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Insight into Cellopentaose Binding Mode in Glycone and Aglycone Binding Site of Beta-Glucosidase B: A Molecular Dynamics Approach

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Abstract: Paenibacillus polymxca β-glucosidase B (BglB) which belongs to GH family 1 is a monomeric enzyme that acts as an exo-β-glucosidase hydrolyzing cellobiose and cello oligosaccharides of higher degree polymerization by cleaving the β-1, 4 glycosidic linkage between glucosyl residues. This study is based on the binding modes of cellopentaose consisting of five glucosyl residues in the active site of BglB. A Molecular Dynamics (MD) simulation was performed at 300 K under periodic boundary condition for 5 ns using the complex structures obtained from previous reported docking study. The residues that are responsible for recognizing the glycone and aglycone subsites include Gln22, Glu167, Glu409, Glu356, Tyr298, Trp410 and Glu225, Tyr169, Asn223, Trp412, His181, Ala360, Arg243, Gln316, respectively. The findings of this study support the fact that the interaction of subsite-1 and OH_2 is the most crucial in the enzyme-substrate complex stabilization.

Key words: β-glucosidase, aglycone, glycone, molecular docking, molecular dynamics, simulation

INTRODUCTION

β-glucosidase is derived from the GH1 family. It belongs to the GH-A, GH5 and GH30 families which share a similar $(\beta/\alpha)_8$ barrel structure. There are two conserved carboxylic acid residues on β-strands 4 and 7. One is acting as the catalytic acid/base and the other acts as the nucleophile (Henrissat and Bairoch, 1996). The catalytic domains of this enzyme ranges from 440-550 residues depending on the loops at C-terminal ends of the β-strands of the $(\beta/\alpha)_8$ barrel (Isorna *et al*., 2007). These enzymes form a wide range of quaternary structures such as dimers, tetramers, hexamers and octamers. The β-glucosidase active site can be divided into several subsites with sufficient space to bind a monosaccharide unit. The subsites that bind the glycone and aglycone regions of the substrate are prefixed by - and +, respectively.

Structural data has shown that within each subsite, the side chain of apolar amino acids forms a platform which acts as a supporting base for the ligand (Marana *et al*., 2001). Polar amino acids can also be found distributed around the ligand forming hydrogen bonds. The crystallographic studies of β-glucosidase with either bound substrate or inhibitor revealed that the residues that are present within subsite -1 formed hydrogen bond interactions with the glycone hydroxyls (Marana, 2006). There are numerous crystallographic structure submitted for cello-oligosaccharides in order to investigate the aglycone specificity of β-glucosidase (Isorna *et al*., 2007; Nijikken *et al*., 2007; Chuenchor *et al*., 2008). The aglycone specificity of the maize, sorghum and wheat has been intensively studied, this is by mutagenesis and structural studies of mutants with bound substrates or inhibitors (Barrett *et al*., 1995; Czjzek *et al*., 2001; Verdoucq *et al*., 2004; Sue *et al*., 2005; Seshadri *et al*., 2009). These studies suggest that a conserved tryptophan (basal platform) is responsible for substrate aglycone binding. As for glucose residues, the binding affinity tends to be higher at subsite +1 due to the noncovalent interaction formed with the polar groups of the glycone. However, there is yet a general rule on how to identify the specificity of β-glucosidase towards the aglycone substrate. The current study is a continuation from a previous reported research (Khairudin and Mazlan, 2015).

MATERIALS AND METHODS

The crystallographic structure of β-glucosidase B was obtained from PDB website (http://www.pdb.org)

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PDB's ID 2O9T[2]. The 2 Dimensional (2D) ligand structure of Cellopentaose (CE5) was obtained from PubChem (http://www.pubchem.org) with CID 440949.

The starting structure for β-glucosidase B docked with Cellopentaose (BglB-CE5) was obtained from previous docking study (Khairudin and Mazlan, 2015). The similar MD simulation protocol was applied for two different systems; BglB (without substrates) and BglB-CE5. The MD simulation was conducted using Gromacs by employing forcefield 45a3 (Lins and Hunenberger, 2005). The substrates topology was prepared using ProDRG webserver (Schuttelkopf and van Aalten, 2004). The simulation was set for 5 ns and the coordinate were saved for every 2 ps. All graphical molecular representations presented in this study were generated using molecular viewer programs such as USCF Chimera (Pettersen *et al*., 2004) and Pymol.

