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Original Article

STABILITY OF OMEGA-3 COMPOUNDS COMPLEX WITH PPAR-γ RECEPTOR AS AN ANTI-OBESITY USING MOLECULAR DYNAMIC SIMULATION

IDA MUSFIROH[1*], GINNA MEGAWATI[2,3], DEWI M. D. HERAWATI[2], MUS IFAYA[1,4]

[1]Department of Pharmaceutical Analysis dan Medicinal Chemistry, Faculty of Pharmacy Universitas Padjadjaran Bandung Indonesia, [2]Division of Medical Nutrition, Department of Public Health, Faculty of Medicine Universitas Padjadjaran, Bandung, Indonesia, [3]Doctoral Study Program, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia, [4]Department of Pharmacy, Faculty of Science and Technology, Universitas Mandala Waluya, Indonesia
*Email: ida.musfiroh@unpad.ac.id

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ABSTRACT

Objective: Obesity is a major contributor to comorbid diseases based on low grade chronic inflammation. Omega-3 fatty acids have a role in inflammation so it is thought to prevent obesity. This study was conducted to analyze the stability of omega-3 fatty acids with the PPAR-γ receptor using molecular dynamic simulation to investigate the relationship of macromolecule interactions to biologically relevant as an obesity comorbid.

Methods: The methods consisted of ligand acquisition, molecular dynamic simulation, and analysis of dynamic molecular results using Gromacs 2016.3 software and the results of the MD analysis were carried out by simulating time with VMD software and graphing the results of MD data analysis using Microsoft Excel.

Results: The result showed that docosahexaenoic acid (DHA), docosapentaenoic acid (DPA), and heneicosapentaenoic acid (HPA) have good stability. Average RMSD values of DHA, DPA, and HPA were 0.347 Å, 0.464 Å, and 0.706 Å with similar pattern of fluctuation across the region. DHA forms a hydrogen bond to Tyr347 and Leu343. Meanwhile, DPA binds to Asn52 and HPA bind to Arg213. DHA, DPA, and HPA have an average SASA of 233.91 nm^2, 231.47 nm^2, and 225.52 nm^2, respectively. DHA has the lowest total binding energy (-129.914 kJ/mol) compared to DPA (-102.018 kJ/mol) and HPA (-115.992 kJ/mol).

Conclusion: Based on the molecular dynamics simulation approach, omega-3 compounds, DHA, DPA, and HPA showed that DHA has good stability compared to DPA and HPA. DHA, DPA, and HPA can be used as lead drugs to bind to PPAR-γ receptors to prevent and treat obesity.

Keywords: Molecular dynamic simulation, Obesity, Omega-3 fatty acids, PPAR-γ

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INTRODUCTION

Obesity is an excess of normal adiposity due to excessive adipokine secretion. It is a major contributor to the pathophysiology of comorbid diseases such as diabetes mellitus, insulin resistance, dyslipidemia, hypertension, and atherosclerosis [1]. Omega-3 has been proven to reduce the accumulation of body fat. Omega-3 fatty acids are vital contributors to the inflammatory process and control of lipid metabolism. The mechanism of omega-3 fatty acids in preventing obesity remains unclear. However, it has been reported that omega-3 fatty acids are able to improve insulin sensitivity by stimulating the activation of peroxisome proliferator-activated receptor protein PPAR-γ [2, 3]. Currently, full agonist PPAR-γ drugs are known to have cardiovascular side effects [4].

Drug discovery and development innovation require a very long and expensive research process. The computational method opens opportunities. *In silico* study, strategies include molecular docking simulation, Absorption, Distribution, Metabolism, and Excretion (ADME) prediction, 3D-pharmacophore modelling, and molecular dynamic simulation [5].

The previous study, three omega-3 fatty acids, docosapentaenoic acid (DPA), docosahexaenoic acid (DHA), and heneicosapentaenoic acid (HPA) have affinity for PPAR-γ. DPA and DHA are compounds that are predicted to have the highest affinity indicated by the Gibbs energy values of-9.26 kcal/mol and-8.92 kcal/mol, respectively. DPA and DHA are predicted to be partial agonists and total agonists of PPAR-γ. The partial agonist ability of DPA was demonstrated by hydrogen bond interactions at Ser342, while DHA had hydrogen bond interactions at residue Ile281. Meanwhile, HPA has a Gibbs energy value of-8.11 kcal/mol without any hydrogen bond interaction at the PPAR-γ residue [6].

