ANALYSIS OF A MOLECULAR DYNAMICS SIMULATION OF THE ACETYLCHOLINESTERASE ENZYME AND ITS COMPLEX WITH (AXILLARIDINE-A) INHIBITOR

[*]Hassan Hadi and [**]Ammar J. Mohammed
[*]Chemistry Dept., College of Science , University of Baghdad.
[**]Biochemical Eng. Dept., Al-Khawarizmi College of Eng., University of Baghdad.

Abstract

Molecular dynamics (MD) simulations were carried out in order to investigate the binding mode of axillaridine-A at the active site of human acetylcholinesterase (AChE) enzyme. 2.0 nanosecond of MD simulations was made for the protein and the complex to dynamically explore the active site and the behavior of the ligand at the peripheral AChE binding site. These calculations for 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 and Tyr72 is almost perpendicular to the Trp286 ring and forms a stable π-π interaction. The size of the active site of the complex decreases with time due to increase the interaction. Axillaridine-A forms in addition to van der Waals interactions, stable π-π interaction with the aromatic ring of Tyr117 residue which is responsible for the inhibition of the catalytic activity of the enzyme. Finally because of the biological relevance of the target studied here, the present results can be of interest for the rational design of molecules potentially useful in the treatment of the Alzheimer's disease.

Introduction

According to the cholinergic hypothesis, the memory impairment in the patients with senile dementia of the Alzheimer type result from a deficiency in cholinergic function in the brain[1, 2]. Hence, the most promising therapeutic strategy for activating central cholinergic function has been the use of cholinomimetic agents. The enzyme acetyl-cholinesterase (AChE) has long been an attractive target for the rational drug design and discovery of mechanism-based inhibitors, for the treatment of Alzheimer's disease (AD). The aim of acetylcholinesterase inhibitors is to boost the endogenous levels of acetylcholine in the brains of AD patients and, thereby, to boost cholinergic neurotransmission.

A 10-ns molecular dynamics (MD) simulation of mammalian AChE (mAChE) has already been carried out. This has revealed the complex nature of the gorge fluctuations.[3] Collective motions on many scales contribute to the opening behavior of the gorge, and two distinct states, one narrow and one wide, were found. correlation results identified the motions of many protein residues within the mAChE moiety that includes the gorge apparently move away from the gorge entrance when the gorge opens. The opening of alternative passages to the active site was found to be infrequent, since less than one-hundred of the frames collected showed opening of alternative passages. These alternative passages are the back door bounded by residues Trp 86, Tyr 449 and Ile 451[4] and the side door, bounded by residues Thr 75, Leu 76 and Thr 83.[5, 6]

Molecular dynamics (MD) has proved to be an optimum numerical recipe applicable to problem with many degrees of freedom from quite different fields of science. The knowledge of the energy or potential landscape of interacting particles, like electrons and atoms, enables one to calculate the forces acting on the particles, and to study the evolution of the system with time. As long as classical mechanics is appropriate to describe the dynamics of the individual constituents (i.e., atoms or molecules), the Newtonian equations of motion can be related to the statistical mechanics of the (classical) particles by using the equipartition theorem. Although the euipartition theorem holds only for classical particles the combination of classical and statistical physics has also been used to simulate small molecules at low

temperatures without critically discussing so far the limitations when applying to very small quantum mechanical systems. The forces are then simply related to the gradients of the potential energy surface (PES) of either the classical or the quantum mechanical *N*-particle system.[7]

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[8-17] and theoretical considerations[18-20] 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[21], hemoglobin cooperativity[22], immunoglobulin action[23]). To obtain a more complete understanding of protein, it is essential to have a detailed knowledge of their dynamics. Inspite of the considerable effort directed toward protein folding[24], very little has been done to investigate the motions of a protein in the neighborhood of its equilibrium configuration. These displacements can give us information about the changing in the conformation of the protein or the complex along a suitably chosen co-ordinate and during the time, sine the crystal structure is one and fixed conformation, and then the comparison with it helps more in this field. It has been demonstrated that empirical energy functions can be used to analyze the motion involved.

The steroidal alkaloids isolated from the plant *Sarcococca saligna* have already been showed very potent inhibitory activities against the enzyme acetyl as well as butyrylcholinesterase and axillaridine-A is one of the same class. This novel steroidal alkaloid was first isolated from *Pachysandra axillaries* and its structure was identified as 20*α*-dimethylamino-3-benzoylamino-5*α*-pregn-2(3)-en-4-one[25] as shown in Fig. (1).

