Phosphorylation and aging mechanisms of mipafox and butyrylcholinesterase: A theoretical study

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Phosphorylation and aging processes between butyrylcholinesterase with mipafox have been studied at the B3LYP/6-311G(d,p) level of theory. The calculated results indicate that the phosphorylation process employs a two-step addition-elimination mechanism with the addition (the first step) as the rate-limiting step. Two different calculation models revealed that the catalytic triad of butyrylcholinesterase plays an important role in accelerating the reaction. This is the same mechanism as the phosphorylation reaction of acetylcholinesterase by sarin reported by Wang et al. However, the energy barrier of the rate-limiting step in the present reaction is higher than that in phosphorylation reaction of acetylcholinesterase by sarin. This indicates the differences in the phosphorylation activity of sarin and mipafox. The aging process occurs through a two-step addition-elimination mechanism similar to the phosphorylation process with the addition as the rate-limiting step. The solvent effects have been evaluated by using a CPCM model and the results show that the stationary structures and the negative charges around some important atoms involved in the two processes are not significantly different. However, the energy barrier of the phosphorylation process is remarkably decreased, revealing that this process is feasible in solution.

acetylcholinesterase, butyrylcholinesterase, quantum chemical calculations, reaction mechanism, solvent effect

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Cholinesterases are classified into two major subfamilies based on their substrate and inhibitor specificities: acetylcholinesterase (AChE; EC 3.1.1.7) and butyrylcholinesterase (BChE; EC 3.1.1.8). AChE, a serine hydrolase, is a membrane bound enzyme present in cholinergic neurons which plays an essential role in the central and peripheral nervous systems [1,2]. Massoulie et al. [3] reported that AChE can hydrolyze acetylcholine released at the synaptic cleft and the neuromuscular junction in response to nerve action potential. In contrast to AChE, the physiological function of BChE is unclear [4,5]. Despite having no identified endogenous substrate, BChE can efficiently degrade succinylcholine and cocaine and plays an important role in

detoxification [6]. The X-ray crystal structures of AChE [7] and BChE [8] indicate that they have amino acid sequence homology of 65% and amino acid sequence identity of 54%. The same Ser-His-Glu catalytic triad is found at the bottom of a deep gorge in both AChE [9] and BChE [8].

As one of the groups of inhibitors of cholinesterase, organophosphorus (OP) compounds are used as pesticides in agriculture, as antihelminthics in medical practice, as additives to hydraulic fluid and jet engine oil in the airline industry, and as chemical warfare agents [10]. Moreover, cholinesterase inhibitors have been also confirmed to play an important role in the treatment of Alzheimer's disease (AD). They can inhibit both AChE and BChE by phosphorylation of the active site serine residue. The hydroxyl oxygen of the active site serine attacks the phosphorus atom of an OP compound to displace the primary leaving group and form

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the OP adduct through a covalent bond. Although it remains unclear whether phosphonylated BChE can result in toxicity directly [11], this process is of considerable toxicological interest. Kropp and Richardson [12] reported that the extent of inhibition and the chemical composition of the OP adduct can change with time depending on both the rate of reactivation and the rate and nature of aging. Therefore, investigation of the mechanisms of inhibition and aging of both AChE and BChE by OP compounds are important for accurately predicting the conjugates formed on both AChE and BChE resulting from OP compound exposure, developing biomarkers of exposure to these agents and understanding toxicity. Kropp and Richardson [12,13] reported the products of both inhibition and aging processes between cholinesterases and the organophosphorus compound mipafox. As shown in Scheme 1, both AChE and BChE form similar conjugates with mipafox. Then, the aging of the mipafox-BChE conjugate is initiated by water followed by release of one of the isopropylamine groups. In contrast, the mipafox-AChE conjugate releases both isopropylamine groups during the aging process. However, to date, the fundamental reaction mechanisms for both the phosphorylation and aging processes are not defined.