RESULTS AND DISCUSSION

From the MD trajectories, the substrate CE5 was observed to form interactions with residues His218, Val317, Gln316, Gly327, Trp328, Ala360, Lys416, Ala360, Ala359, Tyr298, Gly358, Tyr297, Glu356, Asn296, Asn223, Gln22, Glu167, Asn166, His122, Trp123, Tyr169, Trp410, Glu409, His181 and Arg243 as shown in Fig. 1. As observed, the subsite +1 and +2 were to form hydrogen bonds with Glu167, Trp328, Asn22 and Ala360, Glu225, Tyr169, Arg243, respectively. On the other hand, the subsite +3 and +4 were observed to form hydrogen bonds with Arg243, Gln316, Trp328, Thr299 and Gly327, His318, Gln316 and Val317, respectively.

The residue Ala360 was observed to form a stable hydrogen bond with OH_6 of CE5 (subsite +1) with occupancy of 45.5%. However, there was no formation of stable hydrogen bond within the subsite +2 to +4. In comparison to other glycosidase enzymes from GH1 that share similar active site with *Paenibacillus polymyxca* BglB, the rice β-glucosidase (Bglu1) showed a stronger binding at subsites +2 and +3 when compared to subsite +1 which preferred to hydrolyze cellotriose over cellobiosee (Chuenchor *et al*., 2008). In a similar case with Bglu1, the barley β-glucosidase (BGQ60) also showed high level of affinity for the third glucosyl residue of cellotriose at the subsite +2 (Hrmova *et al*., 1998). This is due to the fact that all GH1 enzymes behave similarly in glycone binding site (subsite -1) but shows great variation in aglycone binding (subsite +1 to +4) for each enzyme.

From the docking data, it was observed that the catalytic residues Glu356 to did form a strong hydrogen bond with OH_2 (subsite -1) from substrate beta-D-glucose and cellobiose (Mazlan and Khairudin, 2014). From MD analysis, the residues that had stable hydrogen bond occupancy of more than 10% with OH_2 were His 122, Asn 166 and Asn 296. Schubert *et al.* (2007) had suggested that for glycosidase, histidine might be involved in pH tolerance and is responsible in keeping the appropriate protonation state of both nucleophile and proton donor in the hydrolysis reaction by switching between charged and neutral states. On the other hand, the mutation of Asn206 (equivalent to Asn166) in GH1 β-glucosidase CelB had showed a major decrement in the rate of hydrolysis towards glucose by 10 fold (Schubert *et al*., 2007). Thus, it can be suggested that these three residues (His122, Asn166 and Asn296) might play significant roles in the determination of the glycone specificity in BglB enzymes. Badieyan *et al.* (2012) had reported that residue Tyr315 of rice BGlu1 (PDB code 3F4V) in the position equivalent to residue Tyr298 in BglB gave the highest relative energy profile out of all the active residues suggesting its crucial role in the catalytic process.

The mutational studies on ZmGlu1, Verdoucq *et al*. (2003) indicates that the interaction formed in the subsite +1 contributed towards stabilizing the enzyme-substrates complex in glycosylation step as the interactions might affect the glycone positioning within subsite -1. The important residues that interact within subsite +1 were reported to be Asn223 and Trp412 that formed hydrogen bond with OH_6 and OH_3 of cellotriose, respectively (Isorna *et al*., 2007). Similar position of Asn223 was also observed to be present in the previous docking study (Mazlan and Khairudin, 2014). It is quite interesting to observe that the residues of BglB have formed interactions with OH_6 (subsite +1) with hydrogen bond occupancy of more than 10%. These residues were Trp412, His181, Asn223 and Ala360. The residues Arg243 and Glu225 were both observed to form important hydrogen bond interactions in subsite +2 in all the experimental, docking and MD simulation studies. As for the remaining aglycone binding sites, there was no sign of stable hydrogen bond occupancy of more than 10% recorded in both subsites +3 and +4. Isorna *et al.* (2007) reported that residues Gln316 and Glu180 within subsite +3 formed hydrogen bond interactions with OH3 and OH6 of CTT, respectively. By observing the MD trajectory, the aglycone within subsite +3 was also making hydrogen bond interactions that involved residues Trp328, Thr299 and Gly327. In both experimental and docking studies, there were no hydrogen bondformation within subsite +4. However in the MD simulation study, this aglycone was

