Docking Study of β-glucosidase B (BglB) from P. Polymyxca with Cellobiose and Cellotetrose

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***Abstract*—Beta-glucosidase (3.2.1.21) plays an essential role in the removal of non-reducing terminal glucosyl residues from sacharides and glycosides. Recently, beta-glucosidase has been of interest for biomass conversion that acts in synergy with two other enzymes, endo-glucanase and exo-glucanase. However, there is not much information regarding the molecular interactions of beta-glucosidase with cellobiose. Thus, this study reports on the binding modes between beta-glucosidase from glycoside hydrolase family 1 namely BglB with cellobiose and cellotetrose via molecular docking method. Further analysis on the hydrophobic interactions revealed the key residues involved in forming hydrogen bonds (h-bond) with the substrates. The active residue were identified to be Gln22, Glu167, Glu356, Glu402 and Trp402 .These findings may provide valuable insigths in designing beta-glucosidase with higher cellulose-hydrolyzing efficiency.**

***Index Terms*—beta-glucosidase, binding site, molecular docking, cellobiose, protein ligand interaction**

I. Introduction

Cellulose is a highly unbranched polymer consisting of glucose residues linked together by β-1, 4-glycosidic bonds with cellobiose as its structural unit. The glucose chains in cellulose are very tightly packed to form insoluble crystallite which is impenetrable to any molecules including water. The structure of lignocelluloses is very complex where cellulose forms a skeleton which surrounded by hemicelluloses and lignin like matrix compose.

Cellulose is one of the most abundant polysaccharide compound in nature and is thought to be a promisingly renewable biomass resource for alternative fuels [1]. The chemical formula of cellulose is $(C_6H_{10}O_5)_n$ and the structure of one chain of the polymer is shown in fig. 1.

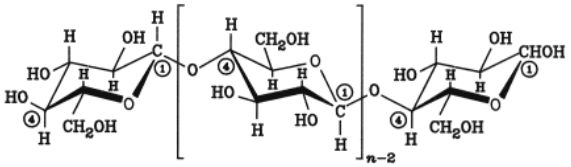

Figure 1. Chemical structure of cellulose unit

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The cellobiose unit in cellulose structure can be further degraded into glucose monomer unit through enzymatic hydrolysis which is performed by cellulases [2]. There are three enzymes in cellulase system that work in synergy to hydrolyze cellulose into glucose monomer which are endocellulase (endo-1, 4-β-glucanohydrolase, EC 3.2.1.4), exocellulase (1, 4-β-D-glucan cellobiohydrolase, EC 3.2.1.91) and β-glucosidase (β-1, 4-glucosidase, EC 3.2.1.21).

The cellulose's biodegradation process starts by endocellulase by cutting randomly at β-1, 4-glucosidic linkages in the cellulose polysaccharides chain producing various lengths of oligosaccharides with the new chain ends. This action caused the breakdown of crystalline structure of cellulase. Afterward, exocellulase cleaves the reducing and non-reducing ends of this new oligosacarides chain generating either glucose (glucanohydrolases) or cellobiose (cellobiohydrolase) as the major products. Finally, β-glucosidase ended this degradation process by hydrolyzing the remaining cellobiose or cellotetraose into glucose monomer unit [3].

Cellobiose which is an intermediate product is also a strong inhibitor for endoglucanase and exoglucanase and it becomes one of the key bottlenecks in enzymatic hydrolysis [4] . In order to prevent this inhibition process [5], cellobiose unit must be immediately removed . Thus, it is important to understand the catalytic activity of β-glucosidase in order to improve the efficiency of this enzyme. This will help in designing an enhanced β-glucosidases. However, little is known about the catalaytic interactions between β-glucosidase and cellobiose.

