STUDIA UNIVERSITATIS BABEŞ-BOLYAI, CHEMIA, LII, 1, 2007

Dedicated to Professor Ionel Haiduc,
President of The Romanian Academy at his 70th anniversary

A PARADIGM FOR O-O BOND CLEAVAGE IN FERRIC-HYDROPEROXO COMPLEXES

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ABSTRACT. Using density functional (DFT) calculations, heterolytic oxygen-oxygen bond cleavage is found to be extremely facile in heme and non-heme ferric-hydrogen peroxide complexes, Fe(III)-OH-OH. These findings question the need to invoke double protonation of the "distal" oxygen atom in Fe(III)-O-OH complexes as the universal mechanism of O-O bond cleavage in biological systems.

Introduction

Controlled activation of oxygen-oxygen bonds in hemoproteins has been at the heart of breakthrough moments in the development of modern science, both in terms of concepts and in terms of methodology. [1-10] In heme and non-heme iron complexes, O-O bond cleavage is generally accepted to occur via $[Fe(III)\text{-}O\text{-}OH]^{2+}$ intermediates, in processes of vast biological significance.[1-12] In biological systems, this decay of Fe(III)-O-OH species is heterolytic; this is presumably due to proton donors placed strategically at enzyme active sites, leading to a doubly-protonated $[Fe(III)\text{-}O\text{-}OH_2]^{3+}$ (oxy-water) state which decomposes to water and a high-valent $[FeO]^{3+}$ species (formally Fe(V)).[12-15] Alternatively, when such proton donors are not available (e.g., in synthetic model compounds), homolytic decay of $[Fe(III)\text{-}O\text{-}OH]^{2+}$ to $[FeO]^{2+}$ (formally Fe(IV)) and hydroxyl radical, •OH, has been reported; the extraordinary toxicity of •OH would justify the choice of a heterolytic mechanism in biological systems.[6,11,16-19] Related to the double-protonation heterolytic mechanism, it is generally believed that alteration of proton delivery, leading to a doubly-protonated $[Fe(III)\text{-}OH\text{-}OH]^{3+}$ species rather than to "oxy-water", is responsible for "uncoupling" processes, whereby the peroxide substrate leaves the active site as H_2O_2, rather than undergo O-O bond cleavage.[8] Reported here are density functional calculations challenging these assumptions, and pointing to $[Fe(III)\text{-}OH\text{-}OH]^{3+}$ species as competent substrates for O-O heterolytic O-O bond cleavage in biological systems.

Results and discussion

Non-heme iron. Activated bleomycin (ABLM) has been characterized as a non-heme Fe(III)-O-OH (ferric-hydroperoxo) complex.[11,19] ABLM oxidatively

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damages nucleic acids, in a process thought responsible for bleomycin's therapeutic action. Although an initial hypothesis was that ABLM's oxidative action was due to cleavage of the O-OH bond and transient formation of a strongly-oxidizing $[FeO]^{2+}$ or $[FeO]^{3+}$ species, it is now known that O-O bond cleavage is energetically unfavourable in Fe(III)-O-OH ABLM.[16,19] By contrast, Figure 1 shows that elongation and cleavage of the O-O bond in a Fe(III)-OH-OH bleomycin model (i.e., a protonated version of the classical ABLM ferric-hydroperoxide) is intrinsically extremely facile.[20] The final products of this O-O bond cleavage reaction are a water molecule and a $[FeO]^{3+}$ (formally Fe(V)) bleomycin adduct, which is expected to be a strong oxidant and may account for bleomycin's therapeutic action. It may be speculated that O-O bond clevage in ABLM is in fact triggered by specific proton donation upon binding to the substrate, DNA. These findings on O-O bond cleavage in protonated ABLM are strongly reminiscent to those recently reported for another non-heme Fe-OH-OH complex, where initial homolytic dissociation of the HO-OH bond was accompanied by a subsequent hydrogen atom abstraction by the leaving OH radical, leading to an iron-oxo moiety and water as the final products.[21]

