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COMMUNICATION

Trispyrazolylborato Iron Cysteinato Complex efficiently mimic cysteine dioxygenase process: mechanistic insights

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**The atomistic details of the dioxygenation process of a cysteinato ligand by an efficient CDO biomimetic model system -1- has been herein investigated by means of first principle calculations. The outcomes of the detailed DFT study show that the reaction proceeds with a very feasible activation barrier via multistate reactivity patterns.**

The addition of molecular oxygen to organic substrates takes part in a broad range of essential biological processes involved in mammalian metabolism. Such reactions are catalyzed by oxygenases which often utilize a transition metal center for the biotransformation of substrates. Two types of transition metal containing oxygenases have been identified, the best known heme[1] oxygenases including Cytochrome P450 and non-heme[2] iron enzymes involved in repair mechanism, biosynthesis as well as biodegradation of compounds. Non heme iron enzymes include a large collection of dioxygenases that are able to transfer both oxygen atoms of molecular oxygen to substrate. A prominent structural motif in these enzymes is a triad of ligands arranged in a mutually cis geometry by which they anchor the metal comprised of two histidines and a carboxylate side chain (2-His-1-Asp facial triad).[3] Unlike most non-heme Fe(II) dioxygenase, there are some members that deviate from this archetype still being able to catalyze dioxygenation reaction.[4] One of these enzymes is cysteine dioxygenase (CDO),[5] which adopts a (His)$_3$ facial triad by substitution of the Asp residue. The effects of such Asp-His replacement were previously reviewed.[6] Moreover, it has been shown that the replacement of the 3 His ligands system by a 2-His-1-Asp triad disrupts the dioxygenation process of cysteine, in particular leading to a weakening of the Fe-S bond.[7] CDO is an essential enzyme able to catalyze the oxidation of toxic cysteine to cysteine sulfinic acid, which is the first major step in cysteine catabolism in mammalian tissues. Such process is vital in human health and the absence or lack of activity of CDO has been linked to a number of disease states correlated with high levels of cysteine in the body, such as Alzheimer's, Parkinson's [8] and motor neuron diseases.[9] Localized accumulation of cysteine and decreased activity of CDO has been observed in patients suffering from the rare neurological disorder Hallervorden–Spatz disease.[10] In addition, a lack of its activity has been observed in malignant tumor cells, [11] as a consequence also CDO behaves as a multitude of enzymes whose expression is altered in cancer cells. Therefore, understanding the catalytic mechanism by which CDO metabolizes cysteine to cysteine sulfinic acid is of great interest even to biotechnological industry for the catalytic dioxygenation of sulphides. The exact mechanism of cysteine oxidation is still controversial even if several works appeared in the last years, focusing on its catalytic bioconversion, from both experimental and theoretical point of views.[12,13] While several mimics of many oxygenating non-heme enzymes were proposed in the last decades,[14] to date there are only few biomimetic models of cysteine dioxygenase available in literature. Recently Limberg and co-workers[15] were successful in obtaining a good realistic model of active site of CDO which meets both structural and function similarities with CDO. They chose a trispyrazolylborato ligand system bound to a Fe(II) center to simulate the (His)$_3$ triad and a chelating cysteinato, so that the coordination of iron resembles that of the same metallic center in native CDO. (Scheme 1)
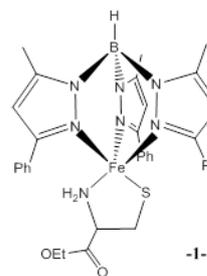
Scheme 1: CDO biomimetic complex [Tp^{Me,Ph} FeCysOEt] [15] **-1-**

The mechanism by which such compound catalyzes the formation of cysteine sulfinic acid is not known but it could show similarities with the CDO enzymatic reaction. The proposed biomimetic complex, meets two important requirement: 1) Structural similarity with the native CDO enzyme; the Tp^{Me,Ph} ligand reliably mimics the His$_3$-coordination sphere of the FeII center, and the cysteine

