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Zaheer ul-Haq, Hassan Hadi, Syed Tarique Moin & M. Iqbal Choudhary

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RESEARCH ARTICLE

Molecular dynamics simulation of Axillaridine–A: A potent natural cholinesterase inhibitor

Zaheer-ul-Haq, Hassan Hadi, Syed Tarique Moin and M. Iqbal Choudhary

Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan

Abstract

Molecular Dynamics (MD) simulations were carried out for human acetylcholinesterase (hAChE) and its complex with Axillaridine–A, in order to dynamically explore the active site of the protein and the behaviour of the ligand at the peripheral binding site. Simulation of the enzyme alone showed that the active site of AChE is located at the bottom of a deep and narrow cavity whose surface is lined with rings of aromatic residues while Tyr72 is almost perpendicular to the Trp286, which is responsible for stable π -π interactions. The complexation of AChE with Axillaridine-A, results in the reduction of gorge size due to interaction between the ligand and the active site residues. The gorge size was determined by the distance between the center of mass of Glu81 and Trp286. As far as the geometry of the active site is concerned, the presence of ligand in the active site alters its specific conformation, as revealed by stable hydrogen bondings established between amino acids. With the increasing interaction between ligand and the active amino acids, size of the active site of the complex decreases with respect to time. Axillaridine-A, forms stable π -π interactions with the aromatic ring of Tyr124 that results in inhibition of catalytic activity of the enzyme. This π -π interaction keeps the substrate stable at the edge of the catalytic gorge by inhibiting its catalytic activity. The MD results clearly provide an explanation for the binding pattern of bulky steroidal alkaloids at the active site of AChE.

Keywords: *MD simulations; Axillaridine-A; π–π Interactions; Acetylcholinesterase; Hydrogen bonding*

Introduction

Acetylcholinesterase (AChE) is a key component of cholinergic brain synapses and neuromuscular junctions. The major biological role of the enzyme is the termination of impulse transmission by rapid hydrolysis of the cationic neurotransmitter acetylcholine (ACh) [1]. According to the cholinergic hypothesis, the memory impairment in patients with senile dementia of the Alzheimer type results from a deficiency in cholinergic functions of the brain [2,3]. Hence, the most promising therapeutic strategy for activating the central cholinergic function has been the use of cholinomimetic agents. The enzyme, AChE has long been an attractive target for the rational drug design and discovery of mechanism-based inhibitors used for the treatment of Alzheimer's disease (AD) [4]. The AChE inhibitors boost the endogenous levels of ACh in the brain of AD patients and thereby boost cholinergic neurotransmission. As far as the active site, responsible for catalytic action of the AChE is concerned, studies have suggested that this site is localized at the bottom of a narrow catalytic gorge [5,6]. The complex nature of the gorge fluctuations has been revealed by carrying out the 10-ns molecular dynamics (MD) simulations of AChE [7]. A collective motions on many scales determine the opening behavior of the gorge; two distinct states, one narrow and one wide, have been found. Correlation results identified the motions of many residues within the AChE moiety including the gorge that apparently move away from its entrance when it opens. The opening of alternative passages to the active site was found to be infrequent, since less than one-hundred of the frames collected were available to create the opening of alternative passages. These alternative passages are the back door bounded by residues Trp86, Tyr449 and Ile451 [8] and the side door, bounded by residues Thr75, Leu76 and Thr83 [9,10].

Address for Correspondence: Fax: +92-21-4819018-19; E-mail: zaheer_qasmi@hotmail.com

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Results of X-ray crystallography provide a picture of a protein in its native conformation as a well defined, densely-packed structure. Other experimental data [11–20] and theoretical considerations [21–23] indicate that there is considerable local motion inside a protein at ordinary temperatures. Moreover, the structural data themselves show that significant residue or subunit displacements have an important role in the activity of proteins (for example, enzyme catalysis [24], hemoglobin cooperativity [25], and immunoglobulin action [26]). To obtain a more complete understanding of proteins, it is essential to have a detailed knowledge of their dynamics. In spite of the considerable effort directed toward protein folding [27], very little has been done to investigate the motions of a protein in the neighborhood of its equilibrium configuration. For certain cases, in which the displacements along a suitably chosen co-ordinate system can be isolated, it has been demonstrated that empirical energy functions can be used to analyze the motions involved.