The studies of the enzymatic reaction mechanisms of AChE [14] and BChE [6,15] show that serine and histidine act as the nucleophilic attacking group and general acid-base catalytic elements, respectively, and the reaction is facilitated by simultaneous proton transfer from serine to histidine. Wang et al. reported the phosphorylation mechanism of sarin and AChE [16] and the oxime-induced reactivation of sarin-inhibited AChE [17] using density functional theory (DFT). However, no reports have been published on the reaction mechanism between BChE and its inhibitors based on quantum chemical calculations. In the present study, we constructed a methanol model as a representation of the catalytic serine of BChE to theoretically explore the

phosphorylation and aging mechanisms between BChE and mipafox by density functional theory. The effect of aqueous solvation was considered via the CPCM model at the same calculation level to evaluate the solvent influences on the reactions.

1 Computational details

All quantum chemical calculations were performed with the Gaussian 03 program package [18]. The density functional theory (DFT) with Becke's three-parameter (B3) [19] exchange functional along with the Lee-Yang-Parr (LYP) [20,21] nonlocal correlation functional (B3LYP) was applied in the present study, and the standard valence triple- ζ basis set, augmented with d-type polarization functions for heavy elements and p-type polarization functions for H, 6-311G(d,p) [22], was used. All of the calculation models were fully optimized by analytic gradient techniques. Harmonic vibrational frequencies were calculated to check whether the obtained species is minima (with all real frequencies) or transition state (with one and only one imaginary frequency). For each transition state, the intrinsic reaction coordinate (IRC) calculations were performed to guarantee its correct connection to the minima. To evaluate the solvation effects on the reactions, the CPCM model with a dielectric constant $\epsilon=78.39$ was employed to perform the structure optimization and IRC calculation at the B3LYP/6-311G(d,p) level. In the CPCM model, the electrostatic interactions between the charges on the solute molecules and the induced charges on the polarizable solvent molecules are solved self-consistently and a simple scaled conductor boundary condition is used which improves the precision and the efficiency [23–26]. All Gibbs free energies were calculated at room temperature and 1 atm (1 atm=1.01 $\times 10^5$ Pa) pressure. Natural Population Atomic (NPA) charges

Scheme 1 Pathways of inhibition and aging of mipafox for AChE and BChE.

were determined at the same level with the Natural Bond Order (NBO) analysis [27–30]. In this paper, all energies mentioned are the Gibbs free energies. In order to simply our discussion, the energies of reactants are set to be zero for reference purposes.

2 Results and discussion

The phosphorylation of BChE by mipafox occurs by nucleophilic attack on the phosphorus atom by the hydroxyl oxygen atom in the catalytic serine, displacing the primary leaving group, fluoride, and generating the same *N,N'*-diisopropylphosphorodiamido adduct. Then, the adduct undergoes the aging process to eliminate an isopropylamine group [12]. It is difficult to investigate these reaction mechanisms using DFT theory if the whole enzyme system is considered, thus, for a simplified model, methanol CH_3OH was chosen to represent the active site serine in the enzyme. This enzyme model was also applied in the studies of the reaction mechanism between sarin and AChE by Wang et al. [16].

2.1 Phosphorylation mechanism

The nucleophilicity of the catalytic serine in the phospho-

nylation reaction can be enhanced by proton transfer to the imidazole nitrogen of the catalytic histidine residue in the catalytic triad. Thus, both the deprotonated CH_3O^- anion and neutral CH_3OH were considered to explore the catalytic effect of the catalytic triad.

2.2 Pathway A

Pathway A describes the reaction between CH_3O^- and mipafox when the proton in the hydroxyl group of the serine residue has absolutely transferred to the imidazole nitrogen of the histidine residue in the catalytic triad. The structures of reactant, intermediate, transition states and product are shown in Figure 1. The transition states are determined with only one imaginary frequency and IRC calculations were performed to confirm that the transition states connect the corresponding minima. The profile of pathway A is shown in Figure 2. The NPA charges of several important atoms by NBO analysis are listed in Table 1.

Anionic CH_3O^- and mipafox, which form a stable complex by two hydrogen bonds in the reactant Ra, generate a covalent intermediate Ia through the nucleophilic attack. Subsequently, the P–F bond is broken, resulting in the generation of the final product Pa. Obviously, this is a two-step addition-elimination reaction.

