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Interaction Dynamics of Caffeine in the Human Acetylcholinesterase Binding Pocket

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Abstract: Alzheimer's disease (AD) cases are increasing in Indonesia, with no effective therapy due to multiple hypotheses about its causes. The cholinergic hypothesis, focusing on acetylcholinesterase (ACHE) inhibition, is a key therapeutic approach. Caffeine, a natural compound, shows a potent activity as an AChE inhibitor. This study used computational methods to investigate the interaction dynamics of caffeine with the AChE's active site. This study performed 100 redocking simulations of donepezil to validate the docking protocol followed by 100 molecular docking simulations of caffeine. The 50-ns molecular dynamics (MD) production phase simulations of donepezil and caffeine were performed to study the interaction dynamics, such as conformational stability and binding free energies. The interaction hotspots during the simulations were identified using PyPLIF HIPPOS. Our findings reveal that caffeine interacted in the active site during the simulations and the importance of Glu202 and Phe338 in helping caffeine reside within the esteratic site of AChE.

Keywords: Acetylcholinesterase, Caffeine, Interaction Dynamics, PyPLIF HIPPOS

1. INTRODUCTION

The number of Alzheimer's disease (AD) cases in Indonesia is expected to continue increasing each year [1]. There are many hypotheses regarding the causes of AD to date, which makes the main problem of AD the lack of effective therapy [1,2]. One hypothesis that forms the basis of AD therapy is the "cholinergic hypothesis", which states that the progressive degeneration of cholinergic neurons is the main factor contributing to AD. The decrease in acetylcholine concentration is also reinforced by acetylcholinesterase activity [3].

Acetylcholinesterase (AChE) is a cholinergic enzyme that catalyzes the hydrolysis of acetylcholine (ACh) into choline and acetic acid [4]. AChE inhibition is the primary strategy in increasing acetylcholine levels in the brain and addressing cholinergic deficiency [5]. Some AChE inhibitors used in treating mild to moderate Alzheimer's dementia symptoms are donepezil, galantamine, and rivastigmine [6]. Another strategy is the development of natural substances as more potent Alzheimer's drugs [7].

Caffeine is an active alkaloid compound and central nervous system stimulant predominantly found in coffee and tea leaves [8]. Caffeine produces potent antioxidant, anti-inflammatory, and anti-apoptotic effects against various neurodegenerative disease models [9]. Caffeine is clinically known

to be used in slowing Alzheimer's pathology [10]. Additionally, caffeine is known as an AChE inhibitor [11].

Molecular dynamics (MD) studies targeting AChE show caffeine can act as a non-competitive AChE inhibitor [12]. Molecular mechanics Poisson-Boltzmann/surface area (MM-PBSA) calculation is a practical MD approach in computing binding free energy as the binding affinity of biomolecular complexes [13]. Several software programs can run MD simulations and perform MM-PBSA calculations, such as YASARA [14-16]. The latest version of YASARA-Structure allows binding energy analysis on each trajectory. Furthermore, PyPLIF HIPPOS can help identify protein-ligand interactions as supporting data for the MM-PBSA analysis module in YASARA [14].

This study aimed to identify the interaction of caffeine in the AChE active site using an in silico perspective. The study involved 100 molecular docking simulations, followed by 5-ns equilibrium phase and 50-ns production phase MD simulations. To identify the interaction hotspots during the simulations, we analyzed the MD results using PyPLIF HIPPOS 0.2.0, featuring its direct interaction fingerprint (IFP). The aim was to provide in silico perceptions about the molecular mechanisms of caffeine in inhibiting the AChE enzyme.

2. MATERIALS AND METHODS

2.1. Protein-ligand Preparation

The crystal structure of human acetylcholinesterase in complex with donepezil (PDB ID: 7E3H) was obtained from https://www.rcsb.org using YASARA-Structure. Aside from molecule A, the other molecules were omitted with a command of "DelMol !A". The solvent (Hoh) was also deleted from the system. The lost amino acid from crystallographic structure based on the SEQRES data was added using YASARA module of "Edit > Build > loop" and "Edit > Build > C-terminal loop". Next, the system pH was set to 7.4. Terminal cap was added with a command of "AddCapObj 1,Type=ACE+NME,Location=all". The structure was checked using module of "Edit > Clean > All". The energy minimization of the human acetylcholinesterase-donepezil complex was conducted using YASARA module of "Options > Choose experiment > Energy minimization". The corrected structure was saved as a YASARA-Object file with a file name of "7e3h-corr.yob" for redocking simulations.

