

Differences between Acetylcholinesterase Inhibitors

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Abstract

The goal of this project is to compare two structures of human acetylcholinesterase (AchE), focused on active sites. Each of the structure binds different ligand. The selected ligands are inhibitors used in the treatment of the Alzheimer's disease. The idea is to compare the active site interactions and point out the differences between them. The chosen structures are 4EY6 and 4EY7. The ligand in the first structure is galanthamine (GNT), in the second structure is dopenezil (E20).

Introduction

Alzheimer's disease (AD) is a chronic, progressive, neurodegenerative disorder of the brain characterized clinically by deterioration in the key symptoms of activities of daily living (ADLs), behavior, and cognition. Based on the cholinergic hypothesis¹, the cognitive decline in AD is a result of the deficits in central cholinergic neurotransmission resulting from a loss of acetylcholine (Ach).

Normally, the actions of Ach are terminated by a specific mechanism to keep the target cells from becoming overactivated. Acetylcholine is destroyed by an enzyme², acetylcholinesterase (AchE), that is located in every Ach synapse. The defect course of enzymatic hydrolysis of Ach is considered to be one of the possible reasons of AD². Cholinesterase inhibitors enhance central cholinergic function, and their usage remains the standard approach to the symptomatic treatment of AD³.

In this work, we focus on two commonly used AChE inhibitors for the symptomatic treatment of AD, galanthamine and dopenezil. Mainly, we focus on the binding site interactions of these inhibitors with human AchE, and we will point out the differences between these interactions.

Methods

Used structures

Two molecular structures of human AchE binded with different ligands were used. PDB code of protein-ligand complex AchE with galanthamine (PDB code: GNT) is 4EY6. PDB code of complex AchE with dopenezil (PDB code: E20) is 4EY7. AchE is a homodimer. Each chain contains binding site for the selected ligands. Both structures were crystalized using the same

approach in the study presenting several crystal structures of AchE in complexes with drug ligands⁴. Resolution of 4EY6 is 2.4Å, resolution of 4EY7 is 2.35Å. Based on PDB validation report, quality of both structures are reasonably good and in all studied metrics result above average quality.

Besides the mentioned ligands GNT and E20, the structures contain other ligands. The structure 4EY6 contains besides GNT also PE8, NAG, EDO and NO3 ligands. The structure 4EY7 contains besides E20 also NAG, EDO and NO3 ligands. Presence of these ligands is not mentioned in the original study⁴. Possible explanation of presence of PE8, EDO and NO3 might be that these are crystallization artifacts. The bonded oligosaccharide NAG might be result of post-translational modifications of the protein. As our study is focused on the interaction of GNT (respectively E20) and these additional ligands do not interact with our focused protein-ligand interaction, we do not investigate these interactions more profoundly.

Methods and Workflow

Acetylcholinesterase structures were investigated using the program PyMOL. First, we aligned the structures to verify its similarity. Then, we hid one of the chains (as it is homodimer), and we showed the AchE surface focused mainly on the binding side where the selected ligands bind. Furthermore, we found the polar contact between the protein and the ligands, and we measured the distances of the bounds. Finally, we filtered out the protein surroundings and focus only on the structure around the ligands. The finding of the polar contacts, the measurement of the distances as well as visualizations were done by the appropriate functions in PyMOL.

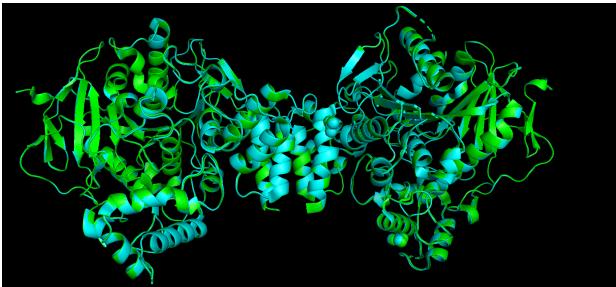


Figure 1: Structure Comparison: Comparision of the structures 4EY6 (green) and 4EY7 (blue).

