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Comparative Analysis of Mice Acetylcholinesterases by Functional Amino Acid Residues and Molecular Screening.

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ABSTRACT

In this paper, we have measured the distances between the reference amino acid residues of the acetylcholinesterase (AChE) active site, which showed that the existing three-dimensional models either have no differences or have differences within the structures resolution. Using the method of molecular docking in AutoDock, we have carried out the AChE screening with ligands, which denied the result of a previous calculation and showed that the affinity energy of one ligand (e.g.: donepezil) with 23 structures of enzyme is in the range of -6 to -12 kcal/mol, which is impossible for those structures having no significant differences. Following this line of reasoning, we can make two different conclusions: firstly, the existing AChE models are similar in general, and the choice of a model for work shall be based on the structure resolution, and a variety of screening results are just special cases of interaction with the side radicals of amino acid residues; secondly, the amount of insignificant changes in the spatial structure of each protein contributes largely to the screening result.

Keywords: molecular screening, docking, protein spatial structures, acetylcholinesterase, ligands, molecular interactions.

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INTRODUCTION

Acetylcholinesterase (AChE) is an important enzyme of the nervous system that splits a neurotransmitter - acetylcholine [1]. Inhibition of the enzyme is carried out to extend the nerve impulse generation in various diseases, including neurodegenerative ones, such as Alzheimer's disease. Initial work in the inhibitor analysis is usually conducted on laboratory animals, so the structures of mice AChE, found in Protein Data Bank (PDB), have served as object for this study [2]. The existing three-dimensional structures of proteins differ little in primary sequence (they have differences only at the beginning and end of the corresponding polypeptide sequences). Therefore, it is interesting to get an answer to the question of whether there are differences in the three-dimensional models of these proteins. To address this problem, we used two approaches:

- Measuring the volume of cavities in the mice AChE structures in the amino acid residues of the enzyme active site; and
- Analyzing the affinity energy of enzyme-ligand complexes by molecular screening. Screening was conducted with the known AChE inhibitors [3], the potential inhibitors [4, 5], and the ligands with known interaction types and low molecular weight compounds present in the cells.

METHODS

In our study, we used the mice AChE structures obtained from Protein Data Bank (Table 1), and analyzed the structures of 40 enzymes.

The ligands used were 10 new synthetic pyridoxine derivatives; 7 AChE inhibitors, used in medicine; an AChE substrate — acetylcholine; a covalent irreversible inhibitor - sarin; 11 molecules of pyridoxine derivatives of different structure from the PubChem database and 10 molecules differing in their structure from the pyridoxine derivatives.

We performed measurements between the atoms of the amino acid residues of the active site of Ser203, Trp86, Asp74, which play an important role in the interaction with ligands [6-9]. Amino acid residues have more or less movable parts. The first are atoms taking no part in the formation of the peptide bond, among which the side radicals are the most movable, and the second are C and N atoms forming a peptide bond. On this basis, we performed two types of measurements: between both movable and immovable parts. We used Asp74, Trp86, Ser203 amino acid residues for measurements (Fig. 1); for some enzymes, numbers of these amino acid residues vary insignificantly. We took the peptide bond-forming N atoms to serve as reference points of the immovable parts. For measuring the movable parts, the following atoms were taken: O atom of Ser203 hydroxyl group, O atom of Trp86 carboxyl group, and the O atom closest to active cavity of the Asp74 side radical.

Molecular screening

We performed molecular screening in AutoDock [10] by using its vina.exe applications. The study was conducted with the use of Python-written scripts. During the screening, we determined the affinity energy and position of a ligand in the enzyme active cavity. We used only those AChE structures in screening, which were obtained without a covalent inhibitor: there were 23 of them.

