QM/MM study on the spontaneous reactivation mechanism of (±)methamidophos-inhibited-acetylcholinesterase

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ABSTRACT

As an organophosphorus pesticide with high toxicity, methamidophos has been used globally to control a variety of pest insects. It can inhibit acetylcholinesterase (AChE) of nerve cell and cause respiratory failure or even death. In this work, the spontaneous reactivation mechanism of (−)-MeP-inhibited human AChE (Path A) and (+)-MeP-inhibited human AChE (Path B or path C) was studied with QM/MM method. All the intermediates and transition states were optimized at the B3LYP/6-31G(d)//CHARMM22 level, and single point energies of these optimized geometries were calculated at the B3LYP/6-311++G(d,p)//CHARMM22 level. This study reveals that the spontaneous reactivation mechanism is composed of three steps, i.e., the nucleophilic attack on the P atom by a water molecule, the reorganization step, and the dephosphorylation step. The nucleophilic attack is the rate-determining step. All the intermediates of the three paths exhibit the trigonal bipyramidal character. The highest energy barriers of path A, path B and path C are 22.7 kcal/mol, 20.0 kcal/mol and 25.6 kcal/mol, respectively. This indicates that path B initiated by H_2O^{ω} is the dominate spontaneous reactivation pathway of (+)-MeP-inhibited human AChE. Compared with path A, the relatively lower energy barrier of path B indicates that (+)-MeP-inhibited human AChE is more feasible to be spontaneously reactivated than (−)-MeP-inhibited human AChE.

1. Introduction

Methamidophos (O, S-dimethyl phosphoramidothioate, MeP) is a highly efficient and broad spectrum organophosphorus pesticide, which has been applied globally to agricultural production to control a variety of pest insects. MeP is soluble in water (>2 kg/l, 25°) and has a shorter half-life in anaerobic soils (<5 days) than that in aerobic soils (>41 days) [1]. Due to the acute toxicity for humans and animals, MeP has been restricted or prohibited from application to limited crops such as cottons, tomatoes and potatoes. However, it is still used in many countries and results in high contamination of soil [2] and aquatic [3] environment. MeP can firmly combine with Ser203 in the acetylcholinesterase (AChE; EC 3.1.1.7) and inhibit the hydrolysis function for the neurotransmitter acetylcholine (ACh). Successive accumulation of acetylcholine can cause muscle fasciculation and respiratory failure leading to death [4].

There are four possible stages for the reaction of MeP with AChE. The first stage is the movement of MeP to the active site of AChE through a certain channel. The second stage is the covalent linkage between MeP and Ser203, which is also called phosphorylation. Then, the AChE–MeP complex can either go through the

aging process, making the inhibited-AChE harder to be reactivated, or go through dephosphorylation process, which is the P–O bond scission between MeP and Ser203. After dephosphorylation, the next stage is the dissociation of the product from the active site to the outsider of AChE. The rate constants of the inhibition, spontaneous reactivation of MeP-inhibited AChE and aging reaction are 1900 M^{-1} min^{-1}, 0.239 h^{-1} and 0.071 h^{-1}, respectively [5].

The X-ray crystallography [6] and the chromatography [7] have been used to explore the valuable insights in the reaction between MeP and AChE. The reported studies indicated that the optical isomer (−)-MeP combines with AChE much easier than (+)-MeP in vitro assays. However, in the biological target, such as houseflies [8], (+)-MeP might be more toxic than (−)-MeP. Thus, the reaction mechanism of each optical isomer of MeP with AChE should be studied. As mentioned above, the rate constant of spontaneous reactivation of MeP-inhibited AChE is much larger than that of aging reaction. It seems that spontaneous reactivation is the dominate pathway after phosphorylation rather than aging reaction. In addition, fully understanding the spontaneous reactivation mechanism is helpful for the design of new antidotes against other highly deleterious organophosphorus compounds. In this work, the spontaneous reactivation mechanism of (−)-MeP and (+)-MeP inhibited-acetycholinesterase are investigated by using QM/MM method. Details of the reaction pathways, such as the exact

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location of some critical water molecules, the structure information of the intermediates and transition states, and the function of the critical amino acids in the active site, are clearly revealed. Computational results also provide valuable information about energy profile of each reaction path.

