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Research Article

Molecular and Interactions Modelling of polyethylene terephthalate hydrolase (PETase) and Its Variant with Different Types of Crosslinker in Enzyme Immobilization

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ABSTRACT

Plastics are made from non-renewable resources and due to the tremendous production, use and indiscriminate dumping of plastics nowadays, they can lead to high levels of pollution. Biodegradation of plastic by utilizing enzymatic catalytic reaction is an environmentally friendly strategy that produce less or no negative carbon footprint. PETase from *Ideonella sakaiensis* (*Is*PETase) is an enzyme that able to degrade polyethylene terephthalate (PET), a building block of plastic. However, free enzyme has several limitations such as unstable in harsh conditions and lack of reusability. One of the strategies to overcome this drawback is through enzyme immobilization that able to improve the enzymatic properties. A suitable crosslinker is very important as it would determine the interactions of the enzymatic particles. Crosslinker should be chosen before performing the enzyme immobilization and this can be accomplished by molecular docking. Thus, the purpose of this research is to determine the suitability of glutaraldehyde, chitosan, dialdehyde starch (DAS) and ethylene glycol as the crosslinker for *Is*PETase and its variant through molecular docking analysis. Three-dimensional structure of the enzymes was built and docked with different types of crosslinkers. Binding affinity and interactions between the enzymes and the crosslinkers were analyzed and it was found that chitosan has the lowest binding affinity (-7.9 kcal/mol) and the highest number of interactions. This is followed by DAS, ethylene glycol and glutaraldehyde. By using computational analysis, suitable crosslinker for *Is*PETase could be determine and this would a cost-effective practice in enzyme immobilization strategy.

INTRODUCTION

In recent years, there are a lot of news that have been broadcasted about plastic pollution which affected living organisms especially the aquatic life. Plastics are synthetic materials which primarily developed from inorganic products such as natural gas, coal, salt, sand, crude oil and also other possible constituents (Rogers, 2015). One bacterium, *Ideonella sakaiensis* 201-F6 has been reported to able to hydrolyze polyethylene terephthalate (PET) plastic and utilizing it as a major energy and carbon source (Yoshida et al., 2016). Upon further investigation, it was found that the bacterium secreted an enzyme known as PETase which converted PET into major product which is mono(2-hydroxyethyl) terephthalic acid (MHET).

The uses of enzyme in industrial scale are worldwide as it has high reaction rates, selectivity, high product purity and green properties. Despite their useful attributes and extensive industrial applications, their usage is still hindered by the lack of long-term operating stability

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and shelf-storage. These limitations usually can be resolved by using enzyme immobilization and protein engineering (Homaei et al., 2013).

Immobilization of enzyme is a biological technology in which the enzyme is attach to a carrier or by restricting the enzyme movement (Li, 2017). This biotechnology strategy is effective in improving the enzymatic properties, activity,sand stability. To ensure high efficiency of enzyme immobilization, the enzyme should contain high composition of external lysine residues, or the technique will be less effective and problematic due to less formation of interactions between the crosslinker and enzyme (Nawawi et al., 2020). Strong intermolecular covalent bonds between amine group of lysine and aldehyde group of crosslinker is crucial in the formation of CLEAs. A study by Lucero and Kagan (2006) showed that lysine residue is the favorable amino acid to create binding between an enzyme (Lysyl Oxidase) and the crosslinker (collagen). In addition, a research by Salem *et al.* (2010) reported that the cross-linking of glutaraldehyde is considered to be an extremely non-specific mechanism affecting most lysine residues on the surface of the protein.

