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Density Functional Theory Calculations on Fe-O and O-O Cleavage of Ferric Hydroperoxide Species: Role of axial ligand and spin state

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Abstract

Density Functional Theory (DFT) calculations are performed on thiolate bound hydroperoxide complexes. O-O and Fe-O cleavage reaction coordinates, relevant to the active sites of Cytochrome P450 and Superoxide Reductase enzymes, were investigated for both high and low spin states and for cis and trans orientations of the thiolate ligand with respect to the hydroperoxide ligand. The results indicate that the presence of a thiolate ligand produces significant elongation of the Fe-O bond and reduction of Fe-O vibrational frequency. While the fate of the O-O cleavage reaction is not significantly altered, the presence of a thiolate induces a heterolytic Fe-O cleavage irrespective of the spin state and orientation which is very different from results obtained with a trans ammine ligand.

Introduction

Thiolate bound hydroperoxide species are proposed intermediates in a number of biological processes.[1,2] In cytochrome P450 a low-spin thiolate ferric hydroperoxide species (compound 0) results after electron and proton transfer to the initial dioxygen bound intermediate.[3] This species either undergoes a protonation assisted heterolytic O-O bond cleavage or a protonation assisted loss of H₂O₂ in an uncoupled reaction.[4] It has been also proposed that it may undergo a homolytic O-O bond cleavage and result in direct H-atom abstraction.[5] In superoxide reductases (SOR) a high-spin thiolate bound Ferric hydroperoxide species results from the reaction of the reduced active site with its superoxide substrate.[6] This intermediate decays by protonation assisted release of the H₂O₂ product. [7] Similar thiolate Fe^{III} hydroperoxide species are also possible intermediates involved in the active sites of P450 NO synthases and cysteine dioxygenases.[8,9] Thus understanding their diverse reactivities and the factors affecting these have been a major focus of several research groups.[10–16]

Experimental and theoretical modeling of these key intermediates has been attempted.[1,2] Kovacs *et.al.* reported two synthetic analogues of SOR where hydroperoxide intermediates were trapped and characterized.[15,16] In one case the hydroperoxide complex was low-spin with a thiolate ligand cis to the hydroperoxide,[16] while in the second system the

hydroperoxide was high-spin with a trans thiolate.[15] The later species was characterized to have a low Fe-O (420 cm^{-1}) and a high O-O (898 cm^{-1}) vibrational frequency and it quickly lost H_2O_2 as in the SOR enzyme. Goldberg *et.al.* has reported a series of low-spin Fe^{III} hydroperoxide complexes with trans arylthiolate ligands.[12,17] The substituents on the aryl group were systematically varied which produced changes in the Fe-O and O-O stretches.[18] Significant reductions in Fe-O vibrations were observed due to the trans effect of the thiolate ligand.

X-ray absorption spectroscopy (XAS) and Density functional theory (DFT) calculations on active sites of P450 and SOR revealed that the anionic nature of the thiolate and the very covalent $\text{Fe}^{\text{III}}\text{-S}_{\text{thiolate}}$ bond enhances the protonation affinity of these active sites.[19,20] In the case of P450 the protonation, guided by 2nd sphere H-bonding, occurs on the distal O atom of the hydroperoxide ligand and leads to heterolytic O-O cleavage.[20] However in case of SOR, protonation occurs at the proximal O atom and triggers H_2O_2 release from the active site.[19] It was further suggested that the presence of the thiolate did not affect homolytic O-O bond cleavage of a low-spin trans thiolate Fe^{III} hydroperoxide species. In this paper several theoretical models of high and low-spin ferric hydroperoxides with cis and trans thiolate ligands are evaluated. Their relative O-O and Fe-O cleavage reactivities are compared. Comparison to systems with trans axial ammine ligand provides valuable insights into how the reactivities of ferric hydroperoxide species are tuned by spin state, by the presence of an anionic thiolate ligand and by its orientation relative to the hydroperoxide ligand.

Methods

All optimizations were performed using 6-311g* basis set for Fe, S, N, O and H atoms in Gaussian 03 program ver. C02[21] employing the B3LYP[22,23] hybrid functional. Single point calculations were performed using the same functionals and a 6-311+g* basis set in the gas phase (for wavefunctions), and additionally a PCM[24] with an $\epsilon=4.0$ and radius of solvent =1.4 was used for obtaining the total energies. The potential energy scans were performed by incrementally increasing the relevant bond by 0.2 Å and optimizing rest of the molecule. Then a single point on the optimized geometry was performed to obtain the energy and the wavefunction. Frequency calculations were performed to ensure a stable minimum for the fully optimized structures.

