unit. The other natural magnetic orbital corresponds to the radical center. That antiferromagnetic coupling is a consequence of this ligand-based radical is apparent from the NMOs. The phenolate-based radical has a nonzero overlap integral with one member of the FeO π* set. This bonding interaction favors spin pairing, producing the $S = {}^1/_2$ ground state.

Calculations that include the proximal arginine, in the form of a methylguanidinium, show a dramatic change in the location of the radical, the nature of the electronic coupling, and the structure of the intermediate. Oxidation now occurs at the porphyrin (Figure 2). In agreement with experiment, the porphyrin-based radical couples ferromagnetically to the $S = 1$ ferryl moiety ($J = 50$ cm^{-1}), and the phenyl ring shows no rotation about the C−O bond. We also find ≈20% of the radical's spin density on the

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(14) Natural magnetic orbitals are obtained by taking linear combinations of the coupled natural orbitals. Natural orbitals (NOs) are obtained by diagonalizing the SCF density matrix. The manipulations are identical to those performed in ref 7. (See references therein.) In this case, the three "singly" occupied NOs have occupation numbers of 1.18, 0.82, 1.00.

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Table 1. Ground State, Coupling Constant, Bond Distances, and NMO (SCF) Spin Densities of Phenolate-Ligated Compound I Intermediates

			distance[b]			spin density[a,c]			average spin density[a,c]			
proximal interaction	ground state	J cm^{-1}	Fe−L	Fe−O	Fe−N_{av}	L	Por	FeO	N	C_m	C_α	C_β
none	$S = {}^1/_2$	−90	2.17	1.64	2.02	−0.92	−0.02	1.94	0.001	−0.005	0.000	0.000
						(−0.93)	(−0.13)	(2.06)	(−0.016)	(−0.014)	(−0.001)	(−0.001)
$CF_2(NFH)_2$	$S = {}^3/_2$	22	2.17	1.64	2.02	(0.43)	(0.49)	(2.08)	(0.089)	(0.187)	(−0.067)	(−0.004)
K^+	$S = {}^3/_2$	34	2.12	1.65	2.02	(0.39)	(0.53)	(2.08)	(0.098)	(0.199)	(−0.073)	(−0.005)
methylguanidinium	$S = {}^3/_2$	50	2.12	1.65	2.02	0.21	0.83	1.96	0.072	0.120	0.003	0.003
						(0.22)	(0.70)	(2.08)	(0.124)	(0.255)	(−0.091)	(−0.005)

[a] NMO (SCF) spin densities. [b] K^+ structure not optimized. The K^+ was simply positioned at the coordinates of the central carbon of the methylguanidinium, for both the doublet and quartet states. The 3-21G basis was used for K. [c] Natural orbital analysis was not performed on K and $CF_2(NFH)_2$ systems.

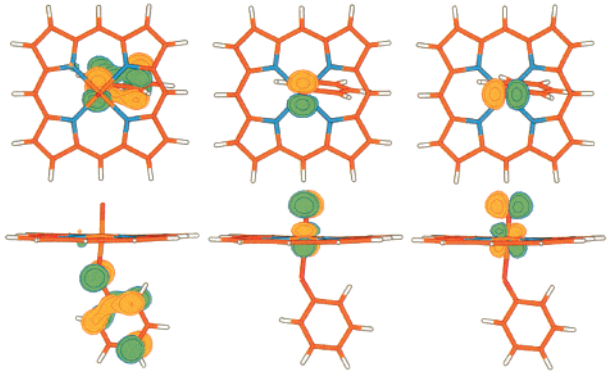

Figure 1. Natural magnetic orbitals of the $S = 1/2$ phenolate compound I intermediate. Top and side views are shown at a contour value of 0.05.

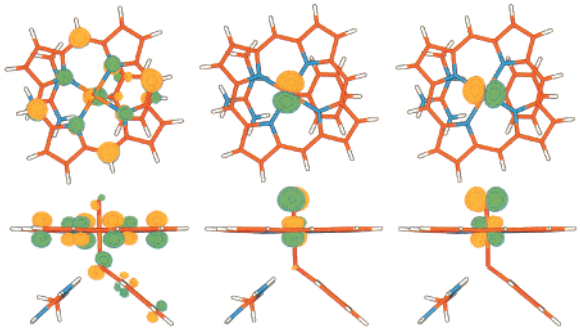

Figure 2. Natural orbitals of the $S = {}^3/_2$ methylguanidinium-bound phenolate compound I with occupation numbers of 1.0. Top and side views are shown at a contour value of 0.05.

phenolate ligand, in agreement with resonance Raman experiments that suggest some radical density on the proximal tyrosine.[15]

Calculations were also performed in which the methylguanidinium cation was replaced with (1) a K^+ and (2) a neutral $CF_2(NFH)_2$. This was done to separate the effects of cationic charge from those of hydrogen bonding. The results, shown in Table 1, indicate that H-bonding and the cationic charge have similar effects. Both of them shift a majority of the radical character to the porphyrin but leave a significant amount of spin density on the axial ligand. Neither the K^+ nor the $CF_2(NFH)_2$ has the full effect of the methylguanidinium.

Figure 3 displays experimental and computational J values.[7b] Notice that chloroperoxidase (CPO-I) is the only experimental

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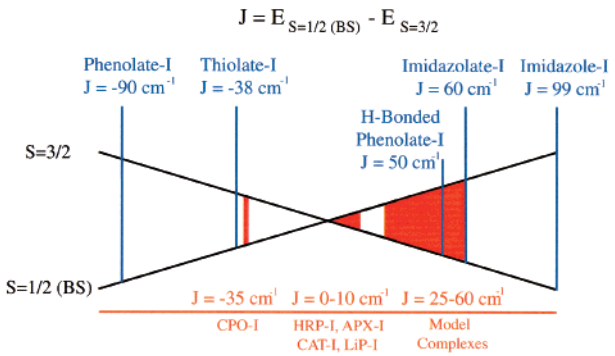

Figure 3. Experimental (red) and computational (blue) J values. The computational J values were obtained by optimizing each spin state at the B3LYP/6-311G level.

system that exhibits antiferromagnetic coupling. We have explained this antiferromagnetic coupling in terms of an axial-ligand based radical.[7a] Our findings for the phenolate-ligated system support this explanation. As noncovalent interactions shift the radical from the ligand to the porphyrin, the sign of the coupling is reversed.

Although the calculated ferromagnetic couplings are too large, the correct trends are observed. The protein mimics (the imidazolate- and H-bonded phenolate-ligated species) have weaker ferromagnetic couplings than the model for synthetic systems (the imidazole-ligated species).[7b] The weaker ferromagnetic couplings obtained for the protein mimics are a result of increased axial ligand spin density, relative to that of the imidazole-ligated species.

In conclusion. our calculations suggest that the tyrosinate ligand is oxidized only in the absence of the proximal charge-relay. As a result, it seems unlikely that the BLC tyrosine radical is ligand-based. The absence of interactions with the charge-relay results in a rotation of the phenyl ring and strong antiferromagnetic coupling, neither of which is observed experimentally. The inclusion of the proximal arginine, in the form of a methylguanidinium, shifts the radical from the ligand to the porphyrin, resulting in ferromagnetic coupling. In agreement with experiment, no rotation of the phenyl ring is observed. Thus, it appears the charge-relay raises the potential of the axial ligand, forcing oxidation at the porphyrin ring, and, in doing so, determines the structure of compound I.

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