The mechanism of the reaction of intradiol dioxygenase with hydroperoxy probe. A DFT study.

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INTRODUCTION

Intradiol dioxygenases are non-heme Fe(III)-dependent dioxygenases found in soil bacteria that catalyze oxidative cleavage of a catecholic substrate. They specifically cleave the aromatic ring between the two carbon atoms bound to hydroxyl groups. The dioxygenase nature of these biocatalysts manifests in that two oxygens originating from O_2 are incorporated into the product. In the resting state the ferric ion is coordinated by five ligands (two tyrosines, two histidines and OH group) arranged in the trigonal bipyramid geometry. During the substrate binding both water and axial tyrosine dissociate from the metal making space for the dianion of the substrate, which chelates Fe(III) with its both oxygens. Experimental evidence concerning the mechanism of the catalytic steps following substrate binding is scarce. It was suggested that binding of dioxygen leads to species with a peroxide bridge between the ferric ion and catechol substrate. There are two possible mechanisms, the first where the proton is released, the O-O bond cleavage yields species, which features a reactive oxoferryl group and an alkoxyl radical (Figure 3) or the second, which assumes the proton remains in the active site, the peroxide oxygen atom which is proximal with respect to Fe(III) is protonated (Figure2). The first mechanism has recently been proposed by Xin and Bugg based on their experimental findings obtained for 2-hydroperoxy-2-methylocyklohexanone which is a mechanistic probe reacting with intradiol dioxygenase.

PURPOSE

In this work we studied, by means of theoretical methods, the reaction mechanism for intradiol dioxygenase reacting with the mechanistic probe. The theoretical studies were focused on finding proper reaction pathway explaining the experimentally observed product **R5**.
All steps for reaction proposed by Xin and Bugg were considered using different models, starting with O-O homlytic cleavage in the peroxo group of substrate bound to active site.

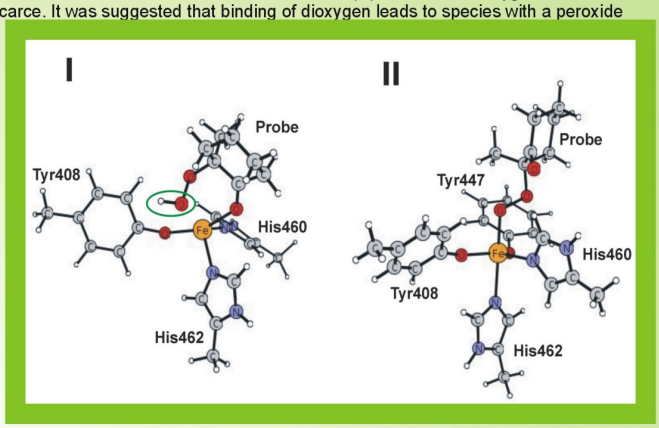

Figure1. The optimized structures for the models I and II of the enzyme-probe complex

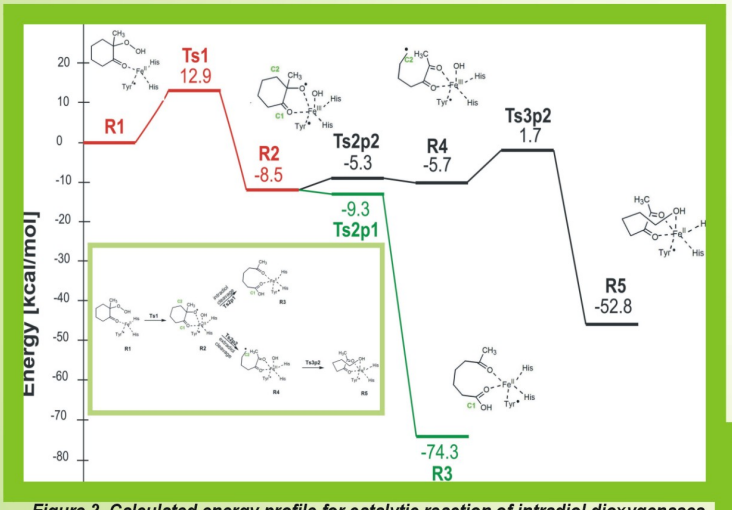

Figure 2. Calculated energy profile for catalytic reaction of intradiol dioxygenases with the mechanistic probe, as derived from the study for the model I.

