IJFAC
Indonesian Journal of Fundamental and Applied Chemistry
Article http://ijfac.unsri.ac.id

Analyses of Non-bonding Length, Partial Atomics Charge and Electrostatic Energy from Molecular Dynamics Simulation of Phospholipase A2 – Substrate

Nirwan Syarif[1]* and Dedi Rohendi[1]

[1] *Department of Chemistry, University of Sriwijaya, Inderalaya 30662 South Sumatera*
[*]*Corresponding Author: nnsyarif@gmail.com*

Abstract

This paper reports molecular dynamics simulation of phospholipase A2 (PLA2)– substrate that has been done. Non-bonding length, partial atomic charge and electrostatic energy were used to evaluation the interaction between PLA2 and substrate. The research was subjected for three types of PLA2 of different sources, i.e, homo sapien, bovinus and porcinus, by using computer files of their molecular structures. The files with code 3elo, 1bp2, dan 1y6o were downloaded from protein data bank. Substrate structure can be found in 1y60 and was separated from its enzyme structure and docked into two other PLA2 structures for simulation purpose. Molecular dynamics simulations were done for 30000 steps with constant in number of molecules, volume and temperature (NVT). The results showed the existing of flip-flop mechanism as basic feature of PLA2 – substrate reactions. Interaction length analysis results indicated the presence of water molecules on the structures of 1bp2 and 3elo at the time of the simulation was completed. The existence of aspagine at the reaction site confirmed the theory that this amino acid is responsible for the survival of the reaction. the electrostatic energy increased substantially in the interaction after homo sapien PLA2 (3elo) and Bovinus (1bp2) with the substrate. Inverse effect took place in the PLA porcinus (1y6o).

Keywords: flip flop, inflammation, in-silico, simulation

Abstrak (Indonesian)

Telah dilakukan penelitian tentang simulasi dinamika molekuler pada Situs Reaksi Phospholipase A2 (PLA2) dengan substratnya. Analisis panjang non-ikatan, muatan atom parsial dan energi elektrostatis digunakan untuk menilai interaksi antara PLA2 dan substratnya. Penelitian dilakukan pada tiga jenis sumber PLA2, yaitu homo sapien, bovinus dan porcinus dengan menggunakan file komputer untuk struktur molekul dengan kode 3elo, 1bp2, dan 1y6o. Pada file 1y60 terdapat struktur substrat yang dapat ditemukan secara alamiah. Kedua file lainnya tidak mengandung struktur molekul substrat. Simulasi dinamika molekul dilakukan untuk 30.000 langkah dengan konstan dalam jumlah molekul, volume dan suhu (NVT). Hasil penelitian menunjukkan keberadaan mekanisme flip-flop sebagai fitur dasar reaksi PLA2 - substrat. Hasil analisis panjang interaksi menunjukkan bahwa kehadiran molekul air pada struktur 1bp2 dan 3elo pada saat simulasi dilakukan. Keberadaan aspagine di lokasi reaksi menegaskan teori bahwa asam amino ini bertanggung jawab untuk kelangsungan hidup reaksi. energi elektrostatik meningkat secara substansial dalam interaksi setelah homo sapien PLA2 (3elo) dan Bovinus (1bp2) dengan substrat. efek terbalik terjadi di porcinus PLA (1y6o)

Keywords: flip flop, inflamasi, peradangan, in-silico, simulasi

Article Info

Received 1 July 2016
Received in revised 26 August 2016
Accepted 8 September 2016
Available online 12 November 2016

IJFAC

Indonesian Journal of
Fundamental and Applied Chemistry

Article http://ijfac.unsri.ac.id

INTRODUCTION

Chronic inflammation strongly relate with cellular mutation that has character in initiating cancer [1]. Severely inflammation in blood vessel contribute to develop flake in artery. However, inflammation is a defense mechanism of human body against infections and repairs body tissues. Continuos exposure (chronic) of inflammation can cause tissues damage and develop into diseases [2,3]. Inflammation is initiated by the changed in blood vessel that increase leucocyte recruitment and liquid transfer within plasmatic protein inside the tissues [4].

Phospholipase (PLA), both PLA type 1 (PLA1) and type 2 (PLA2) were activated to transform phospholipids to arachidonic acid in the tissue [5]. Part of arachidonic acid is changed to prostaglandin by cyclooxygenase. The other part of arachidonic acid is changed to leucotriene by lypoxgenase. Therefore, Both of these substances are responsible for most of the symptoms of inflammation [6].

