Conformation Analysis of T1 Lipase on Alcohols Solvent using Molecular Dynamics Simulation

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Abstract. Biodiesel usually is produced commercially via a transesterification reaction of vegetable oil with alcohol and alkali catalyst. The alkali catalyst has some drawbacks, such as the soap formation during the reaction. T1 Lipase enzyme had been known as a thermostable biocatalyst which is able to produce biodiesel through a clearer process. In this paper the performance of T1 lipase enzyme as catalyst for transesterification reaction in pure ethanol, methanol, and water solvents were studied using a Molecular Dynamics (MD) Simulation at temperature of 300 K for 10 nanoseconds. The results have shown that in general the conformation of T1 lipase enzyme in methanol is more dynamics as shown by the value of root mean square deviation (RMSD), root mean squared fluctuation (RMSF), and radius of gyration. The highest solvent accessible surface area (SASA) total was also found in methanol due to the contribution of non-polar amino acid in the interior of the protein. Analysis of MD simulation has also revealed that the enzyme structure tend to be more rigid in ethanol environment. The analysis of electrostatic interactions have shown that Glu359-Arg270 salt-bridge pair might hold the key of thermostability of T1 lipase enzyme as shown by its strong and stable binding in all three solvents.

Keywords: T1 Lipase enzyme, molecular dynamics simulation, alcohol solvents, biodiesel

1. Introduction
One type of renewable energy sources is biodiesel. Biodiesel is basically a diesel engine fuel made from natural products (vegetable oil) and consists of alkyl esters of fatty acids. Production of biodiesel can be obtained through many routes of reaction, for example by using alkali (alkali metal) catalyst. Biodiesel that produced via transesterification of vegetable oils with methanol and alkali catalyst has some drawbacks, such as saponification which contaminates the end products [1]. One alternative to avoid this problem is by using other type of catalyst i.e lipase enzyme, which can be used as a biocatalyst to accelerate the transesterification reactions through a clean and thermostable reaction.
A good biocatalyst has to maintain its structure and should not dissolve in a solvent while directing a specific reaction to produce the desired product. Lipase enzymes become popular nowadays due to its ability to maintain its structure, even at relatively high (industrial) temperature, so the separation process between the biodiesel and side products become easier [2,3]. However, the use of lipase as biocatalyst leaves one problem, alcohol such as methanol might destabilize lipase enzyme quickly and halt the biodiesel production process. Therefore, the study of lipase and alcohol solvent interaction is very important to optimize and speed up the biodiesel production process.

One of the thermostable lipases that already have a 3D structure is T1 lipase with PDB code 2dsn.PDB. T1 lipase is a thermostable enzyme and was isolated from Palm Oil Mill Effluent (POME) in Malaysia [4,5]. Besides resistance to high-temperature, T1 lipase also has resistance to alkalies solvent. This is due to its environment contains many alkalies. T1 lipase comprises of two independently chains, A and B. Each of them consists of 388 amino acid with missing the first residue. T1 lipase belonging of lipase that has two lids that cover active site [4]. According to Rahman et al [6], the lids are alpha-helix 6 and 7, but according to Wang et al [7], the lids are alpha-helix 6 and 9. The activity of lipase depends on the flexibility of lids covering the active site.

Researchers had previously conducted a study to see the opening mechanism of lid that covering active site [7] and examined the T1 lipase flexibility in the variation of temperature [8]. This paper is aimed to analyze the stability of the T1 lipase enzyme [5] in some alcohol solvents (ethanol and methanol) and water by observing the change in their geometrical and energetical parameters by using a Molecular dynamics simulations of T1 lipase enzyme in various solvents at temperature of 300 K and 10 ns simulation.

