ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Molecular Dynamics of the Pyridoxine Derivative in the Acetylcholinesterase Active Cavity.

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ABSTRACT

Acetylcholinesterase (AChE) is a key enzyme in central nervous system, responsible for the regulation of nerve impulse transmission through the rapid hydrolysis of the acetylcholine neurotransmitter. In recent years, the issue of AChE specific interaction with ligands to regulate its activity becomes more and more popular. In particular, it is necessary to develop specific AChE inhibitors - potential new drugs for the treatment of neurodegenerative diseases, which will have a greater efficacy and fewer side effects. Currently, the methods of molecular modeling are actively used for the development of new drugs. In this paper we studied the insilico structure of a mouse AChE, since the study of enzyme activity in preclinical tests was carried out on mice. Pyridoxine derivative was used as ligands for which anticholinesterase symptoms were shown during the initial experiments invivo, and its position in the active center during the docking was similar acetylcholinesterase inhibitors used in medicine nowadays (proserin, physostigmine). The use of molecular dynamic simulation method allowed to evaluate the drug potential of inhibitors by the most cost-effective way. The study was conducted using the software package NAMD 2.8 and the force field AMBER 99. The study showed that the spatial position of ligand is favorable for AChE inhibiting. As the result of the molecular dynamics, the distance between the oxygen of the hydroxyl group Ser203 and the carbon atom of derived pyridoxine fragment carbamylation decreased from 6.4 Å to 3.8 Å, which contributes to their interaction to form a bond. The spatial position of the ligand is supported by the weak link between the tertiary nitrogen of carbamylation fragment and the oxygen of hydroxyl group Tyr124 AChE. Moreover, the ligand is held in an active cavity of the enzyme by hydrophobic interaction of its heterogenic cycle with Trp86 AChE. This state of the ligand structure may provide a long-term anticholinesterase of pyridoxine effect producers.

Keywords: acetylcholinesterase (AChE), pyridoxine derivative, molecular dynamics, specific interaction of enzyme with ligand.

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ISSN: 0975-8585

INTRODUCTION

Acetylcholinesterase is an enzyme (AChE, EC 3.1.1.7) from the family of cholinesterase, which dates back butyrylcholinesterase (BChE, EC 3.1.1.8). It is one of the key enzymes of the central nervous system, responsible for the regulation of a nerve impulse transmission through the rapid hydrolysis of acetylcholine neurotransmitter (kcat /KM=1.6×108M-1s-1) [1]. Besides its classic functions - the catalyst one, the enzyme carries out a number of non-classical features, including the participation in the lymphatic system operation and in embryonic development [2]. The enzyme is localized in central nervous system, in sympathetic and parasympathetic ganglia (peripheral nervous system), in lymphatic system and embryonic tissues. Among the clarified nonclassical AChE functions its participation in the growth of axons, synapse formation [3,4] and the growth of malignant tumors [5-7] were discovered.

The interest in this class of enzymes is explained by the fact that the signs of cholinergic system failure are observed in such diseases as glaucoma, myasthenic syndrome, and neurodegenerative diseases (Alzheimer's disease) [8-10].

In a review devoted [11] to the currently used and studied AChE inhibitors, their characteristics, differences and similarities, the therapeutic effects of drugs on their basis are described. However, a significant disadvantage of all inhibitors is the presence of a large range of side effects. The fundamental difference between existing products from one another is the type of binding to the enzyme. Tacrine, velnacrin, huperzine and donepezil are non-covalent inhibitors with a high affinity; metrifonate forms an irreversible covalent compound with the substrate. Physostigmine, prozerine are reversible inhibitors. Tacrine and velnacrin are unbeatable inhibitors, donepezil has competitive and unbeatable features. Galantamine is a competitive and a reversible AChE inhibitor, as well as an allosteric modulator of nicotinic choline receptors [12]. The action of metrifonate starts with a competitive inhibition, but over time it is transformed into a non-competitive inhibition [13].

The purpose of this study was to clarify the general mechanism of AChE enzyme interaction with pyridoxine derivatives based on the analysis of the enzyme-ligand complex molecular dynamics.