The program utilized for molecular docking in this study is GOLD [6]. A large number of conformations of protein ligand complexes were generated by this program. Each conformation is positioned at active site in a variety of orientation known as pose whereas poses were ranked by the scoring function to determine the best overall poses. The scoring function incorporated in GOLD uses classical molecular mechanic pose force field. GOLD employed genetic algorithm on the whole molecule and hydrogen bonding and van der Waals interactions to determine the conformation and orientation of the ligand [7]. Since little information is known about the catalaytic interactions between β-glucosidase and cellobiose, computational docking study was performed to explore the binding mode interaction of this protein and ligand.

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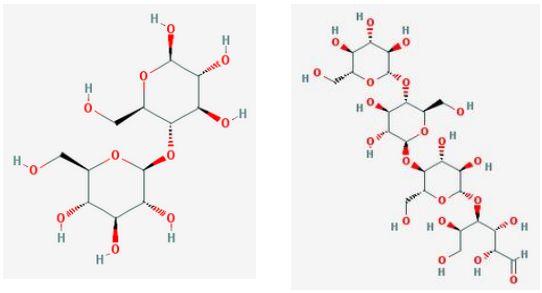
II. Material and Methodology

The x-ray crystal structures of β-glucosidase B (BglB) was obtained from the RCSB Protein Data Bank (http://www.rscb.org) with accession code 209T [8] . Waters, cofactors and originally bounded ligand in BglB was manually removed from the original PDB file. A 2-dimensional (2D) structure of cellobiose and cellotetrose were obtained from PUBCHEM (http://www.pubchem.org) with chemical identification code 10712 and 170125 respectively as shown in Fig. 2.

The three-dimensional cellobiose and cellotetrose structures were built using the Builder module in MOE version 2011.10 (Molecular Operating Environment). MOE software was installed in Macintosh machine running on Intel Core i5 processor with 2GB RAM and 500GB hard disk with Mac OSX Lion version 10.7.5 as operating system. The multi conformation structural data of the ligands was generated using LowModeMD [9] in MOE 2011.10 with the default parameters ; rejection limit = 100, RMSD gradient = 0.005, iteration limit = 10000, MM Iteration Limit, RMSD Limit = 0.25, energy windows = 7 and conformation limit = 10000. The top ten ligand conformations were saved in *.mol2* format and were used in docking simulation by GOLD.



(a) (b)

Figure 2. Chemical structure of (a) cellobiose and (b) cellotetrose

The computational docking was performed in machine running on Intel Core i7 processor with 4GB RAM and 500GB hard disk with CentOS version 5 as the operating system. The binding site was set at coordinate 66, 28, and 38 in x, y, and z respectively. The active site was defined within 10 Å and ligand-binding interactions were analyzed using Gold Score (GS) scoring function. The default settings were applied for all other parameters: population size (100); selection-pressure (1:1); number of operation (10,000); number of island (1); niche size (2) and operator weight for migrate (0), mutate (100) and crossover (100) .

From GOLD docking results, the top ten conformation pose were selected and saved in *.pdb* format for further use in protein ligand interaction analysis. The analysis of these complexes was performed using Protein Ligand Interaction Fingerprint (PLIF) and Ligand Interaction modules in MOE 2011.10.

III. Result and Discussion

A. Gold Score

In this study, cellobiose and cellotetrose were docked into β-glucosidase active site using GOLD docking program. GOLD used genetic algorithm (GA) for docking flexible ligands into protein with flexible hydroxyl groups. In most cases the protein is considered to be rigid. This may lead a good choice since the binding pocket may contain amino acids that form hydrogen bonds with the ligands.

GOLD scoring function is a molecular-like function made up with four terms: (i) Protein ligand hydrogen bond energy (external H-bond); (ii) Protein-ligand vanderwaals energy (external vdw); (iii) Ligand internal vanderwaals energy (internal vdw); (iv) Ligand intamolecular hydrogen bond energy (internal H-Bond). By default, the external vdw score is multiplied by a factor of 1.375 during the computation of total fitness score [9]. Equation 1 shows the fitness function that has been optimized for the prediction of ligand binding position.

$$GF = Shb\_ext + Svdw\_ext + Shb\_int + Svdw\_int \quad (1)$$

where GF is Goldscore Fitness, S_{hb_ext} is the protein-ligand hydrogen bond and S_{vdw_ext} is the protein-ligand van der Waals score . S_{hb_int} is the contribution to the fitness due to the intermolecular hydrogen bonds. S_{vdw_int} is the contribution due to intermolecular strain in the ligand [7].