Within the molecular modeling framework, here we use molecular dynamics (MD) simulations to determine the binding mode(s) of Axilliridine-A with AChE. We performed another MD simulation of AChE complexed with inhibitor to investigate the structural and dynamical effect of inhibitor binding on AChE, and compared the results of the two calculations like the AChE properties.

Method

All simulations were based on the crystal structure of the human AChE refined at 2.76 Å resolution (PDB IP 1B41) [26]. Hydrogen atoms were added to the protein amino acides, and the atomic partial charges from the all-atom amber force field were loaded[27], and the Sander module implemented in the AMBER7 package[28]. The MD trajectories were analyzed by means of the carnal module of the same package. The internal as well as van der Walls parameters of axillaridine-A were determined using an analogy criterion.

The three dimensional model of axillaridine-A was geometrically optimized by means of the PM3 semiemperical Hamiltonian as implemented in the Sybyl graphic interface to MOPAC[29] version 9.0. Atomic partial charges for axillaridine-A molecule were calculated by carrying out single point ab initio calculated at the HF/6-31 G(d) level using the Gaussian 98 package and the then applying the restrained electrostatic potential (RESP) procedure[30].

The ligand /enzyme complexes were immersed in a solvation box of about 13500 TIP3P water molecules[31]. Six K^+ counter ions were added to keep electrostatic neutrality on the whole system. The dielectric constant was set to 1. Short-range coulomb and van der Waals interactions were estimated within an 8 A cutoff. The Shake algorithm was used to constrain all bonds involving hydrogen atoms[32].

An approximately 2.0 nanosecond trajectories was provided for this project as a continuation of the work performed by Tara et al[33]. We need more calculations efforts and more efficient computers to go far or for longer time (may be future study). All the pictures are taken by using VMD1.8.3 program after loading the output files of Amber program for 2ns period.

Discussion

MD solves Newton's equations of motion for atoms in the system to give a trajectory that defines how the positions of the atoms vary with time. Snapshots taken from the trajectory

may then be subjected to minimization in order to generate a sequence of minimum energy conformations.

The geometry optimization for the ligand axillaridine-A was calculated by using the g98 software and PM3 semiemperical method. The ligand molecule is a steroidal conformation with an aromatic ring Fig (1).

MD simulations were calculated for the protein alone and for the complex with Axilliridine and the aim was to obtain more precise enzyme inhibitor models in the state close to natural condition, to further explore the binding modes of the inhibiter and to make comparison between the complex and the enzyme to know the changes occur for the active site and the hole protein during the binding.

The active site of the AChE enzyme is located at the bottom of a deep and narrow cavity (aromatic gorge) whose surface is lined with rings of aromatic residues. The three-dimensional structure of AChE after 2.0 nanosecond simulations is shown in Fig. (2).

There is a variety of amino acids in the active site depending on the kind of the interaction with ligand, the residues Trp86, Ile451, Gly448, Tyr449 and Ser229 are the most important residues in the active site for van der Waal interactions, Trp86 functions by forming π-π interactions with ligand acyl group (when available), while the other residues define the shape of the gorge base serving to discriminate accordingly to ligand shape. In the upper gorge area and the acyl binding pocket residues Tyr124, Phe295, Phe338 and Phe297 are responsible for providing hydrophobic contacts. The ring of Tyr72 is almost perpendicular to the Trp286 ring and forms a blocking wall to prevent the ligand ring from moving away from the position where it forms a stable π-π interaction with the Trp286 ring Fig. (3).

Phe295, Phe297, Val365 and Glu292 form another wall on the other side of the gorge stretching from the acyl pocket towards the peripheral active site.

Residues Tyr133, Glu450, Ile451, Ala127, Ser128, Tyr133, Ile118 near Trp86 and the "oxyanion hole" residues Gly121 and Gly122 are important in providing electrostatic interactions in the active site. Tyr325, Asp74, Thr83 and Asn87 are the primary electrostatic contrbutions in the gorge area. Gly342, Leu76, Glu285, Trp286, His287 and Glu292 are probably helpful in enhancing the activity of ligands with polar groups oriented in this area Fig. (4).

One atom pair was identified to extent that determines the gorge size, the distance profile of the pair Glu70 and Trp275 plotted over time in Fig.(5), is very similar to the main gorge fluctuations throughout the simulation. The time series shows that the distance between the two residues decreases with time due to increase the interaction between the ligand and the active site.

The presence of the ligand in the active site change the geometry of the active site and this is clear from Table (1) which contains the stable hydrogen bonds between the amino acids in the active site for the protein and the complex.

