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THE FLUCTUATION ANALYSIS OF CONFORMATIONAL MOBILITY FOR PYRIDOXINE DERIVATIVES IN THE ACTIVE SITE OF ACETYLCHOLINESTERASE

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Abstract:

Acetylcholinesterase (AChE) is the main enzyme of the nervous system that is responsible for the regulation of a nerve impulse transfer by a rapid hydrolysis of the neurotransmitter - acetylcholine. The most important aspect in the studies related to AChE is the issue of specific interaction with ligands for its activity regulation. In most cases it is necessary for AChE site-specific inhibitors design for development of potential new drugs for the treatment of neurodegenerative diseases with more efficiency and less side effects. Currently the methods of molecular modeling are widely used for new drugs design.

In this study AChE structure of 2JEY mouse served as biological target. The derivatives of pyridoxine were used as ligands, with anticholinesterase effect shown in vivo. Moreover the position of these ligands in AChE active center during docking was similar to AChE inhibitors used in medicine (proserin, physostigmine). A combination of molecular docking and Molecular Dynamics methods allowed us to estimate the drug potential of inhibitors more efficiently. The study was carried out using the following software packages: AutoDock.1.5.6 (Vina application) and NAMD 2.8 with AMBER 99 force field.

The result showed that the ligand position in the active cavity of an enzyme may vary significantly even for related compounds and these positions depend on ligand modification. The analysis of molecular dynamics revealed that the ligand mobility and respective inhibitory activity depend on an inhibitor size and on ligand affinity to AChE. The number of interatomic interactions between molecules and probabilities of their interactions in an active site of the enzyme were used for analysis. It was shown that the molecules with a high affinity for the enzyme active cavity not always will be able to get into active cavity by reason of their size. The hydrophobic interactions of ligands with uncharged amino acid residues allows them to bind more efficiently to the active center of AChE. Thus the structural

features of the ligand exactly define long-term anticholinesterase effect.

Keywords: acetylcholinesterase (AChE), pyridoxine derivatives, screening, molecular docking, Molecular Dynamics, a specific interaction of enzyme with ligand.

1. Introduction

Acetylcholinesterase is the enzyme (AChE, KF 3.1.1.7) of cholinesterase family, one of key enzymes of nervous system. It is involved in regulation of nerve signal transmission by hydrolysis of neurotransmitter acetylcholine (kcat/KM = 1.6 × 108M-1s-1) [1]. The enzyme is localized in the central and in the peripheral nervous systems (in sympathetic and parasympathetic ganglia), in the lymphatic system and in embryonic tissues. Nonclassical AChE functions are known, such as the participation in the lymphatic system working and in embryonic development [2]. The first identified nonclassical enzyme functions were its participation in the growth of axons, synapses formation [3,4] and the growth of malignant tumors [5-7]. The increased interest in this class of enzymes is explained by the fact that the defects in AChE activity are observed in such diseases as glaucoma, myasthenic syndrome, and other neurodegenerative pathologies (e.g. Alzheimer's disease) [8-10]. The review devoted to AChE inhibitors gives the description of their properties, differences and similarities, the therapeutic effects of drugs on basis of these inhibitors [11]. A significant problem in this field of research is a wide range of side effects existence in inhibitors. An important difference of described agents from each other is the type of binding to an enzyme. Metrifonate develops an irreversible covalent linkage to the substrate; donepezil, tacrine, huperzine and velnakrin are the non-covalent inhibitors with a high affinity. Galantamine is a competitive and a reversible inhibitor of AChE, as well as an allosteric modulator of nicotinic cholinergic receptor [12]. Physostigmine and proserin are reversible action inhibitors. Donepezil has competitive and unbeatable features, velnacrine and tacrine are unbeatable inhibitors. The effect of metrifonate begins with a competitive inhibition, but eventually it is transformed into a non-competitive inhibition [13]. The Molecular Dynamics method is used in various fields of structural biology and physics [14,15] and allows to evaluate the features of the interaction between molecules and their structure mobility. The Molecular Dynamics of AChE with pyridoxine derivative showed some properties of ligand interaction with this enzyme [16,17].

The present study is devoted to elucidation of the ligands modifications influence on the mechanism of AChE interaction with pyridoxine derivatives based on the analysis of molecular docking and Molecular Dynamics of enzyme-ligand complex.

