In Silico Modification Of Thermostable Lipase from *Geobacillus* sp. Strain T1

Mohd. Basyaruddin Abd. Rahman[1], Chang Kok Khan[1], Mahiran Basri[1],
Abu Bakar Salleh[2], Raja Noor Zaliha Raja Abdul Rahman[2]

[1]*Department of Chemistry, Faculty of Science, Universiti Putra Malaysia, 43400 Serdang, Malaysia;*
[2]*Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 Serdang, Malaysia.*

ABSTRACT

Structural modification through molecular modeling on T1 lipase was analyzed in details to gain insight for their role and biological function in certain reactions. A total of 65 pocket cavities were found in T1 structure by pvSOAR. 1KU0 and 1JI3 structures from PDB were found to have highest similarities with T1 lipase. Nine largest pockets were selected as potential binding sites for ligand docking. Ten chemical ligands were screened from PDB for automated docking in T1 lipase. Ligands PHE and PDO posted their lowest final docked energies, -6.71 kcal/mol in pocket 62 and -4.69 kcal/mol in pocket 60, respectively. Ligand BEN posted -4.04 kcal/mol, its lowest final docked energy at pocket 60. Hydrogen bonds and hydrophobic interactions played a major role to distinguish the differences of final docked energy of ligands in different pockets.

Keywords: thermostable, lipase, modified protein, molecular docking, *Geobacillus*

INTRODUCTION

Lipase (triacylglycerol acylhydrolase, EC 3.1.1.3), acts on an ester-water interface, can be used to catalyze the hydrolysis of long chain triglycerides with the formation of triglycerides and free fatty acid chains, as well as the reverse reaction of the synthesis of esters formed from fatty acids and glycerols [1]. Advanced research for biocatalyst showed that only microbial thermostable lipases are commercially significant for their potential use in industries, such as specialty organic syntheses [2-4], hydrolysis of fats and oils, modification of fats, flavor enhancement in food processing [3], resolution of racemic mixtures, and chemical analyses [5-7]. In recent years, thermostable lipases from extreme thermophiles have led to a special focus due to their inherent thermostability and resistance against chemical denaturants, such as extremely acidic and alkaline conditions, detergents, chaotropic reagents, organic solvent and protein inhibitors [8].

Thermostable lipases are potentially exploitable for modification and to enhance the properties of lipase. Enhancement of an enzyme could be solved by *in silico* modification through molecular modeling. Molecular modeling has been used to predict the capability of the enzyme to distinguish between the enantiomers of a series of substances. It was applied in redesigning enzyme such as *Candida rugosa* by site-directed mutagenesis in order to test the importance of a selected couple of amino acids selected on the basis of the computational results [9].

Docking programs are computationally very efficient, which allow the screening of large databases of compounds searching for new 'hits' able to interact with the target protein. Nonetheless, the success of *in silico* screening and generally of docking techniques greatly depends on the knowledge of fine structural details of the recognition site. This shows that docking strategies are often unable to detect the binding of a drug to a protein, whose structure has been determined bound to a different ligand. This suggests that, at least for some proteins, multiple sets of ligand-protein coordinates should be considered to account for the range of configurational space accessible in the binding site[10-11].

A highly thermostable lipase from *Geobacillus* sp. Strain T1 [12] was selected to undergo modification through molecular modeling. The coordinates of the actives sites and pocket cavities are derived from Brookhaven Protein Database Bank (PDB) and pvSOAR internet servers. Since the structure of T1 lipase is not available in PDB, we have to seek an alternative to define the pocket cavities which is available through pvSOAR. This useful tool can take a structure either uploaded by a user or obtained from the PDB, and identifies similar surface patterns based on geometrically defined pockets and voids [13]. Well-formed concave surface regions in the form of pockets and voids are examined to identify similarity relationship that might be directly related to protein function [14].

Molecular modeling programs were used to exploit the structural information of the enzyme. Docking programs such as AutoDock 3.05 [15] was used to bind or attach specific ligands or metals into the pocket cavities of enzyme. In this paper, we reported the potential ligand binding sites for putative chemical ligands and metals which are capable to manipulate the pathway for substrate to bind at active sites of the lipase.

AutoDock 3.05 is a valuable tool in the X-ray structure determination process itself; given the electron density for a ligand, AutoDock can help to narrow the conformational possibilities and help identify a good a structure.

EXPERIMENTAL

I. Determination of pocket cavities of T1 lipase in PDB file format.

The PDB format of T1 lipase was uploaded onto pvSOAR, an Internet based server to determine the total pockets in T1 lipase and enzymes in Protein Database Bank (PDB) that have similarities with T1 lipase structure. pvSOAR provided two selections, first, querying a surface pattern against a database and second, querying a whole structure against a database. The first selection compared a single surface pattern on T1 lipase which referred as pocket with the selected surface database while the second selection compared the surface patterns on an entire structure with the database.