2. Material and Methods

2.1. Material

The materials used in this study is the crystallography data from Geobacillus Zalihae lipase (PDB code 2DSN) known as T1 lipase [4], which can be downloaded from the Protein Data Bank website (http://www.rscb.org/pdb). The equipment used in this study consist of hardware with the specification of a computer with 8 GB RAM, Quad Core Processor (Intel Corei7), NVIDIA Card GTX-760, Intel Corei7 and LINUX operating system Ubuntu version 14.04. The software used for the simulation process is AMBER 12 [9] and VMD (Visual Molecular Dynamics Program) version 1.9.2 [10] for visualization and analysis of the simulation result. The moving average technique for data smoothing were done using VBA program. For plotting the results, MS Excel 2010 is used to create the graphs.

2.2. Methods

T1 lipase was then solvated in water, ethanol, and methanol box using Packmol program were used for solvent preparation. 14658, 4542, and 6514 particle of water, ethanol and methanol respectively was used to build 10 Å boxes. Amber force field ff12SB was used parameterize amino acid. TIP3P was used to modelling the water [11] and the parameter for alcohol solvent, ethanol and methanol were obtained from R.E.D Server. The periodic boundary condition (PBC) was also used to eliminate the effect of surface tension and to achieve a condition with uniform density and pressure [12]. The SHAKE algorithm with a tolerance of $10^{-5}$ is applied to the system to curb the entire bond containing hydrogen atoms [13]. The electrostatic energy systems were calculated using the particle mesh Ewald (PME) [14] whereas the van der Waals interaction was calculated using the Lennard-Jones potential with a cutoff distance of 12 Å [15]. To control the stages of minimization, heating and the production run the NVT ensemble (constant number, volume and temperature) was used to the desired temperature where the system is coupled to the thermostat velocity rescaling the coupling constants of 0.1 [16]. To control the equilibration stage maintained at NPT Ensemble (constant number, pressure and temperature) and using langevin dynamics for temperature regulation [17].
Molecules that have solvated then minimized to avoid the van der Waals contacts that do not match (bad contact) and to minimize the steric effects of high energy. Each simulation starts with minimization process which is aimed to put the protein in its lowest energy (native conformation). In the heating process, protein was heated gradually until it reach the intended simulation temperature, 300K. To secure the stable conformation of protein, the equilibration processes were done using Langevin protocol. The final process is the production run at 300 K for 10 ns in three different solvents environment, where protein free to move without any constraint. The molecular dynamics results then analyzed and discussed.

3. Results and Discussions

3.1. Global Descriptor Analysis

The stability of a protein can be analyzed by a number of parameters such as, root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (Rg), solvent accessible surface area (SASA), and Secondary Structure. Figure 1 shows the fluctuations of RMSD, and RMSF value at 300 K and for 10 ns simulation in water, ethanol, and methanol environment.

RMSD is the average deviation between the protein conformation at a particular time and the reference structure. A seen in figure 1(a), at 5 ns first movement of RMSD in the water is relatively less than in Ethanol and Methanol. But after that, the movement of RMSD molecule in ethanol is relatively as the same as RMSD in the water. The RMSD molecule in methanol up to 10 ns which keep moving is higher than in ethanol and water as shown in figure 1(a). Dynamics of RMSD in methanol solvent which is higher than in the water and ethanol, allegedly is because of relative methanol polarity (0.762) is closer to water polarity (1) than ethanol polarity (0.654). The relative methanol polarity allegedly has hydrophilic and hydrophobic residues to keep it moving. The high movement in ethanol solvent at 5 ns allegedly is the movement of polar residues on the suppressed surface encountering non-polar solvent.

High peaks of amino acid residues show on RMSF graph showed the residue flexibility. The amino acid residue in water and methanol showed higher flexibility than in ethanol (Fig. 1b). Residue of Thr74, Thr93, Gly104, Ser201, Arg230, and Ser333 is polar residue, which located on the surface and has high flexibility when it was in the water than in methanol and ethanol. However, there were two hydrophobic residues Pro217 and Val294 which have higher flexibility in water than in methanol and ethanol. Although Pros217 is a hydrophobic residue but the position is squeezed by surrounding 3 polar residues, so the RMSF value became higher in water than in methanol and ethanol. The hydrophobic residue Val294 is on helix 9th which adjacent to active center lid and the position Val294 is shoved by Arg179 residue which polar residue. The RMSF result also showed that the fluctuation of amino acid atomic in ethanol solvent is generally more rigid than in water and methanol (figure 1b).

