STUDIA UNIVERSITATIS BABEŞ-BOLYAI, CHEMIA, L, 1, 2005

DISCONTINUUM BETWEEN FERROUS-SUPEROXO AND FERRIC-PEROXO IN HEME $[FEO_2]^9$ COMPLEXES?

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ABSTRACT. Low-spin $[FeO_2]^9$ complexes (where the superscript denotes the sum of iron d electrons and dioxygen π^* electrons), generally described as ferric-peroxo or ferrous-superoxo, have been implicated in the catalytic cycles of heme and non-heme proteins. Experimental observation of these complexes, mainly by ENDOR spectroscopy, has led to two distinct descriptions of heme $[FeO_2]^9$, depending on whether or not hydrogen bonding to the oxygen ligand is available. Hydrogen-bonded $[FeO_2]^9$ were described as ferric-peroxo, while non hydrogen-bonded $[FeO_2]^9$ were described as ferrous-superoxo. Reported here is a DFT investigation of the effects of hydrogen bonding on the structure of heme $[FeO_2]^9$ complexes. Calculated bond lengths, spin densities and hyperfine couplings all argue against a ferrous-superoxo/ferric-peroxo discontinuum. Possible implications of this finding are discussed.

INTRODUCTION

S=1/2 $[FeO_2]^9$ complexes,[1] generally described as ferric-peroxo or ferrous-superoxo,[2-13] have been implicated in the catalytic cycles of heme and non-heme proteins. Experimental observation of such hemoprotein complexes, mainly by cryoradiolytic ENDOR spectroscopy,[3, 5-9, 14, 15] has led to two distinct descriptions of heme $[FeO_2]^9$, depending on whether or not hydrogen bonding to the oxygen ligand is available. Hydrogen-bonded $[FeO_2]^9$, as observed in cytochrome P450,[6-9] nitric oxide synthase,[5] horseradish peroxidase,[14, 15] hemoglobin and myoglobin,[4, 16] were described as ferric-peroxo. Non hydrogen-bonded $[FeO_2]^9$, as observed in a hemoglobin variant and in model compounds,[3] were described as ferrous-superoxo. Intrigued by this apparent discontinuum, we report a DFT investigation of the effects of hydrogen bonding on the structure of heme $[FeO_2]^9$ complexes. Calculated bond lengths, spin densities and hyperfine couplings all argue against a ferrous-superoxo/ferric-peroxo discontinuum.

RESULTS AND DISCUSSION

Figure 1 shows models examined in the present study,[17-19] mimicking the active sites of thiolate- and histidine-ligated $[FeO_2]^9$ previously characterized via cryoradiolytic spectroscopies.[3, 5-7, 14, 16] Calculated bond lengths, spin densities and charges for these models (cf. Table 1) suggest a strong superoxo character for the dioxygenic ligand, consistent with previous theoretical findings.[10, 13] An increased peroxo character, manifested in longer O-O bond lengths, higher oxygen charges, and lower oxygen spin densities, is seen upon addition of hydrogen bond donors and upon inclusion of solvation effects. There is no evidence, however, for a superoxo-peroxo *discontinuum*.

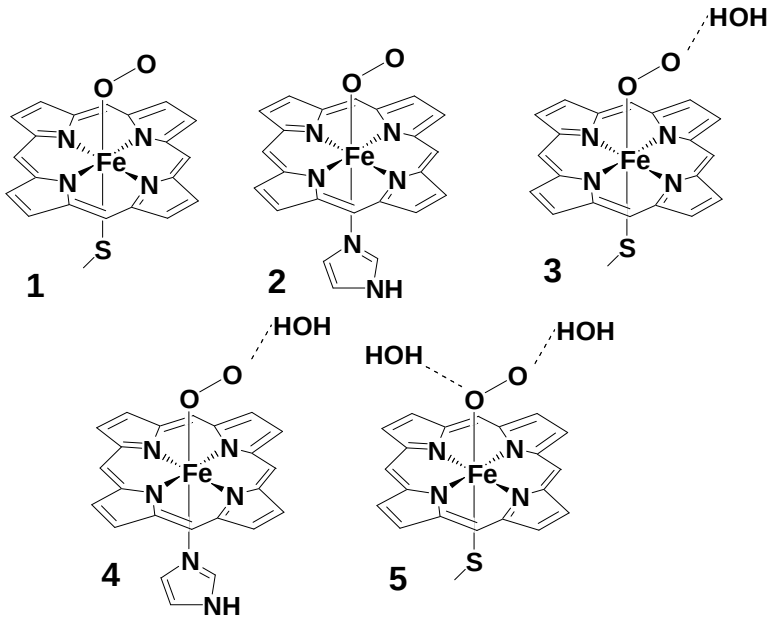

Figure 1. $[FeO_2]^9$ models employed in the present study.[20]

Table 1.

