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ACETYLCHOLINESTERASE: MECHANISMS OF COVALENT INHIBITION OF H447I MUTANT DETERMINED BY COMPUTATIONAL ANALYSES

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Abstract

The reaction mechanisms of two inhibitor TFK⁺ and TFK⁰ binding to H447I mutant mouse acetylcholinesterase (mAChE) have been investigated by using a combined *ab initio* quantum mechanical/molecular mechanical (QM/MM) approach and classical molecular dynamics (MD) simulations. TFK⁺ binding to the H447I mutant may proceed with a different reaction mechanism from the wild type. A water molecule takes over the role of His447 and participates in the bond breaking and forming as a “charge relay”. Unlike in the wild-type mAChE case, Glu334, a conserved residue from the catalytic triad, acts as a catalytic base in the reaction. The calculated energy barrier for this reaction is about 8 kcal/mol. These predictions await experimental verification. In the case of the neutral ligand TFK⁰, however, multiple MD simulations on the TFK⁰/H447I complex reveal that none of the water molecules can be retained in the active site as a “catalytic” water. Taken together our computational studies confirm that TFK⁰ is almost inactive in the H447I mutant, and also provide detailed mechanistic insights into the experimental observations.

Keywords

ab initio QM/MM; molecular dynamics; free energy calculations; charge relay

Introduction

Acetylcholinesterase (AChE, EC 3.1.1.7) is a hydrolytic enzyme that belongs to the serine hydrolase family. It plays important roles during the course of signal transmission at cholinergic synapses. The principal biological role of acetylcholinesterase is the termination of impulse transmissions by rapidly hydrolyzing the neurotransmitter, acetylcholine (ACh) [1–3]. Dysfunctions of AChE or other components of cholinergic synapses are involved in several human diseases, including myasthenia gravis, glaucoma, Alzheimer's and Parkinson's Diseases [4–9]. As a result, AChE has become an important target for rational drug design.

The crystal structure of AChE is characterized by a deep narrow gorge which penetrates halfway into the enzyme and contains the catalytic site located near the bottom, *ca.* 20 Å deep

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[10]. Kinetic studies have revealed that AChE possesses a remarkably high activity, with an ACh turnover rate of about 10^4 s^{-1} under physiological conditions, approaching the diffusion-controlled limit [11–13].

Similar to many other proteases, the catalytic triad in AChE consisting of Ser203(200) [14], His447(440) and Glu334(327) is believed to be essential to hydrolysis.

However, recent experimental mutagenesis studies have brought new challenges to all the above proposed reaction mechanisms. TFK^+ (m-(N,N,N-trimethylammonio) trifluoroacetophenone (TMTFA)) (see Fig. 1 (b)), a common inhibitor to AChE, can still react with the mouse AChE (mAChE), even with the replacement of His447 by a hydrophobic Ile. In contrast, its neutral analog, TFK^0 , shows no apparent binding activity to H447I mutant, while it shows slightly reduced binding to the wild-type mAChE as compared to TFK^+ (Table 1).

In order to explore the enzymatic activity of H447I mutant mAChE, we have performed computational studies on both TFK^+ and TFK^0 binding to the wild-type and H447I mutant mAChEs, using a combined *ab initio* quantum mechanical and molecular mechanical (QM/MM) approach, as well as multiple MD simulations. In our study, a water molecule is found to play an essential catalytic role in place of His447 in the binding reaction of TFK^+ to the H447I mutant. Along with this water molecule, Ser203 and Glu334 form a new stable catalytic triad. During the reaction, Ser203 delivers a proton to the water molecule while the water molecule serves as a charge relay to pass one proton to Glu334. The QM/MM free energy barrier for the reaction is lower than 8.0 kcal/mol. On the other hand, the water triad was unable to be retained in the corresponding TFK^0 /H447I complexes from multiple MD simulations, indicating that TFK^0 might not be able to stably bind to the H447I mutant. To further validate our QM/MM calculations, we also used thermodynamic integration (TI) calculations to investigate the binding energy differences between TFK^0 and TFK^+ in both the wild-type and H447 mutant enzymes. The TI calculations also suggest that the binding of TFK^+ to both enzymes is much stronger than the neutral analog TFK^0 , which is consistent with experimental observations as well as the above QM/MM calculations.