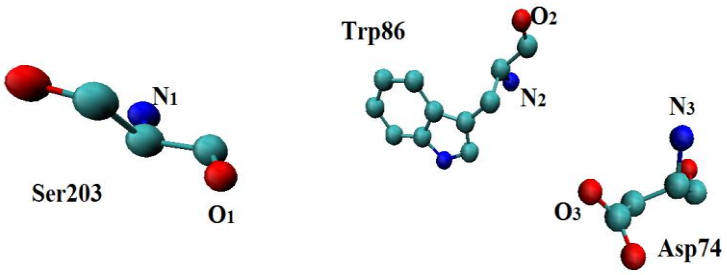

Figure 1: Amino acid residues of the AChE enzyme active site

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Table 1: Mice AChE enzymes

Code	Enzyme
2jey	Mus musculus acetylcholinesterase in complex with hlo-7
2jez	Mus musculus acetylcholinesterase in complex with tabun and hlo-7
2jf0	Mus musculus acetylcholinesterase in complex with tabun and ortho-7
2jge	Crystal structure of mouse acetylcholinesterase inhibited by non-aged methamidophos
2jgf	Crystal structure of mouse acetylcholinesterase inhibited by non-aged fenamiphos
2jgi	Crystal structure of mouse acetylcholinesterase inhibited by non-aged diisopropyl fluorophosphate (dfp)
2jgj	Crystal structure of mouse acetylcholinesterase inhibited by aged methamidophos
2jgk	Crystal structure of mouse acetylcholinesterase inhibited by aged fenamiphos
2jgl	Crystal structure of mouse acetylcholinesterase inhibited by aged vx and sarin
2jgm	Crystal structure of mouse acetylcholinesterase inhibited by aged diisopropyl fluorophosphate (dfp)
2whp	Crystal structure of acetylcholinesterase, phosphonylated by sarin and in complex with hi-6
2whq	Crystal structure of acetylcholinesterase, phosphonylated by sarin (aged) in complex with hi-6
2whr	Crystal structure of acetylcholinesterase in complex with k027
2wls	Crystal structure of mus musculus acetylcholinesterase in complex with amts13
2wu3	Crystal structure of mouse acetylcholinesterase in complex with fenamiphos and hi-6
2wu4	Crystal structure of mouse acetylcholinesterase in complex with fenamiphos and ortho-7
3dl4	Non-aged form of mouse acetylcholinesterase inhibited by tabun- update
3dl7	Aged form of mouse acetylcholinesterase inhibited by tabun- update
1j06	Crystal structure of mouse acetylcholinesterase in the apo form
1j07	Crystal structure of the mouse acetylcholinesterase-decidium complex
1ku6	Fasciculin 2-mouse acetylcholinesterase complex
1maa	Mouse acetylcholinesterase catalytic domain, glycosylated protein
1mah	Fasciculin2-mouse acetylcholinesterase complex
1n5m	Crystal structure of the mouse acetylcholinesterase-gallamine complex
1n5r	Crystal structure of the mouse acetylcholinesterase-propidium complex
1q83	Crystal structure of the mouse acetylcholinesterase-tz2pa6 syn complex
1q84	Crystal structure of the mouse acetylcholinesterase-tz2pa6 anti complex
2c0p	Aged form of mouse acetylcholinesterase inhibited by tabun
2c0q	Non-aged form of mouse acetylcholinesterase inhibited by tabun
2gyu	Crystal structure of mus musculus acetylcholinesterase in complex with hi-6
2gyv	Crystal structure of mus musculus acetylcholinesterase in complex with ortho-7
2gyw	Crystal structure of mus musculus acetylcholinesterase in complex with obidoxime
2h9y	Crystal structure of mouse acetylcholinesterase complexed with m-(n,n,n-trimethylammonio)trifluoroacetophenone
2ha0	Crystal structure of mouse acetylcholinesterase complexed with 4-ketoamyltrimethylammonium
2ha2	Crystal structure of mouse acetylcholinesterase complexed with succinylcholine
2ha3	Crystal structure of mouse acetylcholinesterase complexed with choline
2xud	Crystal structure of the y337a mutant of mouse acetylcholinesterase
2y2u	Nonaged form of mouse acetylcholinesterase inhibited by vx-update
2y2v	Nonaged form of mouse acetylcholinesterase inhibited by sarin-update
4a23	Mus musculus acetylcholinesterase in complex with racemic c5685