Axillaridine-A is one of the cholinesterase inhibitors that binds at the active site and is responsible for inhibiting the catalytic action of AChE. In the previous papers, we reported the isolation, characterization and biological evaluations of a series of steroidal alkaloids including Axillaridine-A isolated from *S. saligna* [28] as well as described our efforts toward the better understanding of interaction of Axillaridine-A in the active site of AChE enzyme obtained from *Torpedo californica* [29]. Axillaridine-A was first isolated from *Pachysandra axillaries* and its structure was identified as 20α-dimethylamino-3-benzoylamino-5α-pregna-2(3)-en-4-one [30]. This paper is an attempt to study the interactions of Axillaridine-A on the binding site of AChE as well as to determine the structural and dynamic effects of Axillaridine-A present at the catalytic gorge of the AChE.

Methods

MD simulation is one of the most versatile and widely applied computational techniques for the study of biological macromolecules. Particularly, it is valuable for understanding the dynamic behavior of proteins at different timescales, from fast internal motions to slow conformational changes or even protein folding processes.

MD simulations were performed on AChE protein, based on the crystal structure details obtained from protein databank (PDB code: 1B41) [31] refined at 2.76 Å resolution in complex with fasciculin. Fasciculin and other non-proteinic residues were removed from the complex and missing atoms of the residues were added using Biopolymer module of SYBYL7.2 (Tripos Inc, St. Louis, MO). Hydrogen atoms were added to the protein amino acids and atomic partial charges for the all-atom Amber force field were loaded. The three dimensional model of Axillaridine-A was geometrically optimized by means of PM3 semi-empirical Hamiltonian and atomic partial charges were derived using the Restrained electrostatic potential (RESP) charge fitting procedure [32] by carrying out single-point *ab initio* calculation at the HF/6-31G(d) level using Gaussian 98 [33]. MD simulations were carried out on the protein as well as on its complex with Axillaridine-A using the *cornell* et. al. [34] force field for the protein, general amber force field (GAFF) [35] for Axillaridine-A and the sander module implemented in AMBER 7 [36]. To ensure electro-neutrality of the protein and its complex, eight sodium ions were added with the subsequent solvation with TIP3 water boxes requiring a 12 Å thick solvent shell in all directions resulted in a system of dimension 93 × 92 × 88 $Å^3$ containing about 13500 water molecules [37]. Minimizations were carried out, starting with 500 steps and harmonic restraints of 25 kcal mol^{-1} $Å^{-2}$ on protein or complex and counterion positions. During the following 500-steps minimizations, the restraints on the counterions were relaxed faster than on the protein or complex. Finally, 500 steps of unrestrained minimization were carried out. A similar procedure was applied for the equilibration process. The system was heated from 50 to 300 K during 10-ps under constant-pressure conditions and harmonic restraints. Subsequently, the restraints were once again relaxed and finally an unrestrained 5 ps equilibration was carried out. After this procedure, the systems were subjected to constant temperature that was kept constant at 300K. General simulation parameters were kept constant during whole simulation: 2-fs time step, SHAKE algorithm to constrain bonding involving hydrogen atom [38], 9 Å cutoff and 0.00001 convergence criteria of the Ewald summation was used for part of the non-bonded interactions. The structural information was collected every 1000 steps (2-ps). The simulations were performed on eight processors of a Xeon 2.66 GHz Linux Cluster running under SUSE 9.2. The resulting MD trajectories were analyzed by means of carnal module of the same package.

