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Study on binding modes between cellobiose and β-glucosidases from glycoside hydrolase family 1

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ABSTRACT

The hydrolysis of cellobiose by β-glucodisases is an important step of cellulose biodegradation. However, the interactive mechanism between cellobiose and β-glucosidases is still unclear until now. Thus, in this study, we explored the binding modes between cellobiose and three β-glucosidases from glycoside hydrolase family 1 by means of molecular docking. The three β-glucosidases were named as TmGH1 (from bacterium *Thermotoga*), SsGH1 (from archaea *Sulfolobus solfataricus*) and TrGH1 (from fungus *Trichoderma reesei*) respectively, according to the monophyletic groups they belong to. Molecular dockings were performed between cellobiose and the three β-glucosidases, resulting in three optimum docking complexes, that is TmGH1-cellobiose, SsGH1-cellobiose and TrGh1-cellobiose complexes. Our docking results indicated that there were non-bonded interactions between cellobiose and the three β-glucosidases. The binding affinities of the three complexes were −13.6669 kJ/mol, −13.2973 kJ/mol and −18.6492 kJ/mol, respectively. Then the detailed interactions were investigated, which revealed the key amino acid residues interacted with cellobiose by hydrogen bonds (H-bonds) or hydrophobic interactions. It was observed that most of the key residues involved in the non-bonded interactions were equivalent and conserved for the three complexes, and these residues were a glutamine, a histidine, a tyrosine, a phenylalanine, three glutamics, and four tryptophans. This information is of great importance for designing β-glucosidase with higher cellobiose-hydrolyzing efficiency.

Cellulose is a major component of municipal solid waste.[1,2] It is a highly unbranched polymer consisting of glucose residues linked together by β-1,4 glycosidic bonds.[3,4] Its structural unit is cellobiose. The glucose chains in cellulose are very tightly packed to form insoluble crystallites, which makes cellulose a recalcitrant polymer[4,5] in the treatment of municipal solid waste.[6] Furthermore, cellulose is one of the most abundant polysaccharide compound in nature[7,8] and is thought to be a promisingly renewable biomass resource for alternative fuels.[9–11] In view of these two reasons, cellulose biodegradation has been brought into focus as a solution of dealing with municipal solid waste and energy crisis.

Due to the superiority of biodegradation to physico-chemical degradation, enzymatic hydrolysis of cellulose has caught much attention.[5,9,12] The enzymatic hydrolysis of cellulose is performed by cellulase. Cellulase is a series of cellulolytic enzymes that work synergistically as a system to hydrolyze cellulose into glucose. Among cellulase system, three major enzymes are endoglucanase (endo-1,4-β-glucanohydrolase, EC 3.2.1.4), exoglucanase (1,4-β-D-glucan cellobiohydrolase, EC 3.2.1.91) and β-glucosidase (β-1,4-glucosidase, EC 3.2.1.21). The first two cellulases attract the crystal in cellulose fiber, producing free chain-ends, and then cut cellobiose units from the chain-ends. After these two steps, the cellulose crystal is hydrolyzed to cellobioses, which is an important intermediate product during the cellulose hydrolysis. Finally, β-glucosidase hydrolyzes cellobioses into glucoses.[5,10,13] In the process of cellulose biodegradation, cellobiose is a strong inhibitor for endoglucanase and exoglucanase,[14] and it has become one of the bottlenecks in the biodegradation. Due to the reversibility of the inhibition, it is of great help to remove cellobioses in time to eliminate the inhibition.[15] So it is essential to improve the hydrolysis activity of β-glucosidase in order to improve the efficiency of cellulose biodegradation. But most of previous researches in relation to cellulose degradation focus on the hydrolysis of crystalline cellulose to cellobiose rather than the hydrolysis of cellobiose.[16–18] In this article, we attempt to explore the binding modes between cellobiose and three β-glucosidases at molecular level by employing molecular docking method. This allows for a better understanding of the interactions between cellobiose and β-glucosidases.

