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The Ligand Behavior on the Surface of Acetylcholinesterase.

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ABSTRACT

Acetylcholinesterase (AChE) is an important enzyme of a man's central nervous system responsible for the regulation of a nerve signal transmission by the hydrolysis of acetylcholine. For the development of AChE inhibitors the regulation of its activity at specific interactions with different ligands is a relevant one. The clarifying of ligand and AChE interaction details on a structural level, will allow to develop its specific inhibitors - the potential compounds for the treatment of neurodegenerative diseases. These compounds are more effective. One of the methods for potential inhibitor and enzyme interaction studying is the molecular dynamics. In this work the molecular dynamics of the enzyme-ligand complex was performed, wherein the ligand (pyridoxine derivative) is located approximately near amino acid residues forming the "gates" for an active center channel. The study was conducted with the help of the software package NAMD 2.8 and a power field AMBER 99. The results of the molecular dynamics showed the mobility of the amino acid residues forming the "gates" of a channel. The data about the size and the mobility of the "gate" led to the conclusion that a ligand is capable of entering into the channel of an enzyme active cavity.

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

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INTRODUCTION

Acetylcholinesterase is an enzyme of cholinesterase family (AChE, KF 3.1.1.7). It is a key enzyme of a nervous system responsible for the regulation of a nerve impulse transmission by rapid hydrolysis of acetylcholine neurotransmitter [1,2]. The interest for this class of enzymes is explained by the fact that the signs of cholinergic system deterioration are observed during such diseases as glaucoma, myasthenic syndrome and neurodegenerative diseases (Alzheimer's disease) [3-5]. The process of AChE inhibiting leads to increased concentrations of acetylcholine in the synapse and the extension of its impact on the receptor that lets pass the signal along a nervous system. The derivatives of pyridoxine in preliminary experiments in vivo [6] showed some anticholinesterase activity. The previous studies [7] showed that the ligand interacts non-covalently with AChE in the active center of an enzyme. At that its covalent interaction is not excluded. Also the role of catalytic triad in the active site of an enzyme was shown during the interaction with the ligand [8]. In the studies using computer modeling techniques various characteristics of an enzyme are considered and its interaction with ligands, as well as the modification of enzymes to achieve the desired properties [9]. In this study, the molecular dynamics method reveals the issue of interaction of ligand and amino acid residues interaction located at the entrance of the channel leading to the active center of AChE.

METHODS

AChE structure of the mouse 2JEY was used from the protein structure bank PDB. Pyridoxine derivative "a" was used as the ligand [6.7]. The molecular dynamics was carried out in the program NAMD 2.8 using the force field AMBER99.

The initial position of the ligand on the protein surface was obtained during the molecular docking using the program AutoDock, after which the ligand structure was parametrized in the ANTECHAMBER application.

The molecular dynamics was performed using periodic boundary conditions, the integration step made 2 fs, the temperature was 300 K. The track was recorded for 2 ns. The protein structure was placed in a cell consisting of water molecules with the size 105 × 75 × 150 Å. Before the dynamics the energy of the complex structure was minimized by gradient descent method during 20,000 steps. The cutting potential threshold makes 10 Å. To reduce the calculation time the relations with H atoms were fixed. The static interactions between periodic images were calculated by Ewald summation method (PME).

The analysis of molecular dynamics was carried out using VMD program VMD and scripts written by the programming language Python.

RESULTS AND DISCUSSION

The initial position of the ligand (Figure 1.1) on the enzyme surface near the entrance to the active site of a channel received during docking, shows the presence of stacking interaction with amino acid residue Trp286. This position of the ligand is energetically favorable, but its orientation does not allow the passage in the active center channel. However, the coordinates of the ligand atoms in this position were chosen as the initial ones for the dynamics, to determine whether the ligand may remain in this position, or its position would change.

