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Zn(II)-Zn(II) and Cd(II)-Cd(II) Metal-Substituted Phosphotriesterase (pdPTE): Theoretical Analysis of the Structure the Active Site and Interaction with Phosphate Triester Paraoxon

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Abstract: Phosphotriesterases (PTEs) are enzymes with an active site that contains a binuclear metal center composed of ions such as Zn^{2+}, Cd^{2+}, Co^{2+}, Mn^{2+}. These enzymes catalyze the hydrolysis of a vast array of organophosphate triesters, which are chemical species frequently used as insecticides and agricultural pesticides and as chemical warfare nerve agents. [1] Since there is great interest in developing technologies to facilitate degradation of organophosphate contaminants, the study of such enzymes is relevant. [2] In the present work, we investigate the structural properties of PTE/Zn(II)-Zn(II) and PTE/Cd(II)-Cd(II) and how they are influenced by the presence of the substrate Paraoxon, as well as substrate-enzyme interaction, in order to support a more detailed study of the hydrolysis mechanism of organophosphate triesters catalyzed by these enzymes. To this end, we have employed DFT to model the enzymes' active sites based on their crystal structures (PDB codes 1HZY and 1JGM for PTE/Zn(II)-Zn(II) and PTE/Cd(II)-Cd(II), respectively), where the first shell ligands are His55, His57, His201 e His230, Lys169 e Asp301. For the PTE/Zn(II)-Zn(II) system there is one water molecule coordinated to the active site metal ion most exposed to the solvent and for the PTE/Cd(II)-Cd(II) system there are two. The active sites were modeled as cluster structures, in which the His were modeled as methylimidazoles, Lys169 as carboxylated methylamine and Asp301 as acetate. We used functionals B3LYP, X3LYP, TPSSh and M06 and bassets 6-31+G(d) and 6-311++G(d,p) for C, H, O and N atoms and LANL2DZ for Zn and Cd valence electrons. Zn and Cd core electrons were treated with LANL2DZ pseudo potential. We optimized the active site model with and without constraining coordinates of the ligands' methyl carbon atoms. We also used the ONIOM approach in which the active site was treated with DFT while the remainder of the protein was treated at molecular mechanics level for PTE/Cd(II)-Cd(II). Excluding the ONIOM calculation, all calculations were done both for gas and solvated phases. The solvent effect was modeled by IEFPCM, with dielectric constants $\varepsilon = 4$ (standard protein cavity medium value) and $\varepsilon = 80$ (standard aqueous medium value). The influence of the substrate on the enzymes' structural properties was evaluated by a potential surface scan using DFT optimizations at theory level B3LYP, with pseudo potential and basset LANL2DZ for Zn and Cd atoms and basset 6-31G(d,p) for C, H, O,

N and P atoms. System energy, structural data and atom charges were evaluated as a function of the enzyme-substrate distance. Atom charges were calculated by Mulliken and CHELPG methods. Substrate-enzyme interaction was investigated by molecular conformational analysis, which was performed by employing DFT at theory level B3LYP 6-31G+(d) to calculate system energy values for different substrate geometries as it interacts with the enzyme. The resulting conformation was then used in a potential surface scan analogous to the prementioned one. Active site model adequacy was evaluated by RMSD comparison with the crystal structure. Taking into account computational cost, adequacy, and information available in the literature, we chose as best, for both systems, the model optimized at gas phase at theory level B3LYP 6-31G+(d) with the fixed atoms constraint. We found that applying fixed atom constraints did not result in significant change of RMSD for bond lengths. However, the overall RMSD for relevant angles of the constrained system was 6.30, while for the unconstrained system it was 16.61. Our results indicated that constraining the system emulates the effect of the enzyme structure external to the active site that is not modeled in the cluster structure. Based on our active site model, for the Zn system, the distance between the water hydrogen and the bridging hydroxyl oxygen in the active site was found to be 1.845 Å while its distance to the water oxygen was found to be 0.987 Å. For one of the water molecules in the Cd system, theses distances were found to be 1.504 Å and 1.035 Å, respectively. This suggests that these water molecules are not very labile and could thus participate in the hydrolysis mechanism. From the potential surface scan, we found that for PTE/Zn(II)-Zn(II), system energy increases as the distance enzyme-substrate decreases. Contrastingly, for PTE/Cd(II)-Cd(II) we have observed an energy decrease as this distance decreases. It is therefore suggested that PTE/Cd(II)-Cd(II) catalysis is favorable over the Zn enzyme. Furthermore, for PTE/Cd(II)-Cd(II), we observed a significant decrease of distance between the water hydrogen and the hydroxyl oxygen as the substrate approaches de enzyme. At r = 4 Å, this distance is 1.66 Å, whereas at r = 2.2 Å, the distance is 1.61 Å and the hydrogen is acidic, since its charge is positive. We could not conclude the conformational analysis for the abstract submission date, but believe that it will be finished for the poster presentation. We also intend to compare the result with molecular docking. From our study, in addition to modeling the active site, which is fundamental for a more detailed mechanism investigation, we were able to analyze structural properties that support a hydrolysis catalysis mechanism in which there is participation of the coordinated water molecule.

Key-words: Organophosphates, Phosphate Triesters, Reaction Mechanisms
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