In addition, the previous 3D-pharmacophore modelling illustrated that DPA, DHA, and HPA could be used as lead compounds against PPAR-γ with pharmacophore fit scores of 36.56, 36.59, and 36.56, respectively. The carboxylate functional group becomes an active functional group that forms hydrogen bond interactions. While the alkyl chain is the part that can be modified to increase its activity [7].

However, the binding stability of omega-3 compounds remains unknown, so the aim of this study was to determine the stability of omega-3 compounds on the active site of the PPAR-γ receptor through molecular dynamics simulation. Molecular dynamic simulation is a drug discovery method capable of describing the movement of atoms and their interactions with surrounding atoms or proteins in a regulated environmental system [8].

MATERIALS AND METHODS

Ligand and receptor acquisition

The PPAR-γ receptor (PDB ID: 4R06) was downloaded from the protein database https://www.rcsb.org/. Ligands that consisted of DHA, HPA and DPA as omega-3 compounds were prepared using ChemDraw 16 PerkinElmer Inc as shown in fig. 1. Telmisartan is the positive control for PPAR-γ was also prepared using ChemDraw 16 PerkinElmer Inc. as a positive control. The MD results are based on our the results of docking conducted previously by Megawati *et al.*, 2020 with the lowest binding energy of DHA-8.92 kcal/mol, DPA-9.26 kcal/mol and HPA-8.11 kcal/mol. The application uses the GROMACS versions on a single-socket 8-core Core-i7 5960X desktop with one NVIDIA GTX980 GPU. With SIMD, GPU and OpenMP acceleration, the desktop achieves close to 200 ns/day for the VSD.

Molecular dynamic simulation

Molecular dynamic (MD) simulation was carried out using the Gromacs 2016.3 software with the AMBER99SB-ILDN force field

software is free licensed. Topology and ligand parameters were made using ACPYPE. The electrostatic force was set using the PME method. The system was neutralized by carried out the Na^+ and Cl^- ions into the complex system. The solvation of the system was adjusted by the model of TIP3P water cube. The minimization stride in the preparation stage includes the heating to 310 °K, temperature and pressure equilibration, and simulation process. Furthermore, 100 ns of MD production was performed with a 2 fs timestep. After the simulation, were calculated by g_rms, g_rmsf, and g_rg functions.

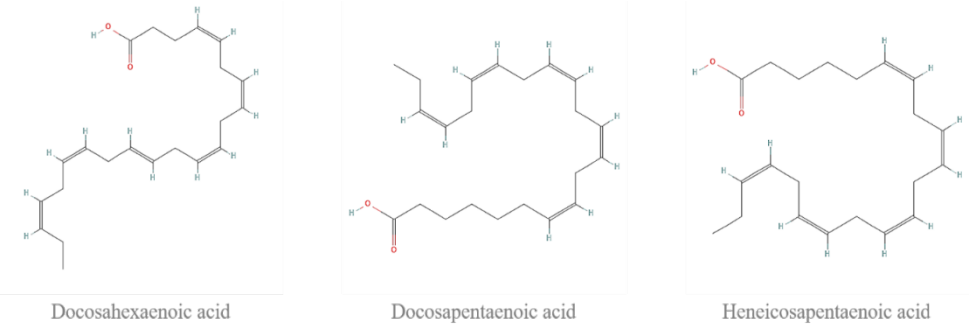

Fig. 1: Structure of docosahexaenoic acid (DHA), docosapentaenoic acid (DPA), and heneicosapentaenoic acid (HPA)

Analysis of molecular dynamic result

Post-MD simulation analysis of MD results using VMD and Microsoft excel was performed by calculating the root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (Rg), and binding free energy using MM-PBSA method, and then the SASA analysis and PCA analysis for the detection of the direction and amplitude of the dominant motions were analysed [9].