TABLE I. List of Highest Gold Score Value

	BglB-Cellobiose	BglB-Cellotetrose
Gold Score	81.74	91.54
S (hb_ext)	23.22	29.09
S(vdw_ext)	47.60	50.28
S(hb_int)	0.00	0.00
S(int)	6.93	-6.69

The list of highest gold score for both BglB-cellobiose and BglB-cellotetrose complexes were tabulated in Table I. It is clearly shown that cellotetrose give higher score than cellobiose in respect to the longer chains structure in cellotetrose. The binding mode poses of both ligands is shown in Fig. 3.

B. Hydrogen Bonding Interaction

Molecular interaction especially hydrogen bonding plays an important role in forming enzyme-substrates complexes. Both cellobiose and cellotetrose were stablilized by BglB residues via hydrogen bond and pi-pi interactions. By utilizing ligand interaction mode in MOE 2011.10, the protein ligand interaction had been maping out in fig. 4. MOE was able to visualize solvent exposed ligand atoms as well as sidechain and backbone acceptor and donor interactions.

Seven hydrogen bond and one pi-pi interaction was observed in BglB-cellobiose complex as shown in Table II which involve residues Cys170, Glu356, Glu409, Glu167, Trp410, Gln22 and Tyr298. Whilst another eight

hydrogen bond reported in BglB-cellotetrose complex as shown in Table III, interacting with residue Glu167, Glu409, Glu225, Glu180, Gln22, Trp410 and Arg243.

Glu409 was found to form hydrogen bond for both BglB-cellobiose and BglB-cellotetrose complexes suggesting Glu409 play as essential role in pathway of enzymatic hydrolysis. This glutamate residue is highly conserved among family glycoside hydrolase 1. The glutamate side-chain can adapt its position to an axial O4 and have ability to recognize galacto-configured substrates due to its conformational freedom characteristic [10]. In other studies, Tiwari et al (2012) [11] also reported that glutamate is important in reducing the energy barrier of the glycosylation step. Thus it is ensure that glutamate plays important residue in catalytic mechanism of cellobiose hydrolysis.

