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INTERPRETING THE EFFECT OF METHYL GROUP AT THE THREE CARBON BRIDGE OF (-)-HUPERZINE A ON ITS ANTICHOLINESTERASE ACTIVITY BY MOLECULAR DYNAMICS METHOD

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Abstract: Based on the recently resolved crystal structure of complex (-)-huperzine A-AChE, we simulated the interaction between (-)-huperzine A analogues and AChE using molecular dynamics method. It was revealed that the methyl group at the three carbon bridge of (-)-huperzine A can form a weak hydrogen bond with the phenol hydroxyl oxygen of Tyr121 and the main-chain oxygen of Gly118 of AChE, respectively.

(-)-Huperzine A (HupA) **(1)** is a potent reversible acetylcholinesterase(AChE) inhibitor extracted from Chinese herb *Huperzia serrata* (Figure 1). There have been a lot of structural modification works aimed to explore the substituent groups effect on its biological activity [1-3]. It is now generally accepted that the bridge methyl group inserts into a highly aromatic environment when binding with AChE receptor [4]. However, Terashima *et al* have recently synthesized three novel fluorinated analogues of (-)-huperzine A **(2)**, **(3)** and **(4)** [5]. The *in vitro* AChE inhibitory activity shows that analogue **(2)**, **(3)** and **(4)** are 40-, 200- and 300-fold less potent than (-)-huperzine A, respectively. The X-ray crystal structure of complex HupA-AChE elucidates that an unusually short (3.0 Å) hydrogen-bond is formed between the C-14 methyl group of (-)-huperzine A and the main-chain oxygen of His440 (a member of the catalytic triad) [4]. The biological activity decrease due to the introducing of CF_3 at R_2 position can be explained by this unusual hydrogen-bond. As to the case of analogue **(2)**, the reason of its activity decreasing is not very clear. In order to interpret this phenomenon, we embarked to study the interaction between (-)-huperzine A analogues and *Torpedo* AChE with molecular dynamics simulation method to find out the above unclear reason.

The initial HupA-AChE complex structure is taken from the X-ray crystal coordinates. The *Torpedo* AChE receptor includes 537 amino acid residues. For the sake of saving the computational time and disk space required by molecular dynamics simulation, we only extracted those residues encircled by 20 Å radius around (-)-huperzine A for MD simulation.

All MD simulations were performed with SYBYL 6.2 software on Indigo R4000 workstation. The simulation temperature is 300K and the step is 1fs. The molecular dynamics simulation process endured for 40ps under NTV atmosphere with Gasteiger-Huckel charge in Tripos force field. Firstly, we simulated the interaction of (-)-huperzine A with the active site residues of *Torpedo* AChE. Then, the C-12 methyl group

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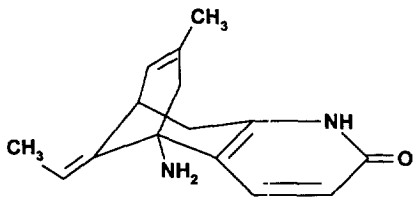
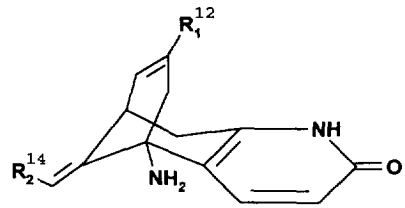
of (-)-huperzine A was substituted with CF_3 to generate compound **(2)** and MD simulation was performed on compound **(2)** directly without any further geometry minimization.



(-)-Huperzine A **(1)**



(±)-12,12,12-triflurohuperzine **(2)**: R_1=CF_3 R_2=CH_3

(±)-14,14,14-triflurohuperzine **(3)**: R_1=CH_3 R_2=CF_3

(±)-12,12,12,14,14,14-hexaflurohuperzine **(4)**: R_1=CF_3 R_2=CF_3

Figure 1. Structures of (-)-huperzine A **(1)** and its fluorinated analogues **(2)**, **(3)**, **(4)**