RESULTS AND DISCUSSION

RMSD and RMSF analysis of ligand-receptor complex

The ligand-receptor complexes were analyzed by molecular dynamic simulation during 100 ns simulation using GROMACS 2016. The stability of the system during 100 ns simulation was successfully measured through the RMSD and the RMSF (fig. 2). RMSD analysis was measured to predict the stability of the complex over time as compared to the starting point, meanwhile RMSF analysis was measured to assess the stability of each amino acid residues [10].

DHA the best docking score of metabolites, was simulated by molecular dynamic. The complex stability was compared to 2 other compounds, i.e. the DPA and HPA, which are inhibitor of PPAR-γ receptor. DHA in the complex with PPAR-γ receptor shows high similar fluctuation with DPA and HPA. Meanwhile, the average of RMSD fluctuations for each system, i.e. DHA, DPA, HPA, and telmisartan were 0.347 Å, 0.464 Å, 0.706 Å and 0.370 Å, respectively. The RMSD average indicated that DHA showed the lowest fluctuation compared to the reference ligand. The amino acid fluctuation of the receptor complex systems calculated by RMSF showed similar patterns in all regions. At residues number 62, 92, 126, 175, 216 and 338 in PPAR-γ receptor, it presented higher fluctuation than in another residue. These residues were seen in the amino acid chain that is responsible for the loop region.

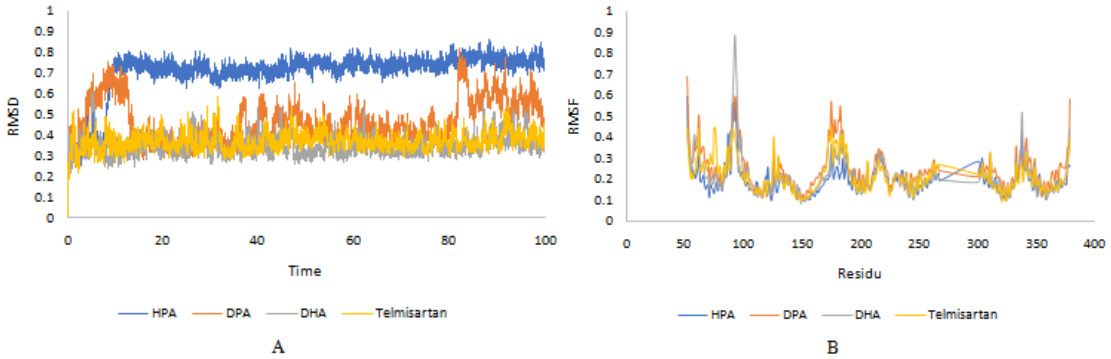

Fig. 2: RMSD (A) and RMSF (B) value of complexes of heneicosapentaenoic-PPAR-γ (blue), docosapentaenoic-PPAR-γ (red), docosahexaenoic-PPAR-γ (grey) and telmisartan-PPAR-γ (orange)

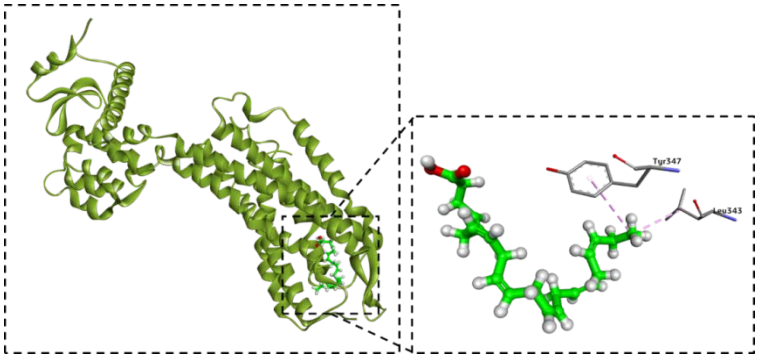

Fig. 3: Docosahexaenoic-PPAR-γ after being simulated for 100ns

Conformational analysis across selected trajectories

For gaining more insight regarding the newly adopted ligand-protein conformations by each ligand within the late MD simulation runs, the selected frames of each system were extracted and minimized to a gradient of 0.001 kcal/mol/A2 [11]. Fig. 3, fig. 4, and fig. 5 show the conformational changes of the receptor due to the binding of DHA, HPA, and DPA.

