MODELING BIMETALLIC PP5 ENZYME*

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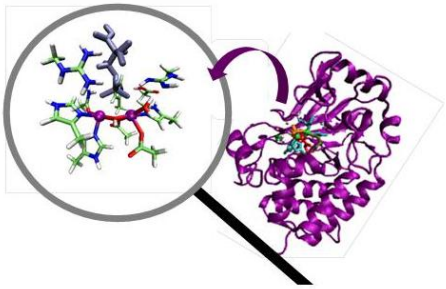
The catalytic mechanism of the Mn(II)-Mn(II) containing Ser/Thr phosphatase 5 (PP5), has been investigated by means of a cluster model approach at DFT level. According to our results, the reaction occurs through an inline concerted transition state with no intermediates formed.



Active site cluster model extracted from the X-ray structure of an enzyme

Metalloenzymes are widespread proteins, ubiquitous in all life kingdoms, being involved in various biosynthetic processes. They require one or more metal ions for full activity and represent approximately one-third of the known enzymes[1]. Among metalloproteins, binuclear hydrolases have received in the last years a considerable attention[2,3,4,5,6,7,8]. They use binuclear metal ion centers to catalyze the hydrolysis of amides and esters of carboxylic and phosphoric acids. Members of this family have been recognized as potential targets for the development of chemotherapeutics, for drug design against a wide variety of human disorders and represent also promising candidates in bioremediation. Although considerable progress in enzyme catalysis has been realized by experimental and theoretical investigations, it is still challenging for both chemists and biochemists to unravel the detailed catalytic mechanism of natural enzymes also for their potential applications[9,10,11]. Actually, a complete and profound comprehension of how enzymes display incredible catalytic efficiency and selectivity together with the exploration of principles of structure and reactivity provide a wealth of opportunity to the creation of new materials such as catalysts and biosensors.
One clear trend in the computational modeling of enzymatic reactions in recent years has been to use relatively small cluster models of enzyme active sites and apply accurate quantum chemical methods to study their reaction mechanisms[12,13,14,15]. With such a model (≈150 atoms), it is

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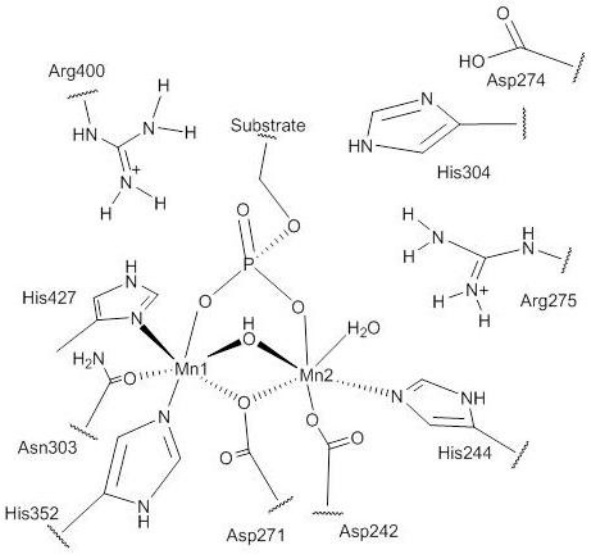
generally possible to identify all structural changes in the model during a reaction and make certain that these changes are not artifacts of the model. A quantum chemical study of a reaction mechanism implies the determination of all intermediates and transition states along the reaction path. The approach of modeling small clusters has proven to be particularly fruitful in the modeling of the catalytic reaction mechanisms of metalloenzymes, since all the important chemical steps take place at the metal ions and their immediate environment. Actually, a large portion of the catalysis is dictated by the electronic structure of the metal ions. A correct model of a metal active site should hence represent the electronic structure of the metal correctly.

In order to elucidate the catalytic mechanism of the Mn(II)-Mn(II)-containing serine/threonine protein phosphatase 5 (PP5), we presented a density functional theory study with a cluster model approach[16].

Ser/Thr phosphatase (PP5) catalyzes the removal of a phosphoryl group from a phosphoserine or phosphothreonine residue of the target protein. The details of the reaction are not known, but the available crystallographic structure with a coordinated phosphate in the active center[17], together with the data on similar enzymes[18], suggest a catalytic mechanism similar to other phosphatases.

The available X-ray structure of the enzyme (PDB: 1S95) [17] was used to build a cluster model of the enzyme-substrate complex. The cluster is composed by the primary coordination sphere of metal ions and some residues of the second coordination sphere that in some extent can be considered as part of the scaffold, since their properties can strongly influence the reactivity of the metal ions. The resulting cluster consists of 128 atoms and includes: Mn(II) ions, two water molecules bound to the Mn ions, the phosphoserine substrate, and the side chain of the ten catalytic residues capped at the α-carbon atom (Asp242, His244, Asp271, His352, His 427, Arg275, His304, Arg400, Asn303, Asp274) (Scheme 1).