According to the ligand-protein interactions revealed by the X-ray crystal structure of complex HupA-AChE, a strong hydrogen-bond (2.6Å) is formed between the carbonyl group of (-)-huperzine A and the hydroxyl oxygen of Tyr130 [4]. Inspecting the statistics results in Table 1, the shortest distance between the oxygen of carbonyl group of (-)-huperzine A and the hydroxyl oxygen of Tyr130 is 2.33Å, which is very close to the experimental data. The shortest and mean distances between C-14 methyl group of (-)-huperzine A and the main-chain oxygen of His440 are 2.09Å and 2.86Å, respectively, which demonstrate that an unusually short hydrogen-bond can be formed between C-14 methyl group and His440 residue. This kind of unusual hydrogen-bond has previously been reported both in small molecules [6, 7] and in proteins [8]. Meanwhile, the shortest and mean distances between C-12 methyl group of (-)-huperzine A and the phenol hydroxyl oxygen of Tyr121 are 2.19Å and 3.09Å, respectively, which imply that the C-12 methyl group could form a hydrogen-bond with the phenol hydroxyl oxygen of Tyr121 residue. Besides, the shortest and mean distances between C-12 methyl group of (-)-huperzine A and the main-chain oxygen of Gly118 are 2.21Å and 2.98Å, respectively, which imply that the C-12 methyl group would likely form another hydrogen-bond with the main-chain oxygen of Gly118 residue. If all the hydrogen atoms of C-12 methyl group are substituted with fluorines, these two hydrogen-bonds will disappear.

Although (-)-huperzine A has three potential hydrogen-bond donor and acceptor sites, only one strong hydrogen-bond is found between the pyridone oxygen of (-)-huperzine A and residue Tyr130. The ring nitrogen of (-)-huperzine A forms a hydrogen-bond with a water molecule which hydrogen-bonds with Gly117, Tyr130 and Glu199. The hydrogen-bond between primary nitrogen of (-)-huperzine A and protein are mediated by at least two water molecules. The crystal structure of the complex shows that there exists hydrophobic interaction between C-12 methyl group of (-)-huperzine A and residues from Gly118 to Ser122. Moreover, the C-H...O hydrogen-bond between C-14 methyl group of (-)-huperzine A and His440

of AChE is also very important for its anticholinesterase activity [4]. Our MD simulation result reveals that C-12 methyl group of (-)-huperzine A could form another two C-H...O hydrogen-bonds with the phenol hydroxyl oxygen of Tyr121 and the main-chain oxygen of Gly118 of AChE, respectively. This result can interpret the anticholinesterase activity decreasing of compound **(2)**.

Table 1: Statistics information of MD simulation on (-)-huperzine A and its analogue **(2)** (unit: Å)

Distance Type	(-)-Huperzine A				Analogue (2)			
	mean	**std-dev**	**high**	**low**	**mean**	**std-dev**	**high**	**low**
His440D	2.86	0.41	4.19	2.09	2.95	0.51	4.86	2.14
Tyr130D	3.08	0.34	4.44	2.33	3.07	0.41	4.78	2.28
Tyr121D	3.09	0.56	5.95	2.19	3.27	0.41	4.55	2.36
Gly118D	2.98	0.43	5.67	2.21	3.26	0.44	4.95	2.29

Notes: **His440D** is the distance between C-14 methyl group and the main-chain oxygen of His440, **Tyr130D** is the distance between the oxygen of carbonyl group of ligand and the hydroxyl oxygen of Tyr130, **Tyr121D** is the distance between C-12 methyl group and the phenol hydroxyl oxygen of Tyr121, **Gly118D** is the distance between C-12 methyl group and the main-chain oxygen of Gly118.

In conclusion, with molecular dynamics simulation method, we have studied the interaction between (-)-huperzine A analogues and *Torpedo* AChE receptor. It was found that the methyl group at the three carbon bridge (C-12 methyl group) of (-)-huperzine A could form hydrogen-bonds with the phenol hydroxyl oxygen of Tyr121 and the main-chain oxygen of Gly118, respectively. This finding gives us clues that introducing hydrophobic groups which can also form hydrogen bonds with residue Tyr121 and Gly118 of AChE at the C-12 position of (-)-huperzine A might improve its anticholinesterase activity. The structural modification work of (-)-huperzine A based on this idea is now in progress in our laboratory.

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