METHODS

AChE structure of the mouse 2JEY from Protein Date Bank was used in the work (the structure resolution makes 2.7 Å), and a synthetic derivative of pyridoxine "a". The inhibitor "a" is the founder of pyridoxine derivatives, differing from each other by the length of the radical tail [14]. The coordinates of inhibitor used in this work were obtained during docking [15], which was conducted in AutoDock program, providing good results in various assays [16].

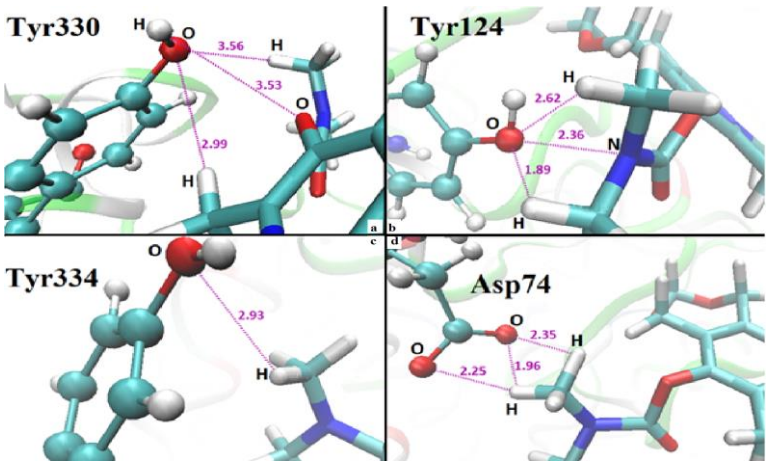

Figure 1: Interaction of AChE amino acid residues with ligand

In order to study the interaction of the ligand with AChE the molecular dynamic simulation was conducted using the software package NAMD 2.8 [17] and AMBER 99 force field [18]. AChE complex with

pyridoxine derivatives obtained during docking was selected as the starting position. Protein structure was placed in a cell consisting of water molecules, obtained in such a way that its borders were placed at the distance of 8 Å from the protein surface (the cell was composed of 32135 water molecules).

The initial position of the ligand structure in protein was stabilized mainly due to hydrophobic interactions between amino acid residue Trp86 AChE and inhibitor "rings". The methyl fragment with heterocyclic carbon is stabilized by hydrogen bond with the oxygen of Tyr330 (2,99 Å) hydroxyl group (Fig. 1.a). The hydrogen atoms of the radical group Tyr330 form a hydrogen bond with the atom O of pyridoxine derivative (3,36Å and 3,53Å). The structure of carbamylated fragment is stabilized by hydrogen bonds between the hydrogen atoms (N-(CH3)2) and the oxygen atoms of hydroxyl groups with amino acid residues (fig. 1. b-d). Figure 1 shows the interaction of Tyr124 oxygen atom with two hydrogen atoms of the inhibitor (at the distances of 1,89 Å and 2,62 Å, respectively). For the oxygen of the amino acid residues Tyr334 the interaction occurs at a distance of 2,93 Å. Three hydrogen atoms of carbamylated inhibitor fragment interact with the two oxygen atoms of the amino acid residue Asp74, at the distances of 2.35 Å and 3,66, 1.96 and 2.25 Å, 3.41 Å and 3,60, respectively. The oxygen atom of the amino acid residue Tyr124 hydroxyl group forms a non-covalent bond with the N atom at the distance of 2,3 Å.

In general the structure of pyridoxine derivative is well stabilized in the active center of the enzyme, which indicates the possibility of a covalent bond development between the ligand and the amino acid residue Ser203 AChE.

Dynamics performance terms

Dynamics was carried out using periodic boundary conditions, the integration step made 2 fs, the modeled system temperature made 300 K. The track recording was carried out for 2 ns at each 800-th step of the dynamics. The mesh size made 105 × 75 × 150 Å. Before the dynamics the complex structure was minimized by gradient descent method during 200 steps. The threshold of potential cutting makes 10 Å. The relations with H atoms were recorded In order to reduce calculations. Static interaction between periodic images was calculated by Ewald summation (PME). The protein structure was placed in a cell consisting of water molecules, obtained in such a way that its borders were at the distance of 8 Å from the protein surface (the cell was composed of 32135 water molecules).