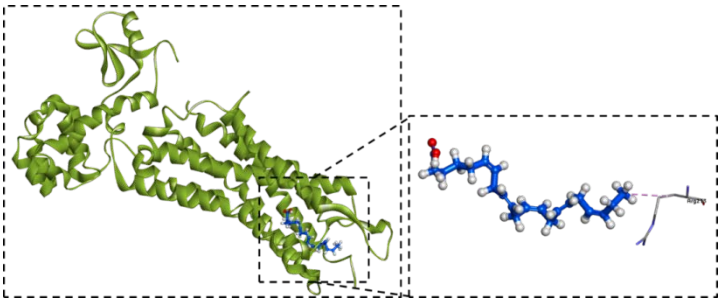

Fig. 4: Heneicosapentaenoic-PPAR-γ after being simulated for 100ns

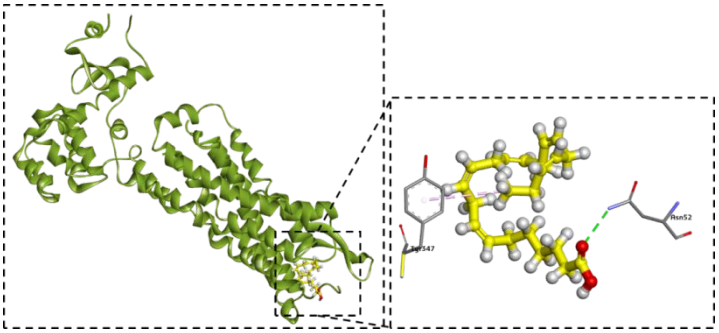

Fig. 5: Docosapentaenoic-PPAR-γ after being simulated for 100ns

The conformational changes that occur at the receptor for DHA and DPA indicate that there is a polar bond formed through hydrogen bonds in the amino acid Tyr347, which indicates the stability of the two compounds. The interaction of amino acids in Leu343 with DHA showed a conformational form with good polarity. Meanwhile, the conformational form of DPA with Asn52 interaction showed good polarity by the receptor. The HPA compound showed poor basicity by not forming a fairly good polar bond from the amino acid Arg 213, which showed the instability of the conformation of this compound.

Solvent accessible surface area (SASA)

The identification of SASA was carried out to predict the protein conformation changes during the simulation, which can be accessed by water molecules [12]. SASA was analyzed during 100 ns of MD trajectory simulation, which is shown in fig. 6. The SASA of the ligand-PPAR-γ receptor complex were revealed.

In the DHA-PPAR-γ complex, the ligands showed high similarity in fluctuation compared to DPA, HPA, and telmisartan. Furthermore, the average value of DHA, DPA and HPA were 233.91 nm^2, 231.47 nm^2, 225.52 $nm^2$and 230.56 nm^2, respectively. The lower value of SASA analysis was provided by HPA and followed by DHA and DPA. The lower value of SASA, the more stable the system complex [12]. This analysis is not correlated with the RMSD value that presented DHA has better stability in PPAR-γ receptor than DPA and HPA.

Principal component analysis (PCA)

PCA was analyzed for significant fluctuations in protein-ligand complexes [13]. The direction and amplitude of the eigenvectors, which are responsible for the motion, and complex dynamics analyzed in 2D projection of trajectory plot generation in PCA. We choose the first of two principal components, namely PC1 and PC2. Fig. 7 illustrates projections of the two eigenvectors of the DHA, DPA, and HPA in complex with PPAR-γ receptor. The less space occupied by the clusters indicates a more stable complex, whereas those that occupy more space indicate a less stable protein-ligand complex [14]. DHA occupies less phase space than the DPA and HPA patterns. The plot indicated that DHA has stable during 100 ns simulation in the binding site of PPAR-γ receptor.