2.2. Redocking Simulation of the Native Ligand

The redocking of donepezil as the native ligand was conducted following the provided procedure using an in-house plug-in [7,17]. The plug-in run 100 redocking with 7e3h-corr.yob as the MacroTarget. The plug-in also generated a file named rmsd_bestpose_all.txt, which stored the root mean square deviation (RMSD) value of the best-docked pose of each simulation compared to the corrected crystallographic donepezil.

2.3. Molecular Docking Simulation of Caffeine

The molecular docking was also conducted following the provided procedure using an in-house plug-in [7,17]. The molecular docking simulations were performed in a different working directory. The "MacroTarget_receptor.sce" and "MacroTarget_config.mcr" files were copied to the working directory. The three-dimensional structure of caffeine was built using its SMILES code, i.e., CN1C=NC2=C1C(=O)N(C(=O)N2C)C. The system's pH was adjusted to 7.4, and the system's energy was minimized. The structure was then saved as 7e3h-corr.yob and was used as the MacroTarget to

perform 100 molecular docking simulations. The RMSD value resulted from the best-docked pose of each simulation compared to the best-docked pose from the first simulation.

2.4. Molecular Dynamics Simulation

The three best poses of each redocking and molecular docking based on the lower free energy of binding (FEB) were selected as the input for interactions stability assessment using 55 ns molecular dynamics (MD) simulation, comprising five ns equilibrium phase and 20 ns production phase. The system was added with a Simulation Cell using the command "Cell Auto, Extension=10.0, Shape=Cube - OK" with "Periodic" boundary types. The system was then saved as a YASARA-Scene file named "7e3h-don1-r2md.sce". The YASARA-Scene file was used as the input, and the MD simulation was performed using the server from CAD3BNP (Computer-aided Drug Design & Discovery of Bioactive Natural Product). The MD simulation was performed using the macro "md_run-7e3h-55ns.mcr," modified from the default macro md_run.mcr. The simulation duration was 55 ns, with a timestep of 2.5 fs, snapshots taken every 100 ps at a temperature of 310K and water density of 0.993 g/mL, using AMBER14 as the force field, and utilizing 8 CPU Threads. The MD simulations were done in three replicates.

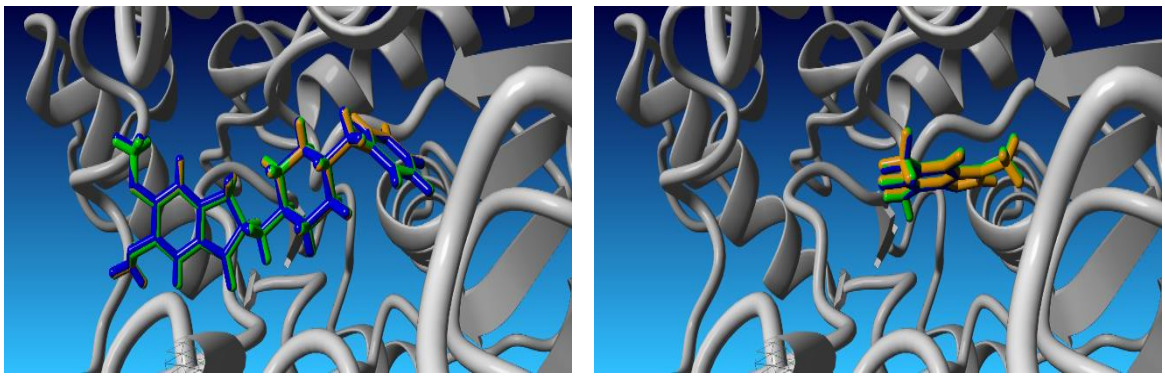
The first five ns duration was treated as the equilibration run, while the last 50 ns was treated as the production run. The first ten ns were used to evaluate conformation stability from the production run, while the whole production run was used to assess the FEB and the identification of interaction hotspots. The resulting snapshots were analyzed using "md_analyze.mcr" to obtain the backbone RMSD and ligand movement [14].