Results and Analysis

Geometries and Vibrations

The optimized geometries of the high and low spin thiolate ferric hydroperoxide species (Fig. 1, left) in both cis and trans configurations are presented in Table 1. In general a change of spin state from $S=5/2$ to $S=1/2$ results in a shortening of the metal ligand bonds. For the Cis configuration the Fe-S shortens by 0.04 Å, the Fe-O by 0.09 Å, the Fe-N by 0.18 Å and the O-O elongates by 0.03 Å. The changes in the Fe-O and Fe-S bonds for the Trans configuration are 0.06 Å and 0.08 Å, respectively. No significant change in the O-O bond is observed.

The change of spin state causes significant perturbations in the vibrational frequencies (Table 1, right). Both the Fe-O and Fe-S shift to higher energy consistent with the calculated decrease in their bond lengths upon change of spin state from $S=5/2$ to $S=1/2$. This is indicative of stronger bonding interactions in the low-spin state relative to the high-spin state. Interestingly, the O-O vibrations are significantly lower in the low-spin state relative to those observed for the high-spin state. Thus strengthening of the Fe-O bond in the low-spin state is associated with a simultaneous weakening of the O-O bond. This is similar to

the results obtained in alkylperoxo systems with trans ammine and hydroxide ligands. This is mainly due to donation from the O-O π bonding orbital in to the empty d_{z^2} orbital on the $S=1/2$ Fe center at short Fe-O distances. This shifts electron density out of the occupied bonding orbital of the O-O fragment leading to weakening of the O-O bond.[25]

In the HS state the trans effect of the thiolate weakens the Fe-O bond as evident from its elongated bond length (1.94 Å) relative to that in the Cis configuration (1.90 Å). This leads to a weakened Fe-O vibration. It does not lead to a significant change in the O-O bond length. The trans effect leads to major structural changes in the low spin state as well. The Fe-O bond is longer in the Trans configuration relative to the Cis configuration by 0.05 Å. This leads to dramatic lowering of the Fe-O vibration from 566 cm^{-1} in the Cis to 532 cm^{-1} in the trans configuration. Note that simultaneously the O-O bond is longer and the O-O vibration is weaker in the Cis configuration.

Reactivity

A) O-O

The O-O bond cleavage reaction (represented in Fig. 1, top) is investigated for both the high-spin and low-spin states.

i) High Spin—The potential energy scan (PES) of O-O bond cleavage of a high spin $S=5/2$ trans ferric thiolate species is shown in Fig. 2A. The reaction is overall endothermic by 22 Kcal/mol. In addition to this increase in energy of the products there is an additional barrier of 11 Kcal/mol at $\sim 2\text{ Å}$. The Mulliken spin densities on the atoms involved in the process are shown in Fig. 2B. The spin densities on the Fe center decreases from 4.1 to 3.35 (Fig. 2B, grey triangles) along the reaction coordinate while that on the distal O of the bound hydroperoxide ligand synchronously increases from ~ 0 to 0.9 (Fig. 2B, filled diamonds). This is characteristic of homolytic O-O bond cleavage resulting in the formation of a $\text{Fe}^{\text{IV}}=\text{O}$ and a OH species. The spin density on the thiolate S (Fig. 2B, filled squares) decreases from 0.33 to ~ 0.0 and that of the proximal O of the bound hydroperoxide increases from 0.21 to 0.65. This indicates stronger π bonding of the Fe^{III} center with the thiolate relative to the hydroperoxide in the reactant while the π bonding is dominated by the oxo ligand in the product $\text{Fe}^{\text{IV}}=\text{O}$ species.

A similar O-O cleavage PES with a neutral ammine ligand is also endothermic by 22 Kcal/mol and has an additional barrier of 9 Kcal/mol. These results are similar to earlier theoretical estimates of O-O bond cleavage of a HS ferric hydroperoxide species with a pentadentate neutral ligand.[25] Note that the highest point on the O-O bond cleavage PES (i.e. transition state) occurs at $\sim 0.1\text{ Å}$ shorter O-O distance in the trans thiolate ligand relative to the trans ammine ligand.

ii) Low Spin—The PES of a low-spin thiolate hydroperoxide species shows that it is endothermic by ~ 20 Kcal/mol (Fig. 3, diamonds). The spin densities on the Fe, S and O centers (data not shown) indicate a homolytic O-O bond cleavage. Note that unlike in the high spin state there is no additional barrier involved in this step. These results are similar to those reported for neutral ammine containing low spin ferric hydroperoxides.[26]

We have not considered the heterolytic O-O bond cleavage for this set of ligands. This process requires protonation of the distal O of the bound hydroperoxide ligand. It has been shown that this process is energetically unfavorable ($> +200$ Kcal/mol uphill) for a non-heme active site relative to the heme active site in cytochrome P450 (-40 Kcal/mol).[19] This is because delocalization of one oxidizing equivalent over the dianionic porphyrin macrocycle plays a major role in stabilizing the resultant highly oxidized Fe site.[19,20]