Results and discussion

MD simulations were analyzed for the AChE protein and its complex with Axillaridine-A. AChE obtained from protein databank (PDB code: 1B41) and its complex with Axillaridine-A obtained from previous docking studies [29] were chosen as starting point for MD simulation. Docking studies revealed that only the ligand was treated in a flexible way. Therefore, in order to relax the Axillaridine-A / AChE complex and to evaluate the dynamic behaviour of the system at 300 K, exhaustive protocols of MD simulations were applied. The solvation of the system and the induced-fit at the binding site, as well as other aspects of the ligand/enzyme interaction, lead us to assess the feasibility of the binding modes as identified by Autodock [39]. For this purpose 2-ns of MD simulations sampling the NPT ensemble is carried out on the Axillaridine-A / AChE complex. During MD simulations, Total Energy, Potential Energy, Density and Temperature were monitored such that they reached stable values for the complex after a few hundreds of picoseconds.

Figure 1 shows a 3D structure of Axillaridine-A that is pregnane-type steroidal alkaloid obtained from *Sarcocca saligna,* optimized at the HF/6-31G(d) level. Axillaridine-A interacts at the active site that is located at the bottom of a deep and narrow cavity (aromatic gorge) whose surface is lined with rings of aromatic residues, after 2-ns MD simulation as shown in Figure 2. The active site of AChE contains a number of amino acids, which perform different types of interactions with Axillaridine-A. Van der wall interactions likely occur with the ligand by the residues like Trp86, Ile451, Gly448, Tyr449 and Ser229, present in the active site as shown in Figure 3. Trp86 may perform π -π interactions with ligand acyl group (if present), while the shape of the gorge base is defined by other residues that serve to discriminate between ligands. In the upper gorge area and the acyl binding pocket, residues Tyr124, Phe295, Phe338 and Phe297 are responsible for making hydrophobic contacts. Tyr72 is almost perpendicular to Trp286 ring so it forms a blocking wall by causing a stable π -π interaction to prevent the ligand ring to move away from its actual position as shown in Figure 5. Additionally, Phe295, Phe297, Val365 and Glu292 form another wall on the other side of the gorge, stretching from the acyl pocket toward the peripheral active site. The oxyanion hole residues, Gly121 and Gly122, as well as other residues like Tyr133, Glu450, Ile451, Ala127, Ser128, Tyr133, Ile118 near Trp86 contribute to the formation of electrostatic interactions in the active site, whereas

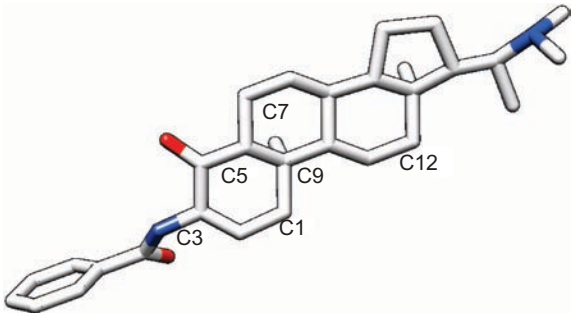

Figure 1. Natural AChE inhibitor, Axillaridine-A.

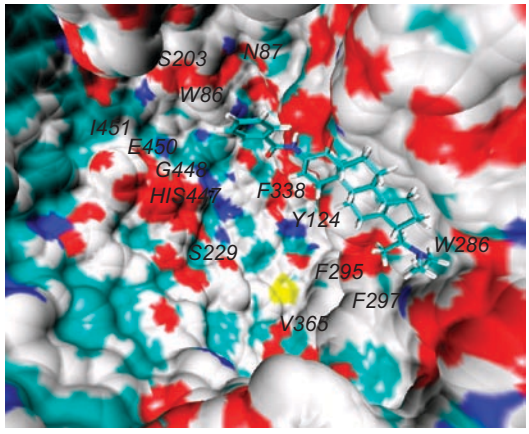

Figure 2. Axillaridine–A located at the aromatic gorge of AChE (PDB code: 1B41) after 2-ns MD simulation. Only the active site amino acids are labeled for the sake of clarity.