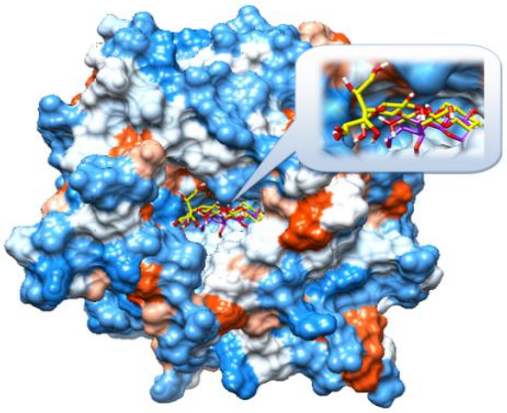
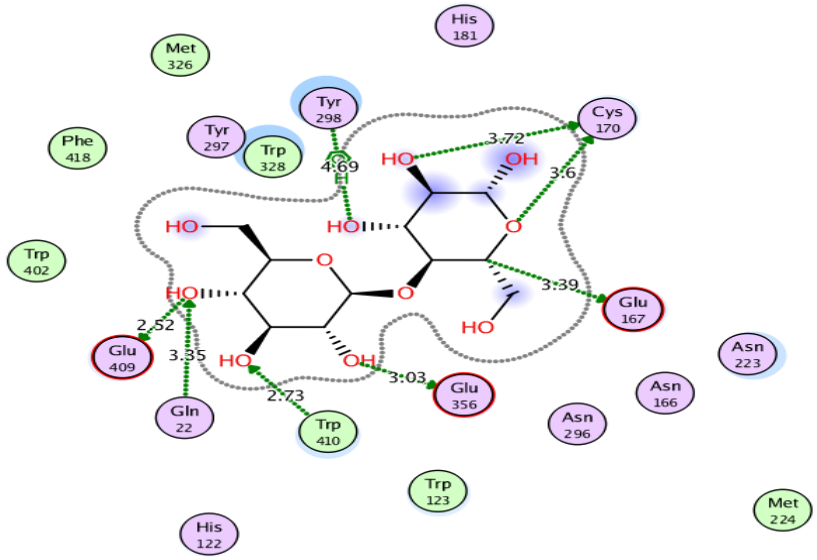
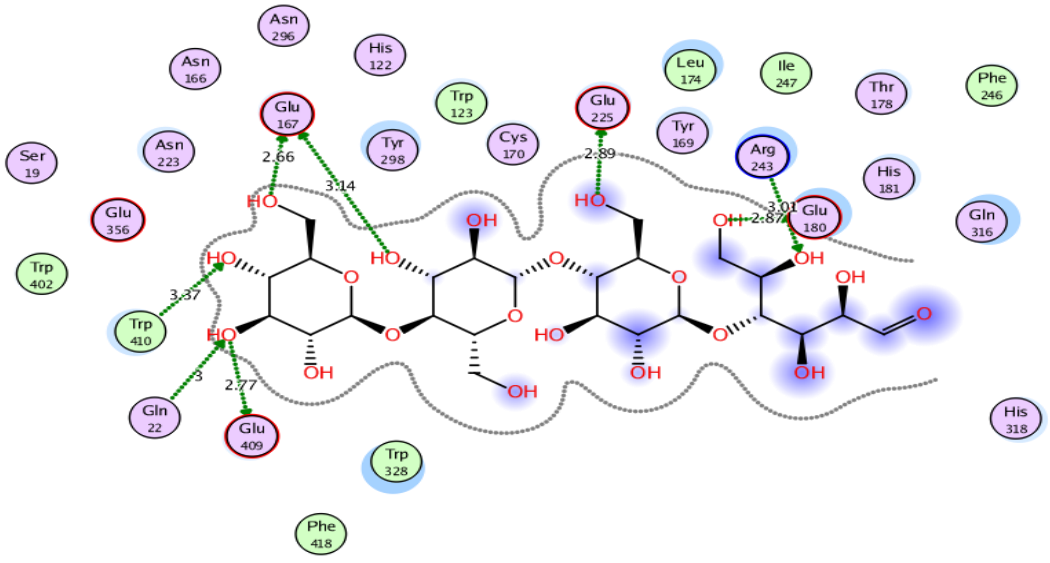


Figure 3. BglB docked conformation poses with cellobiose (purple) and cellotetrose (yellow) view in Chimera using hydrophobicity surface mode.



(a)



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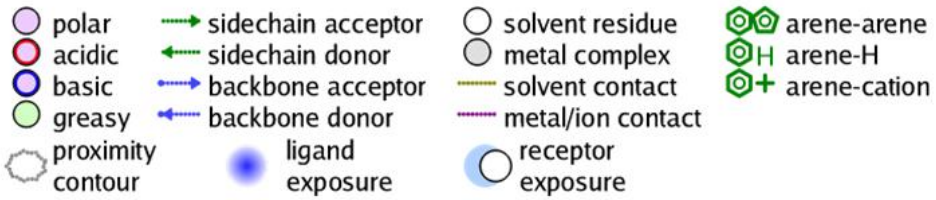


Figure 4. The 2D depiction rendered from MOE 2011.10 shown the ligand interaction of docking complexes conformation (a) BglB-cellobiose and (b) BglB-cellotetrose within the binding site of BglB (PDB ID: 2O9T) .