Figure 1 Optimized structures of the phosphorylation reaction for pathway A. The values out of and in square brackets are for the gas phase and solvent phase, respectively. Distances are in angstroms and atomic angles are in degrees.

Figure 2 The potential energy surfaces of pathways A and B for the phosphorylation reaction. The values out of and in square brackets are for the gas phase and solvent phase, respectively.

In Ra, weak hydrogen-bond interactions are formed between the O1 atom of anionic CH_3O^- and the H2 and H3 atoms in the two amino-group of mipafox to stabilize the two separated reactants. The hydrogen bond lengths are 1.63 and 1.59 Å, respectively, and the bond angles are 161.3° and 161.2°, respectively. The atomic distance between O1 and P is 3.32 Å, which indicates there is no interaction between the two separated species.

The transition state corresponding to serine's nucleophilic attack, TSa1, has only one imaginary frequency of 173i cm^{-1} . The vibrational mode corresponding to the imaginary frequency indicates the direct nucleophilic attack on the P atom of mipafox by O1 of the anionic CH_3O^- from the backside of the F atom. The angle of the O1-P-F atoms is 162.8°, indicating an almost linear attack. The distance between the O1 and P atoms in TSa1 is 2.51 Å, about 0.81 Å shorter than that in Ra. The bond length of the P-F bond increases from 1.64 Å in Ra to 1.68 Å in TSa1, suggesting its weakening. The NPA charges determined at the same theoretical level show the O1 atom in anionic CH_3O^- has strong nucleophilic character with a negative charge of -0.66 au. The Gibbs free energy barrier of this step is 22.1

kcal/mol at 298 K and 1 atm pressure.

The intermediate Ia, with a trigonal bipyramidal structure, is formed in this pathway. It lies 7.0 kcal/mol above the reactant Ra, indicating its lower stabilization than Ra. The O1-P bond is formed with a distance of 1.83 Å and the bond length of P-F increases from 1.68 Å in TSa1 to 1.77 Å in Ia.

The transition state TSa2 has only one imaginary frequency, 160i cm^{-1} , and connects Ia and the product. The vibrational mode corresponding to the imaginary frequency shows that the F atom is away from the P atom and is surrounded by two side groups of mipafox. In TSa2, the P-F bond is broken and the atomic distance is 2.34 Å. The P-O bond is strengthened because its bond length is shortened to 1.70 Å. The NPA charge indicates that the negative charge distributed around the F atom increases to -0.63 au (-0.51 au in Ia and -0.44 au in TSa1) and the negative charge distributed around the O1 atom decreases to -0.57 au (-0.63 au in Ia and -0.66 au in TSa1). The Gibbs free energy barrier of this step is 5.3 kcal/mol at 298 K and 1 atm pressure.

In Pa, the F atom has strong interactions with mipafox through two hydrogen bonds between the F anion and the two amino-groups in mipafox with hydrogen bonds lengths of 1.62 and 1.56 Å, respectively, and bond angles of 155.5° and 160.9°, respectively. The atomic distance between P and F is 3.26 Å, meaning they no longer interact. The NPA charge indicates that the negative charge distributed around the F atom is -0.64 au. The energy of Pa is 6.7 kcal/mol lower than that in Ra, revealing that the product is more thermodynamically stable than Ra and the reaction along this pathway is exothermic. Pa might be detected by experiments.

To evaluate the solvent effects on this reaction pathway, the structures of the reactant, the transition states, the intermediate and the product were optimized with the CPCM

Table 1 NPA charges of several important atoms involved in phosphorylation reaction by NBO analysis^{a)}