Calculated bond lengths (Å) and Mulliken partial atomic charges and spin densities (the latter shown in parentheses), for Figure 1 models.

model	Fe-O	O-O	Fe-X	Fe	O1[a]	O2[b]
1	1.93	1.32	2.43	1.12 (0.80)	-0.25 (0.41)	-0.23 (0.59)
2	1.90	1.31	2.14	1.27 (0.03)	-0.26 (0.38)	-0.23 (0.40)
3	1.92	1.34	2.42	1.09 (0.39)	-0.28 (0.38)	-0.31 (0.38)
4	1.88	1.33	2.13	1.26 (0.17)	-0.26 (0.40)	-0.26 (0.43)
5[c]	1.94	1.35	2.39	1.05 (0.57)	-0.34 (0.33)	-0.39 (0.34)

[a]iron-bound oxygen atom. [b]non iron-bound oxygen atom [c]solvated model (ε=4.333)

Table 2 shows DFT-calculated hyperfine couplings for oxygen and protons in the Figure 1 models.[21] For reference, we include in Table 1 calculated parameters for free superoxide as well as for a number of related heme adducts[8, 13, 22, 23]: ferric-hydroxo (model of hemoprotein resting states), ferric-hydroperoxo (resulted from protonation of $[FeO_2]^9$), perferryl[24]-oxo (resulted form heterolytic cleavage of the O-O bond in the ferric-hydroperoxo complexes), and perferryl-hydroxo (resulted from protonation of the perferryl-oxo).

Proton couplings shown in Table 1 agree well with experimental observations.[3-7, 16, 25] Thus, protons hydrogen bonded to the "superoxo" ligand in $[FeO_2]^9$ feature couplings of the same order of magnitude as the hydroperoxo protons, all of which are significantly more larger than porphyrin protons.

Table 2.

Calculated isotropic Fermi contact couplings (italics) and anisotropic spin dipole couplings (^{17}O values given in Gauss, ^{1}H values in MHz). Oxygen atoms labeled as in Table 1.

model	O1	O2	H[a]
OO^-	*-8.5* 24.3/22.0/-46.3	*-8.5* 24.3/22.0/-46.3	-
Fe(III)-OH[b]	*-3.50* 8.0/7.2/-15.2	-	*-6.58* -8.9/-7.9/16.8
Fe(V)-OH[b]	*-6.92* 10.7/4.6/-15.3	-	*4.40* -15.0/-14.1/29.1
Fe(V)=O[b]	*-10.07* 30.8/-1.7/-29.0	-	-
Fe(III)-OOH[b]	*-5.37* 13.0/12.0/-25.0	*-1.39* 4.2/3.5/-7.7	*-2.01* -6.5/1.3/5.2
1	*-10.96* 24.8/23.2/-47.9	*-11.81* 30.6/30.4/-61.0	-
2	*-8.97* 22.9/18.4/-41.3	*-9.72* 26.1/21.8/-48.0	-
3	*-10.90* 22.4/20.1/-42.5	*-8.76* 20.2/19.0/-39.2	*-1.15* -8.5/-8.3/16.9
4	*-10.14* 23.4/19.8/-43.2	*-9.61* 23.0/20.2/-43.1	*-2.02* -9.34/-8.9/18.2
5[b]	*-10.35* 19.7/18.5/-38.1	*-7.57* 17.7/17.4/-35.1	*0.10* -4.8/ -4.3/ 9.1 *-0.39* -5.0/-4.9/9.9

[a]hydroperoxo or hydroxo protons, or water protons hydrogen-bonded to the superoxo ligand. [b]heme-thiolate models. [c]solvated model (ε=4.333)

Consistent with expectations based on spin densities (Table 1, Refs.[10, 13]), the magnitude of the calculated ^{17}O coupling increases in the order Fe(III)-OH~Fe(III)-OOH ~perferryl-hydroxo < perferryl-oxo~$[FeO_2]^9$~superoxide. For $[FeO_2]^9$, *no evidence is seen for a discontinuum*, where addition of one or more hydrogen bonds, and/or inclusion of dielectric effects, would induce a shift from a purely ferric-peroxo to purely ferrous-superoxo description of $[FeO_2]^9$.