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RESULTS AND DISCUSSION

The distance between the atoms was measured in VMD program. The distance was measured between: N1-N2, N2-N3, N3-N1; O1-O2, O2-O3, O3-O1 (Fig.2 and 3, respectively). Nitrogen atoms distance fluctuation: N(Ser203) – N(Trp86) is 13.8±0.7 Å, N(Trp86) – N(Asp74) 8.6±0.6 Å, N(Asp74) – N(Ser203) 17.5±1 Å. Oxygen atoms distance fluctuation: O(Ser203) – O(Trp86) is 11.8±0.7 Å, O(Trp86) – O(Asp74) 8.1±1.8 Å, O(Asp74) – O(Ser203) 12.9±2.5 Å. All these values are within the coordinate positioning error, namely within the structures resolution. Hence, there is no differences in spatial positions of these amino acid residues in the investigated structures.

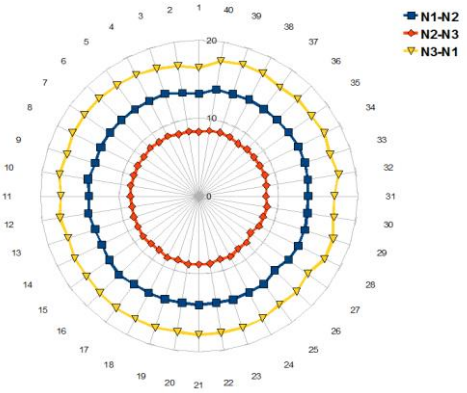

Figure 2: Distance between N atoms

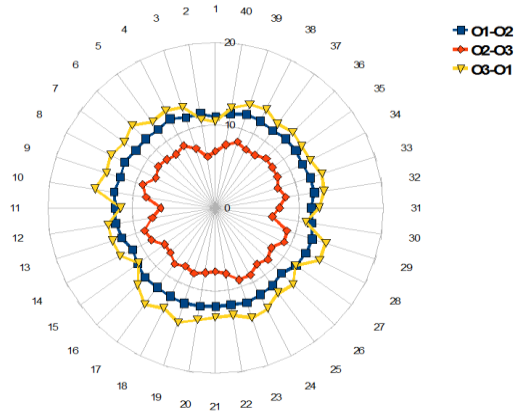

Figure 3: Distance between O atoms

Molecular screening

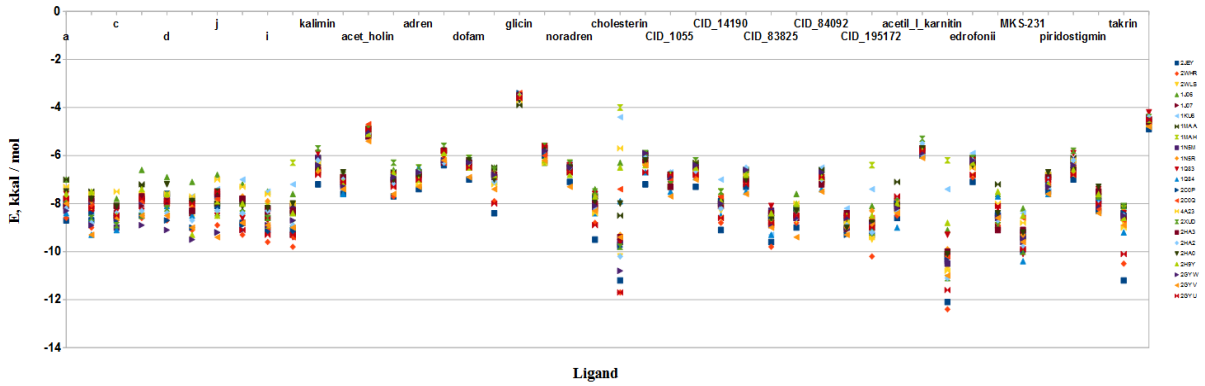

Figure 4: The chart of the affinity energy (*y*) of each ligand (*x*) with each enzyme (see enzyme's name in the right column)

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The screening results are represented in the form of affinity energy between 23 AChEs and 44 ligands (Fig. 4).As we can see from Fig. 4, some ligands have the affinity energy fluctuations equal to 8 kcal/mol (a cholesterol ligand, -4 to -12 kcal/mol). For rest, the affinity energy fluctuations are within 1 kcal/mol (acetylcholine, glycine, sarin). The affinity energy of other ligands ranges 6 kcal/mole.