	O1	O6	P	F
Ra	-0.65 [-0.66]	-0.65 [-0.65]	1.26 [1.27]	-0.39 [-0.40]
TSa1	-0.66 [-0.67]	-0.62 [-0.63]	1.29 [1.30]	-0.44 [-0.44]
Ia	-0.63 [-0.62]	-0.64 [-0.66]	1.31 [1.31]	-0.51 [-0.51]
TSa2	-0.57 [-0.57]	-0.62 [-0.63]	1.27 [1.29]	-0.63 [-0.63]
Pa	-0.53 [-0.53]	-0.62 [-0.63]	1.20 [1.20]	-0.64 [-0.66]
Rb	-0.45 [-0.43]	-0.62 [-0.62]	1.31 [1.31]	-0.35 [-0.36]
TSb1	-0.64 [-0.63]	-0.54 [-0.52]	1.40 [1.39]	-0.38 [-0.41]
Ib1	-0.62 [-0.61]	-0.49 [-0.50]	1.45 [1.45]	-0.43 [-0.44]
TSb2	-0.56 [-0.56]	-0.49 [-0.49]	1.40 [1.43]	-0.45 [-0.48]
Ib2	-0.55 [-0.55]	-0.48 [-0.49]	1.43 [1.43]	-0.48 [-0.48]
TSb3	-0.51 [-0.52]	-0.48 [-0.48]	1.36 [1.37]	-0.58 [-0.59]
Pb	-0.50 [-0.50]	-0.64 [-0.65]	1.27 [1.27]	-0.35 [-0.39]

a) The values (au) out of and in square brackets are got in gas phase and in solvent phase, respectively.

water model and the results are plotted in Figure 1. The NPA charges of several important atoms involved in solvent phase structures by NBO analysis are listed in Table 1 and the profile of this reaction pathway is also shown in Figure 2. By comparing with the results in the gas phase, one can see that the reaction pathway in the solvent phase is very similar to that in the gas phase. However, the conformations of all stationary points and the NPA charges of several important atoms have been slightly changed. The remarkable difference of the reactions in the gas and solvent phases is that the free energy barrier of the rate-limiting step is decreased to 14.3 kcal/mol. Moreover, the whole reaction is exothermic by 10.9 kcal/mol in the solvent phase, which is

4.2 kcal/mol higher than that in the gas phase (6.7 kcal/mol). These changes might be caused by the structure differences in the gas phase and solvent phase. It can be concluded that the solvent effect can increase the rate of the entire phosphorylation reaction.

2.3 Pathway B

Pathway B describes the phosphorylation reaction between neutral CH_3OH and mipafox. The structures of the reactant, intermediates, transition states, and product are shown in Figure 3. The transition states are determined with only one imaginary frequency and IRC calculations were carried out

Figure 3 Optimized structures of the phosphorylation reaction for pathway B. The values out of and in square brackets are for the gas phase and solvent phase, respectively. Distances are in angstroms and atomic angles are in degree.

to confirm that the transition states connect the corresponding minima. The NPA charges of several important atoms involved in this pathway by NBO analysis are listed in Table 1. The profile of pathway B presented in Figure 2 indicates that pathway B is also a two-step addition-elimination reaction mechanism, similar to pathway A.

The neutral CH_3OH forms a stable complex, Rb, with mipafox by a hydrogen bond between the H7 atom of the hydroxyl group in CH_3OH and the O6 atom in mipafox. The bond length is 1.95 Å and the bond angle is 147.9°. The distance between the P and O1 atoms is 3.21 Å and the length of the P–F bond is 1.61 Å.

The transition state TSb1 is determined by only one imaginary frequency of 990i cm^{-1} . The vibrational mode corresponding to the imaginary frequency indicates that the nucleophilic attack on the P atom in mipafox by the O1 atom occurs while the H7 proton of the hydroxyl group in CH_3OH is transferred to the O6 atom in mipafox. The atomic distance between O1 and H7 is 1.31 Å and the bond length of O6–H7 is 1.14 Å. The atomic angle of O1–P–F is 170.9°, which supports the linear nucleophilic attack. The atomic distance between the O1 atom of the hydroxyl group in CH_3OH and the P atom in mipafox is 2.21 Å, which is 0.30 Å shorter than that in TSa1. The bond length of P–F is 1.62 Å, which is 0.08 Å shorter than that in TSa1. The NPA charges display a –0.64 au negative charge around the O1 atom of serine, which is 0.02 au less than that in TSa1. This change means that the nucleophilic ability of the O1 atom of serine in TSa1 is stronger than that in TSb1. The bond length of P–O6 is increased from 1.49 Å in Rb to 1.56 Å in TSb1. The Gibbs free energy barrier of this step is 33.1 kcal/mol at 298 K and 1 atm pressure, which is 11.0 kcal/mol higher than that in pathway A in the gas phase.