Glutaraldehyde which is the most commonly used crosslinker is a bifunctional reagent with capability as a protein crosslinker and an activator of supports (Barbosa et al., 2014). Other than glutaraldehyde, chitosan also has been gaining attention as a crosslinker as it offers numerous desirable characteristics which are non-toxicity, biocompatibility, and extraordinary affinity to proteins (Klein et al., 2016). Besides, dialdehyde starch (DAS) is an alternative for valuable crosslinkers. It is considered as a safe cross-linking agent due to its biodegradability and accepted level of toxicity (Skopinska-Wisniewska et al., 2016). Finally, ethylene glycol is a versatile biocompatible polymer that has many usages in biomedical. It shows high stability, low immunogenicity which does not have any effect on protein molecules even at high concentration and has been use a crosslinker (Oktay et al., 2019). Previous research also has shown that enzyme immobilization performed with a suitable crosslinker will increased the enzymatic properties in terms of stability, storage time and reusability.

Prior to testing in laboratory, the enzyme can be analyzed by using *in silico* analysis (Autodock Vina and PyMOL) to identify their molecular structure behaviour and interactions with the crosslinkers. Computer-based approaches are becoming increasingly important to predict a non-covalent binding of macromolecules or, more frequently, of a macromolecule (receptor) and a small molecule (ligand) (Trott and Olson, 2009). Autodock Vina is a software to estimate the binding affinity between protein and ligand based on the new scoring function (Jaghoori et al., 2016) whereas the main functions for PyMOL is for the enhancement of drug design and visualization (Yuan et al., 2017). Examples of computational analyses include virtual screening for modern drug discovery (Ansari et al., 2017) and molecular docking studies on angiotensin converting enzyme inhibitions and renin activities by hemp peptides (Girgih et al., 2014).

Thus, in this study a few crosslinkers (chitosan, glutaraldehyde, polyethylene glycol and DAS) was tested by using docking analysis with *Is*PETase. Other than that, a modification of lysine was also carried out by substituting a few amino acid residues at the surface of PETase into lysine residue by using PyMOL. Moreover, this mutation technique was performed to determine if there are any improvement in terms of its binding affinity compared to the wild type binding affinity using molecular docking by Autodock Vina. All docking complexes were compared by their binding affinity and interactions analyses to determine the most suitable crosslinker for *Is*PETase. From the study, it was found that chitosan is the best crosslinker for *Is*PETase and genetic engineering improved the interactions between the enzyme and crosslinker in enzyme immobilization. Computational simulation could be carried out prior to enzyme immobilization approach as it will save the time and cost spent screening for the suitable crosslinker.

MATERIALS AND METHOD

Materials

Autodock vina (http://vina.scripps.edu/), Autodock MGL tools (http://mgltools.scripps.edu/) and PyMOL (https://pymol.org/2/) were used to carried out this study. PETase structure from *I. sakaiensis* (*Is*PETase) was obtained from the Protein Data Bank (PDB). Structure of glutaraldehyde, chitosan, dialdehyde starch and polyethylene glycol were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/). The amino acids sequence of *Is*PETase was analysed by using Protparam (https://web.expasy.org/protparam/) tools to identify the percentage of amino acid in the structure.

Preparation of *Is*PETase

Preparation of *Is*PETase was conducted using Autodock Vina. All the water molecules were deleted as it can interfere with the docking analysis whereas hydrogen atoms and Kollman charges were added into the structure. Then, the file was saved in .pdbqt format.

To build PETase variant structure (v*Is*PETase), *Is*PETase structure was analysed using NetSurfP-2.0 (https://services.healthtech.dtu.dk/service.php?NetSurfP-2.0) and the value of relative solvent accessibility (RSA) obtained was compared. Serine-136, Serine-142, Asparagine-73, Serine-103 and Proline-49 were chosen for lysine mutation because those residues have the highest value of RSA. PyMOL was used to mutate those selected residues. All non-biological atoms (Sodium (Na^+) and chloride (Cl^-)) were deleted. After that, the structure was refined using 3D refine online software (http://protein.rnet.missouri.edu/i3drefine/).

2.2 Preparation of crosslinkers

The original file of the crosslinkers (glutaraldehyde, chitosan, DAS and ethylene glycol) which is in .sdf format obtained from PubChem was converted into .pdb file format using PyMOL. Then, the .pdb file was prepared using Autodock Vina and saved as .pdbqt format.