2. Methods

The mouse AChE structure 2JEY from Protein Date Bank (structure resolution 2.7 Å) and 9 derivatives of pyridoxine (denoted as «a», «b», «c», «d», «e», «f», «g», «h», «i», differed by the length of side radicals) were used in the study [18]. The inhibitor coordinates used in this work were obtained from previous docking experiments [19] and used for matching with other ligands of different chemical types [20]. Molecular docking was carried out in the program AutoDock, which provides good results in the various tests [21].

In order to study the interaction of ligands with AChE the molecular dynamic (MD) modeling was carried out in NAMD software package 2.8 [22] using the AMBER 99 force field [23]. The coordinates of high affinity complexes of AChE with pyridoxine derivatives obtained after their docking were selected as initial coordinates for MD simulations.

MD simulation settings

The dynamics was performed using periodic boundary conditions, integration step was 2 fs, the temperature of the simulated system was 300 K. Total simulation time was about 20 ns, 2 ns for each ligand. Protein structure was placed in water box, which boundaries were within the distance of 8 Å from the protein surface. The water box has 105 × 75 × 150 Å dimensions.

The complex structure was minimized by a gradient descent method during 200 steps. Potential cutting threshold is equal to 10 Å. To reduce the calculations bonds with the hydrogen atoms were fixed. Static interactions between periodic reflections were calculated by Ewald summation method (ESM).

3. Results and Discussion

We performed the comparative analysis of the ligands position in the AChE active site relative to the following amino acid residues Trp86, Tyr124, Ser203 (Figure 1). These amino acid residues were selected as the reference ones, since the interaction with them allows a ligand to be fixed near a catalytic triad (Ser203-His447-Glu334) by stacking (Trp86) and electrostatic (Tyr124) interactions.

Distances between the oxygen of the hydroxyl group Ser203 and oxygen of carbamylated fragment (1 on Figure 1), between the oxygen of the hydroxyl group of Tyr124 and the nitrogen of carbamylated fragment (2 on Figure 1) were calculated in the molecular docking (Table 1). These distances were determined at the maximum value of the energetic affinity between molecules. Ligand position in the cavity of an active center was stabilized by stacking interactions with Trp86.

Table.1: The distances between the reference points of the enzyme and the ligands (Figure 1), obtained in the molecular docking study.

Distance (Å) / Inhibitor	O Ser203 and C of carbamylated fragment	O Tyr124 and N of carbamylated fragment
«a»	6.06	3.35
«b»	6.29	3.15
«c»	8.72	5.06
«d»	8.46	4.69
«e»	8.49	4.69
«f»	8.53	4.68
«g»	4.83	6.76
«h»	4.83	6.85
«i»	4.0	7.1

These values of distances characterize the spatial position of the ligand molecule relative to the catalytic triad of an enzyme active center and their stabilization in this space.

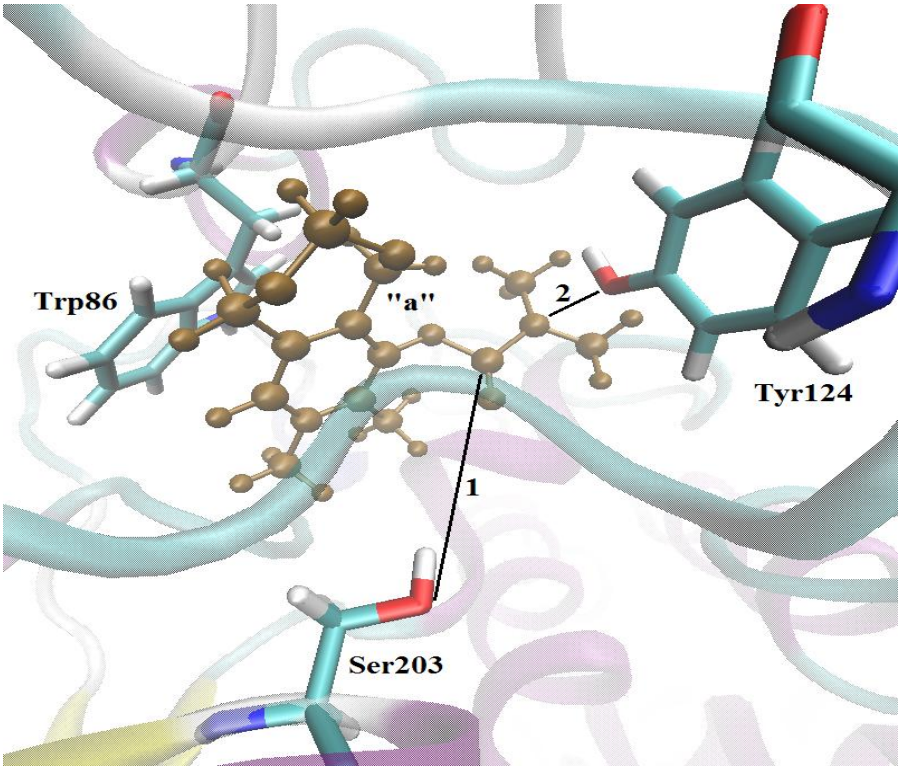

Fig.1. The spatial position of the ligand relative to the amino acid residues of AChE.

