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Acetylcholinesterase: Mechanisms of covalent inhibition of H447I mutant determined by computational analyses

Y.H. Cheng[a,*], X.L. Cheng[a], Z. Radić[b], J.A. McCammon[a,b]

[a] Howard Hughes Medical Institute, Department of Chemistry and Biochemistry, University of California at San Diego, 9500 Gilman Dr. MC 0365, La Jolla, CA 92093-0365, United States
[b] Department of Pharmacology, University of California at San Diego, La Jolla, CA 92093-0365, United States

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ABSTRACT

The reaction mechanisms of two inhibitor TFK^+ and TFK^0 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 relayer". 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 TFK^0, however, multiple MD simulations on the TFK^0/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 TFK^0 is almost inactive in the H447I mutant, and also provide detailed mechanistic insights into the experimental observations.

1. 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 [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

* Corresponding author. Tel.: +1 858 534 0956; fax: +1 858 534 4974.
E-mail address: ycheng@mccammon.ucsd.edu (Y.H. Cheng).

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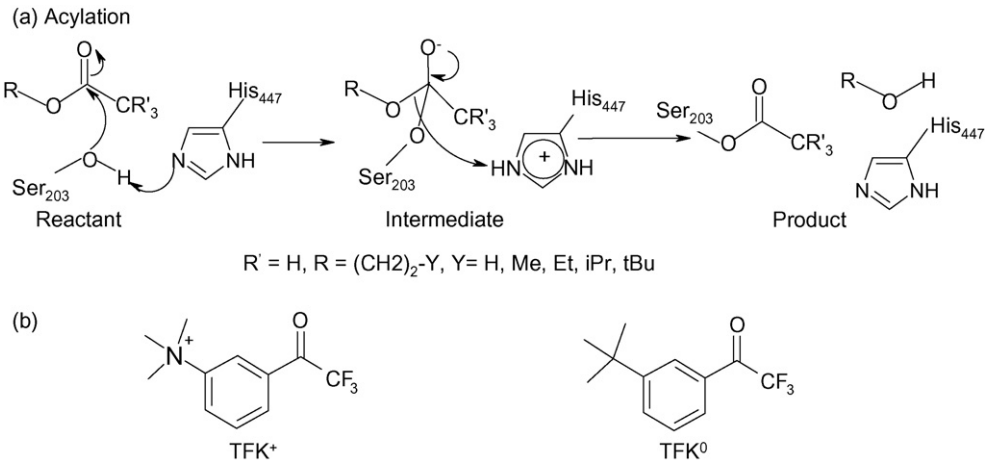

Fig. 1. (a) The acylation mechanism in the wild-type AChE enzyme; (b) the chemical structures of TFK^+ and TFK^0.

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 relayer 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.

2. Computational methods

Since there is no crystal structure of the H447I mutant so far, we tried two approaches to prepare the initial non-covalent complex structure of H447I and TFK^+ (denoted as the [M·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 1000 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 six structures reported. Finally, according to the criteria suggested in Kua et al. [15], the best six 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 kcal/(mol Å^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 non-covalent [W·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 [W–T^+] complex is transferred to Ser203 in the [W·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 seven MD simulations is around 70,000–75,000. Similarly, seven initial models of H447I and TFK^0 ([M·T^0]) were obtained with the same procedure. Additionally, an eighth [M·T^0] model was obtained by directly modifying TFK^+ to TFK^0 in one of the [M·T^+] models. Therefore, a total of eight [M·T^0] models were prepared and subjected to further theoretical investigations.

Table 1
The rate constants for association and dissociation of inhibitors *TFK*$^+$ and *TFK*^{0} with mouse AChEs measured by experiments [22,23]

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

The units of k_{on} and k_{off} are 10^9 M^{-1} min^{-1} and 10^{-3} min^{-1}, respectively.
[a] Unpublished data.
[b] There is no apparent binding affinity detected.

3. Results and discussions

As mentioned in Section 2, seven non-covalent complex [M·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 six of seven 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