2.3 PETase-crosslinker docking simulation

The dimension of the grid box for *Is*PETase / v*Is*PETase was 70 for x-dimension, 104 for y-dimension and 76 for z-dimension. For the centre of grid box, x (-2.575), y (-3.685) and z (-6.758). Then, a config.txt file was created. The value energy (four) and exhaustiveness (eight) are set as default. A command prompt was used to run the docking analysis through Autodock Vina. The location of 'The Scripps Research Institute' containing the vina file was

inserted in the command prompt followed by the .pdbqt file of protein, ligand, config.txt, log.txt and output.pdbqt. The value of the binding energy was analysed after docking has completed.

2.4 Structural analysing

The .pdbqt file of *Is*PETase / v*Is*PETase structure was imported into the Autodock Vina software. The output file from the docking analysis was analysed using 'single molecules with multiple conformation' option. After that, *Is*PETase/ v*Is*PETase .pdbqt file was set as the macromolecules and 'show the interaction' option was selected to analyse every interaction that occurred between the crosslinker and the enzyme structure.

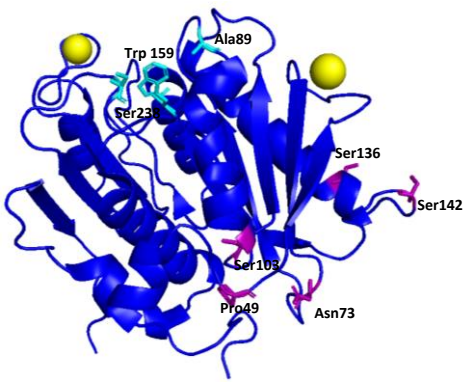
RESULTS AND DISCUSSION

Mutation of *Is*PETase

The structure of *Is*PETase (PDB ID: 6ILW) was obtained from PDB. Based on the research by Liu et al. (2019), the catalytic center of this enzyme was surrounded by hydrophilic residues (Thr88, Ser93, Thr113, Thr116, Arg,123, Gln182, Asp186, Ser214, Ser236, Ser238, Cys239 and Asn241). Amino acids analysis exhibited that serine was the highest composition in the structure at 14.1% and followed by Alanine at 11.1%. The percentage of lysine in *Is*PETase is 2.6% and according to Migneault et al. (2004), glutaraldehyde (crosslinker) is reactive towards lysine residues. Therefore, it was postulated that by mutating few residues into lysine can improve the immobilization interactions especially for glutaraldehyde with *Is*PETase.

An online software, NetSurfP-2.0 was used to analyze the Relative Solvent Accessibility (RSA) of *Is*PETase to identify which residues can be mutated into lysine. Based on the results, residues that are located the furthest from the active site and have the highest score of RSA were selected. These criteria were selected to avoid interactions between the crosslinker and catalytic residues occur at the catalytic site. Residues that were selected to be mutated were Serine-136 (62.6% RSA), Serine-142 (78.2% RSA), Serine-103 (61.0% RSA), Asparagine-73 (71.5% RSA) and Proline-49 (63.1% RSA) (**Figure 1a**). The solvent accessibility is important to measure spatial arrangement during the process of protein folding (Wu et al., 2017). It also defines the surrounding solvent environment and hydration properties which widely used to analyse protein structure.

(a)



(b)

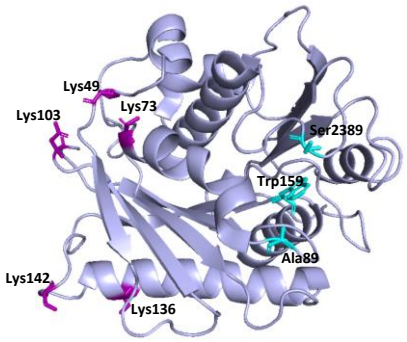


Figure 1 Image of *Is*PETase (a) wild type and (b) refined PETase variant. Active site residues are highlighted in cyan and the selected residues for lysine mutation are highlighted in purple. Yellow ball is non-biological atom (NaCl).