![Figure 1. The pattern of RMSD (a) and RMSF (b) at 300 K of T1 lipase molecule in water (blue line), methanol (green line) and ethanol solvents (red line).]
Initially at simulation of 4 ns, SASA total molecule T1 lipase in methanol is higher than in water and ethanol. After 4 ns until end of simulation, SASA total in methanol and water is relatively balanced showed in figure 2(a). Different result of SASA total showed in figure 2(a) when the molecule was in ethanol solvents. The profile tends to decline and less than SASA total in water and methanol as seen in figure 2(a). The highest average value of SASA total is when the molecule in methanol, 16600 Å², followed by SASA Total in water, 16343 Å², and the last one in ethanol 16185 Å². The high value of SASA total when solvated in methanol is contributed by SASA non-polar which also the highest of the three solvents as seen in figure 2(a). The average value of SASA non-polar is respectively 5264, 5112 and 4729 Å² in methanol, ethanol and water. Calculation of SASA polar for the T1 lipase when in methanol obtained middle value among other solvents that is equal to 11336 Å², while SASA polar in water is 11614 Å² and in ethanol is 11074 Å², showed in figure 2(c). The result of SASA analysis agree with the RMSD data as shown in figure 1(a), which the higher dynamic of T1 lipase in methanol is due to the non-polar residue movement and the quiet enough of polar residue movement. The decreasing of RMSD T1 lipase when in ethanol, showed in figure 1(a), allegedly is that of the SASA polar get shrink and the SASA non polar didn’t changed much after 4 ns.

The radius of gyration of protein which shows the compactness of the protein structure did not show any significant difference in the three solvents. T1 lipase just a little bit experience shrinkage when in ethanol than in water or methanol. This shrinkage show that this structure more rigid compared to structure in water or ethanol. The Radius of gyration not so different when immersed in methanol and water as seen in figure 2(d).

Figure 2. The fluctuation of Solvent Accessible Surface Area (SASA) during the simulation at 300 K for 10 ns (a) SASA total, (b) SASA non-polar, (c) SASA polar, & (d) radius of gyration. Blue, red and green line indicated the molecule immersed in water, ethanol and methanol respectively.
3.2. *Salt bridge Analysis*

Electrostatic interactions are often referred to as ion-pair interactions or a salt-bridge is an interaction between charged amino acid residues. The importance of electrostatic interactions in protein structure were first reported by [18] which stated that this interaction contributes significantly in stabilizing the protein.

The results of our simulation have indicated the occurrence of 14 salt-bridge pairs that appeared in all solvents. There are also 3 salt-bridge that appeared only in water, 2 salt-bridge that appeared in ethanol and 2 salt-bridge that appeared in methanol solvents. Of 14 salt-bridge that appeared in all solvents, four salt-bridge that might play an important role in the rigid conformation of T1 lipase molecule when solvated in ethanol and methanol are Glu132-Lys84, Glu149-Arg134, Asp205-Arg92 and Glu250-Arg330.

The Glu132-Lys84 salt-bridge pair as seen in figure 3(a) appeared to be stable in ethanol and methanol environment. This salt-bridge pair slightly away at 2 to 4 ns in ethanol solvents but showed come up again until the end of simulation. This is indicates that this salt bridge is strong and responsible to the stability of T1 lipase enzyme in ethanol and methanol. Glu149-Arg134 salt bridge pair have intermittent relation since the beginning of simulation until the end of simulation in water environment, showed in figure 3(b). This salt bridge also have a bond distance is far in ethanol but very close enough after 6 ns, as shown in figure 3(b). When this molecule immersed in methanol, this salt bridge pair has a moderate energy around -40 kJ/mol since the beginning of simulation until 8ns. After that this salt bridge has similar profile with salt bridge pair when immersed in ethanol, as shown in figure 3(b). This is indicates that this salt bridge strong enough to make the molecule structure stable in methanol and even more rigid in ethanol.