The trigonal bipyramidal intermediate Ib1, similar to Ia, lies 14.2 kcal/mol above Rb, indicating weak stability. The P–O6 bond length is 1.62 Å, which indicates that the double bond P–O6 in Rb converts to a single bond with H7 transfer. The NPA charge shows that the negative charge of O1 is increased from –0.45 au in Rb to –0.62 au in Ib1 and the negative charge of O6 is decreased from –0.62 au in Rb to –0.49 au in Ib1. This change in charge confirms that the H7 transfer is a proton transfer. The bond length of the P–O1 bond is 1.73 Å and the bond length of the P–F bond is weakened to 1.67 Å from 1.62 Å in TSb1.

Connecting to Ib1, the transition state TSb2 characterizes the rotation of the H7 atom from the side of the O1 atom to the opposite side where the rotated H7 atom is close to the F atom by the analysis of the vibrational mode. The only imaginary frequency of TSb2 is 354i cm^{-1} . The dihedral angle of O1–P–O6–H7 in TSb2 is rotated to –91.6° from –0.2° in Ib1. The bond distances of the P–F bond and P–O1 bond are 1.70 and 1.72 Å, respectively. The Gibbs free energy barrier of this rotation process is 6.6 kcal/mol at 298 K and 1 atm pressure.

The intermediate Ib2, which has the same trigonal bi-

pyramidal structure as Ib1, is calculated to be 4.7 kcal/mol below TSb2. The dihedral angle of O1–P–O6–H7 is –178.2°. The bond length of the P–O1 bond is shortened to 1.69 Å and the P–F bond length is stretched to 1.73 Å. This indicates that the P–F bond has become weaker.

The transition state TSb3 characterizes the P–F bond fission and F anion departure process for the elimination step. The one imaginary frequency is 273i cm^{-1} and the vibrational mode reveals that the leaving F atom is surrounded by certain H atoms in mipafox. The atomic distance between F and H7 is 1.45 Å; however, there is no hydrogen bond formed between them. The P–O1 bond length is shortened to 1.62 Å and the atomic distance between the P and F atoms is calculated to be 2.31 Å. The atomic angle of O1–P–F is 165.6°, indicating that these atoms are almost maintained in a linear conformation. The Gibbs free energy barrier of this step is 11.6 kcal/mol.

The energy of Pb is 20.1 kcal/mol lower than that of TSb3, indicating that it is very feasible energetically to form Pb. Pb is a stable complex because of the hydrogen bond formed between the H7 in the HF molecule and the O6 in mipafox. The hydrogen bond length and angle are 1.60 Å and 166.9°, respectively. The NPA charge shows that the negative charge of O6 is increased from –0.48 au in TSb3 to –0.64 au in Pb and the negative charge of F is decreased from –0.58 au in TSb3 to –0.35 au in Pb, indicating the occurrence of the H7 proton transfer. The P–O6 bond length of 1.50 Å in Pb reveals that the P–O6 bond is converted into a double bond from a single bond in Ib2 with a bond length of 1.63 Å. Pb lies 7.6 kcal/mol above Rb, which indicates that this reaction along Pathway B is thermodynamically endothermal.

To evaluate the solvent effects on this reaction pathway, the structures of reactant, transition states, intermediate, and product were optimized using the CPCM water model and the results are shown in Figure 3. The NPA charges of several important atoms involved in solvent phase structures by NBO analysis are listed in Table 1 and the profile of this pathway is shown in Figure 2. It is evident that the solvent phase pathway has similar characteristics as the gas phase pathway. However, the structures of the stationary points and the NPA charges of several important atoms involved in the solvent phase differ by comparison with the relative structures in the gas phase. The free energy barrier of the rate-limiting step in the solvent phase is 36.3 kcal/mol, which is 3.2 kcal/mol higher than that in gas phase.