TABLE II. LIST OF INTERACTION FOR BGLB-CELLOBIOSE

Ligand Atom	Receptor Atom	Interaction	Distance (Å)	Energy (kcal/mol)
O3	SG CYS170	H-Donor	3.6	-1.1
O5	OE2 GLU356	H-Donor	3.03	-2.9
O7	OE2 GLU409	H-Donor	2.52	-2.6
O8	SG CYS170	H-Donor	3.72	-1.0
C15	OE2 GLU167	H-Donor	3.39	-0.7
O6	NE1 TRP410	H-Acceptor	2.73	-1.2
O7	NE2 GLN22	H-Acceptor	3.35	-0.3
O4	6-Ring TYR298	H-phi	4.69	-0.7

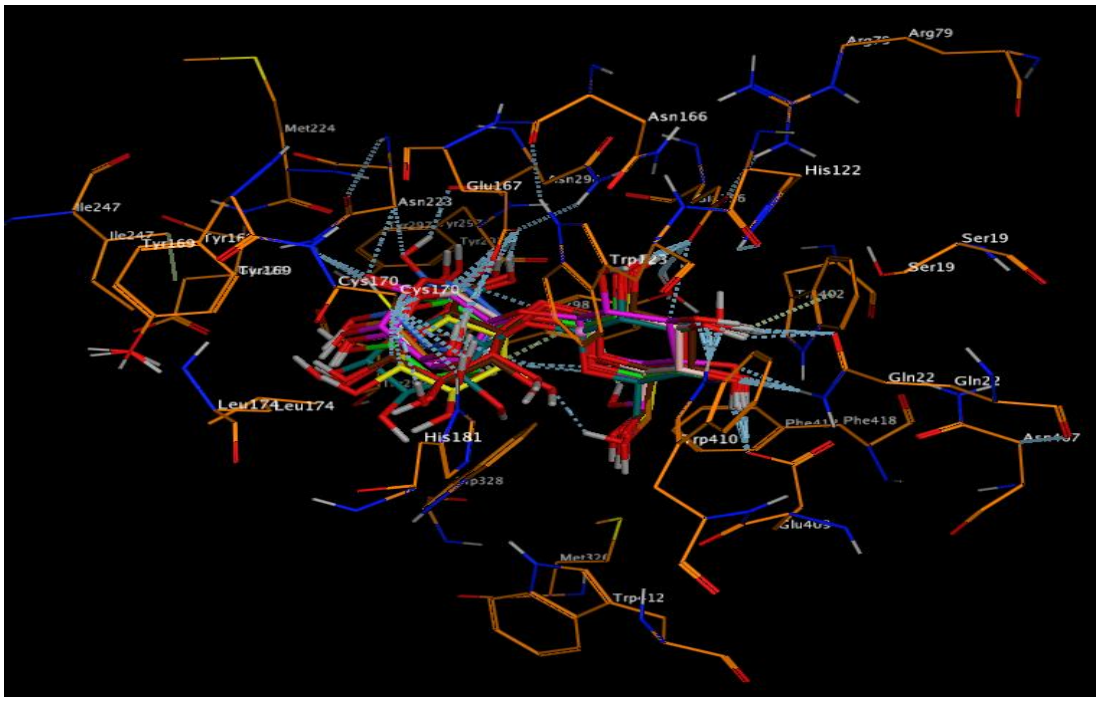
C. Protein Ligand Interaction Fingerprint

The Protein Ligand Interaction Fingerprints (PLIF) application is a method for summarizing the interacting residues between protein and ligands. The top ten ranked conformation complexes from each ligands (refer Fig. 5) were used as input in PLIF program to determine common active residue between these complexes.

From Fig. 6 (a), the common active residue in BglB-cellobiose complexes with score 90% and above were reported to be Gln22, Glu167, Tyr298, Glu356, Trp402 and Trp410. Whilst from fig. 6 (b), the common active residue for BglB-cellotetrose were reported to be Gln22, Glu167, Glu180, Arg243, Glu356, Glu402, Glu409 and Trp410.