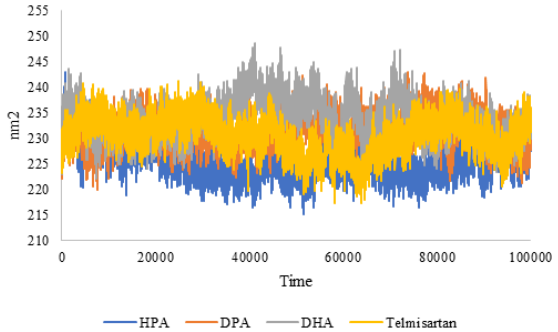

Fig. 6: SASA plot of complexes of heneicosapentaenoic-PPAR-γ (blue), docosapentaenoic-PPAR-γ (red), docosahexaenoic-PPAR-γ (grey) and telmisartan-PPAR-γ (orange)

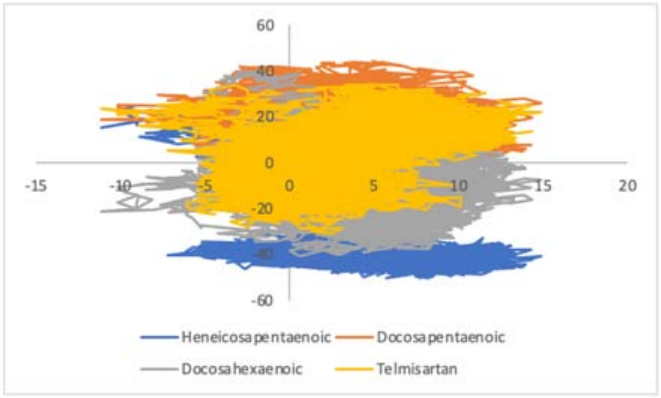

Fig. 7: Evaluation of PCA of complexes of heneicosapentaenoic-PPAR-γ (blue), docosapentaenoic-PPAR-γ (red), docosahexaenoic-PPAR-γ (grey) and telmisartan-PPAR-γ (orange) that represented by 2D projection of trajectory motion during MD simulations

MM-PBSA binding free energy calculations

The binding free energy of the molecular dynamics trajectories of the system complex was calculated using the MM-PBSA method from timestep 0-100 ns [15]. Van der Waals, electrostatic and SASA energies in both complex systems indicated a negative value, while the polar solvation energy showed a positive value (table 1). These results indicated that in both system complex, the polar solvation energy terms opposed the binding, van der Waals, electrostatic and SASA energies favored the binding. The total binding free energy of the ligands shows varying values. DHA provided the lowest binding free energy than DPA and HPA. DHA showed binding energy-124.467 kJ/mol, telmisartan showed energy-129.914 kJ/mol, while HPA and DPA were-115.992 kJ/mol and-102.018 kJ/mol. The MM-PBSA analysis indicated that telmisartan has the strongest affinity against PPAR-γ receptor.

Table 1: MM-PBSA energy summary of ligand-PPAR-γ receptor during 100 ns simulation

Energy components (KJ/mol)	Compounds			
	DHA	DPA	HPA	Telmisartan
van der Waals	-210.978±14.386	-174.449±18.477	-191.092±18.112	-265.439±22.866
Electrostatic	-34.048±13.071	-30.271±19.453	-37.960±9.903	-53.281±22.293
Polar solvation	143.269±22.123	122.495±26.917	134.761±19.374	215.919±36.184
SASA	-22.710±1.047	-19.793±1.326	-21.701±1.278	-27.112±1.563
Binding energy	-124.467±22.141	-102.018±15.845	-115.992±23.979	-129.914±18.047

CONCLUSION

With a molecular dynamic simulation approach, we have identified the stability of omega-3 compounds, namely DHA, DPA, and HPA to determine the relationship of biologically relevant macromolecular interactions. Several parameters were measured including RMSD, RMSF, protein conformation, SASA, PCA, and MM-PBSA binding free energy. RMSD analysis results obtained more stable HPA values, analysis with SASA showed that DHA had more values and PCA values for DHA compounds were the best and MMPBSA data showed that DHA compounds had lower free energy compared to telmisartan. Based on these six parameters, DHA compound can be used as lead drugs to bind to PPAR-γ receptors as a prevention and treatment of obesity.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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