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In silico studies on the role of mutant Y337A to reactivate tabun inhibited *m*AChE with K048

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ABSTRACT

Organophosphorus compound (OP) tabun is resistant to reactivate by many oxime drugs after the formation of OP-conjugate with AChE. The reactivation of tabun-inhibited *m*AChE and site-directed mutants by bispyridinium oxime, K048 (N-[4-(4-hydroxyiminomethylpyridinio)butyl]-4-carbamoylpyridinium dibromide) showed that the mutations significantly poor the overall reactivation efficacy of K048. We have unravelled the lowered efficacy of K048 with the tabun-mutant *m*AChE(Y337A) using docking and steered molecular dynamics (SMD) simulations. The computed results showed some interesting features for the interaction of drug molecule K048 with tabun-*m*AChE(wild-type) and tabun-mutant *m*AChE(Y337A). The SMD simulations showed that the active pyridinium ring of K048 is directed towards the phosphorus atom conjugated to the active serine (SUN203) of tabun-*m*AChE(wild-type). The cradle shaped residues Tyr337-Phe338 present in the choline binding site stabilize the active pyridinium ring of K048 with π–π interaction and the residue Trp86 involved in T-shaped cation-π interaction. However, in the case of tabun-mutant *m*AChE(Y337A).K048 conjugate, the replacement of aromatic Tyr337 with the aliphatic alanine unit in the choline binding site, however, loses one of the π–π interaction between the active pyridinium ring of K048 and the Tyr337. The placement of aliphatic alanine unit resulted in the displacement of the side chain of Phe338 towards the His447. Such displacement is causing the inaccessibility of the drug towards the phosphorus atom conjugated to the active serine (SUN203) of tabun-mutant *m*AChE(Y337A). Furthermore, the unbinding of the K048 with SMD studies showed that the active pyridinium ring of the drug undergoes a complete turn along the gorge axis and is directed away from the phosphorus atom conjugated to the active serine of the tabun-mutant *m*AChE(Y337A). Such effects inside the gorge of tabun-mutant *m*AChE(Y337A) would lower the efficacy of the drug molecule (K048) for the reactivation process. The binding free energy computed for the tabun-*m*AChE(wild-type) and tabun-mutant *m*AChE(Y337A) with K048 showed that the drug molecule prefers to bind strongly with the former enzyme (~30 kJ/mol) than the later one.

1. Introduction

The enzymatic hydrolysis of the neurotransmitter acetylcholine (ACh) into acetate and choline ions at cholinergic synapses in neuromuscular junctions by acetylcholinesterase (AChE, EC 3.1.1.7) is inhibited by organophosphorus compounds (OPs) such as pesticides and nerve agents [1]. The inhibited enzyme can be made free from OP-AChE conjugates through the process called reactivation by strong nucleophilic reactivators like HLö-7, HI6, Ortho-7, TMB-4, K203 and K048 etc [2,3].

Intoxication by OPs based insecticides is a serious health problem and claiming more than 200,000 human lives yearly [4]. OPs based chemical warfare agents such as sarin (GB), cyclosarin (GF), tabun (GA), VX and VR are serious threat to human life due to their possible use in the battle field and terror attacks. The recent terror

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attack in Syria with sarin caused the loss of hundreds of human life [5]. Tabun was first produced in the middle of 1930's [6]. Most of the oximes show the lower reactivity towards the tabun-inhibited AChE conjugates due to their conformational changes in the active site and the presence of bulky dimethyl amine group/alkoxy moiety of tabun [7]. The reason for such resistance to reactivate of tabun-inhibited AChE is not well understood. Therefore, tabun poisoning remains a serious challenge to the experimental and theoretical research groups [7]. K048 has been found to be a potential pre-treatment drug for tabun poisoning [3,8–10].

The oxime groups attached to the drugs attack the electrophilic site of SUN203 (phosphorylated Ser203 residue) to reactivate the AChE. Such reactivation process is facilitated by the proper orientation of the oxime drug in the active site gorge of the AChE [11,12]. The AChE active site gorge involves largely aromatic residues that contribute to its well-defined acyl pocket and choline binding site at the base of the gorge. The active site of *h*AChE is comprised of active triad -Ser203, Glu334 and His447 which are near to the base of deep narrow gorge (~20 Å) [1a,1b,13]. The catalytic anionic binding site (CAS) residues such as Trp86, Tyr337 and Phe338 near the catalytic triad facilitate the orientation and binding of the reactivators to the AChE [14]. The peripheral anionic site (PAS) is another binding site responsible for the drug to move towards active site of the gorge, which is made up of aromatic residues viz. Tyr72, Tyr124, Trp286, Tyr341 and acidic residues viz. Asp74, Glu285 [15]. The structural fluctuation of the enzyme in every few picoseconds allows to open the gorge, which plays a crucial role in drug penetration process [16]. To reactivate OPs-inhibited AChE, nucleophilic strength and orientation of the nucleophile is important in the active site gorge with respect to the conjugated OPs-inhibited AChE. Secondary interactions of the cationic part of the drug and peripheral site of the enzyme have equal importance on reactivation process [2]. The bis-quaternary oximes recently studied have shown to have greater affinity toward the OPs-inhibited enzyme through cation-π and π–π interactions [17]. The extra stability of bis-quaternary oximes as a result of these interactions inside the gorge significantly enhances the potency and efficiency of the drug compared to mono-pyridinium oximes [2].