Fig.(6) shows the relationship between the gorge radius and the distance between Phe326 and Trp80. It is interesting to note that early molecular dynamics simulations had focused on aromatic side chains and revealed such motions in a similar time range [34,35]. The results suggest that such fast motions are important in the activity of acetyl-cholinesterase. In the crystal structures and in the vast majority of the structures seen in the molecular dynamics simulation there is a bottleneck in the channel that prevents entry of the substrate, opening of this gate occurs on the picoseconds timescale which is often enough to allow the enzyme to maintain the high speed of action needed for the destruction of acetylcholine in the function of cholinergic synapses, while in the case of Axillaridine-A which is differ in size it has to wait a long time for a correspondingly larger opening of the gate, this molecule would likely diffuse away from the entrance 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 Tyr117 residue in the protein makes the substrate stay at the gate of the gorge and inhibit its catalytic activity which can assist in the treating of Alzheimer's disease through stopping the action of this enzyme.

Fig.(7) shows the distance between the two rings is changing the begin in the range (4.5-7Å) and to be more stable within time and to reach the minimum after 1.5 nanosecond 3.8 Å and the Figs.(8,9) give the picture of the interaction.

Table (1)
The distances of some stable Hydrogen Bonds in the active site of the complex.

Hydrogen Bond	Distance (Å)	
	protein	complex
Hid 436 : NδH - Glu 323 : Oε2	4.80	2.80
Ser 196 : OγH - Hid 436 : N ε2	2.80	2.90
Asp 68 : NH – Asn 81 : Oδ1	4.20	3.20
Ser 77 : NH – Phe 74 : O	3.00	3.80
Phe 71 :NH - Asp 68 : Oδ2	2.50	4.30
Ser 77 : OγH - Asp 68 : Oδ2	2.65	3.50
Asn 81 : NH - Ser 77 : O	2.90	3.10
Gln 70 : NH - Asp 68 : Oδ1	3.24	3.20
Ser 282 OγH - Asp 281 : O	4.30	3.00
Trp 80 : NH - Ser 77 : O	3.00	3.10
Tyr 330 : OH – Ser 77 : Oγ	2.90	4.80
Tyr 330 : NH - Phe 327 : O	3.40	3.00
Tyr 66 : NH – Tyr 117 : O	3.25	3.05

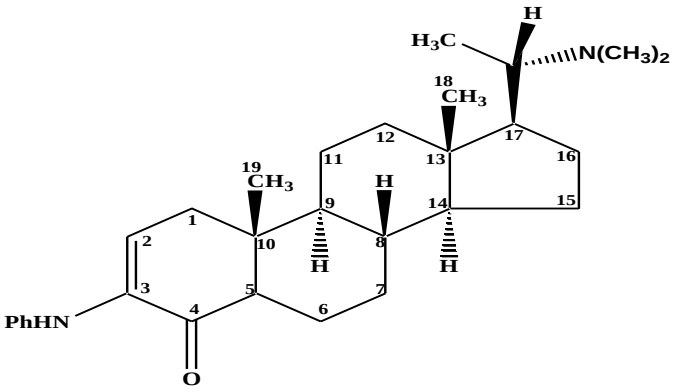

Fig. (1) : Two dimensional representation of AChE inhibitor Axillaridine-A.

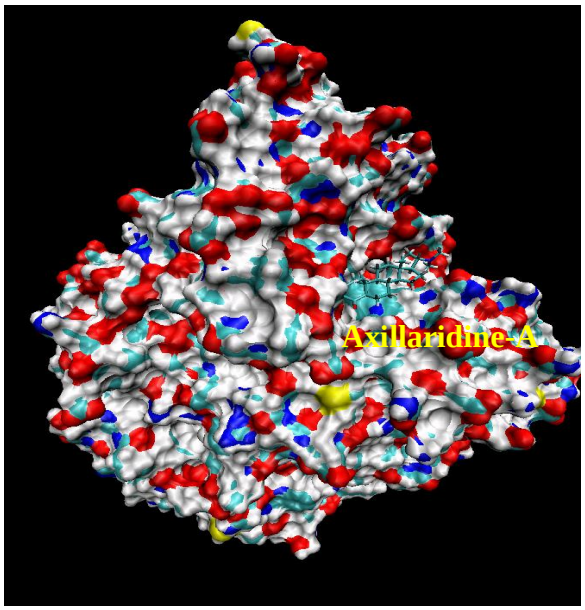

Fig. (2) : The complex of Axillaridine-A with AChE by VMD1.8.3 program.