METHODS

The reaction pathways leading to the two expected products, were characterized for five models of the active site.The models differ in the protonation state of the peroxo group and the presence or absence of the axial tyrosine residue and the OH ligand, which are known to dissociate from the metal when the native substrate binds to the active site. All calculations were performed using density functional theory (DFT) method with a hybrid exchange correlation functional - B3LYP. Geometry optimizations were performed with two programs, Jaguar and Gaussian, employing a double-ζ basis set. For the optimized structures electronic energy was computed with triple-ζ basis set combining *cc-pVTZ(-f)* basis for H,C,N and O atoms, and *lacv3p+* basis for iron. The solvent effects, due to the surrounding protein, were computed with the self-consistent reaction field method implemented in Jaguar. The protein was treated as a macroscopic continuum with a dielectric constant of 4.0, and the solute cavity was obtained with a probe radius of 1.40Å.

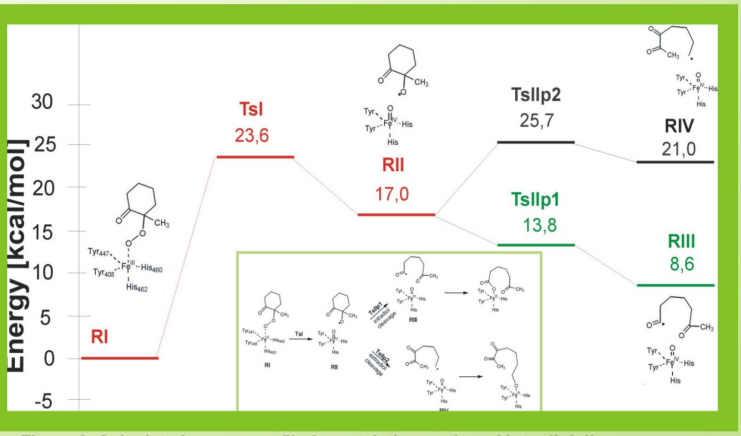

Figure 3. Calculated energy profile for catalytic reaction of intradiol dioxygenases with the mechanistic probe, as derived from the study for the model II.

RESULTS

The reaction was modeled for two spin states: sextet, which is a ground electronic state for all local minima on the reaction path, and quartet, which is a low lying excited state with calculated electronic energy at most 15 kcal/mol higher.
The results obtained for the model I show that if the peroxo group is protonated then the cleavage of the O-O bond is easy (12.9 kcal/mol barrier), the reactive oxoferryl species is not formed, and the preferred pathway for the decay of the alkoxyl radical intermediate **R2** leads to the intradiol cleavage product **R3**. This last observation stays in contrast with the experimental findings showing that **R5** is the sole reaction product. Concluding the results obtained for the model II, when the peroxo group is not protonated, the cleavage of the O-O bond leads to the oxoferryl (Fe(IV)=O) species and an alkoxyl radical derived from the probe. However, the energy barrier for this step is rather high (23.6 kcal/mol), which together with the substantial energy (17.0 kcal/mol) calculated for the radical product indicates that such a process is rather difficult. Like for the model I, the preferred reaction channel leads to the intradiol cleavage products.

CONCLUSIONS

The presence of the proton bound to the peroxo group markedly lowers the activation barrier for the critical O-O bond cleavage step. The lower-barrier path does not lead to the oxoferryl species, but instead to the Fe(III)-OH complex with one of the tyrosine residues oxidized to the tyrozyl radical. Cleavage of the ring in the alkoxyl radical intermediate preferentially leads through the intradiol path, which is consistent with the larger stability of the resulting product. At the present stage there is not enough data on the actual binding mode of the probe within the active site, and thus it is very hard to model the reaction at the required level of accuracy to explain the observed product specificity.