2. Computational methods

2.1. Computational model and classic MD simulation

The initial model of the acetylcholinesterase–methamidophos complex was built on the basis of the X-ray crystal structure of mouse acetylcholinesterase inhibited by methamidophos (PDB code 2JGE) [6]. The two optical isomers of AChE–MeP complex in the crystal structure were separated and then reconstructed into two independent models. Each of the models was repaired by using Modeller [9] to complement the missing residues (258–264, 543–548). The protonation state of residues was checked by using VMD program [11] and the missing hydrogen atoms were added through the HBUILD facility in the CHARMM package [12–14]. In the classic molecular dynamic simulation, the CHARMM22 force field [10] was adopted for protein. Force field parameters for nonstandard protein residue Sgr (Supporting information S1) were obtained from the similar standard parametrized residues. The coordinates of the crystal water molecules were kept unchanged. Together with the crystal water molecules, the AChE–MeP complex was initially solvated in a cubic box with 42875 of TIP3P water molecules [15]. Then the cubic box was modified to a sphere with the diameter 80 Å. The water molecules overlapping within 2.5 Å of AChE–MeP complex was deleted. After this, 1000 steps of constrained MM minimization were performed with constrained AChE–MeP complex and movable water molecules. Then the whole system is neutralized by ten sodium ions at random positions. After that, the system was further relaxed a bit by 3000 steps of MM minimization with no constrains. Finally, the system was heated from absolute 0 to 298.15 K in 50,000 steps (0.001 ps/step) and equilibrated thermally by 500,000 steps (0.001 ps/step) to reach the equilibration state. In addition, no constrains was adopted in 2000,000 simulation steps (0.001 ps/step, the canonical (NVT) ensemble was employed). Equilibration tendency of total energy and the "P···O" distance variation during the simulation dynamics processes are provided in supporting information (Figs. S2, S3, S6–S8). In addition, we compared the average structure which is obtained from last 1 ns simulation dynamics with the structure of last snapshot by using the following equation:

$$P = \frac{R_l^{i-j} - R_a^{i-j}}{R_a^{i-j}} * 100\%$$

P means percentage, R^{i-j} means distance between residue i and residue j (Actually, it is the distance of central alpha carbon in each residue), l means last snapshot, a means average (Fig. S4, S5, S9 and S10). Based on the analysis, the last snapshot is representative enough to be the initial structure for the following QM/MM calculations.

2.2. QM/MM calculations

In the QM/MM calculation, the general additive scheme was adopted, in which, the QM and MM energies are considered complementary and the total energy of the system is obtained by adding them together. And the coupling terms or corrections were added. The hydrogen link atom with charge shift model [16] was used to treat the QM/MM boundary. And the electronic embedding scheme [17] was adopted to treat the interaction between the QM

and MM region. The QM/MM energy expression can de depicted by the following equation [18]:

$$E_{QM/MM}^E = E_{MM}^O + E_{QM}^{I,L} + E_{QM-MM}^{I,O} - E_{Corr}^{I,L}$$

The superscript "E" means entire, "I" means inner, "O" means outer, and "L" means link, separately. In this studies, the QM region was treated at the B3LYP/6-31G(d) level with program Turbomole [19], and the MM region was treated by molecular mechanics with the aid of CHARMM22 [10] force field with software DL-POLY [20]. Package ChemShell [21] was used to integrate these two programs together. The default convergence criteria have been adopted when optimizing all the structures using hybrid delocalized internal coordinates (HDLC) optimizer [22]. The potential energy surface scan (PES) from reactant to product has been performed in searching for the transition state. The corresponding structure of the highest energy point during the PES was extracted and optimized before we confirmed its transition state characteristic.

The initial model in our QM/MM calculations was taken from the last snapshot of the MD simulation which has been discussed in the previous section. Here, the model used in path A was given as an example to clarify the QM/MM partition. The QM region of system A contains residues Gly121, Gly122, Ser203 (modified to Sgr203), functional part of residues Glu202, Ala204, Glu334, His447 and a water molecule. Covalent bonds between QM and MM region were truncated by cutting peptide bonds between Gly120/Gly121, Gly122/Phe123, Glu202/Sgr203; CB–CG bonds of Glu202, Glu203; C–CA bond of Ala 204 and CA–CB bond of His447. This makes the system contain 65 atoms and seven hydrogen link atoms. The rest of the atoms belong to the MM region. During the QM/MM optimization, atoms within 15 Å of atom P were selected and allowed to move freely, whereas other atoms of the system were held frozen as electrostatic environment.

3. Results and discussion

Each of the three reaction paths (paths A, B, and C) comprises three elementary steps. The first step is the nucleophilic attack on the P atom by a water molecule which is assisted by His447; the second one is the reorganization of OP(R1)(R2)(AChE)OH (Scheme 1); the third one is the dephosphorylation reaction which involves a P–O bond cleavage. The details of these three reaction paths are discussed as follows.

3.1. Reactivation of (−)-MeP-inhibited AChE

The reactivation of (−)-MeP-inhibited human AChE is defined as path A. In path A, D8 is 3.37 Å in the reactant (D8 is used to represent the distance between P and O^γ. Data was shown in Scheme 2, Table 1). In the reaction Reactant → TS-1 → INT-1, the changes of D8, D11 and D13 clearly indicate the formation of a trigonal bipyramidal shape structure INT-1 in which the atoms O^γ, P, N^β
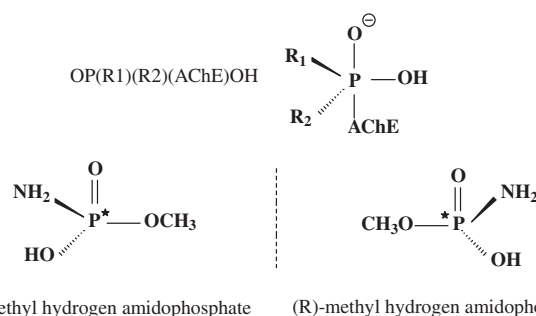
Scheme 1. Structure of OP(R1)(R2)(AChE)OH, (S)-methyl hydrogen amidophosphate, and (R)-methyl hydrogen amidophosphate.