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Theoretical Study of the Phosphotriesterase Reaction Mechanism

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Phosphotriesterase (PTE) is a binuclear zinc enzyme that catalyzes the hydrolysis of extremely toxic organophosphate triesters. In the present work, we have investigated the reaction mechanism of PTE using the hybrid density functional theory method B3LYP. We present a potential energy surface for the reaction and provide characterization of the transition states and intermediates. We used the high resolution crystal structure to construct a model of the active site of PTE, containing the two zinc ions and their first shell ligands. The calculations provide strong support to an associative mechanism for the hydrolysis of phosphotriesters by PTE. No protonation of the leaving group was found to be necessary. In particular, the calculations demonstrate that the nucleophilicity of the bridging hydroxide is sufficient to be utilized in the hydrolysis reaction, a feature that is of importance for a number of other di-zinc enzymes.

I. Introduction

Phosphotriesterase (PTE) is a bacterial enzyme that catalyzes the hydrolysis of a wide range of organophosphate triesters,[1] some of which are employed as agricultural insecticides and chemical warfare nerve agents. The X-ray crystal structure of PTE revealed that the active site contains a binuclear zinc center that is bridged by a hydroxide from the solvent and a carboxylated lysine residue.[2] Four histidines, an aspartate, and a water molecule complete the first coordination sphere around the zinc ions. Three distinct binding pockets at the active site help in orienting the substrate. PTE is a very efficient enzyme, with a turnover number close to 10^4 s^{-1} for the best substrates and a k_{cat}/K_m value of 10^8 M^{-1} s^{-1}, which is close to the diffusion-controlled limit.[3] It has been shown that the reaction proceeds with inversion of the stereochemical configuration of the phosphorus center and that the water oxygen is found in the phosphate product.[4] On the basis of the crystal structure and various biochemical, kinetic, and spectroscopic experiments, a reaction mechanism has been proposed for PTE.[5,6] In this mechanism, the substrate first binds to the more solvent-exposed zinc (Zn_β) by displacing a water molecule. The bridging hydroxide then performs a nucleophilic attack on the phosphate, resulting in the cleavage of the phosphotriester bond in an S_N2-like single displacement reaction. A water molecule then displaces the phosphate product and the active-site hydroxide is regenerated.

In the present work, we have investigated the reaction mechanism of PTE using the hybrid density functional theory (DFT) functional B3LYP.[7] We present a potential energy surface for the reaction and provide characterization of the transition states and intermediates involved.

II. Results

We used the high resolution crystal structure[2b] (PDB code 1HZY) to devise a model of the active site of PTE. The model contains the two zinc ions and their first shell ligands, including the bridging hydroxide, the four histidines (His55, His57, His201, and His230), the carboxylated Lys169, and the Asp301. The ligands were truncated such that in principle only the side chains were kept. The histidines were thus modeled by imidazoles, the aspartate by an acetate, and the carboxylated lysine by a carboxylated methylamine. The total charge of the model is +1. The substrate used in the investigation is dimethyl 4-nitrophenyl phosphate, which was bound to the more solvent-exposed β-Zn site.[8] Consistently with previous quantum chemical optimizations of the PTE active site,[9] the overall geometric parameters obtained from the geometry optimization of the present model of the PTE active site (Figure 1) agree very well

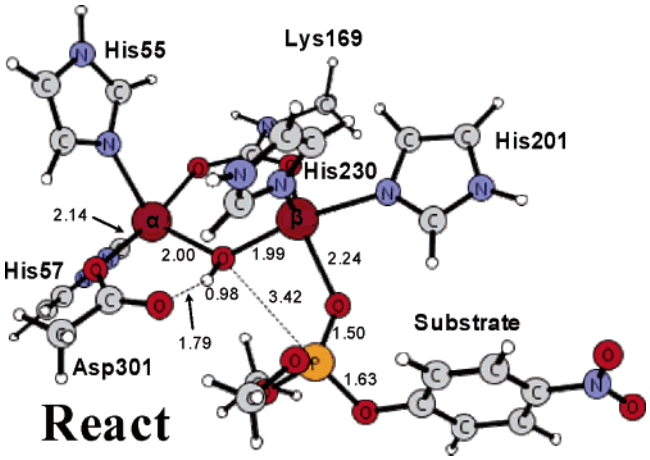

Figure 1. Optimized structure of the PTE active site with dimethyl 4-nitrophenyl phosphate bound. Distances are in angstroms.