II. Screening and selection of suitable chemical ligands from PDB.

There are thousands of chemical ligands exist in the internet directory. Ten ligands were chosen based on their functional groups such as amine, hydroxyl or both. Bidentate ligands were favoured for selection. Size of the ligand must be smaller than the potential binding site in T1 structure in order to be docked into the pockets.

III. Preparation of T1 lipase and selected chemical ligands.

Structures of T1 lipase and chemical ligands were obtained in PDB format. Then, T1lipase.pdb was protonated and solvated using *protonate* and *addsol* tools in AutoDock 3.05 to generate *T1lipase.pdbqs* which was used later as the docking platform for selected ligands. Meanwhile, the selected chemical ligands from Protein Database Bank were transferred into InsightII software to perform structure and force field correction procedures to produce *ligands.mol2* file for each selected ligand. Then, these *ligand.mol2* files were transferred to AutoDock 3.05 and *autotors* tool was used to determine the torsion for each selected ligand. After that, *ligand.pdbq* file was generated which is ready to be used to perform docking process later with *T1lipase.pdbqs*.

IV. Docking of each ligand onto potential pocket cavities in T1 lipase structure.

Each selected ligand was docked into each targeted potential pocket by using AutoDock 3.05, and then final docked energy of each successful docking was recorded for further analysis. Grid map was sized at 60 x 60 x 60 with a grid point spacing of 0.375 Å. A 100 runs were carried out for each ligand at different pockets. Each of the independent run, a maximum of 27000 Genetic Algorithm (GA) operations were generated on a single population of 50 individuals. Operator weight for crossover, mutation and elitism were default parameters (0.80, 0.02 and 1, respectively) [15]

RESULTS AND DISCUSSION

Structural information of T1 lipase

A high thermostable enzyme was extracted from *Geobacillus* sp. Strain T1 which was discovered by a group of researchers from Universiti Putra Malaysia. It was a highly thermophilic and alkaliphilic lipase that withstood denaturation at 65 °C and pH 9.0. The mature lipase was composed of 388 amino acids which correspond to a molecular mass 43.195 kDa and possessed Ca and Zn metal ions in the structure [18].Like other *Bacillus* lipase, an Ala replaces the first Gly residue in the conserved Gly-Xaa-Ser-Xaa-Gly which is conserved among microbial and mammalian lipases [16].

T1.pdb was uploaded onto pvSOAR web based server and it determined that T1 has a total of 65 pockets in the structure. Querying the surface pattern of T1 lipase against the PDB database had generated a list of macromolecule structures which has high similarities with T1 lipase. Two structures with the highest similarities were lipases from *Bacillus stearothermophilus* L1(1KU0) and *Bacillus stearothermophilus* P1(1JI3), respectively.

The 1KU0 and 1JI3 structures were used as a platform to define the surface patterns of T1 lipase because there was no information of T1 structure in PDB which subsequently lead to unavailability of the service of CASTp. Hence, we used the information from 1KU0 and 1JI3 structures to define the location of the pocket cavities in T1 lipase. Only these two structures could be used as a platform for the T1 pockets definition because both structures were almost identical with T1 lipase in term of amino acid residues, total pocket cavities and structural conformation. Besides that, 1KU0 and 1JI3 possessed Ca and Zn metal ions too like T1 lipase which were not usually found in other lipases.

Nine largest pockets from T1 lipase were selected as potential binding sites for ligands docking. Each of the pockets was studied thoroughly for information such as surface area, volume and number of residues within. Pocket 65 was the largest pocket with surface area at 296.1 $Å^2$, volume at 494.3 $Å^3$ and a total 17 amino acids formed the concaved area. Second largest pocket was pocket 64 formed by 12 amino acids followed by pocket 63 with 14 amino acids. Although number of amino acids in pocket 64 was fewer than pocket 63 but the space within pocket 64 was bigger which contributed larger volume of the pocket 64 at 335.9 $Å^3$ than pocket63

at 187.8 $Å^3$. Pocket 59 was formed by 12 amino acids which two of the amino acids, Ser113 and His358 were members of the catalytic triad.

The catalytic triad for T1 lipase is formed by Ser113, Asp317 and His358. The catalytic triad was hidden underneath a helical lid which was in closed conformation. Similar structure like 1KU0 reported that the active site residues were covered by long helix and not accessible to solvent, indicating that the L1 lipase structure is in a closed conformation [17]. When this lid was in closed conformation, the catalytic triad is protected from substrates attack which would cause interfacial activation.

Properties of selected chemical ligands for docking

There were three types of ligands selected which were amine group, hydroxyl group and combination of amine and hydroxyl groups. Ligands with amine group were selected because there is a lone pair electron which could be used for possible binding with the amino acids residue in the pocket cavities.

Same reason went for hydroxyl group but it is expected that hydroxyl group ligands might produce slightly lower final docked energy from amino group ligands because the oxygen in hydroxyl functional possessed two lone pair electrons. An extra lone pair electron might contribute stronger binding with the amino acid residues for successful docking.