Although the general reaction has long been known, but due to the structures of PLA2 are diverse and each of PLA2s have isomer, namely PLA1s, the reaction mechanisms on molecular level are not so clear [7]. The reaction between the enzyme and its substrate involves more specific amino acids that making up the enzyme. Therefore, this study aims to study the effect of PLA variation on reaction dynamics. The knowledges of reaction dynamics and the variation at the molecular level are necessary in designing a drug molecule (inhibitors). Study of the reaction mechanism can be performed by molecular dynamics simulation [8]. Analyses of non-bonding length, partial atomics charge [9] and electrostatic energy [10,11] were used as parameters to evaluate reaction mechanicsm.

MATERIALS AND METHODS

Downloading, File Editing and Creating Topology

The structure of the target protein can be downloaded via website www.rscb.org/pdb. The downloaded enzymes files have water and others, i.e. cofactor or coenzyme molecules structures in its bulk structure. The structures other than enzymes must be eliminated first because it is not required in the simulation. The elimination of water molecules and other molecules can be done using UCSF Chimera application. The result was used for docking process. Separated structure was stored in .pdb format file.

Arachidonic acid substrate structure can be downloaded directly from the PDB website. The obtaining substrate molecules was opened through chimera software and edited its structure to add H atom and partial charge. The structure that has been edited then saved into .mol2 format and uploaded to swissparam.ch page to make topology and parameter files [12]. A zip file that contains data psf and pdb files was appeared after the server stopped the process. Both files are necessary in running simulation with NAMD.

Running Simulation

The simulations were carried out by using the psf and pdb files that have been created, the files were opened with VMD. Furthermore, the simulations conditions were arranged by using NAMDgui and written in the simulation configuration file. The simulation and minimizing enzyme structure were done in 10,000 and 40,000 steps, respectively. The next step will be generating configuration file to be saved with the extension NAMD. The configuration file can be edited by using notepad to accommodate other simulation conditions.

The simulations were carried out by running the NAMD program at the Command prompt mode and performed under conditions explicitly by the addition of water molecules and ions. The addition of water molecules and ions on the structure of the enzyme-substrate can be done by operating the facility and add ion solvation inside VMD. These conditions settings conducted in VMD must be recognized by NAMD in order to have full execution time. Full execution was a time-consuming process and will generate some files, including dcd files, log files, and others. The dcd

files can be used to analysis the interaction of enzymes and substrates.

Data Analysis

Non-bonding length, partial charge analyses were done using the software Ligplot [13] to provide an overview of interaction between enzyme, substrate, water molecules and ions. Electrostatic energy analysis was performed using the facilities NAMD plot provided in the VMD.

RESULT AND DISCUSSION

Some structures of PLA2s were downloaded from www.rcsb.org that having code 1y6o (PLA2 from homo sapiens), 1bp2 (PLA2 from bovine, boss taurus) and 3elo (PLA2 from porcinus). The sequencing analysis of the three enzymes showed the similarity (Figure 1) so that their the interaction with the substrate can be evaluated. Those three are only distinguished from the position of the spiral structures and the sulfur bridge.

Within the three enzymes structures, only 1y6o (Figure 2 (a)) has a substrate (Figure 2 (b)), while the other two only contain some water molecules and ions. The addition of substrate derived from 1y6o into 1bp2 and 3elo structures was carried out by using alignment techniques on VMD. All water molecules and ions contained in the structure of these enzymes were eliminated. However, the waters were re-added back in bulk structures in order to minimize structural error while the simulations took place. The simulations were carried out as many as 50,000 steps on the condition that the molecule number, volume and temperature were constant (NVT) and at temperature of 25 C, in 45.9 x 47.9 x 27.6 A^3 box fit in the size of the enzyme.

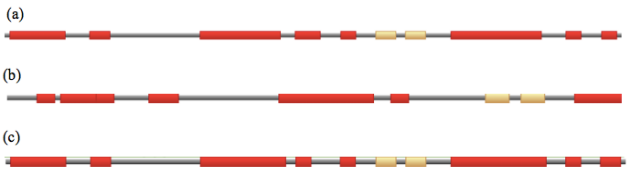

Figure 1. The comparison of the tertiary structures of the enzyme PLA2 derived from (a) pork (b) ox and (c) human. Red represents to spiral part; gray to bar and yellow to sulfur bridge.