2.4. Analysis

The production run simulation snapshot was analyzed using "md_analyzebindenergy.mcr", which had been modified to use Poisson-Boltzmann (PBS) at 310 K to calculate the solvation energy and VINA local search (VINALS) as the algorithm for FEB calculation. The interaction hotspot identification between Human AChE amino acids and the ligands (Donepezil and Caffeine) was analyzed using PyPLIF-HIPPOS version 0.2.0 with the direct_IFP feature [17].

3. RESULTS AND DISCUSSION

The free energies of binding (FEB) values of 100 redocking iterations for donepezil range between -12.070 and -12.194 kcal/mol. The 100 redocking iterations of donepezil as a native ligand show an RMSD value ≤ 2.000 Å, indicating similar docking conformations. This RMSD value also means the docking method was valid and reliable for docking caffeine compounds. The binding energy of 100 redocking iterations for caffeine ranges between -6.726 and -6.848 kcal/mol. The best pose from 100 redocking iterations of caffeine shows an RMSD value ≤ 2.000 Å, indicating similar docking conformations. From both RMSD values of donepezil and caffeine, it can be concluded that there is only one conformation for each ligand, so for the MD simulation process, the three best poses with the lowest FEB values will be used. Figure 1 shows the superimposition of the three best poses, donepezil, and caffeine, inside the acetylcholinesterase binding pocket.



(a) (b)

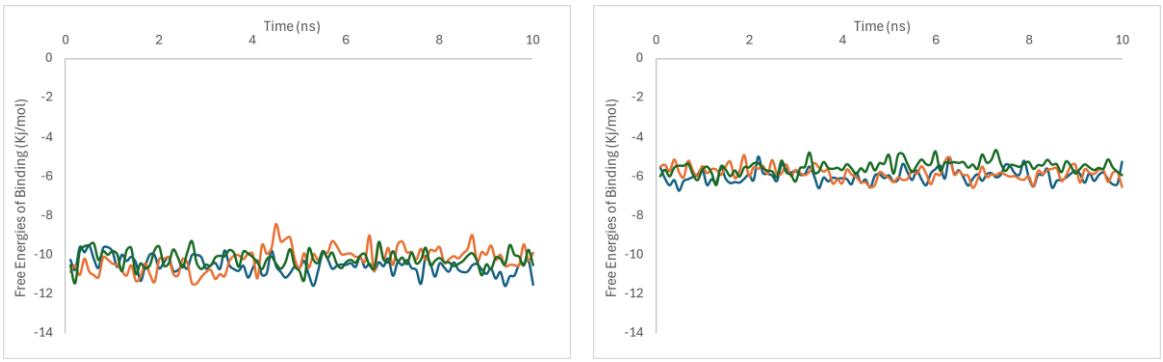
Figure 1. Superimposition of the three best poses of (a) donepezil and (b) caffeine inside acetylcholinesterase binding pocket. Each color represents each best pose: blue for the first-best pose, orange for the second-best pose, and green for the third-best pose.

MD simulation using YASARA-Structure combines the equilibration run and production run into one. In this study, the first five ns of the simulation will be treated as the equilibration run. While the remaining 50 ns will be treated as the production run, the referring for the time will be readjusted from 5-55 ns to 0-50 ns. Based on Liu et al. [18], protein-ligand simulation is considered stable if the ΔRMSD value in the first 5 to 10 ns is ≤ 2.000 Å, and a longer simulation time is optional in further model analysis. Table 1 shows ΔRMSDBb and ΔRMSDLigMove from the 5-10 ns production run. In the human AChE-Donepezil system, the average ΔRMSDBb value is 0.427 Å, and the average ΔRMSDLigMove is 1.092 Å; both ΔRMSD values indicate a stable MD simulation. In the human AChE-Caffeine system, the average ΔRMSDBb value is 0.507 Å, and the average ΔRMSDLigMove is 1.570 Å. These ΔRMSD values show that the human AChE-caffeine system also has a stabilized simulation.