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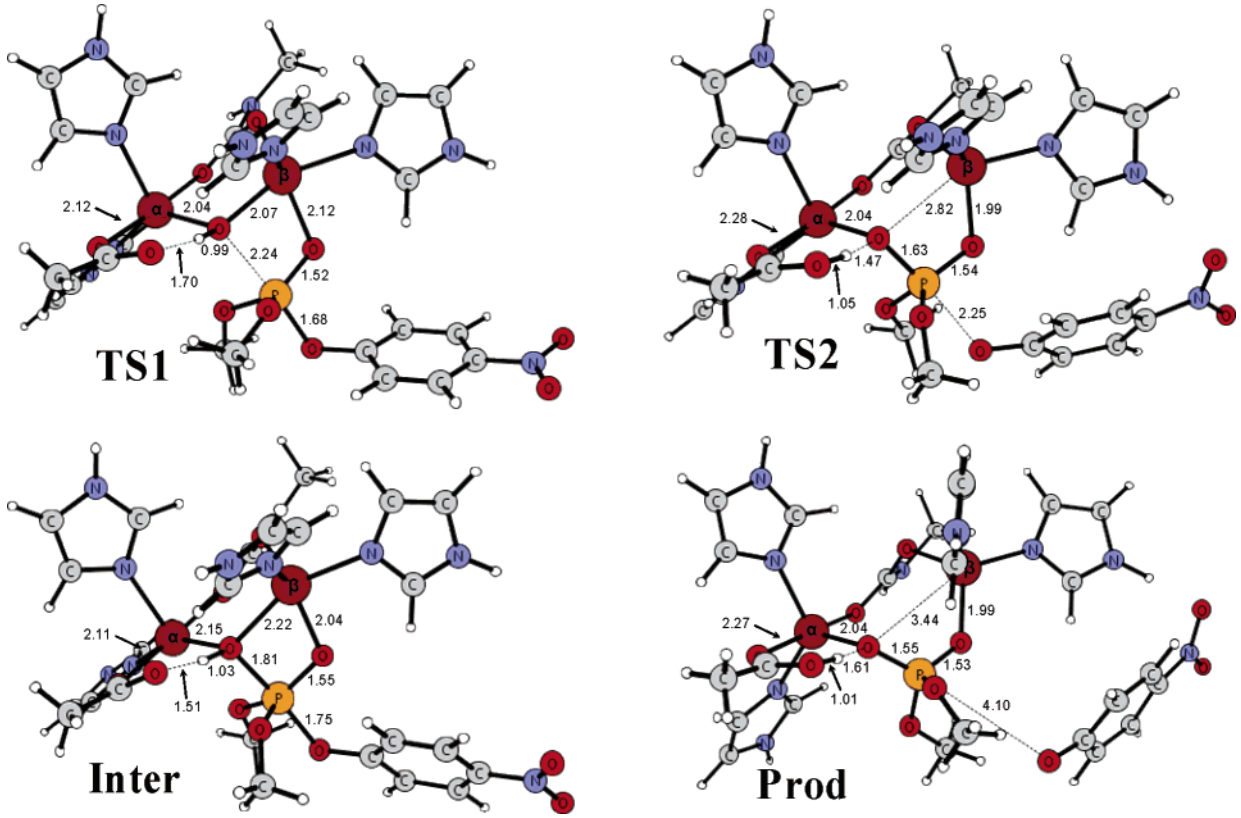

Figure 2. Optimized structures of the transition state for the bridging hydroxide attack on the phosphorus (TS1), the resulting penta-coordinated intermediate (Inter), the following transition state for the P−O_L bond cleavage (TS2), and the resulting enzyme−product complex (Prod).

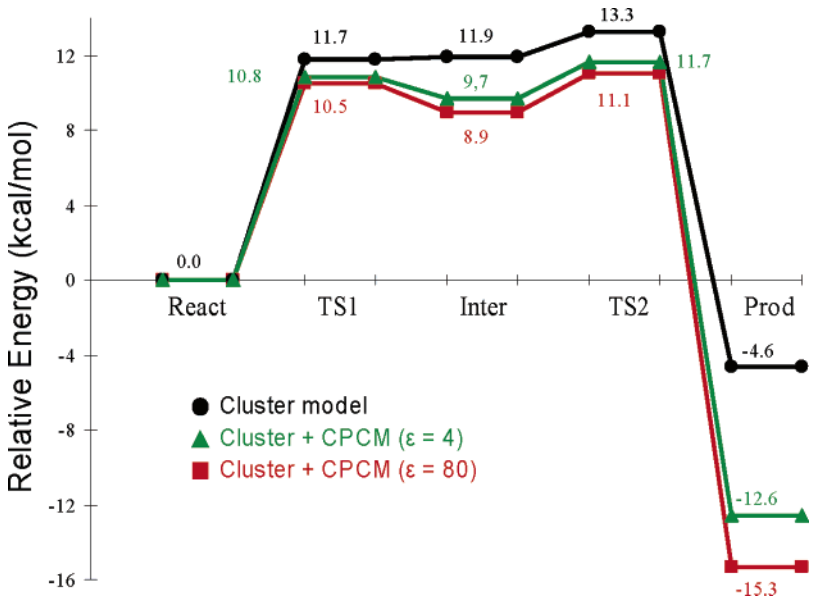

Figure 3. Potential energy profile for the PTE reaction with dimethyl 4-nitrophenyl phosphate as a substrate: (Cluster) B3LYP/6-311+G(2d,2p) energies with zero-point energies included; (CPCM) CPCM solvation effects added with different dielectric constants ($\epsilon = 4$ or 80).

with the X-ray structure. For example, the bond distances of the bridging oxygen to the two zinc ions are calculated to be 2.00 and 1.99 Å, to be compared to the crystallographic distances of 2.0 Å. The distance between the bridging oxygen and the phosphorus center is 3.42 Å.

