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In memoriam prof. dr. Ioan A. Silberg

AN ALTERNATIVE MECHANISM FOR CATALASE ACTIVITY

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ABSTRACT. The ferric heme active sites of enzymes such as catalases and peroxidases react with hydrogen peroxide to generate a “high-valent” (formally, Fe(V)) species, known as Compound I. In peroxidases, Compound I generally decays back to the ferric state by abstracting electrons from various organic substrates or redox proteins. In catalases, the electrons required for Compound I reduction are supplied by a second H_2O_2 molecule. It has long been implied that the reaction between Compound I and H_2O_2 in catalase implies either outer-sphere electron transfer and/or hydrogen/proton abstraction mechanisms. Recent DFT calculations have confirmed that such mechanisms may be viable. However, a recent experimental investigation by Obinger and co-workers now prompts us to investigate an alternative mechanism, whereby the oxygen atom in Compound I is substituted by a second peroxide molecule, generating a formally Fe(V)-peroxo adduct which by electromerism would convert to Fe(III) and liberate the final product, O_2. Reported here are DFT calculations illustrating the theoretical viability of this newly proposed catalase mechanism.

INTRODUCTION

The ferric heme active site of enzymes such as catalases and peroxidases react with hydrogen peroxide to generate a “high-valent” (formally, Fe(V)) species, known as Compound I.[1,2] In peroxidases, Compound I generally decays back to the ferric state by abstracting electrons from various organic substrates or redox proteins.[3] In catalases, the electrons required for Compound I reduction are supplied by a second H_2O_2 molecule, which is in turn oxidized to molecular dioxygen, such that the overall reaction catalyzed is: $2\ H_2O_2 \rightarrow 2H_2O + O_2$.[4,5] Experimental evidence for the mechanism of Compound I + H_2O_2 reaction has not been available. It has for a long time been implied that the reaction between Compound I and H_2O_2 in catalase implies either outer-sphere electron transfer and/or hydrogen/proton abstraction mechanisms. Recent DFT calculations have confirmed that such mechanisms may be viable.[6,7] However, a recent experimental investigation by Obinger and co-workers[8] has suggested an alternative mechanism (cf. Scheme 1), whereby the oxygen atom in Compound I is substituted by the second peroxide molecule, generating a formally Fe(V)-peroxo adduct which by electromerism would

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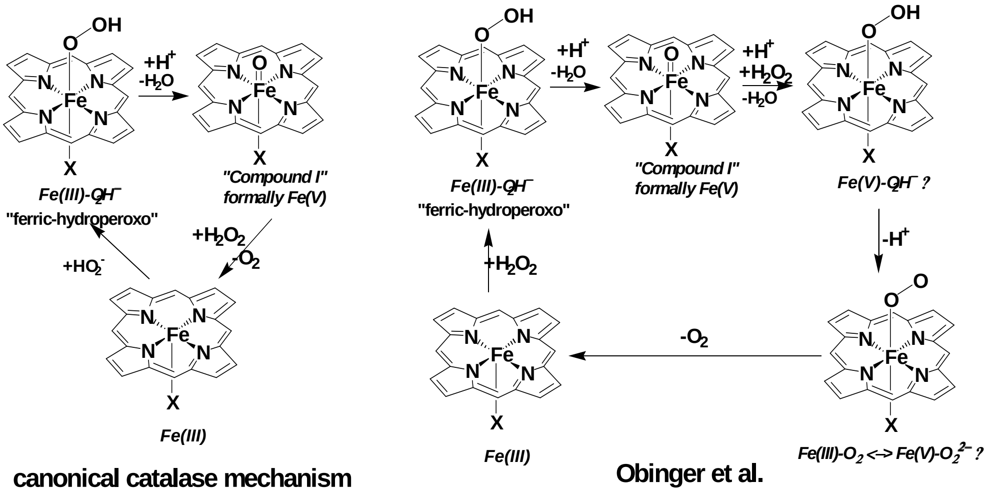
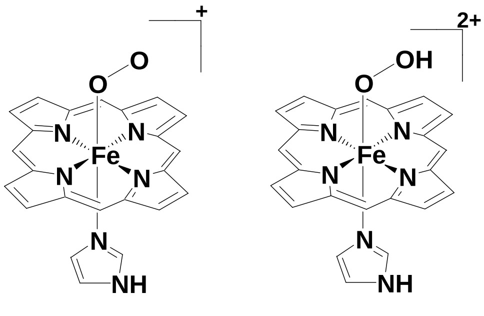
convert to Fe(III) and liberate the final product, O_2. Here we report DFT calculations illustrating the theoretical viability of the newly proposed catalase mechanism.



Scheme 1

RESULTS AND DISCUSSION

The models that we have investigated, shown in Scheme 2, would result following binding of hydroperoxide to Compound I by displacing the “oxo” atom. The nomenclature used in Scheme 2 and throughout the remainder of this discussion stems from the fact that these models can be *formally* described as containing an (Fe(V)-(hydro)peroxo moiety. Calculated geometrical and electronic parameters are shown in Table 1.



$[FeO_2]^{3+}$ $[FeO_2H]^{4+}$

Scheme 2

Table 1.

Calculated bond lengths (Å), partial atomic charges and spin densities (the latter shown in italics) for putative catalase catalytic intermediates.