Table 1. ΔRMSDBb and ΔRMSDLigMove of 5-10 ns production run

System	ΔRMSDBb	SD	CV (%)	ΔRMSDLigMove	SD	CV (%)
Donepezil R1	0.415			0.616		
Donepezil R2	0.393	0.042	9.801	0.922	0.580	53.113
Donepezil R3	0.474			1.738		
Caffeine R1	0.457			1.725		
Caffeine R2	0.455	0.088	17.424	1.425	0.150	9.574
Caffeine R3	0.609			1.559		

Figure 2 shows the FEB value of donepezil and caffeine throughout the ten ns of the production run. The FEB value of donepezil was comparably lower than caffeine, showing more stable interactions. It correlates with the IC_{50} value of donepezil and caffeine. Donepezil is well-known for its AChE inhibition activities with an IC_{50} value of 0.04 μM, while caffeine is a relatively weaker inhibitor with an IC_{50} value of 7.25 μM [19].



(a) (b)

Figure 2. The free energies of binding resulted from each first ten ns MD simulations reflecting interactions of donepezil (a) and caffeine (b) with amino acids of human AChE. Each color represents each best pose: blue for the first-best pose, orange for the second-best pose, and green for the third-best pose.

In Figures 3a and 3b, RMSDBb shows the conformational stability of human AChE backbone atoms and the docked ligands. All systems remained stable for the whole duration of MD simulations. Although the first system of human AChE-donepezil shows a gradually increased RMSDBb value of 2.233 Å at 6.8, the value escalated to 1.389 Å at 6 ns and remained stable until 49.2 ns. Thereafter, the RMSDBb value tended to increase until the end of the simulations.