substrate was only slightly modified by esterification; 2) The function is simulated: treatment with dioxygen was found to lead mainly to cysteine dioxygenase activity, which was confirmed by isotopic labeling experiments.[15] A correct understanding of the several steps involved in the biotrasformation of cystein by compound **-1-** would allow to gain information regarding the performance of the biomimetic model in catalyzing the reaction of the native non-heme enzyme and stimulate future investigations and application of such compound, or to propose new and more efficient systems. Herein, to establish the mechanism of cysteine oxygenation by the biomimetic compound **-1-**, a detailed DFT study also including solvent effects is presented (Computational details are reported in the SI section). All calculations were done for the lowest singlet, triplet and quintet spin states. Such exploration is required since several crossing between the three spin-state surfaces have been previously reported for both CDO and its model complex,[15,16] as well as for other heme and non-heme dioxygenases also showing two-state reactivity.[17] As a consequence, it can be hypothesized that also such biomimetic compound could show multistate reactivity patterns. It is known that the choice of the exchange-correlation (**XC**) functional, in transition metal complexes, could influence the spin-state ordering.[18,19] Accordingly, to establish the ground-state of the reactant molecule **-1-**, that is the complex prior to coordinating the molecular oxygen, preliminary calculations were done and the results are reported in the SI section. All the tested functionals (B3LYP, BP86 and B3LYP-D) predict a quintet spin ground state for the reactant **-1-** (See Table S1 in the SI section). A quintet ground state was also previously found for other structural and functional synthetic models of CDO. [16,20]

The geometrical parameters of the computed quintet ground state are in a very good agreement with those derived from the crystallographic structure. As a result, the DFT-optimized structure is well superimposed to the X-ray structure, as it can be observed in the SI section (Fig. S1).The superposition of the X-ray structure with the optimized singlet and triplet spin states are also reported in the same Figure.

The potential energy profile for the oxygen transfer to the cysteinato ligand of **-1-**, is reported in Figure 1. The oxygen activation process here investigated, begins with the coordination of oxygen to the reactant molecule -1- instead of considering the subsequent iron(II)-superoxo complex as starting point, as previously done for CDO enzymatic reaction.[13,16,20] Actually, the molecular oxygen approaching the reactant is the first step analyzed in the current investigation. All the involved stationary points at their lowest-energy spin states are reported in Figure 2 and their most relevant geometrical parameters are collected in table S2. It is noteworthy that the herein explored dioxygenation process of the cysteinate ligand represent the key step of the whole catalytic process illustrated in the SI section (See Scheme S1). The reaction starts with the formation of the adduct between complex **-1-** and molecular oxygen lying far from it in the second coordination sphere, named **int0**. This adduct was found to have almost the same energy in the case of singlet and quintet spin states although they, obviously, differ from electronic point of view. On the basis of computations, the singlet spin state arises from an antiferromagnetic coupling between two unpaired iron electrons and the unpaired electrons of molecular oxygen (spin density on iron 1,99; spin density on each oxygen atom: -0,99 and -1,01). The quintet spin state arises from the ferromagnetic coupling between these electrons (spin density on iron 1,99; spin density on each oxygen atom: +0,99 and +1,01). This could be the reason why singlet and quintet states have the same energy, having them the same orbital occupation, while the triplet one is located at 10.2 kcal/mol above them.

The oxygen approaches the iron ion in an end-on fashion in the subsequent transition state **ts0**, with an activation barrier of 10.8 kcal/mol, on the lowest-lying quintet spin-state surface. Both singlet and triplet state are found higher in energy. The vibrational mode of **5ts0** clearly indicates the formation of the bond between Fe(II) and one oxygen of the O_2 molecule, at a distance of 2.627 Å (Fig.1b and TableS2).
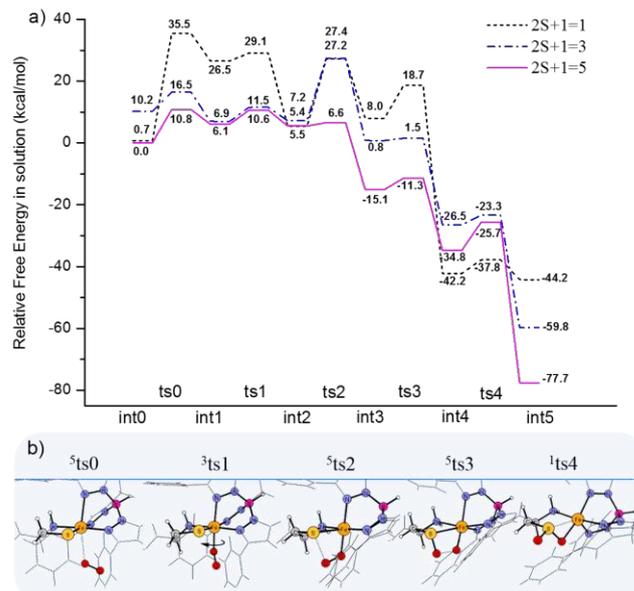

Figure 1: a) Potential energy profile at B3LYP/6-311+G(2d,2p)/SDD/CH_2Cl_2 relative to dioxygen activation by complex **-1-**, starting from int0 complex [**1** + O_2 adduct]; b) snapshots of all the involved transition states on the lowest-lying spin-state surface.