The structure of v*Is*PETase was built by mutating the selected amino acids by using PyMOL. Then, the v*Is*PETase structure was refined by using 3DRefinne to ensure that the built structure mimic or has the same structural agreement/composition with the solved *Is*PETase structure (**Figure 1b**). According to Heo and Feig (2018) and Shabalin et al. (2018) the refining of 3D protein models has appeared as the last breakthrough in the process of structural prediction to achieve parity with experimental precision. Refining 3D models also helps to get them closer to native structures by changing secondary structure units and repackaging side chains. All Cl and Na atom has been removed and the targeted residues for mutation has been mutated to lysine residue.

Docking Analysis of PETase and PETase variant with Different Type of Crosslinkers

Docking with Glutaraldehyde

Based on the result in **Table 1**, the binding affinity for wild type and mutated PETase interacted with glutaraldehyde at conformation 1 is the same (-2.9 kcal/mol). Even though it has the same value of binding affinity, the interactions that occurred for both were different. For the interactions of *Is*PETase, Serine-58 formed a hydrogen bond while other residues (Arginine-59, Phenylalanine-55 and Serine-142) formed hydrophobic interactions (**Figure 2a**).

Table 1 Binding affinity for *Is*PETase wild type and variant with glutaraldehyde

Conformation	Binding Affinity for *Is*PETase (kcal/mol)	Binding Affinity for v*Is*PETase (kcal/mol)
1	-2.9	-2.9
2	-2.9	-2.7
3	-2.7	-2.7
4	-2.6	-2.7
5	-2.5	-2.6
6	-2.5	-2.6
7	-2.5	-2.5
8	-2.5	-2.4
9	-2.4	-2.4

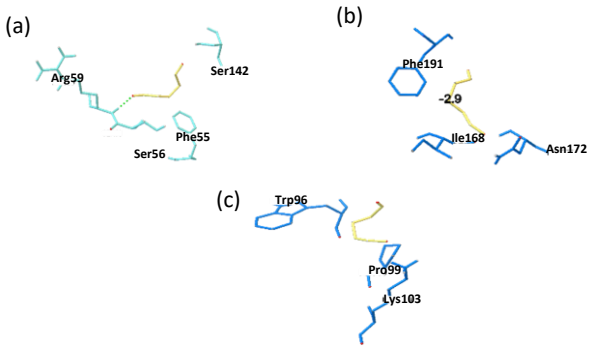


Figure 2 Interactions between (a) *Is*PETase (b) v*Is*PETase and (c) v*Is*PETase at 9th conformation with glutaraldehyde (yellow).

For v*Is*PETase interactions in **Figure 2b**, amino acid residues that interacted with glutaraldehyde were Phenylalanine-191, Isoleucine-168 and Asparagine-172. Docking analysis showed that the interaction between v*Is*PETase and glutaraldehyde only resulted in the formation of hydrophobic Only one residue that has been mutated into lysine which was Lysine-103 interacted with glutaraldehyde at 9th conformation as shown in **Figure 2c**.

Docking with Chitosan

Docking of *Is*PETase with chitosan has resulted in -7.6 kcal/mol binding affinity whilst v*Is*PETase exhibited -7.9 kcal/mol binding affinity (**Table 2**). By comparing the two structures, it was shown that by mutating the amino acids to lysine, the binding affinity towards chitosan increases.