TABLE III. LIST OF INTERACTION FOR BGLB-CELLOTETROSE

Ligand Atom	Receptor Atom	Interaction	Distance (Å)	Energy (kcal/mol)
O7	OE1 GLU167	H-Donor	3.14	-2.5
O17	OE2 GLU409	H-Donor	2.77	-4.4
O23	OE1 GLU225	H-Donor	2.89	-3.5
O25	OE2 GLU167	H-Donor	2.66	-0.7
O32	OE1 GLU180	H-Donor	2.87	-3.5
O17	NE2 GLN22	H-Acceptor	3.00	-0.9
O19	NE1 TRP410	H-Acceptor	3.37	-0.7
O29	NH1 ARG243	H-Acceptor	3.01	-3.4



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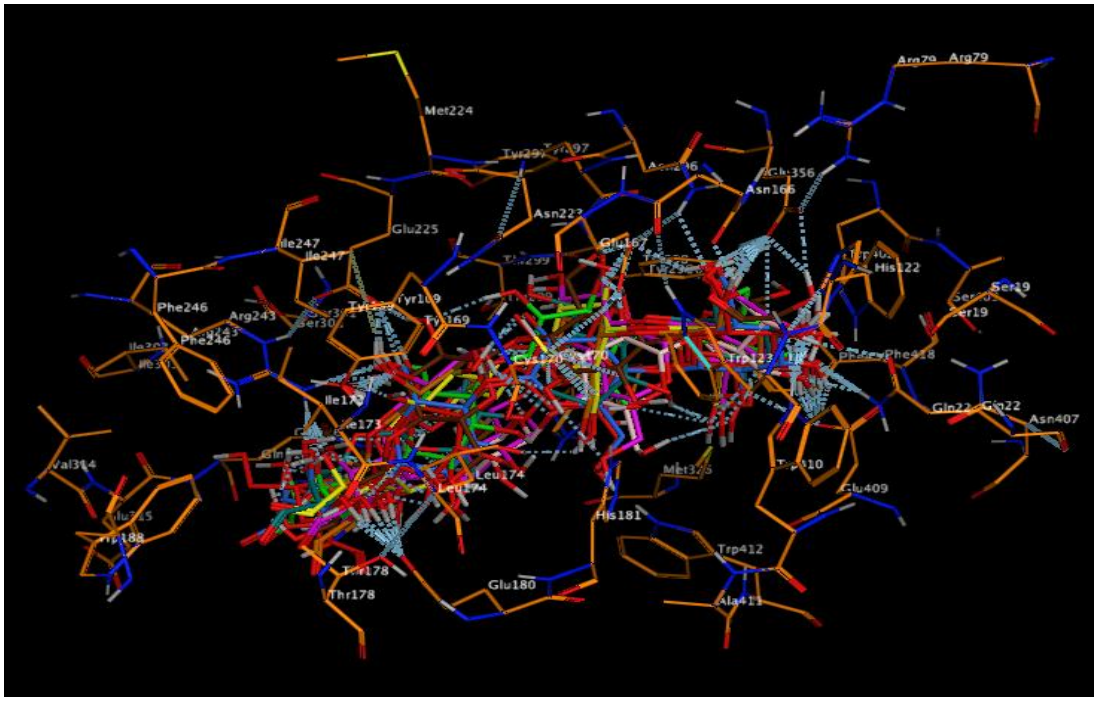
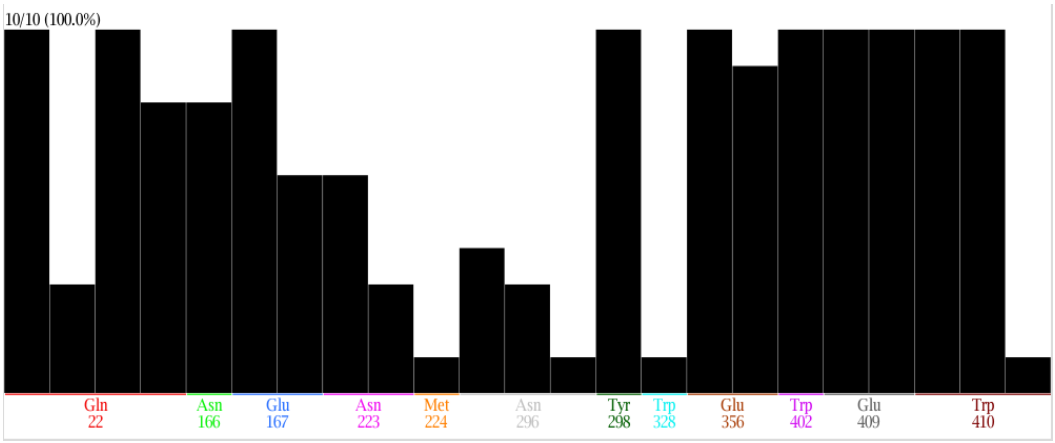
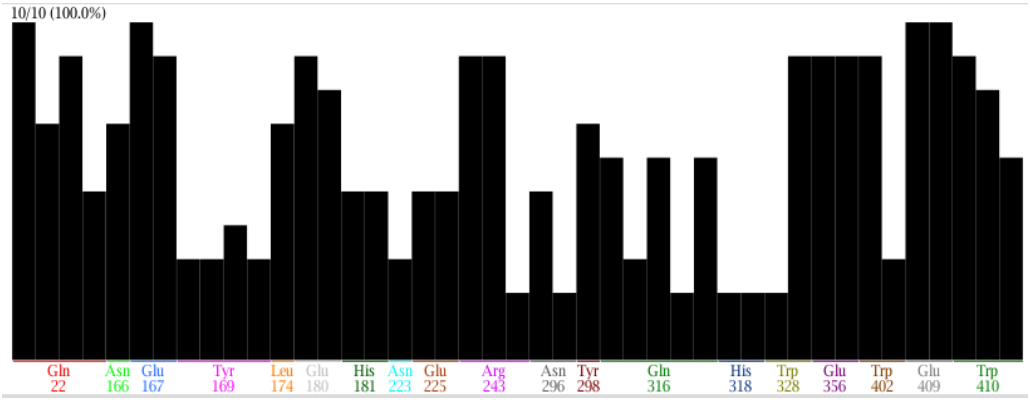