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Table 1
Structural information of TmGH1[a], SsGH1[a], TrGH1[a]

Enzyme Name	PDB ID	Length/aa	Chains	Resolution/Å	Organism	Taxonomy
TmGH1	1W3J	468	A, B	2.00	*Thermotoga maritime*	Bacterium
SsGH1	2CER	489	A, B	2.29	*Sulfolobus sulfataricus*	Archaea
TrGH1	3AHY	473	A, B, C, D	1.63	*Trichoderma reesei*	Fungus

[a] TmGH1, SsGH1 and TmGH1 represent the β-glucosidases from bacterium *Thermotoga maritime*, archaea *Sulfolobus sulfataricus* and fungus *Trichoderma reesei*, respectively.

Molecular docking is initially and predominantly applied in computer aided drug design.[19–21] It aims at predicting the ligand poses and the detailed interaction of a receptor–ligand complex based on the three-dimensional structures of the receptor and ligand,[22–25] which is difficult to study via experiments.[26] Because of the prominent advance, molecular docking has been employed in biodegradation to explore the binding modes between substrates and enzymes.[27] Additionally, there has been a considerable amount of information on the structures and sequences of proteins, and the data continue to increase rapidly,[28,29] which have a promising future for application. These data need systematically further studying, and molecular docking can be used to makes use of them to instruct and interpret experiments.[30]

The chemical three-dimensional structure of cellobiose was obtained from PubChem Compound database (http://pubchem.ncbi.nlm.nih.gov/),[31] and was used as the ligand. The cellobiose structure was downloaded in SDF format with a Compound ID (CID) 10712. Cellobiose is a disaccharide composed of two glucose units linked by a β-1,4 glycosidic bond, and it has four rotatable bonds with a molecular weight of 342.30 g/mol.[31] Then its conformation was optimized before docking.

There are many types of β-glucosidases that can hydrolyze cellobiose. Among various β-glycodidases, three types belonging to family 1 glycoside hydrolases were chosen as the receptors for this study because family 1 glycoside hydrolases were widely studied. The three selected β-glycodidases were from bacterium *Thermotoga maritime* (TmGH1), archaea *Sulfolobus solfataricus* (SsGH1) and fungus *Trichoderma reesei* (TrGH1), respectively. Enzymes in this family share the similar structural features at their catalytic sites.[32] Here, we wanted to know if they also have the similar interactions with cellobiose based on the common structural features. The structures and amino acid sequences of the three β-glycodidases