Table 2 Binding affinity for *Is*PETase wild type and variant with chitosan

Conformation	Binding Affinity for *Is*PETase (kcal/mol)	Binding Affinity for v*Is*PETase (kcal/mol)
1	-7.6	-7.9
2	-7.6	-7.6
3	-7.4	-7.6
4	-7.4	-7.6
5	-7.4	-7.4
6	-7.4	-7.3
7	-7.4	-7.3
8	-7.3	-7.3
9	-7.3	-7.2

For PETase wild type, five amino acids (Aspartic acid- 150, Threonine-151, Alanine-152, Alanine-135 & Arginine-132) formed hydrogen bonds (**Figure 3a**). Other residues (Glycine-35, Alanine-33, Arginine-34, Threonine-77, Proline-31 and Serine-136), formed hydrophobic interactions. PETase variant formed four hydrogen bonds (Asparagine-244, Alanine-248, Lysine-95 and Threonine-88) and hydrophobic interactions (Arginine-280, Glutamine-247, Asparagine-241, Serine-242, Serine-238, Alanine-89 and Tyrosine-87) when docked with chitosan (**Figure 3b**). Among all the docking conformations, two conformations (3rd and 9th) have interactions with mutated residues as shown in **Figure 3c and d**.

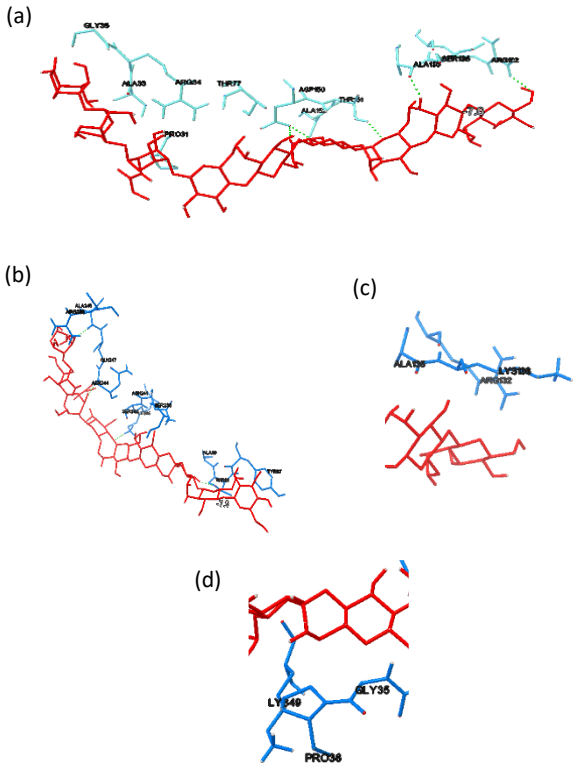


Figure 3 Interactions between (a) *Is*PETase, (b) v*Is*PETase, (c) PETase variant at 3rd conformation and (d) v*Is*PETase at 9th conformation with chitosan (red).

Docking with Dialdehyde Starch

The best binding affinity for *Is*PETase was -3.8 kcal/mol whereas for v*Is*PETase was -3.5 kcal/mol when docked with DAS (**Table 3**). It was exhibited that by mutating the amino acids to lysine, the binding affinity for docked *Is*PETase decreased. Based on **Figure 4a**, the interaction that occurred for wild type was hydrophobic interaction (Serine-168, Isoleucine-166 and Alanine-171). For v*Is*PETase, hydrophobic interaction was formed at residues Serine-160, Tryptophan-159, Histidine-237, Isoleucine-208 and Tyrosine-87 (**Figure 4b**).

Table 3 Binding affinity for *Is*PETase wild type and mutant with DAS

Conformation	Binding Affinity for *Is*PETase (kcal/mol)	Binding Affinity for v*Is*PETase (kcal/mol)
1	-3.8	-3.5
2	-3.6	-3.3
3	-3.5	-3.3
4	-3.5	-3.2
5	-3.5	-3.2
6	-3.5	-3.0
7	-3.5	-3.0
8	-3.4	-3.0
9	-3.4	-3.0

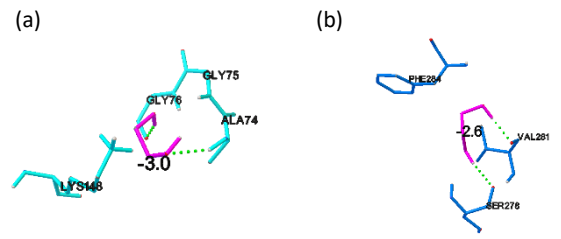


Figure 4 Interactions between (a) *Is*PETase and (b) v*Is*PETase with dialdehyde starch (orange).