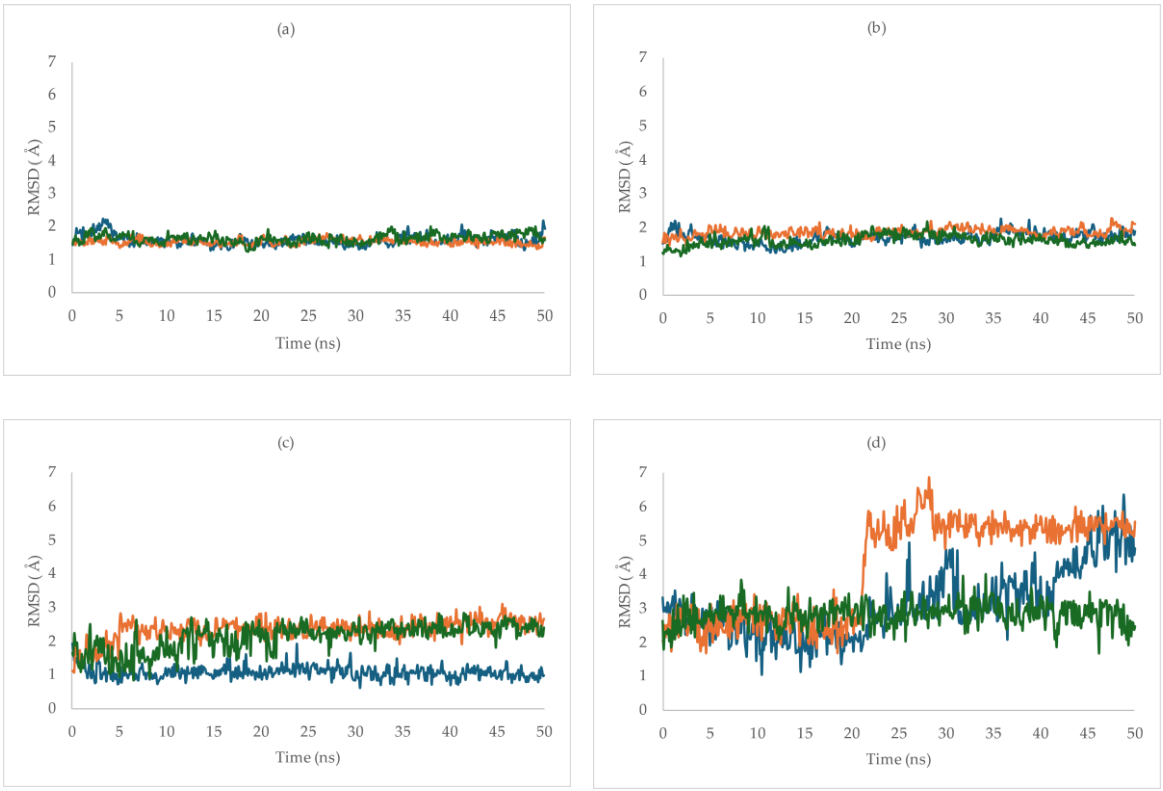


Figure 3. RMSD backbones atom value of AChE with donepezil (a) and caffeine (b), and RMSD Ligand Movement value of donepezil (c) and caffeine (d) throughout 50 ns of production run. Each color represents each best pose: blue for the first best pose, orange for the second-best pose, and green for the third best pose.

The RMSD values of the ligand movement of donepezil are shown in Figure 3c. The movement from the first best pose of the donepezil shows a stable movement throughout the simulations. Although the initial RMSD value decreased from 1.928 Å at 0 ns to 0.894 Å at two ns, it remained stable until 50 ns. The second-best pose of donepezil shows an increased value at the initial production run from 1.113 Å to 2.823 Å at 5.1, and it remained stable until the end of the simulations. The third-best pose of donepezil showed an interesting movement, with a gradually increased RMSD value from 0-ns to 25 ns, and it remained stable until the end of the simulations.

The RMSD values of the ligand movement of caffeine are shown in Figure 3d. The movement from the first best pose of caffeine shows a gradually decreased RMSD value from 3.32 Å at 0 to 1.127 Å at 14.6 ns. Then, the RMSD values gradually increased to 39.4-ns and more until the simulations' end. Interestingly, the second-best pose of caffeine shows a stable RMSD value from the start until 21 ns, which escalated to 6.854 Å at 28.2 ns and remained stable until the end of the simulations. The third-best pose of donepezil showed a stable RMSD value until the end of the simulations. Despite having a higher RMSD value, through visual inspection, the caffeine still resides inside the binding pocket.

PyPLIF-HIPPOS 0.2.0 was employed to identify receptor-ligand interactions such as hydrophobic, aromatic, hydrogen bond, and ionic. The non-hydrophobic interactions identified from PyPLIF-HIPPOS were tabulated in Table 2. PyPLIF-HIPPOS 0.2.0 identified several similar amino acid residues of the active site at the human AChE-donepezil binding pocket. Our findings show that the nitrogen atom of N-benzyl pyridinium moiety formed hydrogen bonds with Tyr337 for more than 20% of production run simulation on the second and third systems of AChE-donepezil. AChE-donepezil system showed that donepezil formed aromatic interaction for more than 50% during the production run with Trp86, Trp286, Tyr337, Phe338, and Tyr341 residues.

Figure 3d revealed that caffeine has RMSD ligand movement values of more than 2 Å throughout the production run simulation. Yet, caffeine retains the aromatic interaction with Trp86, Tyr337, and Phe338 residues. Caffeine also has hydrogen bond interaction with Tyr337 residue and additional hydrogen bonds with Tyr124 and Tyr341 in several snapshots during the production run. Moreover, the nitrogen atom of the imidazole moiety formed ionic bonds with Asp74 and Glu202.

Table 2. Non-hydrophobic interaction hotspots identified by PyPLIF HIPPOS 0.2.0 of human AChE-donepezil and human AChE-caffeine throughout a 50 ns production run.