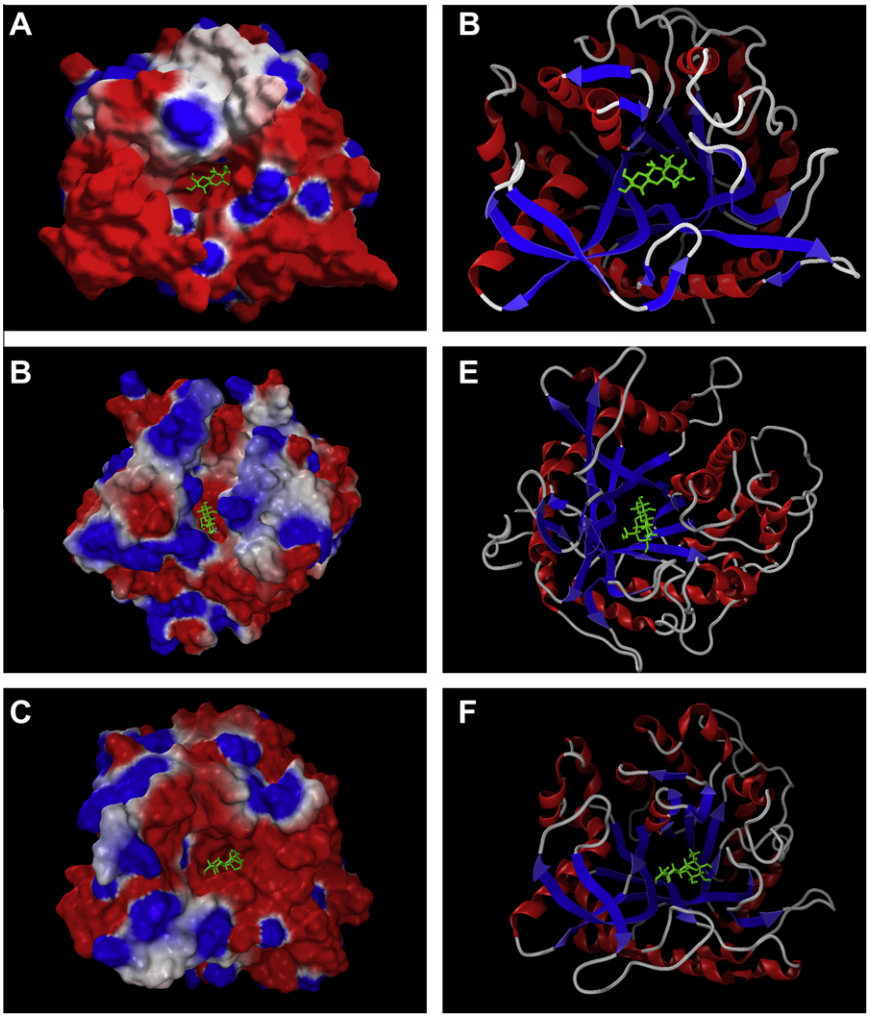

Figure 1. The general views of β-glucosidases-cellobiose complexes. Panels A, B and C show the molecular surfaces of TmGH1-cellobiose, SsGH1-cellobiose, and TrGH1-cellobiose complexes, respectively. Molecular surfaces are colored according to the electrostatic properties. Electropositive surfaces are shown in red, while electronegative in blue. Panels D, E, and F are the secondary structural representation of TmGH1-cellobiose, SsGH1-cellobiose, and TrGH1-cellobiose complexes. α-helixs are shown in red in the backbone, and β-pleated sheet in blue. Cellobiose is shown in stick models.

were obtained from Protein Data Bank (PDB, http://www.rcsb.org/pdb/home/home.do),[33] with the PDB IDs 1W3J,[34] 2CER[35] and 3AHY,[36] respectively, and their detailed structural information was listed in Table 1. Waters, cofactors and originally bounded ligands for each β-glycodidase were removed before docking.

Molecular dockings between cellobiose and β-glucosidases were carried out employing the Molegro Virtual Docker (MVD) Version 4.3.0 program, a graphical-automatic software (http://molegro.com/mvd-product.php). It has been shown to outperform other docking programs for its higher accuracy about 87%.[37,38]

During the docking for each pair of cellobiose and β-glucosidase, firstly, both the receptor and ligand were automatically prepared using the default settings. Namely, the charges were automatically assigned, and the flexible torsions in cellobiose were detected. The bonds, bond orders, atom types and explicit hydrogens were assigned if they were missing. Then the potential active cavities on the β-glycodidase were detected by selecting the 'Detect Cavities' menu option from the preparation menu. For each β-glucosidase, more than one cavity was detected. Finally, docking process was performed by applying the MolDock Score [Grid] as the score function with the grid resolution 0.30 Å and the MolDock SE as the search algorithm.

For each β-glucosidase, docking was performed, focusing on every potential binding cavity detected above, one cavity at a time. Each time, the search space was centered on the center of a cavity with a proper radius according to the size of the cavity. Twenty independent docking runs were carried out without a reference ligand and a maximum 10 top poses were returned. 'Optimize H-Bonds after docking' option was chosen. The search calculation applied a maximum of 1500 iterations with the max population size 50 and the energy threshold 100.00. 'Simplex Evolution' parameters were set at 300 steps with neighbor distance factor 1.0. For every docking, all the parameters stayed unchanged except the centers and radiuses of the search spaces.