B) Fe-O

In this section, the effects of spin state, thiolate ligation and its orientation relative to the OOH ligand on the PES of Fe-O cleavage are evaluated.

i) High Spin

a) Trans thiolate vs Trans Ammine: The PES of Fe-O cleavage of a high spin ferric hydroperoxide species with a trans ammine (Fig. 4A, squares) and a trans thiolate (Fig. 4A, diamonds) ligand shows an endothermic barrier of 16 Kcal/mol and 21 Kcal/mol, respectively. For the trans ammine complex the Mulliken spin densities on the Fe (Fig. 4B, diamonds) and the OOH fragments (Fig. 4B, squares) along the PES show decreasing spin density on the Fe center (4.1 to 3.8) and increasing spin on the OOH (0.5 to 1.0). This indicates that the Fe-O cleavage of the ferric hydroperoxide species with trans ammine ligand is homolytic i.e. results in the formation of $\text{Fe}^{\text{II}} + \cdot\text{OOH}$ species. The spin density on the Fe center along the Fe-O co-ordinate of the ferric hydroperoxide species with trans thiolate ligand remains fairly constant (Fig. 4C, square). Alternatively, the spin density on the hydroperoxide ligand is gradually reduced in the trans thiolate complex (Fig. 4C, triangle) so that it dissociates as an anionic OOH^- ligand. This loss of spin density is compensated by the enhanced spin density on the thiolate ligand (Fig. 4C, diamond). This is indicative of heterolytic Fe-O bond cleavage where the bound hydroperoxide dissociates as OOH^- and the Fe center retains its Fe^{III} state. Note that the Fe-S bond decreases by $\sim 0.1 \text{ \AA}$ as the OOH^- dissociates indicating that the thiolate becomes a much stronger donor ligand to the resulting five coordinate Fe^{III} species.

b) Trans Thiolate vs Cis thiolate: The PES of Fe-O cleavage for a ferric hydroperoxide species with a thiolate ligand Cis to the hydroperoxide (Fig. 5, filled grey diamonds) indicates that it is only ~ 2 Kcal/mol higher in energy than the corresponding trans thiolate (Fig. 5, filled black diamonds). The spin density changes along the PES indicate that this involves a heterolytic Fe-OOH cleavage parallel to the trans thiolate but not the trans ammine system. The Fe-S bond length decreases only by $0.04 \text{ \AA}$ (Fig. 5, empty grey triangles) in this cis thiolate system compared to $0.1 \text{ \AA}$ in the trans thiolate case. In case of the cis thiolate, the Fe-N bond length of the trans ammine ligand is decreased by $0.06 \text{ \AA}$ instead.

ii) Low Spin—The PES for the Fe-O bond cleavage reactions for the low spin Fe^{III} hydroperoxide species indicate that, in general, Fe-O bond cleavage is more endothermic relative to their high-spin counterparts. With the trans ammine ligand, the Fe-O cleavage is endothermic by 35 Kcal/mol which is 15 Kcal/mol higher than that for the high spin state. These Fe-O cleavage results for the cis and trans low spin Fe^{III} thiolates are (Table 2) 41 Kcal/mol and 39 Kcal/mol, respectively. These are 17 Kcal/mol and 18 Kcal/mol higher than the values calculated for their high spin counterparts.

The fate of the Fe-O cleavage for the low spin complexes parallels those for the high spin complexes. The trans and cis thiolate ligand results in heterolytic Fe-O cleavage, while the trans amine ligand results in homolytic Fe-O cleavage.

Discussion

The calculations presented in this study indicate that the thiolate bound ferric hydroperoxides have significant weakening of this Fe-OOH bonds. The Fe-O bond lengths for a model with a trans thiolate are calculated to be $1.94 \text{ \AA}$ and $1.86 \text{ \AA}$ for high spin and low spin states, respectively. These are longer relative to trans ammine bound ferric hydroperoxide species where these values for the Fe^{III} hydroperoxide complexes in the same

spin states are calculated to be at 1.90 Å and 1.83 Å, respectively. Such weakening of the Fe-O bonds of ferric hydroperoxides with trans thiolate ligation is consistent with the weakening of Fe-O vibrations observed experimentally and reflects the trans effect of a strong donor thiolate ligand.[18]

The presence of the anionic thiolate ligand dramatically alters the fate of Fe-O cleavage. With a neutral ammine ligand, dissociation of the OOH ligand is homolytic producing a Fe^{II} and a [•]OOH radical. In the presence of the thiolate, the dissociation is heterolytic producing Fe^{III} and an ⁻OOH anion. This is independent of the orientation of the thiolate relative to the hydroperoxide and also the spin state of the ferric peroxo species. This reflects the enhanced stabilization of the positively charged Fe^{III} center by the anionic thiolate ligand.