The results of the dynamics showed that the initial position of the ligand is unstable (fig. 1.2,1.3). During the intermolecular interaction occurs the discrepancy (repulsion) of the ligand occurs and the side radical of amino acid residue Trp86. At that there is no apparent motion of the ligand to the active center of the channel.

During the data analysis obtained in the course of the dynamics, it is necessary to proceed from the docking results. If the ligand structure during docking will be located close to the channel entrance, it may form the connections with amino acid residues that serve as the channel "gate". At that two options are possible for a channel structure favorable for the penetration into the channel of the ligand structure orientation. First option: the ligand orientation should be such that its carbamoylation radical is directed towards the channel. In this case, the ligand should not be unfold within the channel or an active center cavity,

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to react with an amino acid residue Ser203. The second option: a molecule must be oriented opposite, i.e. carbamoylation radical should be focused "from the channel". In this case the aromatic amino acids of the channel will interact with the aromatic ring of the ligand, "helping" it to pass the channel.

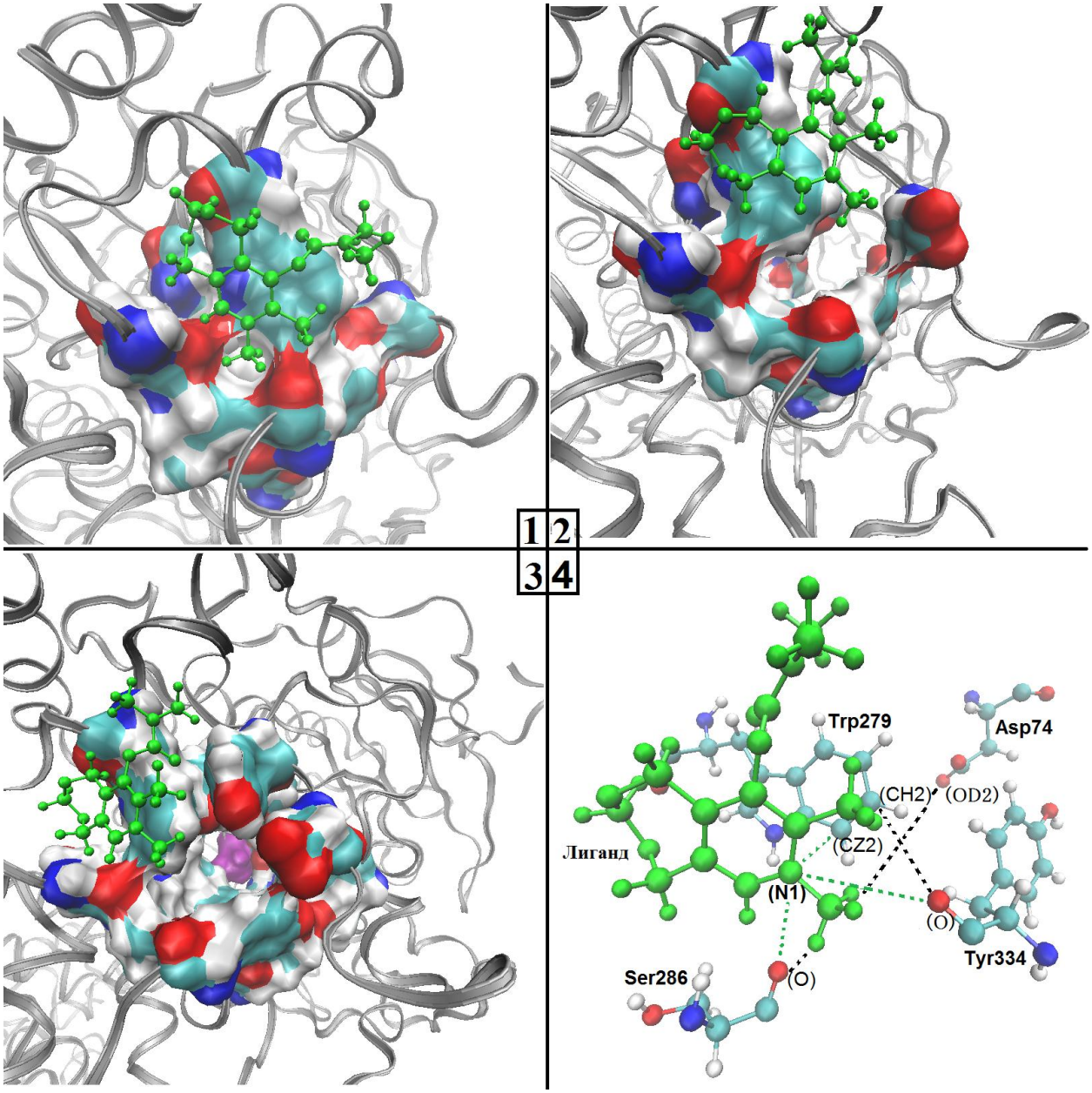

Figure 1: Molecular dynamics of enzyme-ligand complex.

1.1 - ligand original position (indicated by green balloons) with respect to enzyme (all protein is shown in the form of grey spirals, the amino acid residues of the channel and the active center are shown in the form of a surface) with a closed channel; 1.2 - the position of the ligand with an open channel; 1.3 - a look inside the channel (amino acid residue Ser203 is indicated by light purple color); 1.4 - amino acid residues - the channel "gate" in the active center and ligand (black dashed lines between the atoms forming the "gate", the green dashed lines are between the nitrogen atom of the ligand and the reference points for the ligand movement analysis in the dynamics).

Figure 2 shows the distance change dynamics between the atoms. After 50 steps of dynamics the system is stable, the maximum oscillation between the atoms represented by Figure 2 pairs is less or equal to

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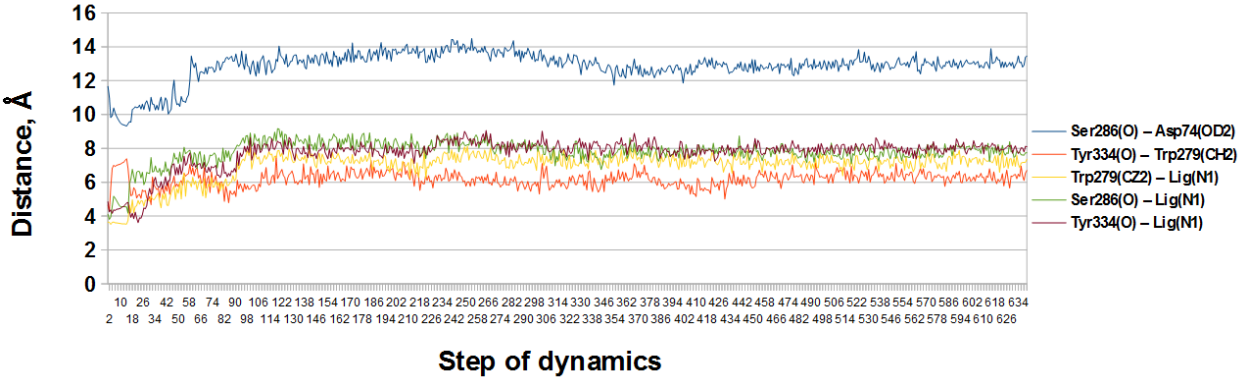
2 angstroms. The blue and orange line shows the changes between the atoms, closing the channel "gates" (Figure 1.4 - the black dotted lines).