The reactivation efficiency of drug molecule depends on the chemical nature of the inhibited conjugated moiety, such as phosphorate, phosphonate, or phosphoramidate and the access of the oxime to the phosphorus atom conjugated to the active serine [18]. It has been perceived that the modification of the active center through mutagenesis can augment the catalytic hydrolysis of OP-inhibited AChE [18]. Such studies have been performed with mutant enzyme and oxime drug to achieve higher efficiency to restore free enzyme from OP poisoning [19–23].

The tabun-mutant *m*AChE(Y337A), however is not always very productive in terms of the reactivation process compared to their wild-type. It has been reported in the literature that the oxime drug K048 for the reactivation of tabun-inhibted *m*AChE is inferior upon mutation (Y337A) of the *m*AChE [24]. The experimental studies performed with K048 assisted reactivation of *m*AChE wild-type showed the overall reactivation rate constant K_r (second order rate constant of reactivation) of 632 mol^{-1} dm^3 min^{-1}. In the case of single mutant Y337A the K_r is 10 times slower than *m*AChE wild-type [24]. The reasons for such poor efficacy of K048 with tabun-mutant *m*AChE(Y337A) is not well understood. In this article, we have explored the poor reactivation of K048 for the tabun-mutant *m*AChE(Y337A) computationally. The crystal structure for non-aged tabun inhibited *h*AChE is not reported in the literature till date. Only the X-ray structure of aged tabun-hAChE is available in the literature [25]. Eugenie Carletti et al. recently reported that however, the non-aged and aged forms of tabun conjugated with *m*AChE crystal structures can be obtained [26]. The crystal structures of tabun inhibited AChE with HLö-7 (2JEZ) and Ortho-7 (2JF0) has been reported, however, the enzyme is *m*AChE [27]. Ramalho et al. have recently theoretically examined the efficiency of different oximes with modelled *h*AChE and *m*AChE [28]. The theoretical results reveal that *m*AChE is a suitable model to predict the thermodynamic and kinetic parameters for the reactivation of *h*AChE with oxime drugs [28].

The cyto- and genotoxicity of K-048 oxime is tested in rats [29]. The compound K-048 applied at 25% of its LD_{50}, which did not affect ChE activity and lipid peroxidation. Further there was no significant change in the superoxide dismutase (SOD) activity plasma. The cytotoxic profile of K048 of such tested dose is acceptable, and did not show any significant DNA-damage. These results show good promise for further evaluation of this drug to cure the poisoned AChE with the OPs [29].

2. Computational methodology

It is available in the literature that the docking-in vitro data correlation is better with 2JF0 (tabun-inhibited *m*AChE.Ortho-7 complex)/2JEZ (tabun-inhibited *m*AChE.HLö-7 complex) than structures without an organophosphorus moiety [27,30]. We have considered 2JF0 for our study. The choice of 2JF0 structure over 2JEZ is due to the following reasons: the drug molecule HLö-7 possesses more number of functional groups (oximes in the active pyridinium ring) whereas, Ortho-7 and K048 drugs possess only one oxime group in the active pyridinium ring. Therefore, the drug molecules Ortho-7 and K048 are more similar in terms of their structural properties and in the both cases the pyridinium rings are connected with simple alkyl chains. Therefore, the change in the enzyme environment in the active site would be less perturbed with Ortho-7 than HLö-7 and would be more appropriate for K048. The PDB structure of tabun-inhibited *m*AChE.Ortho-7 complex (PDB code: 2JF0) was taken from the protein data bank [27], repaired for missing residues and Ortho-7 drug was deleted from the complex to get protein only. The obtained protein was rectified with hydrogens and minimized using macromodel program [31]. The protein system was then mutated in the choline binding site (i.e. Y337A) by using Modeller program [15,32].

2.1. Molecular docking

The tabun-mutant *m*AChE(Y337A) conjugate was considered for the docking study with K048 using a grid based Autodock 4.2 program [33]. Autodock utilizes Lamarckian Genetic Algorithm (LGA) to explore the grid space and performs energy evaluations on the position of the ligand with respect to the target energy grids. The grid box of 70-70-70 Å was used to close the protein and the drug in grid map preparation for the Autogrid simulation. In Autodock simulation, ligands explore six spatial degrees of freedom (i.e. rotation and translation) along with associated torsions, and the interaction energy is calculated at each step until global energy minimum is reached.

2.2. Steered molecular dynamics

For molecular dynamics (MD) simulations, the protein-drug complexes obtained from docking studies were taken using GROMACS 4.6.3 with GROMOS 96 force field [34]. These complexes were soaked in cubic box filled with water molecules with a distance of 10 Å away from the box wall. The required number of positive (Na^+) and negative (Cl^-) ions in the box were added to maintain electro neutrality. The cubic box contains 35511 water molecules for tabun-mutant *m*AChE(Y337A). The solvent and solute were separately coupled to temperature reservoir at 300 K using V-