Computational methods

Since there is no crystal structure of the H447I mutant so far, we tried two approaches to prepare the initial noncovalent complex structure of H447I and TFK^+ (denoted as the $[\text{M}\cdot\text{T}^+]$ model [21]). The first approach is to use the multiple docking approach introduced by Kua et al [15]. His447 in the apo mAChE crystal structure (PDB code: 1J06) was manually modified to Ile, and then TFK^+ was docked into 1,000 snapshots evenly chosen from the last 1 ns trajectory of a 10 ns apo H447I mutant MD simulation. The Autodock 3.0 program [16] was used for all the docking studies. The search method used was the Lamarckian genetic algorithm (LGA) set at level 2 with the top 6 structures reported. Finally, according to the criteria suggested in Kua et al [15], the best 6 complex structures were selected and immersed into explicit water boxes, and subsequent MD simulations were set up to relax each system. To prevent TFK^+ dislocation from the esteratic binding site, a $20.0 \text{ kcal}/(\text{mol}\cdot\text{\AA}^2)$ harmonic restraint between the carbonyl-C of TFK^+ and Ser203- O_γ was applied during simulations. The second approach is to start from the noncovalent $[\text{W}\cdot\text{T}^+]$ structure obtained from our QM/MM calculations of the wild-type enzyme with TFK^+ (see below for details), and then manually replace HID447 (Note: The N_ϵ proton originally in the $[\text{W}\cdot\text{T}^+]$ complex is transferred to Ser203 in the $[\text{W}\cdot\text{T}^+]$ model after the QM/MM run) with Ile. Another MD simulation of the resulting system was then set up by following the same procedure as outlined in the first approach. The total number of atoms in these 7 MD simulations is around 70,000–75,000. Similarly, 7 initial models of H447I and TFK^0 ($[\text{M}\cdot\text{T}^0]$) were obtained with the same procedure. Additionally, an 8th $[\text{M}\cdot\text{T}^0]$ model

was obtained by directly modifying TFK^+ to TFK^0 in one of the $[\text{M}\cdot\text{T}^+]$ models. Therefore, a total of 8 $[\text{M}\cdot\text{T}^0]$ models were prepared and subjected to further theoretical investigations.

Results and Discussions

As mentioned in Methods section, 7 non-covalent complex $[\text{M}\cdot\text{T}^+]$ models of the H447I mutant and TFK^+ were obtained via docking and QM/MM calculations. During the subsequent MD simulations of these models, in 6 of 7 models, a water molecule was observed to diffuse into the center of the triangle formed by Ser203, Ser229 and Glu334 in the first 2 ns simulations; a representative snapshot from the simulation is shown in Fig. 2. Then we extended our MD simulations to 10 ns for each model. The average distance of the hydroxyl oxygen O_γ of Ser203 to the oxygen O_W of the water is 2.72 ± 0.12 Å, the carboxyl oxygen of the Ser229 backbone to O_W of the water is 2.78 ± 0.18 Å, and O_W of the water to one of carboxyl oxygens $O_{\delta 1}$ of Glu334 is 2.66 ± 0.12 Å in all of the 10 ns trajectories. Generally, an effective hydrogen bond can be characterized by the short distance between the heteroatoms (less than 3.50 Å), and the bond angle greater than 135° [17–18]. Using this criterion, the hydrogen bond between the Ser203 O_γ and O_W is found in all the structures in the 10 ns simulations, while the hydrogen bond between O_W and $O_{\delta 1}$ is ~99%, and between O_W and Ser229-O is ~95%. The water molecule is observed to flip between Ser229-O and $O_{\delta 1}$, while the hydrogen bond between O_γ and O_W stays stable.