RESULTS AND DISCUSSION

The visual analysis of an enzyme-ligand complex molecular dynamics shows an approximation of the inhibitor to the amino acid residue of the catalytic triad Ser203.

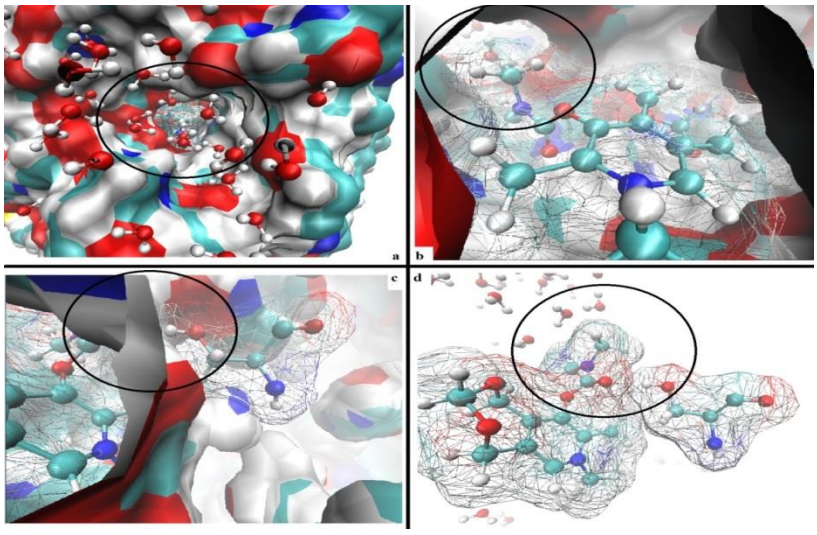

Figure 2: The enzyme-ligand complex (a - a view on the channel to the active center; b - the view from inside the of the active center cavity on the channel; c - the position of the ligand and Ser203, d - the position of water molecules relative to the complex)

ISSN: 0975-8585

Fig. 2.a shows the molecule of water rushing through the channel in the active site cavity. The "border guards" in the channel of the enzyme are 4 amino acid residues Tyr72, Asp74, Trp279, Tyr334 (Figure 3). These amino acid residues form the "gate" the parameters of which prior to dynamics were equal to ≈ 5,11 × 8,42 Å. During the dynamics these parameters varied from 7.11 × 9.98 Å. The maximum value of the parameters reached 8.95 × 10.48 Å. The measurements were performed via a diagonal. The oxygen of the hydroxyl group Tyr72 and the oxygen of the carboxyl group Tyr334 along a long diagonal acted as reference points for measurement, the carbon of the benzene ring Trp279 and the oxygen of the side radical Asp74 along the small diagonal. In its effort to get into the active center cavity the water molecules collide with the ligand which is already in the active cavity (Fig. 2.b, 2.d). The steric hindrance created by pyridoxine derivative does not allow water molecules to get into the free cavities around the amino acid residue Ser203 (Fig. 2.c), whereby the water molecules stop before carbamylated ligand fragment (Fig. 2.d). Presumably, the situation of pyridoxine derivative with respect to the amino acid residue Ser203 leads to the formation of a covalent bond between C (1) of carbamylated ligand fragment and O hydroxyl group Ser203. Then, their interaction involves deacylation according to the mechanism of acetylcholine interaction with AChE. But the process of deacylation in this case will proceed slowly because of the steric hindrances created by the inhibitor.

However, although the probability of a covalent bond development is not 100%, the ligand will be retained in an active cavity by hydrogen bonds and stacking interaction. This leads to long-term antiholinesteratic effect by blocking the active center of the enzyme.