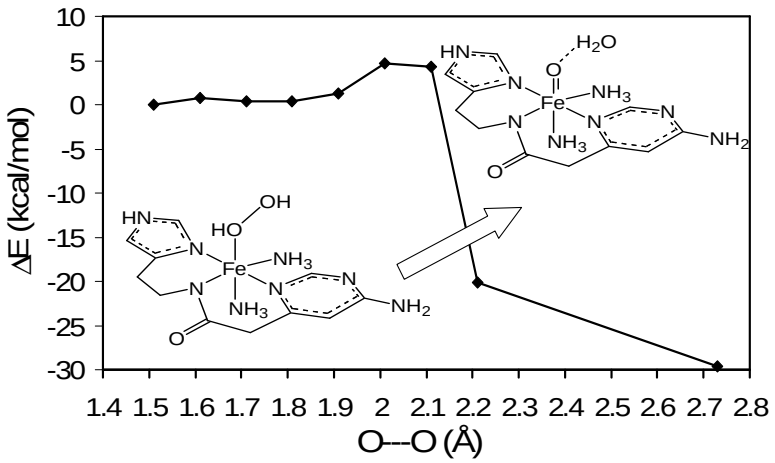

Figure 1. Potential energy surface, following O-O bond elongation in a bleomycin Fe(III)-OH-OH model (structure shown in inset, left lower corner). Geometries were optimized with the HO-OH distance constrained to values indicated in the plots, starting from the equilibrium value of 1.51 Å. Energy differences are plotted for each model, with the energy of the equilibrium structure (far left side of the plot) taken as reference for each model. At an O-O distance of 2.2 Å, both protons originating from the HOOH ligand were found on the leaving oxygen atom, yielding the structure depicted in the inset, upper right corner. Further optimization without any geometry constraints led to a final O-O distance of ~2.7Å (rightmost data point in the plot).

Heme iron. Fe(III)-O-OH complexes are a common point in the mechanisms of all oxygen and peroxide-activating heme enzymes, including peroxidases and heme oxygenase. [1-10] In peroxidases, addition of a second proton to lead to the doubly-protonated $[Fe(III)\text{-}O\text{-}OH_2]^{3+}$ (oxy-water) state is accepted as the dominant mechanism of O-O bond cleavage.[2,12] By contrast, in heme oxygenase proton donation is thought to occur at the iron-bound oxygen atom of a Fe(III)-O-OH intermediate, assisting the departing OH group as it attacks the meso carbon on the porphyrin ring.[22] Figure 2 shows that, similar to the ABLM case, a heme Fe(III)-OH-OH complex is catalytically competent in the heme oxygenase reaction, in line with an independent recent report by Shaik and co-workers.[23] The even more remarkable observation is that, along the reaction coordinate for the heme oxygenase reaction, i.e., as the FeO(H)OH---meso carbon distance is decreased, O-O bond cleavage occurs before any significant interaction between oxygen and carbon develops.

Indeed, as shown in Figure 3, at an oxygen-carbon distance of 2.3 Å the O-O bond is already broken. This implies that the O-O bond in heme $[Fe(III)\text{-}OH\text{-}OH]^{3+}$ systems is *intrinsically* weak. Lifting any geometrical constraints from the model initially optimized with an O---C distance of 2.3 Å leads, as in the case of the $[Fe(III)\text{-}OH\text{-}OH]^{3+}$ doubly-protonated ABLM model, to hydrogen abstraction by the departing OH moiety, yielding a water molecule and a non-protonated $[FeO]^{3+}$ moiety as the final products (cf. Figure 3).