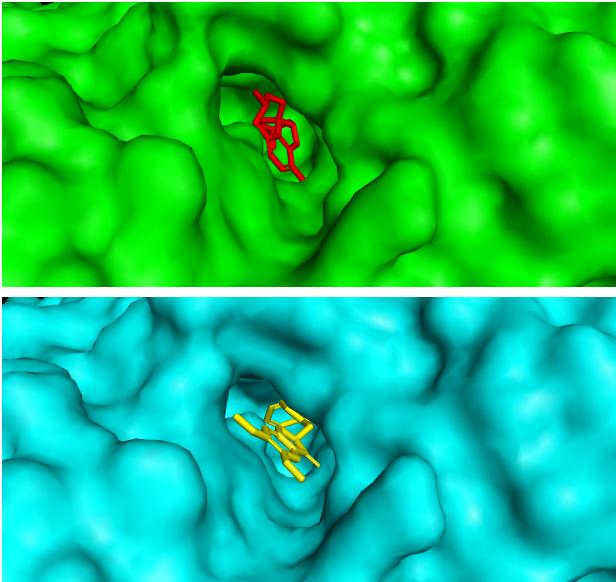


Figure 2: Binding-site Surfaces: Comparision of protein binding-site surfaces of structures 4EY6 (green) ligated with GNT (red) and 4EY7 (blue) ligated with E20 (yellow).

Results

When comparing both structures as whole, we can see almost perfect match between the structures (Figure 1). Both structure models were created for the same study using the same methods, so, the high similarity is not very surprising.

From now on, we focus only on the protein-ligand binding-site. First, we compare the structures based on the surfaces (Figure 2). We can see that the bindig-site is quite narrow and profound. Also, we observe the significant difference of shape and position of the ligands. The ligand GNT is short and circular-like shaped. On the other hand, E20 is much taller and its structure looks more like string. We can better see the difference in shapes of ligands in the figure 3. Also, we need to point out the slight misplacement of the amino acids of the protein in the binding-site. This might be the result of the measurement error as well as different conformation changes cause by the ligation of different ligand.

Next, we focus on the binding interactions of the protein-ligand complex. First, we investigate the structure 4EY6. The ligand GNT is composed of 4 aromatic cycles, so, it is possible that it interacts with the pro-

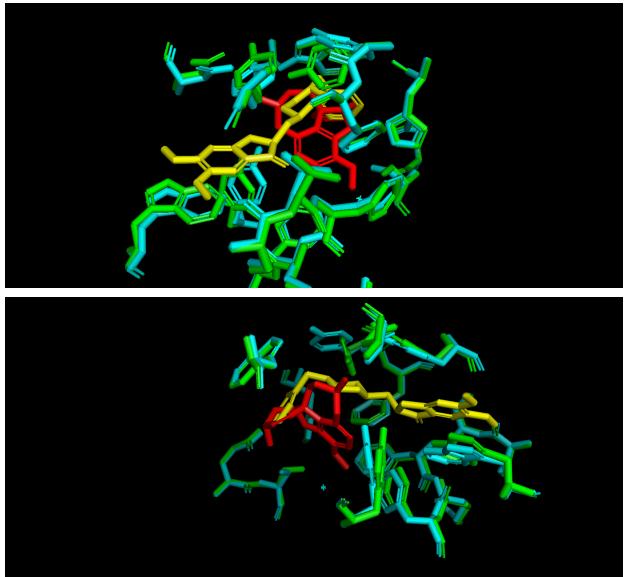


Figure 3: Ligands position comparision Comparision of positions of ligands in the protein binding-site surroundings of the structures 4EY6 (green) ligated with GNT (red) and 4EY7 (blue) ligated with E20 (yellow).

tein by stacking interactions. We observed one such interaction between the ligand and tryptophan. Distance in of the aromatic cycle was measured approximately to 3.7\AA , which is reasonable distance for such interaction. This intaection is visible in figures 4 and 6.