Almost all examined structures of pyridoxine derivatives are well stabilized in an active site of AChE, and for some of them («a», «b», «g», «h», «i») the probability of a covalent bond formation with an amino acid residue Ser203 of AChE is high enough according to docking results.

Molecular Dynamics

The visual analysis of the enzyme-ligand complex during MD simulations indicates the approach to the amino acid residue of catalytic triad Ser203 of certain ligands («a», «b», «c») in comparison with their position during docking. This ligands differ slightly from each other by a side radical length. However, if we evaluate the approximation qualitatively, the ligand «a» reduced the distance between their carbamylated carbon atom and an oxygen atom of Ser203 hydroxyl group almost twice (Table 2).

Table 2. The distances between reference points and enzyme ligands obtained during molecular dynamics.

Distance (Å) / Inhibitor	O Ser203 and C of carbamylated fragment			O Tyr124 and N of carbamylated fragment		
	max	mean	min	max	mean	min
«a»	6.0	3.9	3.5	4.7	3.8	2.3
«b»	8.45	7.5	6.2	4.8	4.2	3.0
«c»	8.7	7.5	6.6	7.0	6.0	5.0
«d»	12.6	11.2	8.4	8.2	6.5	4.5
«e»	12.3	11.6	8.4	7.6	6.7	4.6
«f»	13.6	12.8	8.5	5.3	4.6	4.1
«g»	10.2	9.2	5.6	12.2	10.8	7.5
«h»	13.0	10.1	6.0	9.9	8.8	4.4
«i»	8.3	6.0	4.2	7.1	5.3	3.6

The evaluation of number of the atomic interactions between ligand molecule and the enzyme active center is extremely important for analyzing the trajectory of molecular dynamics (Figure 2). Ligand size determines the number of interatomic interactions, but one should take into account the stereochemical characteristics of the ligand molecule, the ability to form hydrogen bonds, electrostatic interactions, and stacking contacts. In this regard, in order

to select the interacting pairs atoms the threshold value 3.4 Å (the double van der Waals radius of carbon atom) was used. This enables to take into account electrostatic and stacking interactions.

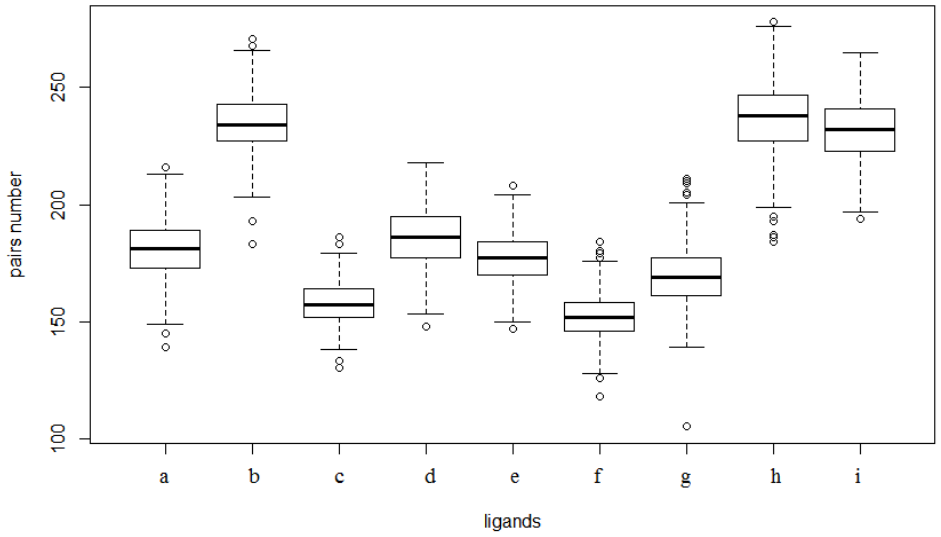

Figure 2. The number of atomic interactions between the enzyme and the ligands in MD simulation.