Dissociation of the ⁻OOH ligand from a low-spin Fe^{III}-OOH species leads to the formation of a five co-ordinate low-spin Fe^{III} center. However an S=5/2 five coordinate center is the thermodynamic ground state. Thus there has to be a two electron spin flip along the reaction coordinate. This spin barrier towards Fe-O bond cleavage for a S=1/2 peroxo species has been raised as a possible contribution towards the experimentally observed slow rate of the energetically feasible uncoupling reaction of P450 (loss of H₂O₂ via an energetically favorable protonation of compound 0).[20] Because there is no spin orbit matrix element to couple two spin states with a $\Delta S = 2$, the role of the intermediate spin state (S=3/2) must be considered. The PES of Fe-O dissociation for the S=5/2 (HS, diamonds), S=3/2 (IS, triangles) and S=1/2 (LS, squares) states are shown in Fig. 6. At the crossing point of the LS and the HS surfaces, the Fe-O distance is 2.45 Å and the energy is 18 Kcal/mol above the bound Fe^{III}-OOH ground state. At this crossing point the S=3/2 surface is only 7 Kcal/mol higher in energy and would provide a mechanism for spin orbit coupling of the S=1/2 and S=5/2 states in 2nd order.

The anionic thiolate lowers the energy of protonation of the hydroperoxide ligand by 40 Kcal/mol relative to a neutral ligand.[19] Protonation of the hydroperoxide ligand prior to dissociation results in the formation of a CH₃SFe^{III}-H₂O₂ complex where the S=5/2 ground state is calculated to be 6.7 Kcal/mol lower in energy relative to the S=1/2 state due to the weaker ligand field of the H₂O₂ ligand relative to a ⁻OOH ligand. In this case the reactant and the product have the same spin states. Thus the protonation step for the non-heme model will involve the spin change and possibly a barrier. However, this is not the case for compound 0 in P450 where the ground state is calculated to remain S=1/2 after protonation of the hydroperoxide ligand leading to a bound H₂O₂. [20] Thus the uncoupled loss of H₂O₂ in P450 will involve a barrier as described above (Fig. 6) where efficient 2nd order spin orbit coupling will be required for the reactant surface to cross over to the product surface.

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Figure 1.
Schematic representation of the Fe^{III} hydroperoxide models and their reactivities investigated.

Figure 2.

A) The PES of O-O bond cleavage of S=5/2 Fe^{III} hydroperoxide species with a trans ammine (small squares) and a trans thiolate (large squares) ligand. B) The Mulliken Spin densities of the relevant centers along the PES for thiolate Fe^{III} hydroperoxide species.

Figure 3.
PES of O-O bond cleavage for $S=5/2$ (filled squares) and $S=1/2$ (filled diamonds) thiolate bound Fe^{III} hydroperoxide species.

Figure 4.

A) PES of Fe-O cleavage of S=5/2 Fe^{III} hydroperoxide species with trans ammine (filled square) and trans thiolate (filled diamonds) ligands. Mulliken Spin densities on relevant centers along the PES for the S=5/2 Fe^{III} hydroperoxide species with B) trans ammine and C) trans thiolate ligand.

Figure 5. PES of Fe-O cleavage of $S=5/2$ Fe^{III} hydroperoxide species with a thiolate ligand Cis (filled grey) and trans (filled black) to the OOH ligand. The change of Fe-S along the PES is indicated with empty triangles (axis to the right).

Figure 6.

The Fe-O PES of the three spin states of a Fe^{III} hydroperoxide complex with a trans thiolate ligand. Triangles ($S=3/2$, Intermediate Spin), Diamonds ($S=5/2$, High Spin) and Squares ($S=1/2$, Low Spin).

Table 1
DFT Calculated Geometry and Vibrational Frequencies of Ferric Hydroperoxide Species

Model	Fe-S	Fe-O	O-O	C-S	Fe-O-O	Fe-S-C	ν Fe-S	ν Fe-O	ν O-O
S=5/2	Trans	2.31	1.94	1.84	113.8	114.5	332	480	914
	Cis	2.31	1.90	1.84	115.2	113.2	326	488	923
S=1/2	Trans	2.25	1.86	1.84	111.5	111.3	364	532	888
	Cis	2.27	1.81	1.83	111.7	107.5	332	566	868

Table 2

Calculated Energies (Kcal/mol) for the Fe-O cleavage

Trans	5/2	21
	1/2	39
Cis	5/2	23
	1/2	41