Apart of these interactions, we can also see non-covalent contacts of sulfur from the GNT and histidine (distance 2.7\AA) on position and nitrogen from the GNT and tyrosine (distance 2.9\AA) on position TYR (337). We can see the visualizations of all interactions in the figures 4, 5 and 6 (each figure is coupled with 4EY7 compound based on the same view angle). At the end, we should mention that there are few other aromatic amino acids from the protein which might also be part of the stacking interactions. Though, we did not visualize them because of the higher uncertainty of existence of these bonds. All adepts for stacking interactions of this structure are the following TRP (86), TYR (124), TYR (337) and PHE (338).

In terms of the structure 4EY7, we see much more adepts of stacking interactions. The stacking interactions are apart of the other figures visualized also in additional figure 7.

Ligand E20 is also composed of 4 aromatic rings and interacts by stacking interactions with tryptophans and tyrosines of the protein (the distances are around 3.6\AA). The adepts for stacking interactions are the following amino acids of the AchE protein TRP (86), TRP (286), TYR (337) and TYR (341). Apart from this interactions, it also interacts with nitrogen in peptide backbone (PHE (295)) in distance 2\AA . Comparision of the interactions between complexes 4EY6 and 4EY7 are shown in the figures 4, 5 and 6.

To sum up, both ligands are surprisingly very distinct in the shape. We cannot state for sure, but it

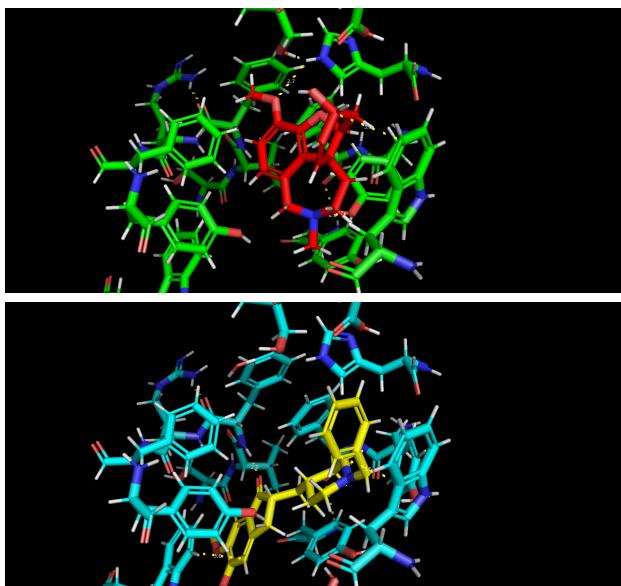


Figure 4: Binding-site contacts: Comparision of protein binding-site non-covalent interactions of structures 4EY6 (green) ligated with GNT (red) and 4EY7 (blue) ligated with E20 (yellow).

looks that both ligands interact with the AchE protein mainly by the stacking interactions of its aromatic rings (mainly the same rings). Despite, the difference of the ligands they both serves the same function, which is inhibition of the AchE resulting in delay of Ach hydrolysis.