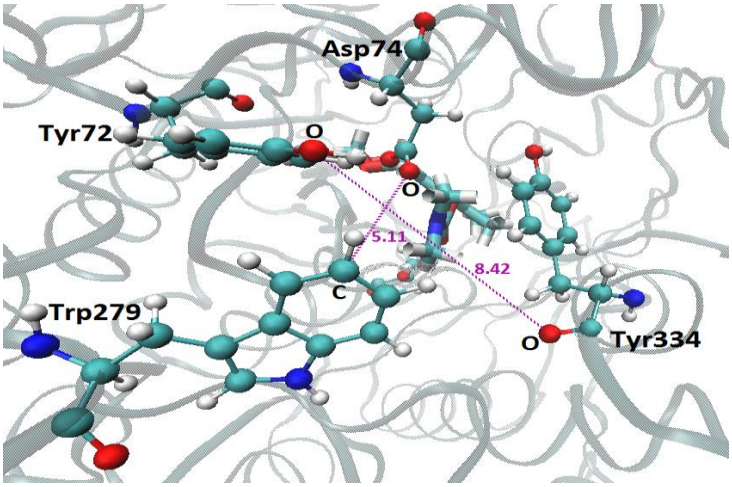

Figure 3: The amino acid residues forming the channel to the active center between Tyr72 and Tyr334 (8.4 Å), and between Trp279 and Asp74 (5.1 Å) (the ligand is depicted in the form of tubes)

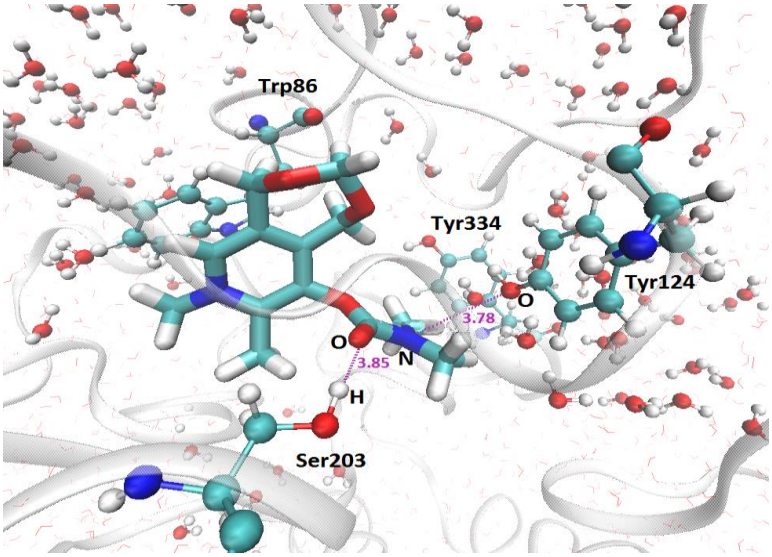

Figure 4: The position of the ligand in the active center after the molecular dynamics

The distance between the oxygen atom O of the hydroxyl group Ser203 and the carbon atom C (1) of derived pyridoxine carbamylated fragment makes 3.8 Å (Figure 4), which promotes the nucleophilic attack by the carbon oxygen and covalent bond development. During the analysis of molecular dynamics this distance between the atoms was observed in 30% of cases, indicating a high probability of such interactions. In general, during the molecular dynamics the minimum value of the distance between the oxygen atom O of the hydroxyl group Ser203 and the carbon atom C(1), of pyridoxine derivative carbamylated fragment made 3,5 Å, the average value made 3.9 Å. The carbamylated fragment retained also due to the developed non-covalent link between its N and O of the hydroxyl group of the amino acid residue Tyr124, the length of the distance makes 3.7 Å (Figure 4). In general, during the molecular dynamics the minimum value of the distance between the nitrogen atoms and oxygen O of amino acid residue Tyr124 made 3,1 Å, an average value made 3.8 Å. The remainder of the ligand is held by stacking interactions of its heterocyclic and amino acid residue Trp86.

CONCLUSION

On the basis of the performed molecular dynamics and its analysis one may suggest that the pyridoxine derivative can inhibit AChE, forming a covalent bond with an amino acid residue of the catalytic triad within the active center Ser203. But we should not exclude the possibility of covalent interactions. Ligand may be maintained in the active site of the enzyme through hydrogen bonding and stacking interactions. At any interaction the mechanism of AChE inhibition by pyridoxine derivatives is that they are steric barrier for acetylcholine, which provides a long term anticholinesterase effect.

SUMMARY

The methods of molecular modeling may predict the behavior of the ligands in the active cavity of the enzyme and suggest the type of their interaction that is important for the creation of specific inhibitors that are in demand in modern medicine.

ACKNOWLEDGEMENT

The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University.

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