During the docking simulation, the items, including MolDock Score, Rerank Score, HBond and so on, were calculated. The corresponding binding affinity which reflected the robustness of a complex was estimated according to an empirical correlation[39]: Binding Affinity = $-19.0155 \times$ C0 + $3.3813 \times$ 'Cofactor (hbond)' $-0.594128 \times$ Csp2 + $0.464056 \times$ HBond $-0.061912 \times$ 'E-Intra (vdw)' + $0.953672 \times$ 'E-Solvation' + $0.483368 \times$ HeavyAtoms $-1.00763 \times$ N + $3.0229 \times$ Nplus + $1.61426 \times$ OH $-3.10696 \times$ OS $-3.9493 \times$ halogen. This is a multiple linear regression expression for calculating the binding affinity measure. The parameters involved in are shown in the docking result files. Among the multiple conformations for a single β-glucosidase-cellobiose complex, the one with the best Rerank Score (in arbitrary units) was chosen for further analysis.[39]

The crystal structures of TmGH1 and SsGH1 are both dimer. In one asymmetric unit there are two monomers (chains A and B) which are sequence-unique. During the structural protein alignment in MVD, there were scarcely any structural differences observed between the two monomers in one unit. When docking cellobiose against the detected cavities of TmGH1, a large number of TmGH1–cellobiose complexes were generated, among which a complex with the Rerank Score −101.816 and the binding affinity −13.6669 kJ/mol led the top hit. This complex was chosen for further study. As to SsGH1, a complex got the top Rerank Score −100.263 with the binding affinity −13.2973 kJ/mol, and it was selected for further analysis. Different from TmGH1 and SsGH1, the structure of TrGH1 is a tetramer, in which the four monomers (chains A, B, C, D) share a unique amino acid sequence. The structures of the four chains are structurally similar. Among the obtained TrGH1-cellobiose complexes, one with the top Rerank Score −108.261 and binding affinity −18.6492 kJ/mol was selected to analyze the detailed interaction.

The docking results showed that binding site of TmGH1 was a long and narrow tunnel-shaped cavity stretching into the core of the enzyme (Fig. 1A). That of SsGH1 was a cavern in the valley between two preiections (Fig. 1B). The binding pocket in TrGH1 (Fig. 1C) resembled that of TmGH1, whereas it was a little wider at the entrance. From the corresponding secondary structures of the three β-glucosidases (Figs. 1D, E and F), we observed that the binding pockets were all bottomed with β-barrels. These suggested that the active site of TrGH1 was the easiest to access due to its