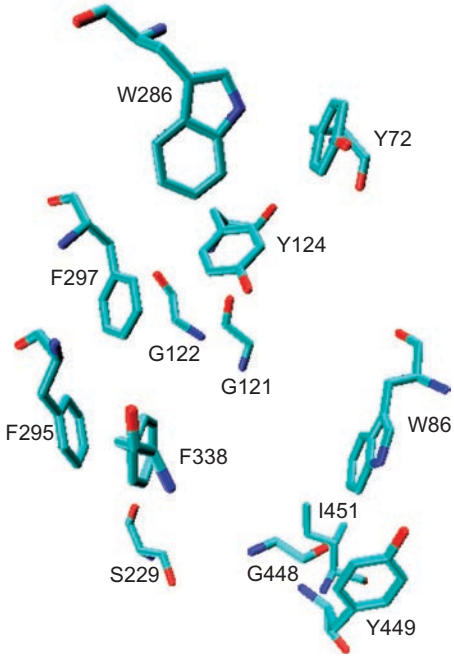

Figure 3. Important amino acid residues within 5.0 Å at the active site of AChE after 2-ns MD simulation. For detail refer to the text.

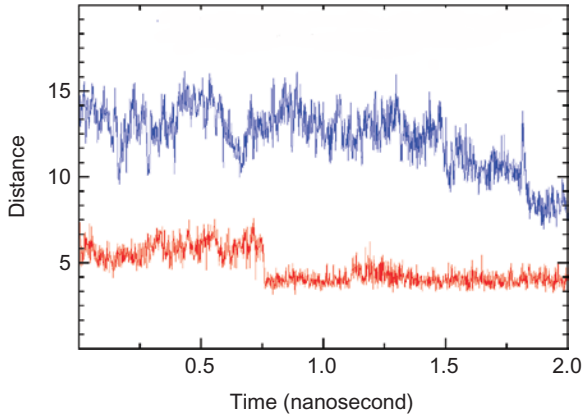

Figure 4. Size (Å) of the aromatic gorge between Glu_{81}/Try_{286} (Upper) and Phe_{338}/Trp_{86} (Lower) during 2-ns AChE-Axillaridine-A simulation.

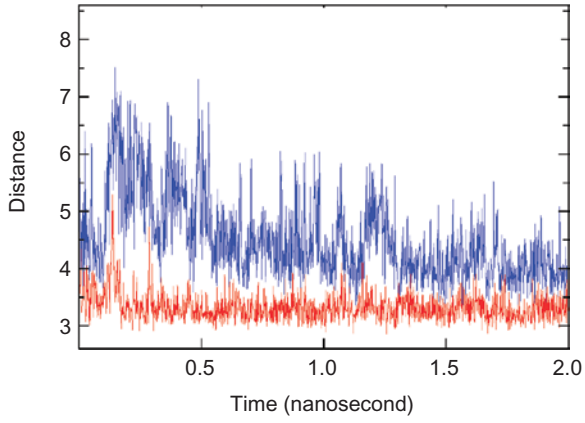

Figure 5. Stable π–π interactions between Trp_{286}/Tyr_{72} (Lower) and the aromatic ring of Axillaridine–A/Tyr_{124} (Upper) as a function of time in the AChE-Axillaridine-A simulation.

the primary electrostatic interactions are caused by residues like Tyr325, Asp74, Thr83 and Asn87 in the gorge area. The activity of ligands having polar groups is probably enhanced by residues like Gly342, Leu76, Glu285, Trp286, His287 and Glu292.