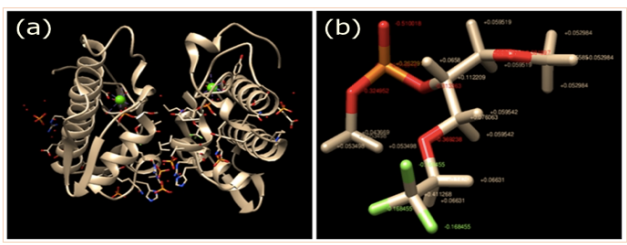

Figure 2. (a) The molecular structure of the PLA2 dimer. Each of monomer consist of PLA, some water molecules, some of Ca molecules (green ball), a phosphate ion, and (b) the substrate is 1-hexadecyl-3-trifluoroethyl-sn-glycero-2-phosphate.

The simulation results show that there are a little movement toward extreme changes both on the enzyme and the substrate along with all the simulations performed. This result due to the application of simulation procedures arrangement. However, the interaction between the enzymes, substrates, water and ions can still be observed by using the same procedure. Figure 3 shows the behavior of molecules that occurred during simulation, i.e. at the beginning, middle and end of the simulation. The simulations are specific to the reaction site needed to be known so that gave detailed picture of the interactions that take place, as shown in Figure 4.

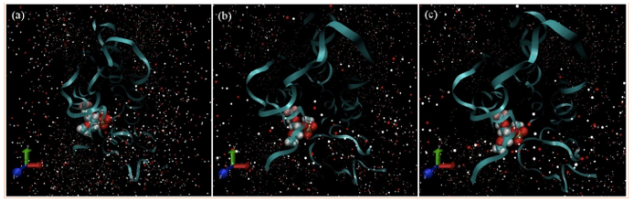

Figure 3. The visualization snapshot of PLA2 enzyme simulation (from 3elo.pdb bulk structure) inserted (docking) with hexadecyl-3-trifluoroethyl-sn-glycero-2-phosphate (from 1y6o.pdb bulk structure) structure

The reaction mechanism between fatty acid and PLA2 has been studied previously by other researchers [13–16]. The reaction of fatty acids - PLA2 from the venom is known to take place as hydrolysis were carried out at the reaction site. The amino acids were considered to be responsible in the reaction, i.e. asparagine and histidine [17]. By performing molecular dynamics simulations, the complete picture of the reaction mechanism can be evaluated.

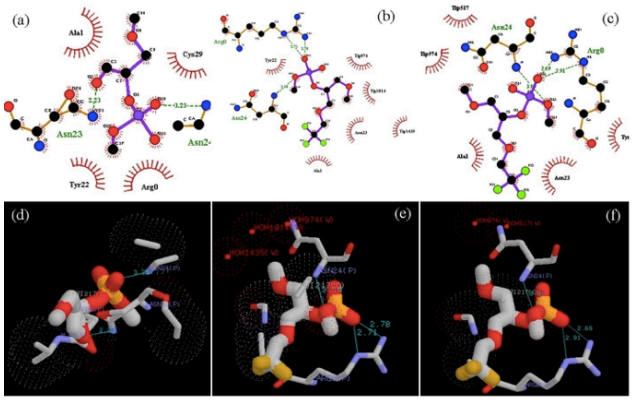

Figure 4. Analyses of length and partial atomic charge of the interaction between substrate molecules and amino acid groups at the PLA2 reaction site. The simulations performed in 700 steps or pose interaction. (a) at step 0 (b) in step 350 (c) in step 700 for length analysis. Figure (d), (e) and (f) for partial charge analysis in 0, 350 and 700 step, respectively.

Figure 4 shows the result of the interaction length and partial atomic charge analyses at the reaction site of 3elo which is a structure human PLA2 enzyme with 1y6o substrate from porcinus. It can be seen in Figure 4 that the reaction site experienced cracking in the midst of the simulation runs. In the other hand, the reaction site experienced shrinking both in the beginning and end of the reaction. Both of these conditions are consistent with the flip-flop mechanism of the enzyme. The existence of aspagine (coded as asn) (Figure 4 (a), (b) and (c)) at the reaction site was confirmed the theory that this amino acid is responsible for the survival of the reaction. The existence of two molecules in step 700, Figure 4 (c) shows the involvement of the molecule provide hydrolysis reaction. The analysis results in Figure 4 (d), (e) and (f) show an increasing in the energy after 350 step and then declined again in 700 step. The same tendency is shown from the electrostatic analysis (Figure 5)

Figure 5 shows that the electrostatic energy increased substantially in the interaction after homo sapiens PLA2 (3elo) and Bovinus (1bp2) with the substrate. Inverse effect took place in the PLA porcinus (1y6o). The differences in these conditions due to the nature of 3elo and 1bp2 hydrophilicity compared with fairly hydrophobic nature of 1y6o [18]. Figure (6) is given to show detail picture of the dynamic behavior of PLA2, substrate, water and ions in the reaction site via the plot results of the electrostatic energy of the enzyme PLA2 of Bovinus (1bp2) in Figure 6 (a), (b) and (c); PLA2 of porcinus (1y6o) in Figure 6 (d), (e) and (f).