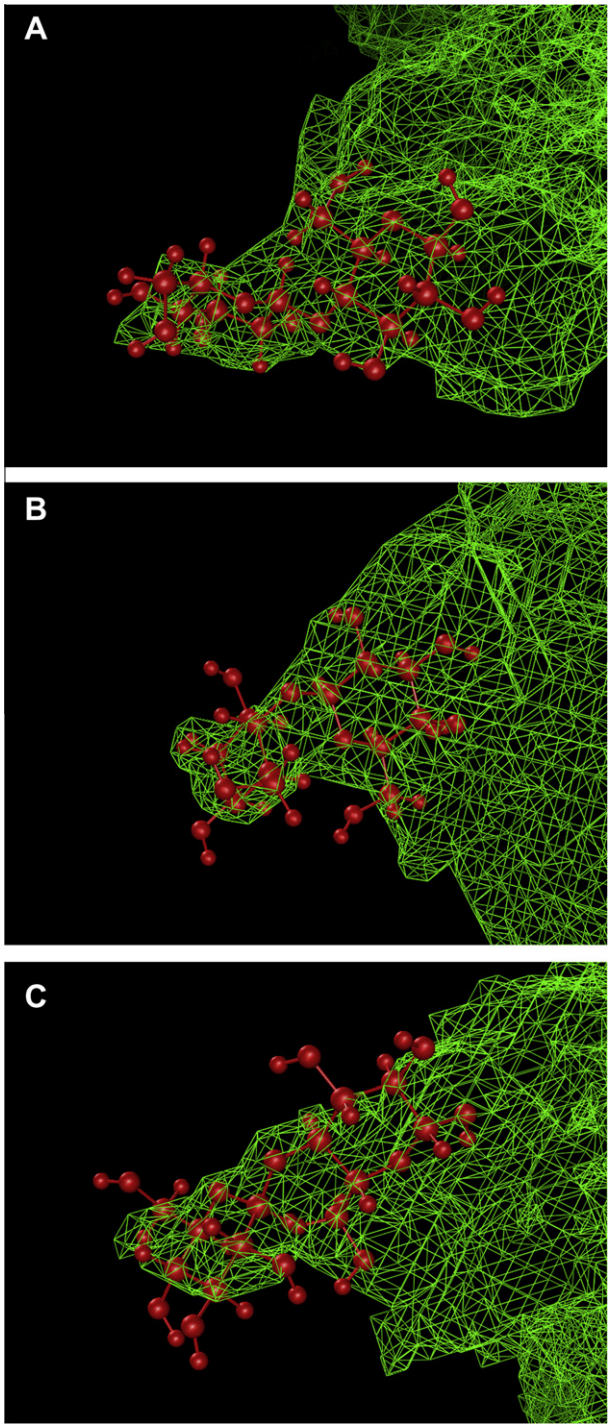

Figure 2. The poses of cellobiose in the binding pockets for complexes of TmGH1-cellobiose (Panel A), SsGH1-cellobiose (Panel B) and TrGH1-cellobiose (Panel C). The green meshed grids are referred to the binding pockets. The red ball-and-stick models are the poses of cellobiose.