(b)

Figure 5. Visualization of top ten ligand structure conformation (a) BglB-cellobiose and (b) BglB-cellotetrose in BglB binding pocket are shown in ball and stick representation whereas light blue dash line represent hydrogen bond interactions. The picture is visualized by MOE 2011.10.



(a)



(b)

Figure 6. PLIF diagram show common interacting residue between top ten ranked conformation protein-ligand complexes.

It has been reported that the residues Glu167 acts as a protonated agent of interglycosidic oxygen atom in hydrolysis mechanism, Glu356 acts as nucleophilic in stabilizing the transition state and finally Glu409 as catalytic residue [8] . Tyr298 which is hydrogen bonded to Glu356 involved in recognition of the inhibitor. In agreement with Isorna *et al* (2007), it is observed that Glu167, Glu356, Glu409 and Tyr298 play an important role in catalytic function for both BglB-cellobiose and BglB-cellotetrose complexes.

IV. Conclusion

The molecular docking studies were conducted in order to further understand the binding modes of β-glucosidases. The docking analysis resulted in identification of important residue in protein-ligand interaction with respect to the binding site namely Gln22, Glu167, Glu356, Glu402 and Trp402. The result from this study will eventually give some idea to experimentalist to design better enzyme for more efficient enzymatic hydrolysis process with higher yields with lower production cost.

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