Figure 2: The distance change between the atoms of amino acid residues that form the "gates" in the course of dynamics



The greatest distance between the atoms of the amino acids Ser286 (O) - Asp74 (OD2) makes 14,49 Å (angstroms), 12,89 Å in average. The distance between Tyr334 (O) - Trp279 (CH2) reaches 7,55 Å, 6,18 Å in average. To analyze the changes of the ligand position the reference points were taken (Figure 1.4 - green dotted lines, Figure 2 - yellow, green, brown lines), the atoms of amino acid residues of the "gate" channel and the nitrogen atom of the ligand (N1). During the dynamics after the stabilization of the ligand structures (N1) it closed slightly to Trp289 (CZ2) from 8 to 7 Å, on average, the maximum value made 6,2 Å; in relation to the points of Ser286 (O) and Tyr334 (O) it showed the corresponding values: the initial -8,9 Å and 8,6 Å, the average 7,8 Å and 7,7 Å, the most distant made 9,17 Å and 9 06 Å, closed most of all by 6,7 Å and 7,1 Å. The "gate» Ser286 (O) - Asp74 (OD2) x Tyr334 (O) - Trp279 (CH2) fluctuations made 12,89 x 6,18 Å on the average, the maximum value at a certain point made 13,89 x 7,18 Å. It is worth noting that during the dynamics performance without a ligand at the entrance to the channel the "gate" dimensions were within the range of 8.95 × 10.48 Å, wherein the ligand parameters make 8,36 × 9,02 Å.

CONCLUSION

The performed study showed that the ligand position on the enzyme surface in the dynamics largely depends on the ligand position during docking. The analysis of the dynamics confirmed the possibility of the ligand penetration in the active channel of the cavity, however, for the corresponding experiment performance the starting position screening is required by the molecular docking method.

SUMMARY

The study showed that the ligand may penetrate into the active cavity of acetylcholinesterase, which allows it to block the enzyme activity. During the molecular dynamics the interaction between the amino acid residues forming the "gate" to the active cavity and the ligand is observed, at that the ligand has the ability to find an energetically favorable position for the penetration of the enzyme channel leading to the active center.

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Conflict of Interests

The author confirms that the presented data do not contain any conflict of interests.

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REFERENCES

[1] Dudel, J. Human Physiology. / Ed. R. Schmidt, G. Tevs. - Moscow: Mir, 1996. - V. 1. – 323 p.

[2] Rosenberry, T.L. Acetylcholinesterase. // Advances in Enzymology and Related Areas of Molecular Biology. – 1975. – v. 43. – p. 103-218.

[3] Rasool, C.G., Svendsen C.N., Selkoe D.J.. Neurofibrillary degeneration of cholinergic and noncholinergic neurons of the basal forebrain in Alzheimer's disease. // Ann Neurol. – 1986. – v. 20 – p. 482-488.

[4] Giacobini, E. Cholinesterase inhibitors: new roles and therapeutic alternatives // Pharmacological Research. – 2004. – v. 50(4). – p. 433-440.

[5] Giacobini, E., Spiegel R., Enz A., Veroff A.E., Cutler N.R. Inhibition of acetyland butyryl-cholinesterase in the cerebrospinal fluid of patients with Alzheimer's disease by rivastigmine: correlation with cognitive benefit // Journal of Neural Transmission. – 2002. – v. 109(7-8). – p. 1053-1065.

[6] Strelnik A.D. Thesis for the degree of of Chemical Sciences candidate. Synthesis and biological activity of some derivatives of pyridoxine. Kazan. 2010. 128 p.

[7] Ayupov, R.H., Akberova N.I., Tarasov D.S. The docking of pyridoxine derivatives in the active center of cholinesterase. // Scientific Rec. of Kazan Univ. Natur. Sciences series - 2011 - V. 153, Book 3. - pp. 107-118.

[8] Ayupov, R.H., Akberova N.I., Tarasov D.S. The interaction of derivated pyridoxine with the active center of acetylcholinesterase // Scientific Rec. of Kazan Univ. Natur. Sciences series - 2012.- V. 154, book 2. – pp. 234-246.

[9] Masson P., Roshu D. Catalytic "biotraps" against toxic esters, an alternative approach for the prevention and treatment of poisoning // Acta naturae. - 2009 - Vol. 1. - pp. 68-78.