The transition state for the nucleophilic attack by the bridging hydroxide on the substrate (TS1, Figure 2) was optimized and confirmed to be the first-order saddle point with only one imaginary frequency (105i cm^{-1}). At TS1, the key distance between bridging oxygen and the phosphorus center (O_μ−P) is 2.24 Å, while the distance between P and the oxygen of the 4-nitrophenyl leaving group (P−O_L) is 1.68 Å. The calculated energetic barrier for this step is the very feasible 11.7 kcal/mol (Figure 3) relative to the reactant complex of Figure 1.

To approximately estimate the energetic effects of parts of the enzyme that are not included in the quantum chemical model, we applied a homogeneous dielectric field according to the CPCM method,[10] with two dielectric constants, $\epsilon = 4$ and $\epsilon = 80$. As seen from Figure 3, this does not change the energetics of the first step significantly. The barrier is slightly reduced to 10.8 and 10.5 kcal/mol, for $\epsilon = 4$ and $\epsilon = 80$, respectively.

The nucleophilic attack results in a penta-coordinated intermediate (Inter, Figure 2), which is calculated to be located in a shallow minimum, ~1.5 kcal/mol lower than TS1. The $P-O_L$ bond breaks with a barrier of only ~2 kcal/mol relative to the intermediate (~12 kcal/mol relative to the reactant species of Figure 1). It turns out that, simultaneously with the $P-O_L$ bond cleavage, the proton of the bridging hydroxide is transferred to the Asp301 residue, resulting in a phosphate anion bridging the two zinc ions in the enzyme−product complex (Prod, Figure 2). The nature of the transition state (TS2, Figure 2) was confirmed by frequency analysis to have a single imaginary frequency of 113i cm^{-1}. At TS2, the critical $O_\mu-P$ and $P-O_L$ bond distances are 1.63 and 2.25 Å, respectively.

In this step, the CPCM solvation effects are more significant, because the leaving group departs as an anion. The combined exothermicity of the two steps is calculated to be 4.6 kcal/mol in the cluster model, and 12.6 and 15.3 kcal/mol when $\epsilon = 4$ and $\epsilon = 80$ are applied, respectively (see Figure 3). It is interesting to monitor how the bond distances of the bridging hydroxide to the two zinc ions change during the reaction. From two symmetric bonds of 1.99 and 2.00 Å in the reactant species, the distances increase to 2.22 and 2.15 Å to β and α metals, respectively, in the penta-coordinated intermediate structure. In the product species, the bond to the β-Zn is broken completely (3.44 Å) while the bond to the α-Zn is maintained (2.04 Å).

Another interesting parameter is the distance between the phosphoryl oxygen and the β-Zn. From a quite weak binding of 2.24 Å in the enzyme−substrate complex, the distance is decreased to 2.04 Å in the penta-coordinated intermediate and 1.99 Å in the enzyme−product complex. These bond lengths indicate that the role of the β-Zn ion is to stabilize the intermediate and product structures, thereby lowering the barriers for the nucleophilic attack and the $P-O_L$ bond cleavage steps. This explains the increased nucleophilicity of the bridging hydroxide, a property that has been questioned.[11]

III. Conclusions

In summary, the calculations presented here provide strong support to an associative mechanism for the hydrolysis of phosphotriesters by PTE, consistently with primary and secondary ^{18}O isotope effects.[12] No activation (protonation) of the leaving group was found to be necessary. We have, furthermore, demonstrated that the nucleophilicity of the bridging hydroxide is sufficient to be utilized in the hydrolysis reaction, a feature that is of importance for a number of other di-zinc enzymes.[13]

IV. Computational Details

All calculations were performed using the density functional theory (DFT) functional B3LYP,[7] which is composed of Becke's three-parameter hybrid exchange functional (B3) and the correlation functional of Lee, Yang, and Parr (LYP). Geometry optimization was carried out with a 6-31G(d,p) basis set for C, H, O, N, and P elements and a LANL2DZ basis set for Zn. On the basis of these geometries, more accurate energies were obtained by performing single-point calculations with the larger basis set 6-311+G(2d,2p) for all elements. Solvent effects were calculated at the same theory level as the optimizations by performing single-point calculations on the optimized structures using the CPCM method.[10] In this model, the surrounding is represented by a constant dielectric medium outside a cavity containing the solute. The dielectric constant (ϵ) was chosen to be 4, which is the standard value used in modeling protein surroundings. In the phophotriesterase (PTE) system, the leaving group pocket is exposed to the solution; therefore, $\epsilon = 80$ is also used to simulate the solution's influence on reaction energies. Frequency calculations were performed at the same theory level as the optimizations to obtain zero-point energies (ZPE) and to confirm the nature of the stationary points. The latter implies no negative eigenvalues for minima and only one negative eigenvalue for transition states. All calculations were performed using the Gaussian 03 program package.[14]

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Supporting Information Available: Cartesian coordinates of optimized stationary points and Mulliken charges of selected atoms. This material is available free of charge via the Internet at http://pubs.acs.org.

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