Three snapshots from the above MD simulations were selected to explore the acylation reaction mechanism in the H447I mutant. The water molecules beyond a 27 Å solvent water sphere, centered on the active site (the hydroxyl oxygen O_γ of Ser203) were removed. The three prepared QM/MM models have 10059 atoms (562 water molecules), 10176 atoms (602 water molecules) and 10170 atoms (600 water molecules) respectively. Each snapshot was first relaxed by the MM method, and then optimized with B3LYP(6-31G*) QM/MM calculations using an iterative minimization approach [19], leading to an optimized structure for the reactant. The QM subsystem consists of the sidechains of Ser203 and Glu334, the water bridge and TFK^+ , with the broken C_β - C_α bonds of Ser203 and Glu334 treated as two pseudobonds [20], resulting in a total of 48 QM atoms. All other atoms are treated by MM. The reaction coordinate (RC) is shown in Fig. 3. Both forward and backward RC driving calculations yield consistent curves and all the reaction paths are smooth and continuous.

All the three QM/MM calculations led to very consistent results, in which the water molecule between Ser203 and Glu334 plays a “charge-relay” role during TFK^+ binding reaction with the H447I mutant. Table 2 presents the relative potential energies in the reactant (non-covalent complex), transition state and product (covalent complex), respectively. The calculated potential energy barriers at the MP2(6-31+G*)/MM level are 8.2, 4.2, 10.6 kcal/mol.

However, the “lock” can be broken easily or even never formed if TFK^+ is replaced by TFK^0 . As compared to TFK^+ , larger RMSDs for TFK^0 have been captured in MD simulations [24].

In conclusion, we explored the TFK^+ and TFK^0 inhibition mechanisms in the H447I mutant mAChE. Despite the replacement of the catalytic base His447, TFK^+ still demonstrates high binding affinity to the H447I mutant. Our computational studies suggest that a water molecule might act as a “charge relay” and facilitate the binding of the ligand to the enzyme while this is not true for the binding of the neutral analogue TFK^0 . We hope that these results will stimulate further experimental studies.

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Figure 1.

(a) The acylation mechanism in the wild-type AChE enzyme; (b) The chemical structures of TFK⁺ and TFK⁰.

Figure 2.
The “water” triad in the active site of the six [M·T⁺] models. The values of the distances in Å are averaged among six models.

Figure 3. Illustration of the reaction coordinate used for the H447I mutant and TFK⁺ reaction, which is $dO_{\gamma}-H_{\gamma}+dO_w-H-dO_{\gamma}-C-dO_w-H_{\gamma}-d_H-O_{\delta}$.

Table 1

The rate constants for association and dissociation of inhibitors TFK^+ and TFK^0 with mouse AChEs measured by experiments [22–23]. The units of k_{on} and k_{off} are $10^9 M^{-1} min^{-1}$ and $10^{-3} min^{-1}$, respectively.

	wild-type mAChE		H447I mutant mAChE	
	k_{on}	k_{off}	k_{on}	k_{off}
TFK^+	980±60	1.1±0.3	$\sim 10^a$	$\sim 1.0^a$
TFK^0	2.2±0.3	15±1	N/A ^b	N/A ^b

^aUnpublished data.

^bThere is no apparent binding affinity detected.

Table 2

The calculated QM/MM potential energy differences (kcal/mol) for three QM/MM models. The geometries are relaxed at the B3LYP(6-31G*)/MM level, and then single-point calculations are performed at three different levels.

	MP2(6-31+G*)/MM	B3LYP(6-31+G*)/MM	B3LYP(6-31G*)/MM
The first snapshot			
Reactant	0.0	0.0	0.0
Transition State	8.2	7.4	8.2
Product	-5.0	-1.1	2.0
The second snapshot			
Reactant	0.0	0.0	0.0
Transition State	4.2	5.8	7.5
Product	-8.0	-2.5	0.0
The third snapshot			
Reactant	0.0	0.0	0.0
Transition State	10.6	10.3	10.4
Product	0.4	3.8	4.4