Model	O-O	Fe-O	Fe-N	Fe	O1[a]	O2[b]	$O_2(H)$[c]	His[d]	P[e]
S=1/2 $[FeO_2]^{3+}$	1.23	3.00	2.14	0.66 *2.57*	-0.01 *0.97*	0.01 *1.00*	0.00 *1.97*	0.23 *0.10*	0.11 *0.36*
S=5/2 $[FeO_2]^{3+}$	1.23	3.00	2.14	0.66 *2.57*	-0.01 *0.97*	0.01 *1.00*	0.00 *1.97*	0.23 *0.10*	0.11 *0.36*
S=3/2 $[FeOOH]^{4+}$	1.41	1.85	2.03	*0.57* *1.25*	-0.17 *0.34*	*-0.17* *0.19*	0.02 0.53	*0.39* *-0.02*	*1.01* *-0.76*

[a]iron-bound oxygen atom. [b]non iron-bound oxygen atom. [c]sum over the OO(H) ligand. [d] sum over the imidazole axial ligand. [e]sum over the porphyrin ligand

The OOH ligand in the protonated model features significant spin density and is essentially electrically neutral – thus being best described as superoxide. The spin density on iron is distinctly larger than 1, and thus consistent with an S=1 Fe(IV) centre. Likewise, there appears to be ~ 1 spin on the porphyrin. The model is thus best described as containing an Fe(IV) bound to superoxide and to a porphyrin cation radical. The S=3/2 state of the $[Fe\text{-}O\text{-}OH]^{4+}$ model was lower in energy by ~20 kcal/mol compared to its cognate S=1/2 and S=5/2, which nevertheless featured essentially identical electronic and structural parameters and are therefore not further discussed.

For the non-protonated model, the S=1/2 and S=5/2 states were essentially degenerate, with the S=5/2 state slightly lower in energy (~5 kcal/mol). This is in sharp contrast with "ferrous-dioxygen" heme complexes, where theory and experiment agree that the low-spin state is distinctly favoured.[9,10] Remarkably, for S=5/2 $[Fe\text{-}O_2]^{3+}$ the Fe-O bond is already broken (i.e., 3 Å), and the O_2 ligand is well described as molecular oxygen, with 2 unpaired electrons and essentially no overall electrical charge. Dissociation of O_2 leaves behind an Fe(III) centre and thus completes the catalase catalytic cycle, cf. Scheme 1. The energy difference between $[Fe\text{-}O\text{-}OH]^{4+}$ and $[Fe\text{-}O_2]^{3+}$ defines the (gas-phase) proton affinity of the latter. This value, at 163 kcal/mol, is lower than the proton affinities of water, hydroxide, or imidazole (171, 411, and 232 kcal/mol, respectively), calculated under the same conditions. This indeed suggests that, should the $[Fe\text{-}O\text{-}OH]^{4+}$ species ever form, either solvent or an active site histidine side-chain might in principle serve as electron acceptors, removing a proton from $[Fe\text{-}O\text{-}OH]^{4+}$ and forming $[Fe\text{-}O_2]^{3+}$, which, as shown above, is best described as Fe(III) + "free" molecular oxygen. An $[Fe\text{-}O\text{-}OH]^{4+}$ species has recently been observed experimentally in a non-heme iron complex.[8] The present data confirms that such a species would be competent in completing the newly proposed catalase catalytic mechanism, cf. Scheme 1.

CONCLUSIONS

An alternative mechanism is proposed for catalases, whereby the oxygen atom in Compound I is substituted by a second peroxide molecule, generating a formally Fe(V)-peroxo adduct which by electromerism would convert to Fe(III) and liberate the final product, O_2. DFT data supporting the viability of this newly proposed catalase mechanism have been presented.

MATERIALS/METHODS

Geometries were optimized at the DFT level in the *Spartan* software package. The BP86 functional, which uses the gradient corrected exchange functional proposed by Becke (1988) [11] and the correlation functional by Perdew (1986),[12] and the DN** numerical basis set (comparable in size to 6-31G**) were used as implemented in *Spartan*. 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.

REFERENCES

1. R. Silaghi-Dumitrescu, *J. Biol. Inorg. Chem.*, **2004**, 9, 471.
2. R. Silaghi-Dumitrescu and I. Silaghi-Dumitrescu, *Rev. Roum. Chim.*, **2004**, 3-4, 257.
3. H. B. Dunford, *"Peroxidases in Chemistry and Biology" (J. Everse, K. E. Everse, and M. B. Grisham edts.), CRC Press, Boca Raton 1991, vol. II., p. 2-17*.
4. D. Metodiewa and H. B. Dunford, *Int. J. Radiat. Biol.*, **1992**, 62, 543.
5. P. Gouet, H. M. Jouve, P. A. Williams, I. Andersson, P. Andreoletti, L. Nussaume and J. Hajdu, *Nat. Struct. Biol.*, **1996**, 3, 951.
6. X. Wang, S. Li and Y. Jiang, *Inorg. Chem.*, **2004**, 43, 6479.
7. F. Buda, B. Ensing, M. C. M. Gribnau and E. J. Baerends, *Chem. Eur. J*, **2003**, 9. 3436.
8. C. Jakopitsch, A. Wanasinghe, W. Jantschko, P. G. Furtmueller and C. Obinger, *J. Biol. Chem.*, **2005**, 280, 9037.
9. P. Rydberg, E. Sigfridsson and U. Ryde, *J. Biol. Inorg. Chem.*, **2004**, 9, 203.
10. K. P. Jensen, B. O. Roos and U. Ryde, *J. Inorg. Biochem.*, **2005**, 99, 45, Erratum on page 978.
11. A. D. Becke, *Phys. Rev.*, **1988**, 3098.
12. J. P. Perdew, *Phys. Rev.*, **1986**, B33, 8822.