The catalytic competence of a Fe(III)-OH-OH intermediate is in agreement with the well-established “peroxide shunt” notion for P450 monooxygenases, i.e. that reaction of ferric P450 with H_2O_2 (presumably via Fe(III)-OH-OH intermediate) and with a hydrocarbon does indeed generate the same product as the “normal” catalytic cycle.[24] Site-directed mutagenesis studies aimed at disturbing the proton delivery network in P450 had the effect of decreasing the enzyme efficiency via H_2O_2 liberation (“uncoupling”) and were interpreted as evidence for increased formation of the Fe(III)-OH-OH tautomer, which, unlike “$Fe(III)\text{-}O\text{-}OH_2$” tautomer, would release H_2O_2 rather than hydroxylate the substrate.[8,12] However, the effects of these mutations can be alternatively interpreted as affecting the polarity and/or sterics at the active site, which in turn are known to affect the reduction potentials and/or spin state of the heme.[25] The calculations reported herein assume an S = 1/2 state for the Fe(III)-OH-OH state. Indeed, coordination of water (a ligand similar to H_2O_2) to P450 is enough to change the spin state from S=5/2 (pentacoordinate) to S=1/2 (aqua).[8] The iron-oxygen bond in S=1/2 Fe(III)-OH-OH is relatively short, apparently allowing sufficient interaction between iron and peroxide orbitals to facilitate O-O bond cleavage. Such orbital interactions would be drastically

diminished in a high-spin state, where a distinctly longer Fe-O bond is predicted.[26] Thus, formation of a certain percentage of *high spin* state for Fe(III)-OH-OH may be the cause of experimentally observed[8] uncoupling in P450. Related to P450 uncoupling, the catalytic cycle of superoxide reductases (SOR)[27-30] involves liberation of H_2O_2 from a ferric-peroxo intermediate. The SOR active site contains a ferric ion with an axial cysteine ligand and four equatorial nitrogen ligands, similar to P450.[31] Unlike the buried porphyrin in P450, the SOR nitrogen ligands belong to neutral imidazoles, and the iron is solvent-exposed.[31] The combination of these structural features appears to result in a distinct preference of the SOR ferric site for high-spin states: even with a carboxylate coordinated to its sixth position, the octahedral SOR site is still high-spin (S=5/2).[32] This preference for high-spin states is presumably essential for SOR in avoiding O-O bond cleavage at *the Fe(III)-OH-OH stage* (which, in light of the results reported herein, may be *the* state where the SOR site must make efforts to avoid O-O bond cleavage, as opposed to previous proposal involving Fe(III)-O-OH as the critical step[28,30,32]).

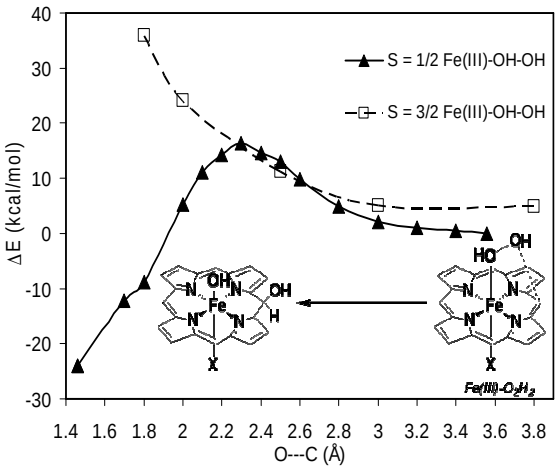

Figure 2. Potential energy surface, following meso-hydroxylation reactions in a Fe(III)-H_2O_2 as well as in a protonated Compound I ("Compound I-H); X = imidazole. Geometries were optimized with the O---C distances (shown as dotted line in the chemical structure on the far right of the diagram) constrained to values indicated in the plots, starting from the equilibrium geometry with C---O ~3.8 Å, and shortening this distance by amounts indicated in the plot. Energy differences are plotted for each model, with the energy of the S=1/2 equilibrium structure (far right side of the plot) taken as reference (zero). No O-O bond cleavage was found to occur for the equivalent S = 3/2 Fe(III)-OH-OH model under the conditions used here (instead, at the left-most point of the S=3/2 plot, O---C = 1.8 Å, the Fe-O bond was irrevocably broken).