Variability of the ligand-AChE affinity energy is represented by the standard deviations (Figure 5). Most ligands had no significant differences in the affinity energy to the enzymes, except of two of them: cholesterol and donepezil (standard deviation - 2.1 kcal/mol and 1.3 kcal/mol, respectively).

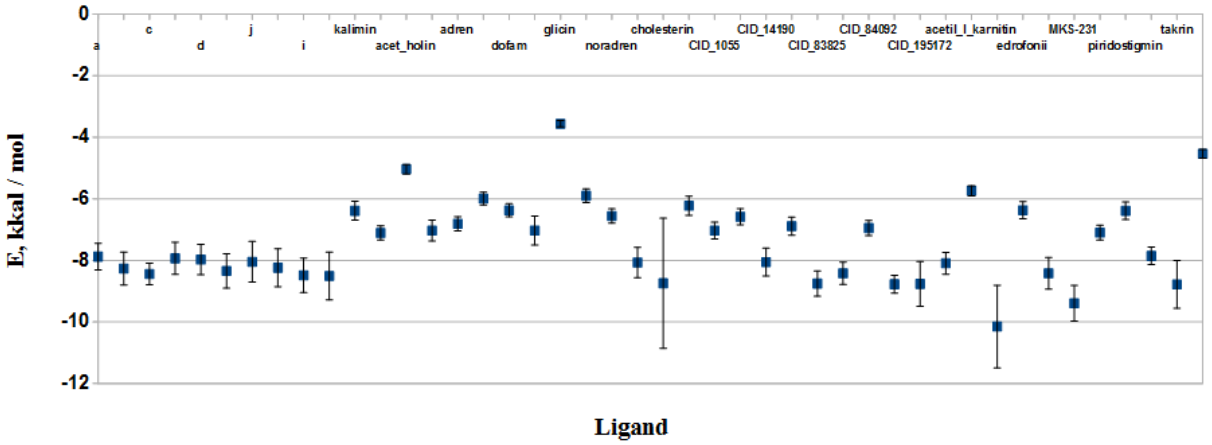

Figure 5: Ligand-protein affinity energies (mean ± standard deviation).

The rest ligands can be grouped by variability into four groups according to their standard deviations: 0.1 kcal/mol to 0.29 kcal/mole, 0.30 kcal/mole to 0.49 kcal/mol, 0.5 kcal/mol to 0.69 kcal/mol, and 0.7 kcal/mole to 0.99 kcal/mole. The first group counts 18 ligands: proserine, acetylcholine, adrenaline, ascorbic acid, dopamine, glycine, glucose, noradrenaline, acetyl-L-carnitine, edrophonium, neostigmine, rivastigmine, sarin, CID_1055, CID_6723, CID_30855, CID_84092, and CID_127221. The second group includes 13 ligands: a, c, e, kalymin, acetylsalicylic acid, phenylalanine, tryptophan, pyridostigmine, CID_1054, CID_14190, CID_83825, CID_83875, and CID_212004. The third group includes 8 ligands: b, d, f, g, h, i, physostigmine, and MKC-231. The fourth - 3 ligands: j, tacrine, and CID_195172.Such clustering of ligands is due to their size, when the largest structures have the greatest variation values during screening.

The molecular screening has shown that, despite a quite identical primary sequence of the proteins, their spatial models have significant differences.

The results obtained in this study can be interpreted in many ways:

- The existing AChE models are similar in general, and the choice of a model for work shall be based on the structure resolution, and a variety of screening results are just special cases of interaction with the side radicals of amino acid residues;
- The existing AChE models have slight differences in their spatial structure, which can be detected during molecular docking;
- The amount of insignificant changes in the spatial structure of each protein contributes greatly to the screening result.

The choice of a model for a protein shall be substantiated by both the resolution degree, and a detailed analysis of the spatial arrangement of amino acid residues in the active site or in another part of the enzyme.

SUMMARY

The obtained results have shown that the existing AChE models are similar in general, and the choice of a model for work shall be based on the structure resolution, and a variety of screening results are due to the

amount of insignificant changes in the spatial structure of each protein contributes largely to the screening result.

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