Scheme 1 - Schematic representation of the cluster model used in this work

Despite the numerous initial proposals for the mechanism of PPPs[19], a nucleophilic attack by a metals-bound water/hydroxide molecule to the phosphorous atom seems the most plausible hypothesis. Nevertheless, while the location of the potential nucleophile is clearly indicated in the crystal, its nature as a water molecule or hydroxide group cannot be distinguished. Moreover, the protonation state of the water molecule bound to Mn2 is also controversial. Thus, we have built three models of the catalytic center of PP5 that differ in the protonation states of the nucleophile and of the second water molecule. As a consequence, the overall charge of the tested models of the active site varied from 2 to 0. This kind of exploration is required to fully understand the

catalytic mechanism since the activation energy of enzymes that catalyze the formation and breaking of phosphoester bonds is very sensitive to the charge balance around the active center[20]. Actually, by using the charged models, we obtained activation energies clearly outside the typical range for enzymatic catalysis[16].

As a result, a zero-charged active centre has been found to be necessary for the reaction to take place with a reasonable catalytic activity. Such a model has been obtained considering both, the nucleophile and the Mn2-bound water molecule, deprotonated.

On the basis of our results, the hydrolysis of phoshoserine residue catalyzed by PP5 enzyme occur through an inline concerted transition state according to a SN2-like mechanism (Fig. 1).

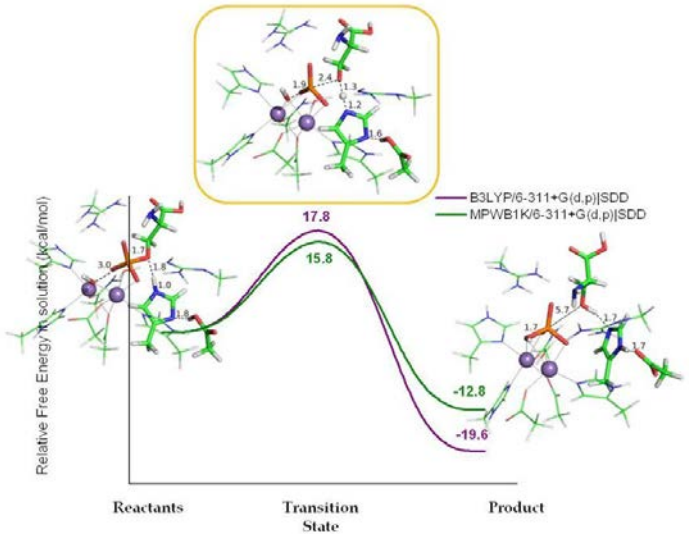


Fig. 1 - Reaction mechanism of Mn(II)-Mn(II) containing Ser/Thr phosphatase

In the ES complex, the substrate results bicoordinated to the metal ions with the nucleophile at 3.01 Å from the phosphorous atom and it is located on the opposite side of the leaving group, as is expected from an inline reaction.

The substrate is further stabilized into the cavity by a strong H-bonds network with uncoordinated His304, Arg 275 and Arg 400 and with the coordinated Asn303.

Arg400 and Arg275, together with the Mn ions, hold the phosphate group in the active center, by counterbalancing the phosphate group's negative charge. Apart from a structural role, manganese and arginines also contribute to catalysis. The energy required to bring together the negative nucleophile to the negative phosphate group would be too high without the counterbalancing charge.

The mechanism proceeds with a concerted transition state in which can be observed the nucleophilic attack performed by bridging metals-coordinated hydroxide to the phosphorus atom of the substrate and the simultaneous departure of the leaving group protonated, at the same time, by His 304.

After the concerted transition state, a non-phosphorylated serine residue is formed. The phosphate ion readopts the tetrahedral conformation, while the coordination sphere of both metals remain unaffected. The serine residue moves away from the active center, while being stabilized by a hydrogen bond with His304. The latter, which has given its proton to the leaving group is protonated back by Asp274.

The reaction activation energy, at the IEFPCM/MPWB1K/6-311+G(d,p)|SDD level and considering the antiferromagnetic coupling, has been calculated equal to 15.8 kcal/mol. This energy is consistent with the experimental data[21] and similar studies on related enzymes[15a]. The reaction has been found to be exothermic, as expected for the breakage of a phosphoester bonds.

In conclusion, our results show that the reaction occurs through an inline concerted transition state, with no intermediate formed, corroborating experimental evidence that showed that PPPs do not form phosphoenzyme intermediates[19]. The role of each residue into the cavity, either catalytic or structural, has been elucidated. We also showed the importance of correctly describe the electronic configuration of the d shell of the binuclear centers, in particular in terms of correctly choosing between high and low spin, and the existence or not of antiferromagnetic coupling. The effect of AFM coupling is usually disregarded in theoretical calculations of enzymatic catalysis, but it can have a significant contribution to the stabilization of the transition-state structure.

On the basis of our results, we think our neutral model captures the real state of the enzyme in the reactants state, and the reaction path obtained for this model is the minimum energy path that PP5 crosses when it catalyzes the phosphate hydrolysis reaction.

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