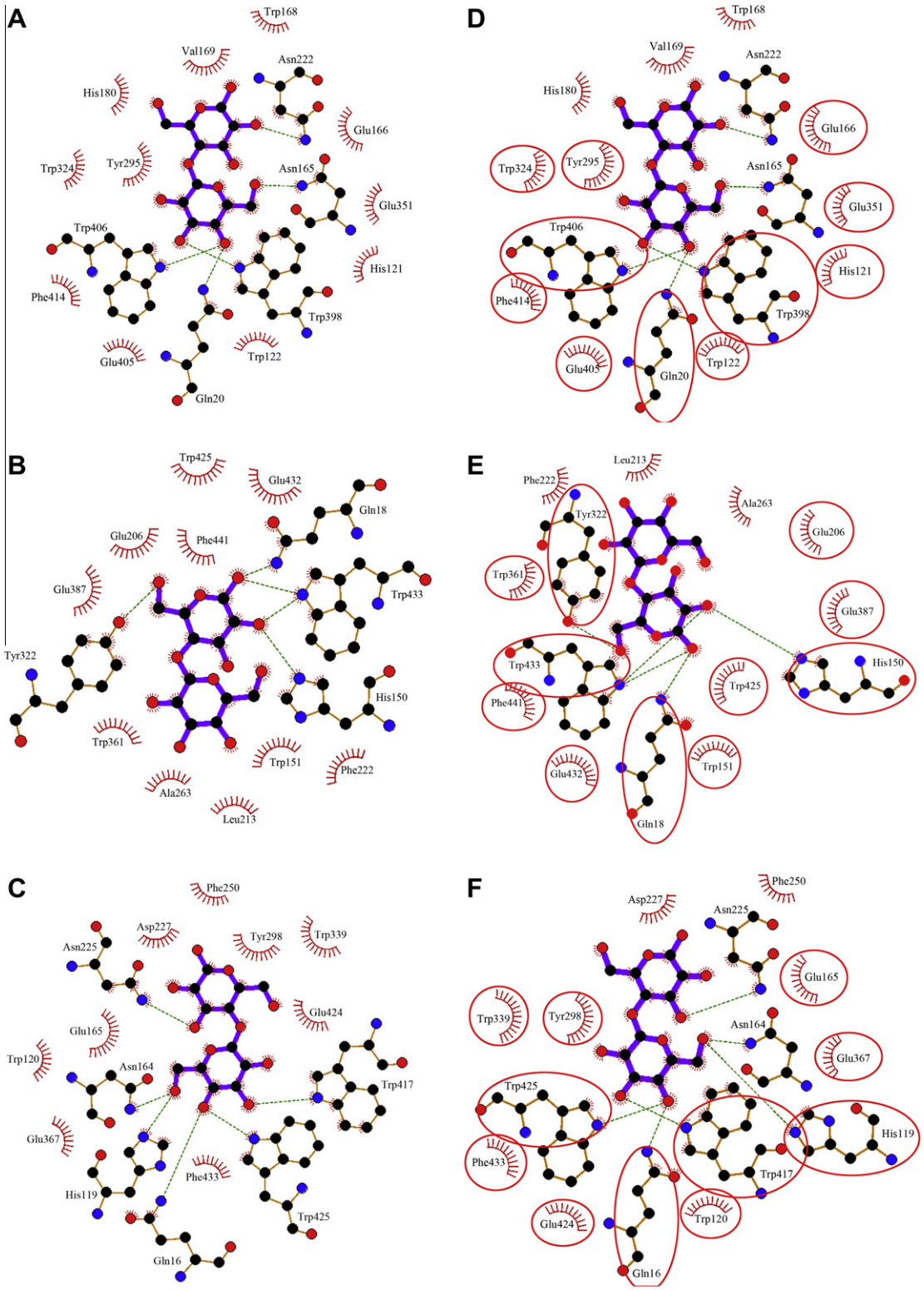

Figure 3. Detailed interactions of the complexes of TmGH1-cellobiose (Panels A and D), SsGH1-cellobiose (Panels B and E) and TrGH1-cellobiose (Panels C and F). The models with solid circles and bold bonds belong to cellobiose. The models with solid circles connected by thin bonds are the amino acid residues contacted with cellobiose by hydrogen bonded residues. Residues with red circles are the equivalent residues for the three complexes. The dashed lines represent the hydrogen bonds between cellobiose and β-glucosidases. The spokewise arcs pointing towards the cellobiose indicate the hydrophobic interactions. The cellobiose atoms with spokes indicate atoms involved in hydrophobic contacts.

wider entrance, while the active site of SsGH1 was the most difficult to reach because it was buried in a canyon. The results also implied that TrGH1 was likely to have higher hydrolysis efficiency than the other two β-glucosidases.[40]

To explore the shape complementation, we showed the poses of cellobiose in the binding pockets of the three β-glucosidases (Fig. 2). In the three complexes, all cellobiose poses were stuck onto the walls of the pockets and embedded into the active sites, showing a very good geometric complementation, which was required for enzymatic reaction.

In order to reveal the key amino acid residues involved in the binding, we analyzed the detailed interactions between cellobiose and each β-glucosidase. After docking, the selected complexes for further investigation were imported into the software LigPlot$^+$ Version V.1.3.2 which is the graphic interface of LigPlot.[41] Then the software calculated the interactions and expressed them in two-dimensional pictures for a better and rapid inspection of the complexes.[41] The results indicated that cellobiose interacted with the three β-glucosidases by non-bonded contacts, namely H-bonds and hydrophobic interactions.

LigPlot$^+$ results showed that the binding pocket of TmGH1 was mainly formed by residues Asn222, Asn165, Trp398, Gln120, Trp406, Glu405, Glu166, Glu351, Trp168, Val169, His180, Tyr295, Trp324, Phe414, Trp122 and His121 (Fig. 3A). Cellobiose is stabilized by the TmGH1 residues via both H-bonds and hydrophobic interactions. And five H-bonds were observed in the TmGH1-cellobiose complexes, involving residues Asn222, Asn165, Trp398, Gln20 and Trp406. Table 2 shows the information of the H-bonds.

Surrounding the binding pocket of SsGH1, four residues, namely Gln18, His150, Trp433 and Tyr322 (Fig. 3B), were related to cellobiose via five H-bonds (Table 3), and the cellobiose was also fixed by some other residues through hydrophobic interactions. The residues involved in hydrophobic interactions were Glu432, Glu206, Glu387, Trp425, Phe441, Trp361, Trp151, Phe222, Ala263 and Leu213 (Fig. 3B).

As for TrGH1, the cellobiose was fixed in the binding pocket composed of residues Asn225, Asn164, His119, Gln16, Trp425, Trp417, Phe250, Asp227, Tyr298, Trp339, Glu424, Phe433, Glu367, Trp120 and Glu165, which were interacted with the cellobiose via either H-bonds or hydrophobic interactions (Fig. 3C). The residues involved in H-bonds were Asn225, Asn164, Glu16, Trp425, and Trp 417 (Table 4).