![Figure 3](image)

*Figure 3.* Electrostatic interaction of Glu132-Lys84 salt bridge pair (a), Glu149-Arg134 salt bridge pair (b), Asp205-Arg92 salt bridge pair (c) and Glu250-Arg330 salt bridge pair (d) Blue, red and green line indicated the molecule solvated in water, ethanol and methanol respectively.
The Asp205-Arg92 salt bridge pair have a strong energy binding in ethanol and methanol solvents and also show weak energy binding after breaking at 1.6 ns in water solvent (Figure 3c). This is indicates that this salt bridge is very strong to make the structure more rigid in ethanol and methanol. The Glu250-Arg330 salt bridge pair also showed intermittent distance in water environment (Figure 3d). From 0-1 ns the pair was away then closed for next 2 ns and then far again until the end of simulation. This salt bridge has electrostatic energy around -30 kJ/mol as shown in figure 3(d). Different situation was happened when this salt bridge appear in ethanol and methanol. In this situation, this salt bridge has energy around -70 kJ/mol. In ethanol, this Glu250-Arg330 pair was experienced a bit far distance at 3-4 ns, also in methanol this salt bridge has a bit far distance at 6.8 to 8 ns as seen in figure 3(d). This salt-bridge is weak in the water, but quite strong in ethanol and methanol environment.

3.3. Secondary Analysis

![Secondary structure evolution during the simulation at 300 K for 10 ns in (a) water, (b) ethanol (c) methanol; α-helix (Purple), β-sheet (Yellow), Turn (Blue) and Coil (Black); α-helix (Purple), β-sheet (Yellow) 310-helix (Blue), Turn (Green) and Coil (White)](image)

Figure 4. Secondary structure evolution during the simulation at 300 K for 10 ns in (a) water, (b) ethanol (c) methanol; α-helix (Purple), β-sheet (Yellow), Turn (Blue) and Coil (Black); α-helix (Purple), β-sheet (Yellow) 310-helix (Blue), Turn (Green) and Coil (White)

Analysis of secondary structures shows that during the simulation, α-helix in ethanol environment only suffered a relatively very small damage as compared to other solvents as seen in figure 4(b). The β-sheet structure relatively stable and constant in all three solvents, showed in Figure 4. At this point, it is too early to state the type of unfolding mechanism in this simulation since we need a longer simulation time (probably in the range of millisecond), but initial indication tend to support the zipper model which start with α-helix destruction [19].

The structure of lipase T1 after 10 ns simulation solvated in ethanol and methanol show a bit conformation change compared to structure in water. The changes occur on polar residue in surface area as expected before, as seen in figure 5.
Figure 5. Conformation of lipase T1 enzyme at 300K after 10 ns solvated in ethanol (a), in water (b), and in methanol (c). Red and Blue indicated negative and positive charge residues respectively, green indicated polar neutral residues and grey indicated as hydrophobic residues.

4. Conclusion
The molecular dynamic simulations have indicated that T1 lipase enzyme is able to maintain its structure integrity in various solvents i.e 100% pure ethanol, methanol, and water. This result is important since the previous assumption stated that T1 lipase enzyme would be dissolved easily in pure alcohols environment. In alcohol environment, our results indicates that the enzyme is more dynamics in methanol, whereas more rigid in ethanol environment. When the conformation is too rigid, the functionality of the enzyme will be reduced, so it is important to let the T1 lipase enzyme structure to have dynamics. This result also suggest that the usage of methanol solvent in biodiesel reaction will be expected well than ethanol solvent. Varying the composition of mixed water and alcohol will be our next tasks.

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