The inclusion of flexibility was an important issue in docking because lock-and-key type binding between rigid bodies was not sufficient to describe all aspects of protein-ligand interactions and significant conformational changes might occur on binding [18]. The most flexible ligand was PSE which has five torsions, followed by PHE, PBZ, PAC, BEN and PDO ligands. POL, EOH and ETA ligands possess one torsion only while PHN has no torsion at all.

Final docked energies from docking program

Four of ten selected chemical ligands showed positive results from AutoDock 3.05 and the ligands are benzamidine(BEN), phenylalanine(PHE), 1,3-propandiol(PDO) and 1-propanol(POL). Among the four successful docked ligands, PHE showed the lowest final docked energy values at pockets 65, 64, 63, 62, 60 and 58 with -6.16 kcal/mol, -4.96 kcal/mol, -5.56 kcal/mol, -6.71 kcal/mol, -6.21 kcal/mol and -5.58 kcal/mol, respectively. The other ligands, BEN showed two lowest final docked energy values, -3.55 kcal/mol and -3.39 kcal/mol at pockets 59 and 57, respectively while PDO showed lowest final docked energy value, -3.81 kcal/mol at pocket 61. Although POL successfully docked into five pockets but the final docked energy generated was higher compared to other putative ligands. Ligands possessed aromatic ring in their structures showed lower final docked energy compare with those ligands without aromatic ring. Such event happened might due to presence of amine group and hydrophobic aromatic ring in ligands contributed to the successful bindings.

PHE shows lowest docking energies compared with other ligands might to its bidentate properties composed of two different functional groups, amine and hydroxyl. PHE has three lone pair electrons which could be used to form hydrogen bonding with amino acid residues of the pockets whereas for BEN has two lone pair electrons from amine group.

Hydrogen Bondings

Ligand BEN shows no hydrogen bonding in most pockets except pocket 59 and 61. Although BEN shows final docked energy in all pockets but not all docked BEN located within the respective pockets. Some bind on the surface area around the mouth of the pocket but determination of hydrogen bonding in this case only applied to ligands and residues within pocket. Therefore, there were no hydrogen bonds in most pockets for ligand BEN.

Number of hydrogen bondings of ligands and residues within pockets alone was not enough to describe the differences of final docked energy between ligands in the same pocket. For instance, ligands PDO and PHE had two hydrogen bonds each in pocket 65 but final docked energy for PHE was much lower than PDO. Also, in pocket 64, mentioned earlier for ligand PHE had one hydrogen bond only while ligand PDO had three hydrogen bonds and yet the final docked energy of PHE was still lower than PDO. Based on these examples, another factor must be brought in to satisfy and prove of differences of final docked energy values between ligands in the very same pocket. Another factor mentioned earlier was hydrophobic interaction.

Hydrophobic Interactions

The term hydrophobic interaction has been used in the context of several closely-related phenomena to hydrophobic species. According to thermodynamics, matter seeks to be a low-energy state, and bonding reduces chemical energy. Water is electrically polarized, and is able to form hydrogen bonds internally, which gives it many of its unique physical properties. But, since hydrophobes are not electrically polarized, and because they are unable to form hydrogen bonds, water repels hydrophobes, in favour of bonding with itself. As one larger area of this kind is energetically more favourable than two smaller ones, thermodynamics favour hydrophobic molecules clustering together, even though hydrophobic molecules are *not* actually attracted to another.

In the case of ligand PDO and PHE in pocket 65, both ligands docked within the pocket had 2 hydrogen bonds. Figure 1 shows the hydrophobic interactions between ligands PDO and PHE with respective residues of the pocket. There are more hydrophobic interactions between ligand PHE and pocket residues compare with

ligand PDO and its pocket residues. Therefore, the difference of number of hydrophobic interaction is an important property to distinguish the final docked energy among several ligands.

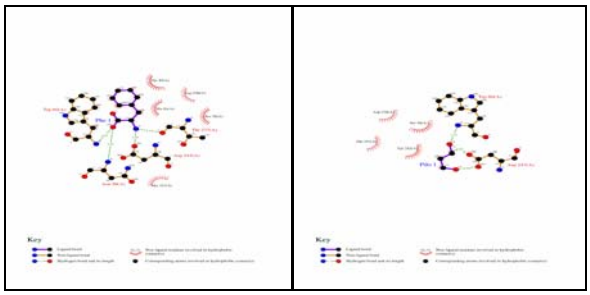

Figure 1: Hydrogen bondings and hydrophobic interactions of ligand PDO and PHE in pocket 65

CONCLUSION

Four chemical ligands selected from Protein Database Bank were successfully docked on nine selected potential binding sites in T1 lipase structure. Ligands PHE, POL and PDO were docked directly within the concave regions in those pockets by using AutoDock. This docking approach could be used to inhibit or activate the target enzyme activity depends of the research objective. Cost saving in automated docking provided an advantage over the laboratory experimental method. This approach would be a potential and handful method to design a semisynthetic enzyme for each specific synthesis in the future.

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