

Cd(II)-Cd(II) Metal-Substitued Phosphotriesterase: Theoretical Analysis of A New Enzymatic Mechanisms to Paraoxon Phosphate Triester Hydrolysis

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Abstract: Among the numerous phosphates, triesters are employed widely as agricultural insecticides and chemical warfare nerve agents. Unfortunately, the extreme toxicity of phosphotriesters makes the experimentations of the chemical destruction for the purposes of chemical defense extremely difficult. Therefore, there is great interest in developing bioremediation technologies to facility the degradation of organophosphate contaminants including the disposal chemical weapons. The enzyme PTE from *Pseudomonas diminuta* is particularly attractive as a biocatalyst because a large number of phosphotriesters can be hydrolyzed. This enzyme has in its native form a active site containing divalent zinc metal cations. The two native Zn^{2+} ions can be substituted with either Cd^{2+} , Co^{2+} , Ni^{2+} , or Mn^{2+} with the restoration of the full catalytic activity [1]. The remarkable enhancement of the hydrolysis of organophosphates catalyzed by the wild-type PTE can be exemplified with paraoxon (diethyl 4-nitrophenyl phosphate) as the best substrate. Hong and Raushel determined the kinetic rate constant for the hydrolysis of paraoxon by wild-type PTE is $k_{cat} = 2100 \text{ s}^{-1}$ at 298 K, which can be translated into a free-energy barrier of $12.9 \text{ kcalmol}^{-1}$. The alkaline hydrolysis of paraoxon in aqueous solution takes place with a second-order rate constant of $7.5 \times 10^{-2} \text{ M}^{-1}\text{s}^{-1}$ at 298 K with a free-energy barrier of $18.9 \text{ kcalmol}^{-1}$ [2]. Several theoretical analyses have been conducted to elucidate the mechanism of phosphotriester hydrolysis in the PTE active site, although, for different reasons, none of them offers a complete picture of the process [2]. In the present work, we investigate the PTE/Cd(II)Cd(II) system, in order to support a more detailed study of the hydrolysis. A new mechanism of organophosphate paraoxon triester is proposed in which the nucleophile is formed in situ through the activation of a water molecule present in the active site of the enzyme. Thus, the utility of a bridging μ -hydroxo as the specific nucleophile in binuclear enzyme centers has been questioned. We have in all calculations employed DFT to model the enzymes' active sites based on their high resolution crystal structure obtained from PDB (1JGM) for PTE/Cd(II)-Cd(II). The five amino acids that is directly



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coordinated to the two metal ions are His55, His57, His201, His230 and Asp301. The metals are bridged by a carboxylated lysine (Lys169) and a μ -hydroxo ion. The two metal ions are designated as α and β . The β metal is more solvent-exposed and coordinated by two water molecules. The active site was modeled as cluster structure, in which the histidines were modeled as methylimidazoles, Lys169 as carboxylated methylamine and Asp301 as acetate. We optimized the active site model with and without constraining coordinates of the ligands' methyl carbon atoms. So, geometries of all reactant complexes, transition states, intermediates and product complexes involved in this study were fully optimized by using density functional theory (DFT). We used functional B3LYP and standard basis set 6-31+G(d) for C, H, O and N atoms and LANL2DZ for Cd valence electrons. Cd core electrons were treated with LANL2DZ pseudo potential. All calculations were done in gas phase. In addition, the solvent effect was modeled by PCM and SMD continuum models, with dielectric constants $\epsilon = 4$ (standard protein cavity medium value) and $\epsilon = 80$ (standard aqueous medium value) using the standard basis set 6-31+G(d) and 6-311++G(2d,2p) using optimized geometries in single-point energies calculations to roughly estimate solvation energies in aqueous solution. All calculations were completed using the Gaussian 09 program package. The most system study is with Zn(II)-Zn(II) metal-substituted phosphotriesterase. Thus, the more elaborated mechanism concerning a proton relay from the μ -hydroxo bridge nucleophile to Asp301 then to His254 and finally to Asp233 has been proposed. In the one of these simulations studies reported a energy barrier of $18.3 \text{ kcal mol}^{-1}$, and the value derived from the experimental rate constant is $12.8 \text{ kcal mol}^{-1}$ for the wild-type PTE. In this work we proposed that only the P=O oxygen is bound to one zinc ion, Zn(II) $_{\beta}$, more exposed to the solvent in the product complex. In contrast to all above theories, Jackson *et al.* claimed that the μ -hydroxo bridge is not a nucleophile but acts as a base for freely exchangeable nucleophilic water molecule. However, the efficiency by the hydrolysis verification by Cd(II)-Cd(II) enzyme type with the pseudo-first-order rate constant $V_{\max}=12900 \text{ s}^{-1}$, $k_{cat}=2500 \text{ s}^{-1}$ and reported a energy barrier of $11.8 \text{ kcal mol}^{-1}$. Our structural studies model with Cd²⁺ containing *pdPTE* active site includes the coordinated water molecules which are stabilized by several hydrogen bonding interactions. In the present study we propose that the nucleophilic hydroxide ion is generated by a proton transfer from the coordinated water molecule to the bridged μ -hydroxo ion and formation of the nucleophile OH⁻ in situ. The free-energy barrier obtained is $10.8 \text{ kcal mol}^{-1}$ in excellent agreement with experimental value reported above. More details of the mechanism and the theoretical methodology will be presented.

Key-words: Organophosphates, Phosphate Triesters, Paraoxon, Reaction Mechanisms.

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