Then, we imported the three complexes into LigPlot$^+$ simultaneously, and the protein structures was alignment by the program automatically. The results showed that the detailed interactions in the complexes TmGH1-cellobiose, SsGH1-cellobiose and TrGH1-cellbiose were similar. It could be easily observed that among the residues involved in the non-bonded interactions, most were equivalent. A glutamine, a histidine, a tyrosine, a phenylalanine, three glutamics, and four tryptophans appeared in all three complexes at the corresponding positions (Figs. 3D, E and F). They were very likely to play critical roles in binding between cellobiose and β-glucosidases.

Table 2
H-bonds between cellobiose and TmGH1[a]

H-bond No.	Ligand atom	Receptor atom	Length/Å
1	O3	Asn222 ND2	3.34
2	O11	Asn165 ND2	3.07
3	O9	Trp398 NE1	2.57
4	O8	Gln20 NE2	2.94
5	O8	Trp406 NE1	2.98

[a] TmGH1 represents the β-glucosidase from bacterium *Thermotoga maritime*.

Table 3
H-Bonds between cellobiose and SsGH1[a]

H-bond No.	Ligand atom	Receptor atom	Length/Å
1	O3	His150 NE2	2.62
2	O5	Try322 OH	2.94
3	O3	Trp433 NE1	3.02
4	O6	Gln18 NE2	2.34
5	O6	Trp433 NE1	3.13

[a] SsGH1 represents the β-glucosidase from archaea *Sulfolobus sulfataricus*.

Table 4
H-Bonds between cellobiose and TrGH1[a]

H-Bond No.	Ligand atoms	Receptor atoms	Length/Å
1	O2	Asn225 ND2	2.97
2	O11	Asn164 ND2	3.10
3	O11	His119 NE2	3.10
4	O8	Gln16 NE2	2.66
5	O8	Trp425 NE1	2.93
6	O9	Trp417 NE1	2.48

[a] TmGH1 represents the β-glucosidases from fungus *Trichoderma reesei*.

Finally, we did a protein sequence alignment for the three β-glucosidases by employing an integrated software package Data Analysis in Molecular Biology and Evolution (DAMBE) Version 5.2.31.[42] The alignment was performed under the default parameters. The alignment result (Fig. 4) showed that the equivalent amino acid residues found by LigPlot$^+$ were all conserved.

Additionally, it has been reported that the catalytic residues in the three β-glucosidases are two glutamics, acting as nucleophile and acid/base catalyst, respectively. For TmGH1, the nucleophile and acid/base catalyst are Glu166 and Glu351.[34] As to SsGH1, Glu387 (the nucleophile) and Glu206 (the acid/base) play as catalytic residues.[35] In TrGH1, the catalyst are Glu367 (the nucleophile) and Glu165 (the acid/base).[36] Our results produced by LigPlot$^+$ and DAMBE showd that these two catalytic residues were all contacted with cellobioses by hydrophobic interactions, and they were conserved in N**E**P and T**E**NG peptide motifs, respectively.

In summary, in this article, we explored the binding modes between cellobiose and three β-glucosidases, that is TmGH1, SsGH1 and TrGH1, and pictured the overall looks of the binding sites. Our results show that there exist non-bonded interactions between cellobiose and each of the three β-glucosidases, and the binding affinities of the complexes TmGH1-cellobiose, SsGH1-cellobiose and TrGH1-cellobiose are −13.6669 kJ/mol, −13.2973 kJ/mol and −18.6492 kJ/mol, respectively, implying that TrGH1-cellobiose complex bonds the most tightly and may have the highest hydrolysis efficiency among the three complexes because of its highest binding affinity. Moreover, most amino acid residues related to the non-bonded interactions are equivalent and conserved in the three β-glucosidases, which suggests that the detailed interactions in the three complexes are of great similarity besides their structural similarity.