In the resulting superoxo complex (**int1**), the bond between iron and oxygen is completely formed. Although such compound is found to be more stable in a quintet spin state, the superoxo species can exist in the close-lying triplet spin state, being very small the energy difference between them. The molecular oxygen coordinated to iron in **int1** requires rotating to be likely to attack the sulfur atom of the cysteinato ligand. Such rotation takes place in the transition state **ts1**, which also in this case, has been found to have a quintet and triplet spin states very close in energy. On the quintet surface it requires an energy barrier to be overcome of about 4.5 kcal/mol, with respect to the previous **int1** point. Such rotation leads to the formation of the **int2** in which the distal oxygen points now toward the sulfur atom. Such intermediate represents the starting point of a mechanistic work done on CDO enzyme.[13g] In analogy to previous indications,[13g] **int2** is also found here as a singlet ground-state. A further spin-crossing from singlet to quintet spin state is thus observed at this stage of the reaction mechanism. It is noteworthy that, the attack of the superoxo group on the cysteinate sulfur atom was proposed as rate determining step of CDO reaction mechanism,[13g] but in that case the coordination of the oxygen to the reactant was not taken in consideration. An alternative intermediate was herein found along the singlet spin surface, that is, a persulfenate intermediate, which however results less stable than **int2** and can be ruled out (see Fig. S2). That kind of compound has been previously detected and proposed in a X-ray study on a CDO enzyme, although it was not able to ascertain whether the persulfenate is an intermediate in the catalytic cycle or a dead-end product from a side reaction.[12c]
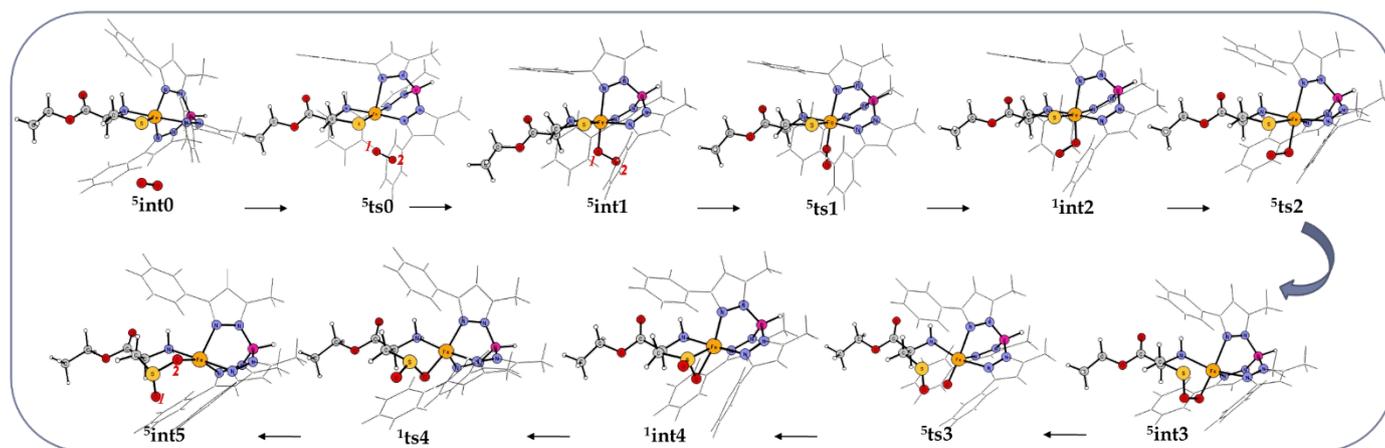
Figure 2. Optimized structures of the stationary points at their lowest-energy spin states for the cysteine sulfinic acid formation catalyzed by Trispyrazolylborato Iron Cysteinato complex -1-