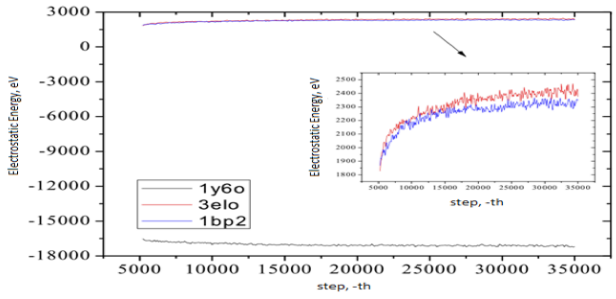

Figure 5. Plot Results of Electrostatic Energy due to the interactions between substrate molecules and amino acid groups at the reaction site PLA2. Simulations performed a total of 700 steps or pose interaction.

It can be seen in Figure 6 that there are differences in the situation and interaction in reaction site of three sources enzyme. The presentation of water molecules on porcinus PLA2 reaction site was not visible in all 700 step simulations (Figure 6 (d), (e) and (f)). This suggests that the hydrophobic enzyme sites which blocked water molecules from approaching. This condition can be understood because the structure still contains its substrate 1y6o.pdb naturally so that the substrates can be detected in the determination of its structure by XRD.

The similar situation was found out in Bovinus PLA2, which water molecules can only be approached about 700 step of the simulation. Therefore, the substrate molecule also cannot found in the structure 1bp2.pdb. All simulations were performed on the these PLA2 structures which are derived from different sources were equally showed the flip-flop mechanism which is the basic mechanism of fatty acid hydrolysis by PLA2

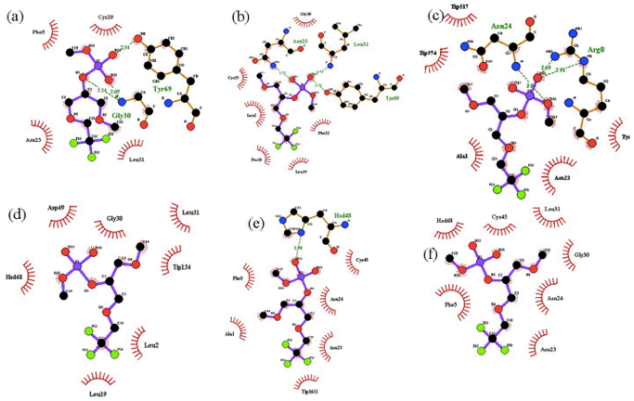

Figure 6. Interaction Length Analysis of the Interaction between Substrate molecules and Amino Acids at the Reaction Site PLA2. Simulations Performed in 700 Steps or Pose Interaction. (a) at step 0 (b) in step 350 (c) in step 700 of PLA2 Bovinus. Figure (d), (e) and (f) for PLA2 porcinus in the same steps.

CONCLUSION

Analysis of the interaction length, partial atomic charge and electrostatic energy can be used to predict and explain the interaction PLA2 and its substrate in the third source (structure) PLA2. The basic feature detected in the simulation is the mechanism of

enzyme-substrate flip flop. Interaction length analysis results indicated that the presence of water molecules on the structures of 1bp2 and 3elo at the time of the simulation was done. The electrostatic energy increased substantially in the interaction after homo sapiens PLA2 (3elo) and Bovinus (1bp2) with the substrate. Inverse effect took place in the PLA porcinus (1y6o).