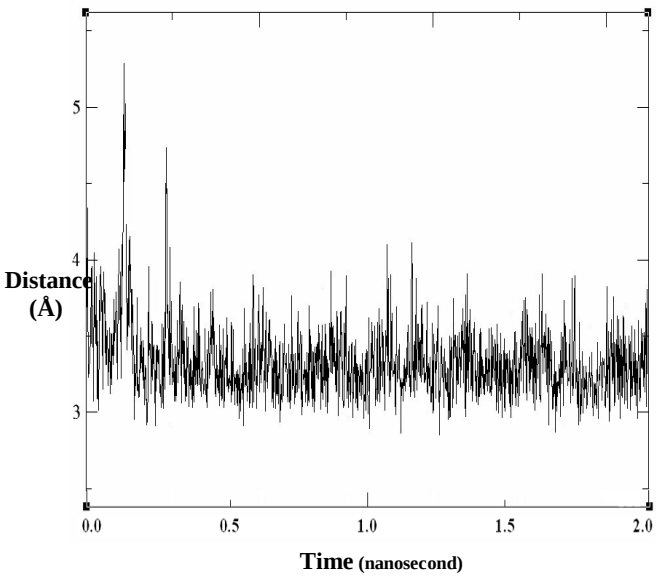

Fig. (3) : The stable π-π interaction between Trp286 and Tyr72 plotted over time.

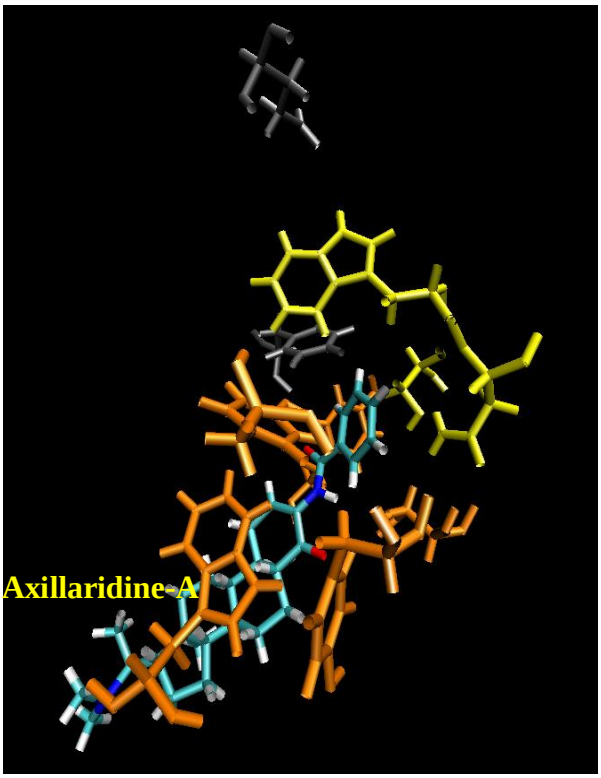

Fig. (4) : Some important residues in the active site of the complex of Axillaridine-A with AChE by VMD1.8.3 program.

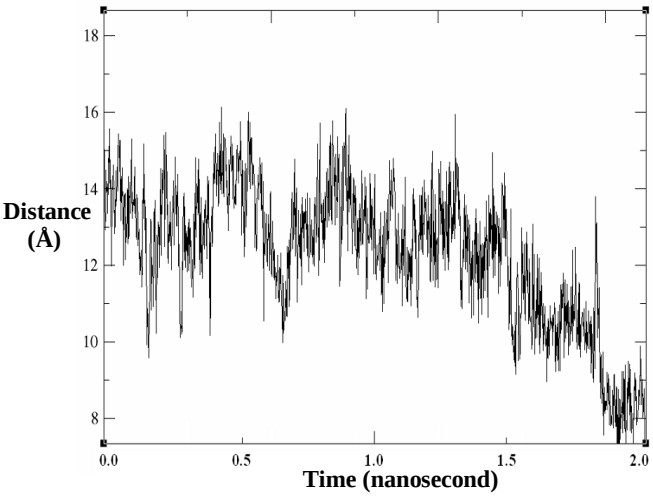

Fig. (5) : Gorge size through the distance between the pair Glu70 and Trp275 plotted over time.