The dynamics of the gorge have already been investigated extensively by MD simulations, given the high flexibility of the gorge which is suggested to be necessary for the enzyme activity. Our simulation results for the complex show that the complication leads to the reduced gorge size due to the interaction between the ligand and the active site that is determined by the distance between the center of mass of Glu81 and Trp286 versus time as shown in Figure 4 The geometry of the active site is also changed by the presence of ligand in the active site that is obvious from the stable hydrogen bondings between the amino acids in the active site both for the protein and the complex, as shown in Table 1. There is a relationship between the gorge radius and the distance between Phe338 and Trp86 as shown in Figure 4. It is interesting to note that early MD simulations had focused on aromatic side chains and revealed such motions in a similar time range [40,41]. The results suggest that such fast motions are important for the activity of AChE. In the crystal structures and in the vast majority of the structures seen in the MD simulations, there is a bottleneck in the channel that prevents entry of the substrate. The opening of this channel occurs in picoseconds, which is often enough to allow the enzyme to maintain the high speed of action needed for the destruction of ACh in the function of cholinergic synapses. In the case of Axillaridine-A, which differs in size, it has to wait for a long time for a correspondingly larger opening of the channel. Axillaridine-A would likely diffuse away from the entrance and back into the surrounding solution rather than reacting. But the presence of the π-π interaction between the aromatic ring of Axillaridine-A and that of the Tyr124 makes the substrate stay and stable at the gate of the gorge, thereby inhibiting its catalytic activity (Figure 6). The distance between the aromatic rings of Axillaridine-A and Tyr124 at the catalytic gorge initially increases in the range of 4.5 – 7.0 Å, approaching to gain stability as time passes and finally reaches at the minimum 3.8 Å after 1.5-ns as shown in Figure 5.

Table 1. Summary of the average distances between heavy atoms (Å) for important stable hydrogen-bonding interactions between active site residues.

	Distance (Å)	
Hydrogen Bond	protein	complex
His436 : NδH – Glu323 : O_2	4.80	2.80
Ser196 : OγH – His436 : N_2	2.80	2.90
Asp68 : NH – Asn81 : Oδ1	4.20	3.20
Ser77 : NH – Phe74 : O	3.00	3.80
Phe71 :NH – Asp68 : Oδ2	2.50	4.30
Ser77 : OγH – Asp68 : Oδ2	2.65	3.50
Asn81 : NH – Ser77 : O	2.90	3.10
Gln70 : NH – Asp68 : Oδ1	3.24	3.20
Ser282 OγH – Asp281 : O	4.30	3.00
Trp80 : NH – Ser77 : O	3.00	3.10
Tyr330 : OH – Ser77 : Oγ	2.90	4.80
Tyr330 : NH – Phe327 : O	3.40	3.00
Tyr66 : NH – Tyr117 : O	3.25	3.05

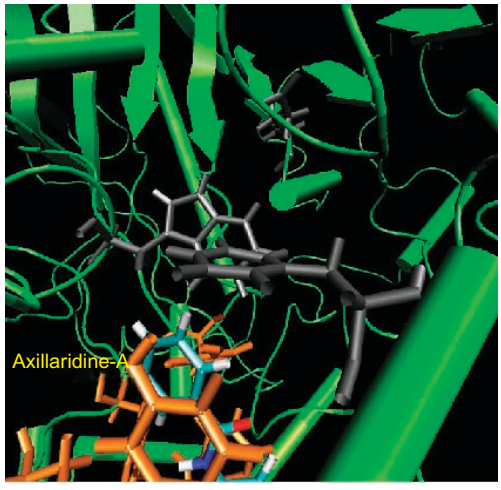

Figure 6. π-π Interaction between the aromatic ring of Axillaridine-A (shown in gray color) and that of the Tyr124 residue (shown in orange color). (Please see colour version online at: www.informa-healthcare.com/enz).

Conclusion

The objective of this study was to provide an explanation of the mechanism of hAChE inhibition, for a relatively bulky substrates like steroidal alkaloids. By means of computational docking, it has been observed that Axillaridine–A, one of the reported steroidal alkaloid binds similarly as other AChE inhibitors into the aromatic gorge. On the other hand, it can not enter deeply into the aromatic gorge as other ligands, which attributed to its bulky steroidal part. MD simulations of the AChE and Axillaridine–A / AChE complex have shown that the complexation of AChE with Axillaridine–A changes the dynamics and size of the gorge. Rigid hydrophobic part of the steroidal alkaloids become sandwich between aromatic residues while the flexible part of the substrate penetrate towards bottom of the active site.

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