The coordination of the distal oxygen atom to cysteinato ligand takes place in the **ts2** (d:1.890Å) in which the vibrational mode indicates the formation of the bond between sulfur and oxygen.(See Table S2). The transition state lies on the lowest quintet spin-state being both **^{3}ts2** and **^{1}ts2** barriers higher in energy, that is over 20 kcal/mol. Such evidence has been also previously found for another CDO biomimetic complex for which the S-O bond formation barrier was found on the quintet spin state surface.[16] This could mean that, although the singlet **^{1}int2** is slightly low in energy, it could have a little effect on the reaction mechanism due to the greater amount of energy required to overcome both **^{1}ts2** and **^{1}ts3**. Actually, also the subsequent **int3**, in which the molecular oxygen is bis-coordinated to metal ion and sulfur atom, lies on the lowest quintet spin state. A significant energy difference with the same stationary point lying on the triplet and singlet spin state surfaces has been found, unequivocally indicating that the ring structure **int3** exists in a quintet state. The O-O bond breaks in the **ts3** point, still lying on the quintet spin surface, with a very feasible energy barrier equal to 3.8 kcal/mol. A spin crossing with the singlet-spin state profile leads to the highly stable **^{1}int4** species in which one oxygen has been transferred on the sulfur atom and the other one assumes a bridging position between it and Fe(II). To reach the final product of the reaction, the bond between iron and sulfur breaks in the last transition state (**^{1}ts4**) generating the cysteine sulfinic acid still coordinated to the Fe^{II} moiety (**int5**). The latter results more stable in the quintet spin state. It is worth noting that a considerably different **int4** was found along triplet and quintet spin surfaces (**^{3}int4'** and **^{5}int4'**) compared with the most stable **^{1}int4** (See Fig. S3). Actually, it was not possible to localize on these surfaces neither a complex similar to that found along the singlet spin state, nor a transition state analogous to **^{1}ts4**. In the intermediate structures, as it can be observed in Fig.S3, the distance between Fe and S increases a lot so that they result not bound at all. Moreover, each of them is coordinated to one oxygen atom, forming a sulfoxide and an iron-oxo specie. On the contrary, along the singlet surface, iron and sulphur are still coordinated and they share a bridging oxygen atom. This structure results accordingly, very low in energy due to a better charge distribution of the positive charge localized on iron center. The subsequent transition state lying on these higher spin surfaces, is characterized by an imaginary frequency clearly showing the formation of the bond between the Fe-coordinated oxygen and sulphur, which then evolve in the cysteine sulfinic acid, already described (See Figure S4). The energy difference between them and

that lying on the singlet spin state profile is large enough to hypothesize that, if the spin crossing between these surfaces is efficient, the reaction mechanism will surely proceed through **^{1}int4** e **^{1}ts4**. The same Fe^{II}-product (**int5**) is formed along the three spin state surfaces with a considerable energy gain, in analogy to what also found for both CDO[13g] and biomimetic models.[16]

The whole process described in the Scheme S1 results highly thermodynamically favored. The ΔG of the reaction starting from the primary catalyst Tp^{Me,Ph}FeCl reacting with cysteine and leading to the cysteine sulfinic acid restoring the catalyst, is computed to be -48.6 kal/mol (See SI section).

Looking at the energetic profile reported in Figure 1, the O$_2$ activation process seems to proceed rapidly with the molecular oxygen coordination to the trispyrazolylborato iron cysteinato complex resulting the rate determining step of the reaction. Computations sustains the hypothesis that the hitherto most realistic model for the active site of cysteine dioxygenase[15] here investigated, efficiently undergoes dioxygenation process leading to the sulfinic acid product.

In summary, the mechanism of [Tp^{Me,Ph} FeCysOEt] dioxygenation process, has been investigated at DFT level. The iron ion enables a stepwise oxygen atom transfer mechanism in which the first step, after the molecular oxygen coordination, is the rotation of it to reach an optimal alignment to perform the S-oxygenation process. The metal, hence, hold the substrate in a tight but specific orientation to allow the formation of a 4-membered ring structure which undergoes dioxygen bond cleavage, and after the iron-sulfur breaking, ultimately leads the cysteine sulfinic acid product.

Conclusions

The O$_2$ activation by a realistic model of CDO active site, i.e. a trispyrazolylborato iron cysteinato complex[Tp^{Me,Ph} FeCysOEt], has been herein investigated at density functional level of theory to gain mechanistic details on the unknown reaction mechanism. The functional model acts, in some extents, similarly to the native CDO enzyme,[13g] indicating that this complex can efficiently mimic the enzymatic process. The reaction proceeds via multistate reactivity patterns on competing singlet, triplet and quintet spin state surfaces. Calculations reveal that the rate determining step of the reaction is the molecular oxygen coordination to the trispyrazolylborato iron cysteinato complex, which requires a very feasible energy barrier to be overcome equal to 10.8 kcal/mol. Accordingly, the

whole process seems to proceed fast, confirming the ability of the biomimetic compound to efficiently activate the molecular oxygen producing the cysteine sulfinic acid product.

Notes and references

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Electronic Supplementary Information (ESI) available: [Relative Energy of the singlet, triplet and quintet spin state of reactant **-1-** , calculated with different XC functional; Superposition of the optimized reactant with X-ray structure; Scheme describing the whole catalytic process starting from the primary catalyst [TpMe]PhFeCl; optimized stationary points at their lowest-energy spin states and most relevant geometrical parameters; Structure of the high-energy persulfenate intermediate; Optimized structure of **int4**' and **ts4'** lying on the higher triplet and quintet spin surfaces; List of gas phase and solution SCF Energy values, Entropy and Enthalpy contributions;]. See DOI: 10.1039/c000000x/

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