Docking with Polyethylene Glycol

The binding affinity for *Is*PETase when docked with polyethylene glycol was -3.0 kcal/mol whilst binding affinity for PETase variant was -2.6 kcal/mol (**Table 4**). There were two amino acids (Glycine-76 and Alanine-74) that formed hydrogen bonds with ethylene glycol whereas Lysine-148 and Glycine-75 formed a hydrophobic interaction in *Is*PETase (**Figure 5a**). For v*Is*PETase, hydrogen bond was formed for Valine-281 and Serine-278 while Phenylalanine-284 residue formed a hydrophobic interaction with polyethylene glycol (**Figure 5b**).

Table 4 Binding affinity for *Is*PETase wild type and variant with ethylene glycol

Conformation	Binding Affinity for *Is*PETase (kcal/mol)	Binding Affinity for v*Is*PETase (kcal/mol)
1	-3.8	-3.5
2	-3.6	-3.3
3	-3.5	-3.3
4	-3.5	-3.2
5	-3.5	-3.2
6	-3.5	-3.0
7	-3.5	-3.0
8	-3.4	-3.0
9	-3.4	-3.0

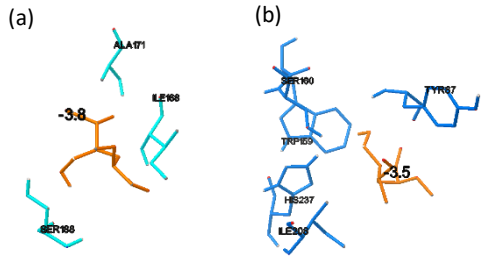


Figure 4 Interactions between (a) *Is*PETase and (b) v*Is*PETase with polyethylene glycol (purple).

Based on the results, it was determined that the 1st conformation for each crosslinker gave the best binding affinity. The score of binding affinity defines the strength of the interaction between enzyme and crosslinker (Kastritis and Bonvin, 2013). The more negative binding affinity, the stronger the interactions, therefore, it was determined that the first conformation in the docking analysis represent the strongest interactions for each crosslinker. This result is similar with Nguyen et al. (2020) that showed conformation 1 from the docking analysis exhibited the highest binding affinity. In addition, chitosan provides the greatest enzyme stabilization as it displayed the highest binding affinity compared to other crosslinker.

Hydrophobic interactions contribute to the enzyme stability whilst hydrogen bonding also facilitates enzyme stabilization, but with a smaller degree than the hydrophobic interactions. Pace et al. (2011) stated that hydrophobic binding is the key determinant of folding structure equilibrium in many native proteins. Therefore, the more hydrophobic interaction and hydrogen bonds formed, the more stable the conformations. For *Is*PETase, due to highest composition of Serine, it was shown that Serine was the most favourable amino acids with crosslinker. Although research by Migneault et al. (2004) stated that glutaraldehyde is reactive towards lysine residues, no lysine residues interaction formed at the best mode conformation.

CONCLUSION

In general, this study is to determine the interactions between *Is*PETase and a few crosslinkers through computational analysis by using molecular docking. Based on the results, the binding affinity for *Is*PETase interactions with chitosan exhibited the highest binding affinity (-7.6 kcal/mol). Similarly, v*Is*PETase docking analysis also displayed the highest binding affinity with chitosan at -7.9 kcal/mol. Lastly, substituting few residues into Lysine did not improve the binding affinity and no mutated residues was found to interact with glutaraldehyde in the first conformation of the molecular docking analysis. Further analysis should be performed to determine the preferred amino acids for each crosslinker to improve the interactions of enzyme immobilization.

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