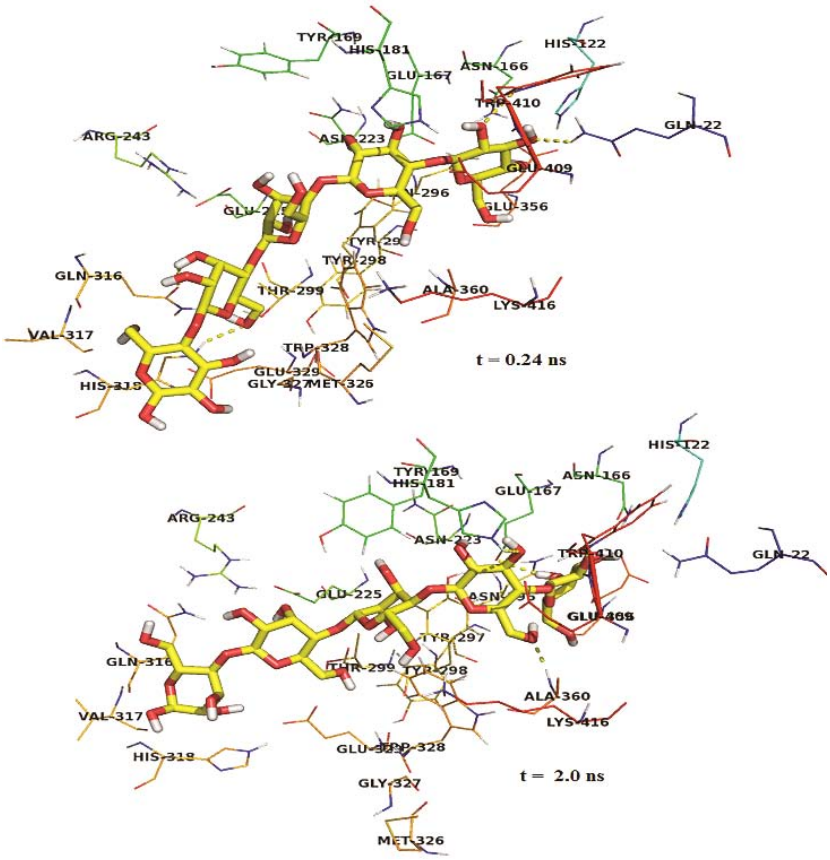

Fig. 1: Stick representation of CE5 at time 0.24 and 2.0 ns

observed to form interactions involving His318, Gln316 and Val317 eventhough it was exposed to the outer surface of BglB.

CONCLUSION

From the MD trajectory inspection, it can be observed that hydrogen bonds between the residues of BglB and the residues of the cellopentaose and keep on forming and breaking throughout the simulation. The hydrogen bond occupancy analysis was performed in order to identify the formation of the important hydrogen bonds during the course of the simulation researches. These residues might be important in recognizing and stabilizing the glyconesubsite-1 and aglycone subsite during the hydrolysis process. This is in agreement with the previous experimental researches whereby there was no formation of stable hydrogen bond within the aglycone subsite +2 to +4 implying that the interaction within these subsites were less significant in the hydrolysis reaction. In summary, all these residues Gln22, Glu167, Glu409, Glu356, Tyr298 and Trp410 indeed play important roles in recognizing the glycone at subsite -1 as they are already conserved in the family of GH1 enzymes. It is a known fact that the interaction of BglB residues with the OH_2 of the glycone (subsite -1) is crucial for the enzyme-substrate complex stabilization. The residues that are responsible in recognizing the aglycone subsites can be found in Glu225, Tyr169, Asn223, Trp412, His181, Ala360, Arg243 and Gln316. Further investigation of these important residues can be done by using extensive Density Functional Theory (DFT) and Quantum Mechanical/Molecular Mechanics (QM/MM) approaches to identify which residues show significant impact on the overall total energy profiles. The role of water within the binding site can also be investigated by looking into the water network in the binding site through Essential Dynamics (ED) simulation.

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