Models **2** and **1** feature ^{17}O couplings essentially identical to free superoxide, consistent with experimental data on non-hydrogen bonded $[FeO_2]^9$.[3] The calculated A_{zz} parameters for free superoxide and for **2** are 20-25 G lower than experimental values[3] but are still >20 G higher than calculated for ferric-hydro(per)oxo complexes - thus reproducing the experimental trend.[3, 5-7, 9, 16]

$[FeO_2]^9$ hemoprotein species, trapped by cryoradiolysis of $[FeO_2]^8$ complexes in various hemoproteins, were clearly described as ferric-peroxo, on the basis of a typical ferric EPR signal and of very reduced ^{17}O hyperfine coupling (only one of the two oxygen atoms in fact exhibited readily detectable coupling, of ~7 G).[3, 5-7, 9, 16] By contrast, with a hemoglobin lacking hydrogen bonding interactions to the dioxygenic ligand, the cryoradiolytically trapped $[FeO_2]^9$ species exhibited a superoxide-like EPR signal, and ^{17}O hyperfine couplings from *both* oxygen atoms, with values essentially

identical to those reported for free superoxide. Responsible for this apparent ferric-peroxo/ferrous-superoxo discontinuum was assumed to be hydrogen bonding to the peroxo ligand.[3] Our results now suggest this assumption to be unwarranted, and intriguingly implicate that with most hemoproteins the *first species* detected upon $[FeO_2]^8$ cryoradiolysis is in fact *already protonated*. A *second species*, formed from the proposed "non-protonated" $[FeO_2]^9$, was also detected with cryoradiolytic spectroscopies, and was assigned as *protonated* $[FeO_2]^9$ (ferric-hydroperoxo).[3, 5-7, 14, 16, 25] A *third species* detected in cryoradiolysis experiments with P450, subsequent to "ferric-hydroperoxo", was a "product-bound" state.[6, 25] Our results now imply either that the second species is an isomer of the first (ferric-hydroperoxo) species, or that the second species in fact represents a state placed *between* "ferric-hydroperoxo" and "product-bound" in the P450 catalytic cycle. For instance, the second species may be a previously unrecognized protonated Compound I – which, as seen in Tables and as pointed out by us elsewhere,[23, 26] would well masquerade as a ferric-hydroperoxo or ferric-hydroxo.

Previous computations on **1** and **2** placed ~one spin unit on the oxygen atoms and negligible spin on the iron. We challenged the obvious ferrous-superoxo assignment[3] with the speculation that **1** and **2** were in fact best described as ferric-superoxo.[13] Results shown here support the latter. The possible meaning of the different degrees of antiferromagnetic coupling/covalence between the ferric center and the porphyrin in **1** and **2** (suggested by Table 1) is currently investigated with more suitable approaches.

EXPERIMENTAL

Geometries were optimized with the BP86 functional, which uses the gradient-corrected exchange functional proposed by Becke (1988),[18] the correlation functional by Perdew (1986),[19] and the DN** numerical basis set (comparable in size to 6-31G**), as implemented in Spartan.[17] For the SCF calculations, a fine grid was used, and the convergence criteria were set to 10^{-6} (for the root-mean square of electron density) and 10^{-8} (energy), respectively. For geometry optimization, convergence criteria were set to 0.001 au (maximum gradient criterion) and 0.0003 (maximum displacement criterion). Charges and spin densities were derived from Mulliken population analyses after DFT geometry optimization. Hyperfine couplings were obtained from UBP86/6-31G**/Gaussian98[21] energy calculations at geometries shown in Table 1.

ACKNOWLEDGMENT. Drs. V. W. Huang and E. D. Coulter (UGA) are thanked for helpful discussions.

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