Both pathways A and B employ a two-step addition-elimination mechanism with the addition process (the first nucleophilic attack step) as the rate-limiting step. The major products in both pathways are consistent with the experimental results [12]. However, the free energy barriers of the rate-limiting step in Pathway A in both the gas phase and the solvent phase are lower than those in Pathway B. This indicates that the phosphorylation reaction along pathway A is more feasible. The differences in the free energy barri-

ers between the two pathways also prove that the catalytic triad of BChE can effectively accelerate the phosphorylation process by accepting the proton from the serine residue as it does in the acylation reaction.

In both the present study and the reported phosphorylation mechanism of sarin and AChE by Wang et al. [16], the same calculation level is employed and a similar methanol CH_3OH model is chosen to represent the active site serine in both enzymes. Both sarin and mipafox are organophosphorus (OP) compounds. Therefore, it is reasonable to examine the phosphorylation mechanisms of cholinesterase by organophosphorus compounds by comparing both research results. One can see that both cholinesterases employ the same two-step addition-elimination reaction mechanism with the addition as the rate-limiting step. In the two different reaction pathways in the present study, the negative charges around the O1 atom of the serine residue at the active site are less than that in the phosphorylation reaction of sarin and AChE by Wang et al. [16], which indicates that the latter has stronger nucleophilicity than the former. This

results in the Gibbs free energy barriers of the present reaction being higher than those in the phosphorylation reaction of sarin and AChE by Wang et al. [16].

2.4 Aging mechanism

A phosphorylated enzyme may undergo aging by water to generate a negatively charged product. In order to understand toxicity and develop biomarkers for determining exposure to these agents, we have theoretically studied the aging mechanism using a calculation model containing the mipafox-enzyme conjugate (the major product of pathway A and pathway B) and a water molecule.

The structures of reactant, intermediate, transition states and the product and the energy profile are shown in Figure 4. In this reaction, H_2O , which is H-bonded to the mipafox-enzyme conjugate in the reactant (Rc), attacks the P atom of the mipafox-enzyme conjugate and forms an intermediate product (Ic), while H9 in H_2O is transferred to the N4 atom of the mipafox-enzyme conjugate. Subsequently, a single

Figure 4 Optimized structures and the potential energy surface for the aging reaction. The values out of and in square brackets are for the gas phase and solvent phase, respectively. The units of distance, angle are angstrom, degree, respectively.

isopropylamine group is released to form the final product (Pc) while the P–N4 bond in Ic is broken. Obviously, this is a two-step addition-elimination mechanism, the same as the phosphorylation reaction.

Rc is stabilized through a weak hydrogen bond between the H9 atom of H₂O and the N4 atom of the mipafox-enzyme conjugate, with a bond length of 2.02 Å and a bond angle of 167.0°. The atom distance between P in the mipafox-enzyme conjugate and O8 in H₂O is 3.92 Å, indicating that there is no interaction between the two separated species.

The transition state corresponding to hydride transfer and nucleophilic attack, TSc1, has only one imaginary frequency of 833i cm⁻¹. The vibrational mode corresponding to the imaginary frequency obviously indicates the direct nucleophilic attack by O8 in H₂O on the P atom of the mipafox-enzyme conjugate. At the same time, the proton transfer from H9 to N4 occurs. The NPA charges calculated in the same theoretical level show that the attacking O8 in H₂O has a negative charge of -0.69 au, which is 0.25 au more than that in Rc, indicating a stronger nucleophilic character. The distance between the P and O8 atoms is 2.14 Å, and the bond length of P–N4 increases to 1.84 Å from 1.71 Å in Rc. The Gibbs free energy barrier of this step is 32.8 kcal/mol at 298 K and 1 atm pressure.

The intermediate Ic, formed through proton transfer and nucleophilic attack, has a trigonal bipyramidal structure similar to Ia and Ib1. It lies 24 kcal/mol above Rc, indicating that it is not as stable as Rc. The bond length of P–N4 is weakened to be 1.97 from 1.71 Å in Rc. Moreover, the P–O8 bond is strengthened as its length is shortened to 1.74 from 2.14 Å in TSc1.

The transition state (TSc2) for the elimination of a single isopropylamine group lies 1.4 kcal/mol above Ic. The one imaginary frequency is estimated as 120i cm⁻¹ and the vibrational mode shows the direct elimination of a single isopropylamine group. The atomic distance of P–N4 is increased to 2.21 from 1.97 Å in Ic, indicating that a weaker interaction may exist between the P atom and N4 atom.