Residue	Interaction Type	Interaction Percentage					
		Donepezil			Caffeine		
		R1	R2	R3	R1	R2	R3
TYR72	aromatic edge-to-face	1.00	8.98	5.59	-	-	-
ASP74	ionic as the anion	-	1.60	-	-	21.56	1.20
TRP86	aromatic edge-to-face	7.39	17.37	17.37	14.37	4.99	-
	aromatic face-to-face	89.82	50.50	74.85	-	38.92	-
TYR124	aromatic edge-to-face	7.58	5.99	10.18	13.17	39.92	17.76
	H-bond donor	-	-	-	7.39	-	2.00
GLU202	ionic as the anion	-	-	-	42.12	14.37	6.19
TRP286	aromatic edge-to-face	9.18	6.39	1.60	-	-	-
	aromatic face-to-face	90.82	93.41	97.60	-	-	-
PHE295	aromatic edge-to-face	0.80	-	-	-	-	-
	aromatic face-to-face	-	-	-	0.40	0.60	0.40

continued Table 2…

PHE297	aromatic edge-to-face	20.56	10.78	13.57	2.20	0.60	-
	aromatic edge-to-face	99.40	94.61	96.41	84.43	92.22	90.22
TYR337	aromatic face-to-face	0.20	0.20	-	-	0.20	-
	H-bond acceptor	-	35.93	23.95	-	-	-
	H-bond donor	-	-	-	5.19	-	1.00
PHE338	aromatic edge-to-face	74.45	26.55	23.95	45.71	34.93	55.29
	aromatic face-to-face	1.40	-	-	7.58	1.20	0.20
	aromatic edge-to-face	42.71	45.91	30.54	5.59	0.40	-
TYR341	aromatic face-to-face	69.66	65.07	69.86	16.37	0.60	-
	H-bond acceptor	-	1.40	-	-	-	-
	H-bond donor	-	-	-	-	0.40	1.20
HIS447	aromatic edge-to-face	36.33	1.20	0.20	25.55	2.79	0.60
	aromatic face-to-face	4.59	-	-	-	-	-

As depicted in Figure 3d, the RMSD ligand movement of the three systems of AChE-caffeine has different dynamics. This may be associated with caffeine's ability to form and maintain aromatic interaction and ionic bonds. The caffeine from the first system only consistently formed ionic bonds with Glu202 from 0 to 21.5 ns production run and formed again at 23.2, 31.3, 31.4, and 31.5 ns. After that, the caffeine forms aromatic interaction with Trp86 and maintains it from 26.1-ns until the end of the simulation. Also, the increased value of RMSD after 39.4 ns may be associated with aromatic interaction with Phe338, as the aromatic interaction does not appear after 40.5 ns. The second system reveals that caffeine forms and maintains the ionic bonds with Glu202 from the initial production run until 20.9 ns. After that, the caffeine forms ionic bonds with Asp74 and maintains it until the end of the simulation. The aromatic interaction with Trp86 also formed after the caffeine could not interact ionically with Glu202 and retain it until the end of the simulation run. The third system shows an interesting result: the ionic bond with Glu202 often appears in the first 2 ns of the production run and rarely appears in the last 10 ns of the production run, while the ionic bond appears more rarely from 25 to 35 ns of the production run. Although they do not have ionic interaction with Glu202, the RMSD ligand movement values are more stable compared to the other system. This might be attributed to aromatic interaction with Phe338, as the caffeine can maintain it throughout the production run simulation. These findings suggest that the critical residues for caffeine residing in the esteratic site of AChE were Glu202 and Phe338. Although each system has different dynamics, the caffeine can stabilize the binding orientation through aromatic interaction with Tyr337 [20], which contributes to more than 80% of MD simulations.

4. CONCLUSION

Molecular docking simulations revealed that donepezil and caffeine have one pose in their interaction with the AChE binding pocket. Molecular dynamics simulations also revealed that donepezil and caffeine interacted in the AChE active site throughout the simulations. Our findings found that the hydrogen bonds and aromatic interaction reinforced caffeine interaction. These findings could be used to comprehend the atomic level of bioactive compounds, i.e., caffeine, in inhibiting the AChE enzyme.

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Conflicts of interest: The authors declare no conflict of interest.

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