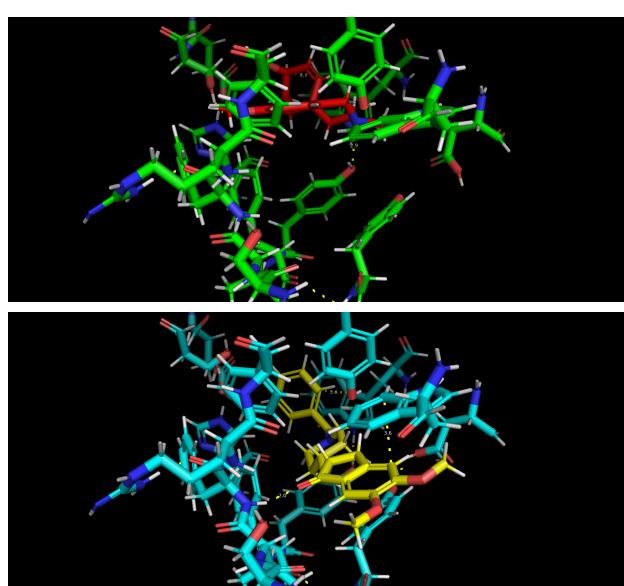


Figure 5: Binding-site contacts: Comparision of protein binding-site non-covalent interactions of structures 4EY6 (green) ligated with GNT (red) and 4EY7 (blue) ligated with E20 (yellow).

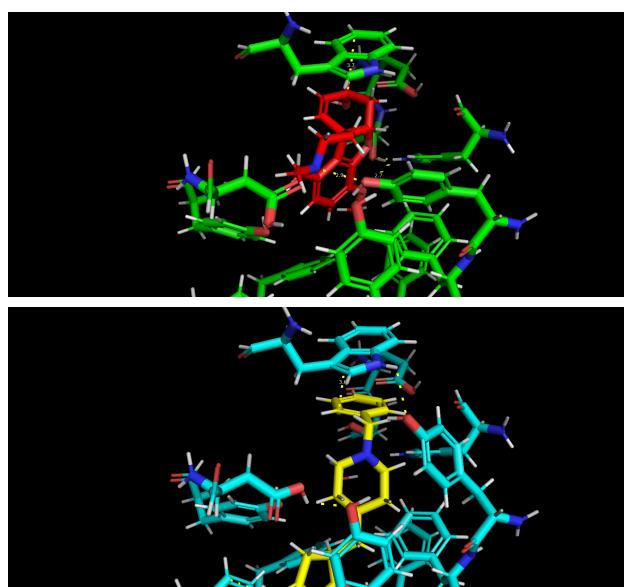


Figure 6: Binding-site contacts: Comparision of protein binding-site non-covalent interactions of structures 4EY6 (green) ligated with GNT (red) and 4EY7 (blue) ligated with E20 (yellow).

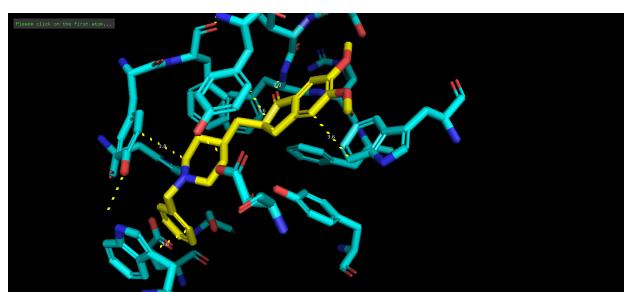


Figure 7: Stacking interactions of 4EY7: Visualization to stress the possible stacking interactions in the 4EY7 complex. Ligand E20 is yellow.

References

- [1] R. T. Bartus, R. L. Dean III, B. Beer, and A. S. Lippa, The cholinergic hypothesis of geriatric memory dysfunction, *Science* **217**, 408 (1982).
- [2] A. Komersová, K. Komers, and P. Zdražilová, (23) kinetics of hydrolysis of acetylthiocholine and acetylcholine by cholinesterases, *Chemico-Biological Interactions* **157-158**, 387 (2005), ISSN 0009-2797, proceedings of the VIII International Meeting on Cholinesterases.
- [3] G. T. Grossberg, Cholinesterase inhibitors for the treatment of alzheimer's disease:: getting on and staying on, *Current Therapeutic Research* **64**, 216 (2003).
- [4] J. Cheung, M. J. Rudolph, F. Burshteyn, M. S. Cassidy, E. N. Gary, J. Love, M. C. Franklin, and J. J. Height, Structures of human acetylcholinesterase in complex with pharmacologically important ligands, *Journal of Medicinal Chemistry* **55**, 10282 (2012), pMID: 23035744, arXiv:<https://doi.org/10.1021/jm300871x>.