The product (Pc) lies 41.3 kcal/mol below TSc2 and 15.9 kcal/mol below Rc, indicating that Pc is more stable than Rc. Two parts of Pc are connected by a weak N4–H10 hydrogen bond interaction, with a bond length of 1.70 Å and a bond angle of 165.8°. The product (Pc) is consistent with the ex-

perimental results [12].

From the discussion above, it is clear that the aging reaction also employs a two-step addition-elimination mechanism with the addition as the rate-limiting step. This reaction is exothermic by 15.9 kcal/mol and hence, thermodynamically feasible. This result may provide a useful guide to study the aging mechanism of the AChE-mipafox adduct.

To evaluate the solvent effects on this reaction, the structures of reactant, transition states, intermediate, and product were optimized using the CPCM water model and the results are shown in Figure 4. The NPA charges of several important atoms involved in the solvent phase structures by NBO analysis are listed in Table 2 and the potential energy surface of the solvent phase pathway is shown in Figure 4. It is evident that the structures of all the stationary points are influenced by the solvent effect and differ from the relative structures in the gas phase. The charges of the O8 atom in both TSc2 and Pc and the charge of N4 in TSc2 in the solvent phase are not in agreement with them in the gas phase and this might be caused by use of the polar solvent. However, the free energy barrier of the rate-limiting step in the solvent phase is 1.9 kcal/mol higher than that in the gas phase indicating that the solvent only slightly influences the aging process.

3 Conclusions

Quantum chemical calculations were performed to theoretically investigate phosphorylation and aging mechanisms of butyrylcholinesterase with mipafox at the B3LYP/6-311G (d,p) level of theory, and the potential energy surfaces of the phosphorylation reaction and aging reaction were identified. In the phosphorylation reaction, two different models (pathway A and pathway B) represent the situation with and without the effect of the catalytic triad of BChE, respectively. Both employ the same reaction pathway which is a two-step addition-elimination reaction with the addition (the first step) as the rate-limiting step. The differences between the two pathways reveal that the catalytic triad of BChE can effectively accelerate the phosphorylation process through the proton transfer. These results are consistent with the phosphorylation mechanism of sarin and AChE by Wang

Table 2 NPA charges of several important atoms involved in aging reaction by NBO analysis^{a)}

	O1	O6	O8	P	F
Rc	-0.52 [-0.52]	-0.57 [-0.57]	-0.46 [-0.46]	1.24 [1.24]	-0.67 [-0.67]
TSc1	-0.58 [-0.58]	-0.57 [-0.57]	-0.69 [-0.71]	1.25 [1.24]	-0.58 [-0.56]
Ic	-0.62 [-0.62]	-0.59 [-0.60]	-0.64 [-0.65]	1.29 [1.28]	-0.50 [-0.50]
TSc2	-0.58 [-0.59]	-0.57 [-0.60]	-0.69 [-0.58]	1.25 [1.28]	-0.58 [-0.51]
Pc	-0.52 [-0.52]	-0.65 [-0.64]	-0.56 [-0.63]	1.23 [1.18]	-0.52 [-0.49]

a) The values (au) out of and in square brackets are got in gas phase and in solvent phase, respectively.

et al. [16]. However, the Gibbs free energy barriers of the present reaction are higher than that in the phosphorylation reaction of sarin and AChE by Wang et al. [16] because the negative charges around the O1 atom of the serine residue in the present study are less. Similar to the phosphorylation reaction, the aging process also employs a two-step addition-elimination reaction with the addition (the first step) as the rate-limiting step. This result may provide a useful guide for studying the aging mechanism of AChE and mipafox.

To evaluate the solvent effects, a CPCM model with a dielectric constant $\epsilon=78.39$ was employed to perform the structure optimizations and IRC calculations at a B3LYP/6-311G(d,p) level. The phosphorylation reaction is strongly influenced by the solvent effects, which lowers the Gibbs free energy barriers and increases the rate of the entire phosphorylation reaction. However, the solvent effects were negligible on the aging reaction.

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