Figure 2 shows the estimate of the number of interacting atoms pairs between ligands and the enzyme at the distance less than 3.4 Å. The maximum number of interacting atoms pairs is revealed for large size ligands («h»,«i»), as well as for a small ligand («b»). In the case of larger molecules it can be explained by their size, but for a small ligand this number of pairs is somewhat surprising. It may be caused by specificity of this ligand interaction with an active center of the enzyme and this requires a further investigation.

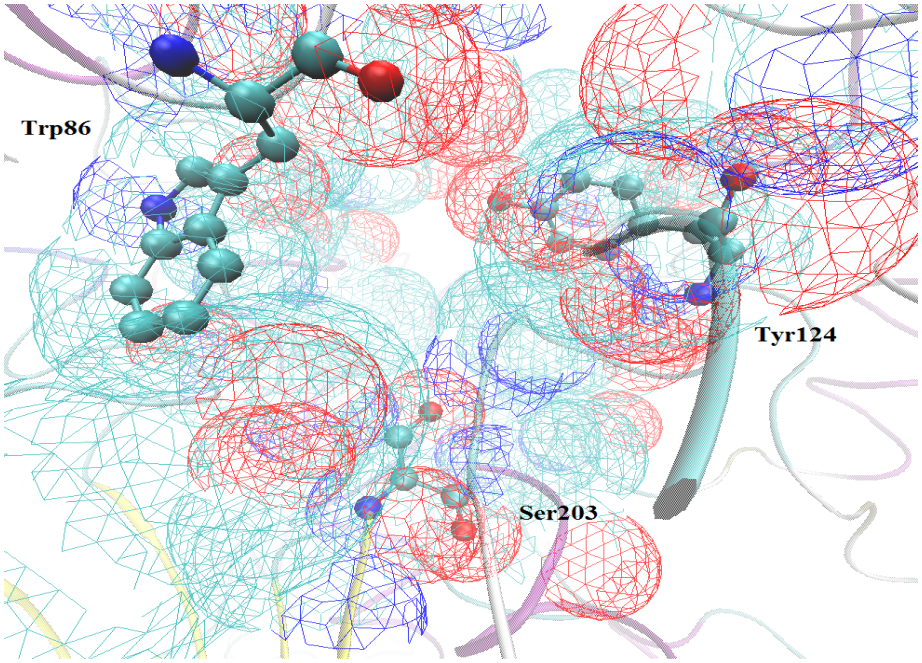

Figure 3. The location of charges in active AChE center. Dark-blue and red show the positively and negatively charged areas, respectively, and uncharged areas are shown by blue.

The cavity of AChE active center is characterized by a large number of hydrophobic regions. Figure 3 shows in blue the hydrophobic parts of an active enzyme cavity, dark-blue and red demonstrate positively and negatively charged areas respectively. The coordinates of amino acid residue atoms Trp86, Tyr124, Ser203 are taken as the reference coordinates. The charged areas are located close to the catalytic triad and an anionic site, which are responsible for the acylation and deacylation of acetylcholine. Hydrophobic regions are responsible for the ligand stabilization. There is a small charged portion (carbamylated fragment) in ligand molecules structure while mainly molecule is uncharged and hydrophobic. Such modification in the ligands structure as elongation of it's side radical group increase it's hydrophobicity. Consequently the largest molecules («h», «i»), move away from the catalytic triad during the molecular dynamics simulations but the stability of their position is ensured by a large number of stacking interactions with the protein hydrophobic cavity.

4. Conclusions

The results of the molecular docking study allow to determine the most probable position of ligand in the active site of AChE. However, the molecular docking itself is not enough for predicting the type of inhibition. Molecular dynamics results showed that not all ligands offered by docking are capable to form covalent bond with enzyme. Ligands «a», «b», «g», «h», «i» were the most closely spaced in the catalytic triad during docking, but only ligand "a" appeared close enough to the amino acid residue Ser203of the catalytic triad in the MD simulation. The other ligands either departed from the catalytic triad («b», «g», «h»), or their positions did not undergo significant changes in the cavity of the enzyme active site («i»).

5. Summary

The combinations of molecular docking and molecular dynamics methods for modeling of enzyme-ligand system behavior allow to define more precisely the position of the ligands in the active cavity of the enzyme and to predict the type of their interaction that is important for the development of specific inhibitors highly required in modern medicine.

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