REFERENCES

1. Hasselbalch HC. Chronic inflammation as a promotor of mutagenesis in essential thrombocythemia, polycythemia vera and myelofibrosis. A human inflammation model for cancer development? Leuk Res. 2013 Feb;37(2):214–20.
2. Pawelec G, Goldeck D, Derhovanessian E. Inflammation, ageing and chronic disease. Curr Opin Immunol. 2014 Aug;29:23–8.
3. Butler J, Jayson GG, Swallow AJ. The reaction between the superoxide anion radical and cytochrome c. Biochim Biophys Acta. 1975 Dec 11;408(3):215–22.
4. Tang PCY, Qin L, Zielonka J, Zhou J, Matte-Martone C, Bergaya S, et al. MyD88-dependent, superoxide-initiated inflammation is necessary for flow-mediated inward remodeling of conduit arteries. J Exp Med. 2008 Dec 22;205(13):3159–71.
5. Hyvönen MT, Öörni K, Kovanen PT, Ala-Korpela M. Changes in a Phospholipid Bilayer Induced by the Hydrolysis of a Phospholipase A2 Enzyme: A Molecular Dynamics Simulation Study. Biophys J. 2001 Feb;80(2):565–78.
6. Rowlinson SW, Crews BC, Lanzo CA, Marnett LJ. The Binding of Arachidonic Acid in the Cyclooxygenase Active Site of Mouse Prostaglandin Endoperoxide Synthase-2 (COX-2): A Putative L-Shaped Binding Conformation Utilizing The Top Channel Region. J Biol Chem. 1999 Aug 13;274(33):23305–10.
7. Aoki J. Mechanisms of lysophosphatidic acid production. Semin Cell Dev Biol. 2004 Oct;15(5):477–89.
8. Linderoth L, Andresen TL, Jørgensen K, Madsen R, Peters GH. Molecular Basis of Phospholipase A2 Activity toward Phospholipids with sn-1 Substitutions. Biophys J. 2008 Jan;94(1):14–26.
9. Dos Santos HF, Paschoal D, Burda JV. Exploring the potential energy surface for interaction of a trichloro(diethylenetriamine)gold(III) complex with strong nucleophiles. Chem Phys Lett. 2012 Oct;548:64–70.
10. Mead FC, Williams AJ. Electrostatic Mechanisms Underlie Neomycin Block of the Cardiac Ryanodine Receptor Channel (RyR2). Biophys J. 2004 Dec;87(6):3814–25.
11. Deponte M. Glutathione catalysis and the reaction mechanisms of glutathione-dependent enzymes. Biochim Biophys Acta BBA - Gen Subj. 2013 May;1830(5):3217–66.
12. King JJ, Manuel CA, Barrett CV, Raber S, Lucas H, Sutter P, et al. Catalytic Pocket Inaccessibility of Activation-Induced Cytidine Deaminase Is a Safeguard against Excessive Mutagenic Activity. Structure. 2015 Apr;23(4):615–27.
13. Köhler GA, Brenot A, Haas-Stapleton E, Agabian N, Deva R, Nigam S. Phospholipase A2 and Phospholipase B activities in fungi. Biochim Biophys Acta BBA - Mol Cell Biol Lipids. 2006 Nov;1761(11):1391–9.
14. Borrelli G, Trono D. Recombinant Lipases and Phospholipases and Their Use as Biocatalysts for Industrial Applications. Int J Mol Sci. 2015 Sep 1;16(9):20774–840.
15. Pan YH, Epstein TM, Jain MK, Bahnson BJ. Five Coplanar Anion Binding Sites on One Face of Phospholipase A $_2$: Relationship to Interface Binding †,‡. Biochemistry (Mosc). 2001 Jan;40(3):609–17.
16. G. R, Simoes-Silva R, M. A, S. G, A. A, S. Caldeira CA, et al. Purification of Phospholipases A2 from American Snake Venoms. In: Calderon L, editor. Chromatography - The Most Versatile Method of Chemical Analysis [Internet]. InTech; 2012 [cited 2016 Jun 11]. Available from: http://www.intechopen.com/books/chromatography-the-most-versatile-method-of-chemical-analysis/purification-of-

phospholipases-a2-from-american-snake-venoms
17. Janssen MJW, van de Wiel WAEC, Beiboer SHW, van Kampen MD, Verheij HM, Slotboom AJ, et al. Catalytic role of the active site histidine of porcine pancreatic phospholipase A2 probed by the variants H48Q, H48N and H48K. Protein Eng Des Sel. 1999 Jun 1;12(6):497–503.
18. Nagel ZD, Meadows CW, Dong M, Bahnson BJ, Klinman JP. Active Site Hydrophobic Residues Impact Hydrogen Tunneling Differently in a Thermophilic Alcohol Dehydrogenase at Optimal versus Nonoptimal Temperatures. Biochemistry (Mosc). 2012 May 22;51(20):4147–56.