In addressing the mechanism of cellulose biodegradation, much work has been done toward the functional mechanisms of endoglucanase and exoglucanase against cellulose, but the mechanism of cellobiose hydrolysis by β-glucosidase, which is an important step in the celloluse biodegradation, still remains unclear. This article employed molecular docking to elucidate the binding modes between cellobiose and three β-glucosidases. Despite the high similarity of these three binding modes described above, there are some differences among them. The shapes of these three binding sites and the binding orientations of cellobiose are different, respectively. Interactional profiles including H-bonds and hydrophobic interactions are also non-identical. It has been well demonstrated that these differences may partly lead to the differences in binding affinities.[27,43] Binding affinities have been speculated to be positively correlated with the efficiency of several enzymes.[43,44] Our recent study also in part supported this finding.[27] Thus, the results in relation to binding affinities and interactional profiles may provide valuable information needed in selecting and designing more effective enzymes to improve the efficiency of cellobiose biodegradation.

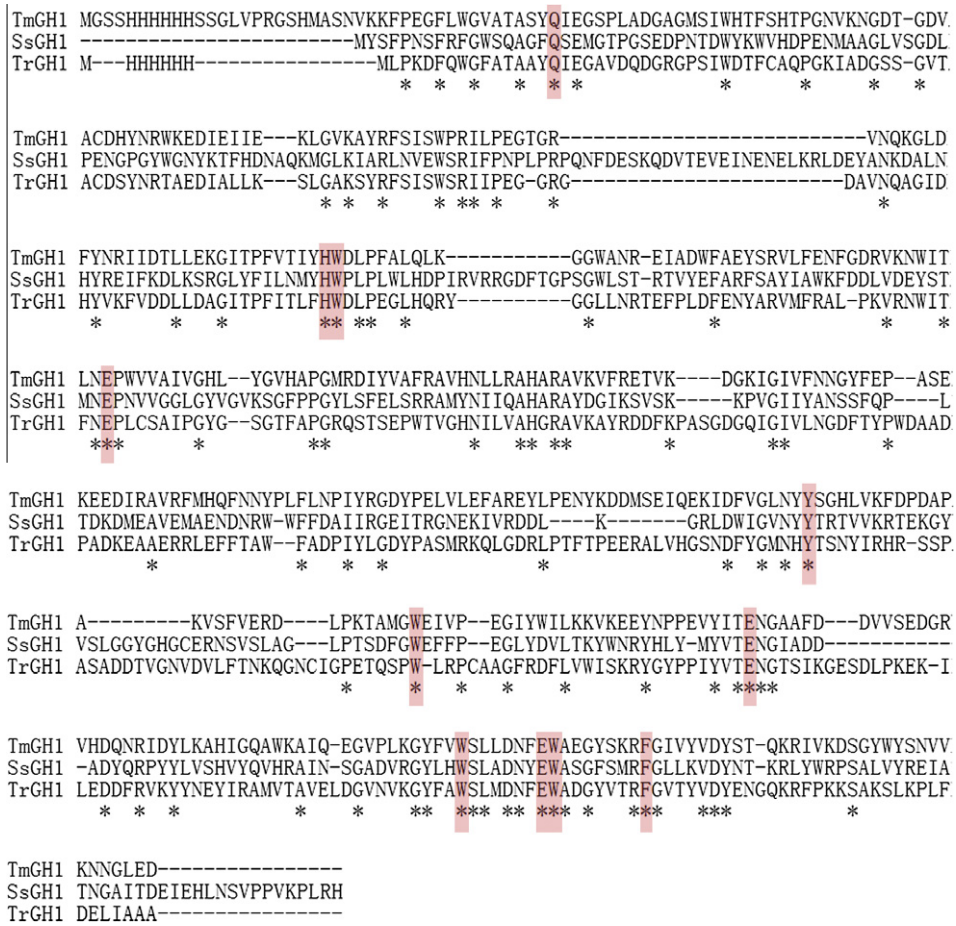

Figure 4. Amino acid sequence alignment of TmGH1, SsGH1 and TrGH1. The residues marked with asterisks below are completely conversed. The residues highlighted with red backgrounds are the equivalent residues showed in the results of LigPlot+.

Acknowledgments

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