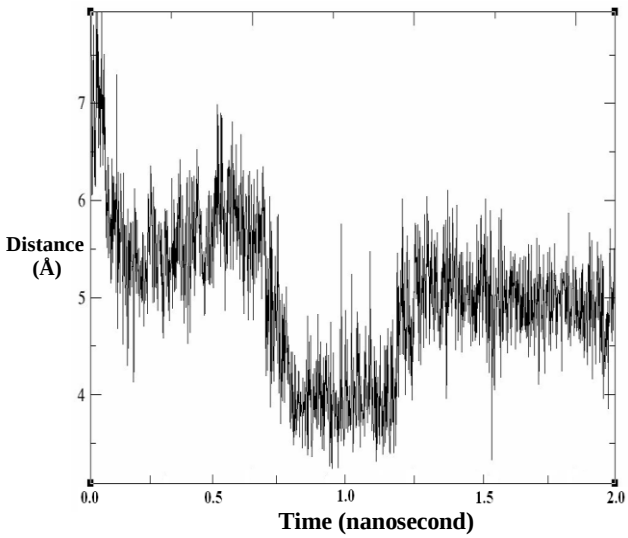

Fig. (6) : The relationship between the gorge radius and the distance between Phe326 and Trp80.

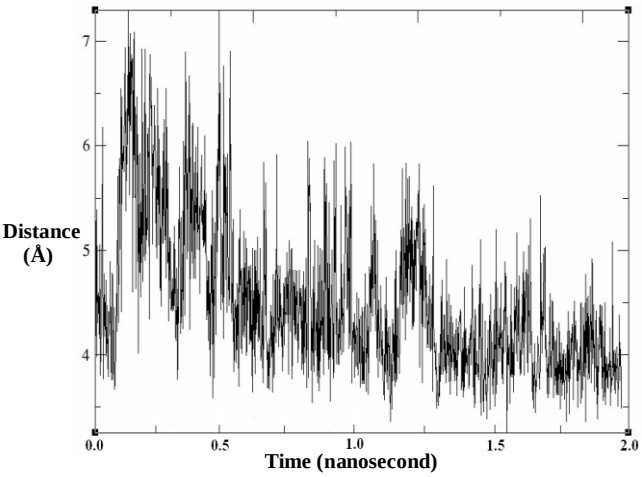

Fig. (7) : The distance of the the π-π interaction between the aromatic ring of Axillaridine-A and that of the Tyr117 residue.

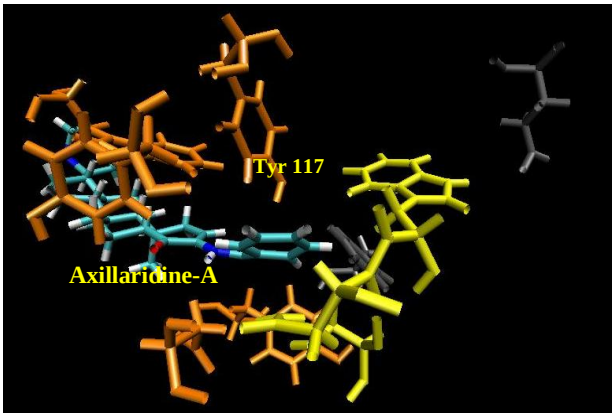

Fig. (8) : Picture of the π-π interaction between the aromatic ring of Axillaridine-A and that of the Tyr117 residue by VMD1.8.3 program.

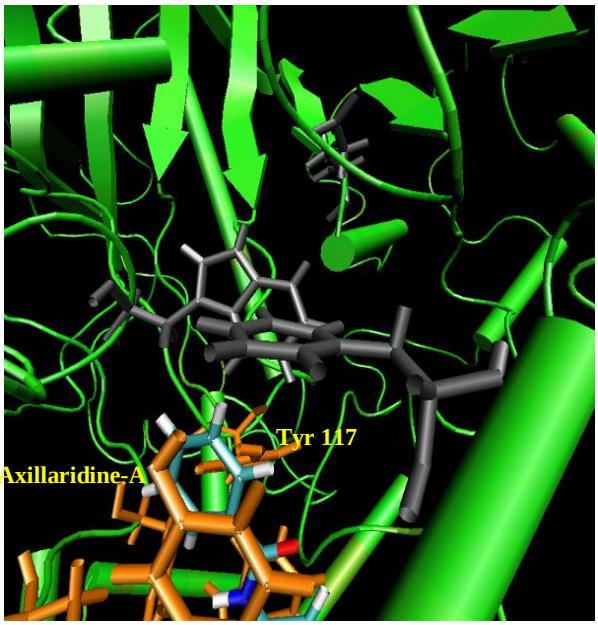

Fig. (9) : Another picture of the π-π interaction between the aromatic ring of Axillaridine-A and that of the Tyr117 residue by VMD1.8.3 program.

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