As illustrated by Figure 2, whereas O-O bond cleavage is extremely facile in low-spin [Fe(III)-OH-OH]$^{3+}$ complexes such as those examined in the present work, it is essentially impossible in higher spin states; this is due to very long (~ 3 Å) initial Fe-O_2H_2 distances which preclude efficient iron-oxygen interaction.[20] This points out to the need for the [Fe(III)-OH-OH]$^{3+}$ state to be low-spin (S=1/2), or perhaps to be preceded by or in equilibrium with a sigly-protonated low-spin [Fe(III)-O-OH]$^{2+}$ state, where the Fe-OOH bond would be significantly shorter.

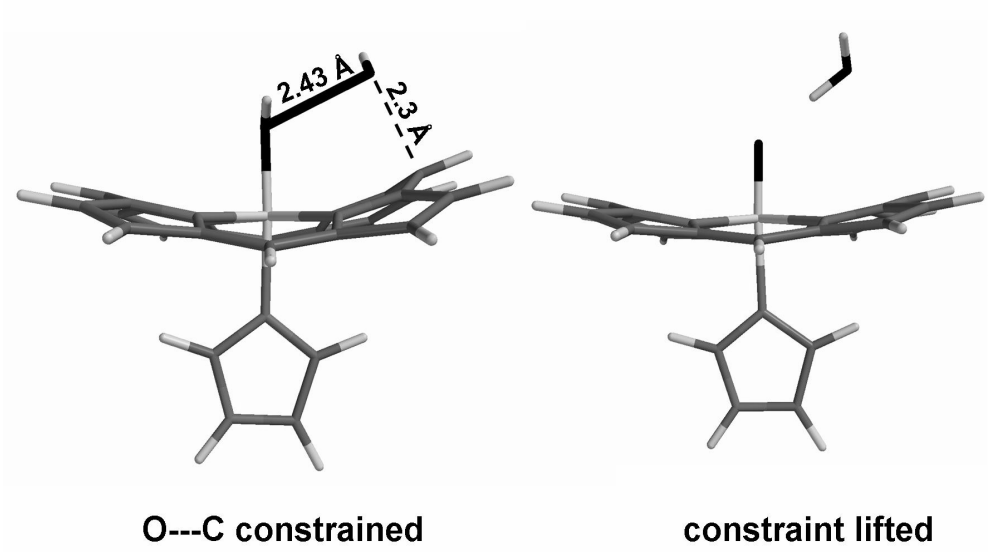

Figure 3. Optimized geometries for heme oxygenase models. Left: Fe(III)-OH-OH adduct, with the O---C distance constrained to 2.3Å, modelling a putative state on the reaction pathway leading to meso-hydroxylation. Right: same model, optimized after lifting the O---C geometry constraint. Colour coding: iron and hydrogen - white, oxygen - black, carbon and nitrogen - grey.

Conclusion

O-O bond cleavage in representative examples of heme and non-heme Fe(III)-OH-OH complexes is extremely facile – perhaps even more facile than in the corresponding Fe(III)-O-OH complexes. These findings question the need to invoke the "oxy-water" state, Fe(III)-O-OH_2]$^{3+}$, as a necessary intermediate in heterolytic O-O bond cleavage within biological systems.

Experimental

Density functional theory (DFT) methods are the main computational tools currently applied to characterization of geometries and electronic

structures of transition metal enzyme active sites and to their reaction mechanisms. For transition metal complexes, DFT usually provides very reliable geometries, but may fail in describing subtle details of spin densities.[3,6,33-36] Geometries for all models in the present work were optimized in the *Spartan* software package[37,38] without any geometry or symmetry constraints, unless otherwise specified. The UBP86 functional, which uses the gradient corrected exchange functional proposed by Becke (1988)[39] and the correlation functional by Perdew (1986),[40] and the 6-31G** basis set (in its numerical variation, DN**) were used as implemented in the *Spartan* software package. For SCF calculations the energy convergence criterion was 10^{-6} hartrees. For geometry optimization, convergence criteria were set to 4.5×10^{-4} hartrees/bohr (maximum gradient criterion) and 1.8×10^{-3} Å (maximum displacement criterion). The unrestricted formalism was used throughout, i.e., the spin-up and spin-down electrons belonging to one given molecular orbital were allowed to have different energies.

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