



QM/MM studies on the glycosylation mechanism of rice BGLu1 β -glucosidase

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ABSTRACT

The quantum-mechanical/molecular-mechanical (QM/MM) method was used to study the glycosylation mechanism of rice BGLu1 β -glucosidase in complex with laminaribiose. The calculation results reveal that the glycosylation step experiences a concerted process from the reactant to the glycosyl-enzyme complex with an activation barrier of 15.7 kcal/mol, in which an oxocarbenium cation-like transition state (TS) is formed. At the TS, the terminal saccharide residue planarizes toward the half-chair conformation, and the glycosidic bond cleavage is promoted by the attacks of proton donor (E176) on glycosidic oxygen and nucleophilic residue (E386) on the anomeric carbon of laminaribiose. Both the nucleophilic glutamate (E386) and acid/base catalyst (E176) establish shorter hydrogen bridges with the C₂-hydroxyl groups of sugar ring, which play an important role in the catalytic reaction of rice BGLu1 β -glucosidase.

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1. Introduction

As the most abundant biopolymers on earth, cellulose and β -glucans of plant cytoderm act as the main sources of energy for a variety of organisms, primarily bacteria and fungi. They not only determine the shape and structural strength of plant cells but also could be degraded by enzymes evolved by bacteria and fungi, including cellobiases and other β -glucan β -glucosidases [1], which hydrolyze β -glycosidic bonds from the non-reducing end of β -glucan-derived oligosaccharides and glycosides. Utilizing the vast sources of cellulosic energy pool, microbial β -glucosidases are typically applied for biomass conversion. At the same times, plants have independently developed their own β -glucosidases (BGLu1), which are necessary for cell growth and metabolic needs with the actions of remodeling of cell walls and releasing glucose.

Up to now, the X-ray crystal structures of β -glucosidases have been determined for bacterial [2], fungal [3], and plants (rice, maize, sorghum and wheat) glycosyl hydrolase family 1 (GH1) enzymes [4–15] that act on cellooligosaccharides. Moreover, the aglycone characteristics of β -glucosidases and their mutants complexed with substrates or inhibitors have been extensively studied by mutagenesis. Analyses indicate that rice BGLu1 (namely Os3bglu7), which evolves from the microbial enzymes, is more closely related to plant enzymes and more efficiently to hydrolyze cello-oligosaccharides [16]. The rice BGLu1, which also belongs to

the glycosyl hydrolase family 1 (GH1) enzyme, is expressed widely in rice organs that have high level of activities, such as tissues, flower and seedling shoot [5,6,17,18].

The substrate hydrolysis mechanism for GH1 β -glucosidases was proposed about sixty years ago by Koshland and Stein [19], which was supposed to proceed in a two-step displacement catalytic mechanism involving a glycosylation and a deglycosylation step (Fig. 1). The catalytic groups are a pair of glutamate residues. In the substrate hydrolysis reaction, one carboxylic acid of these key residues serves as a nucleophile and the other as a general acid/base catalyst, with the reaction proceeding via a covalent glycosyl-enzyme intermediate.

The first step of the reaction is the substrate distortion catalyzed by the protonation of the glycosidic oxygen by the acid/base residue. The other glutamate residue acts as a nucleophile to attack the anomeric carbon in the glycosylation step to generate a covalent intermediate, forming a α -linked covalent glycosyl-enzyme intermediate. The deglycosylation step occurs when the intermediate is hydrolyzed by a water molecule, which is activated by extraction of a proton by the catalytic acid/base residue. The deglycosylation step results in the free enzyme with the protonated glutamate residue and the free sugar.

Previous theoretical calculations were performed on the glycosylation [20] and deglycosylation processes [21] for β -glucosidases mainly via quantum mechanics (QM) using DFT methods. In these calculations, a minimal model was used to mimic the enzymatic reaction. For example, in the study of glycosylation [20], the two catalytic residues were represented by two propanoic acids, and the substrate was modeled by a galactose molecule with a methyl group bound to the glycosidic oxygen. The calculated barriers spanned from 21.1 to 28.7 kcal/mol, depending on the calculation

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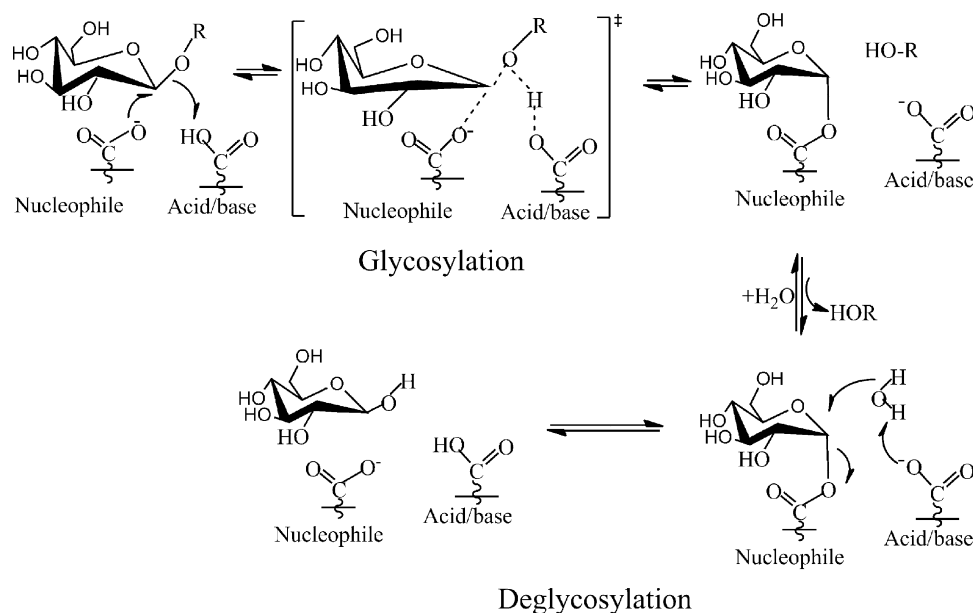


Fig. 1. Proposed double displacement mechanism for the β -glucosidases [19].

method. They found that the hydrogen bridges between the glutamates of the enzyme and the hydroxyl group of the sugar ring imposed a large influence on the energy barrier. Recently, the ONIOM method was also employed to study an enzymatic system of β -galactosidase from *Escherichia coli* [22]. Similar conclusions were obtained.

Although much information has been obtained in the past, the mechanism of glycosylation step and the interactions between the pocket residues and substrate were still not fully understood. In this paper, we reported the first quantum mechanical/molecular mechanical (QM/MM) studies on the rice β -glucosidase glycosylation step. In the modeling, the region that participate in the reaction was defined as QM region and was described by quantum mechanics, while the remaining part of protein and solvent was defined as MM region and was described by molecular mechanics [23–26]. The combined QM/MM method was applied to determine the pathway of catalytic mechanism, which is expected to achieve a better description of glycosylation process.

2. Computational details

2.1. Computational model

The X-ray crystal structure of rice BGLu1 E176Q mutant in complex with laminaribiose (PDB ID code: 3F5L) [16] was taken from Protein Data Bank, as shown in Fig. 2a. First, the E176Q mutation was mutated back to glutamate to generate a functional active site. All the glutamate residues except E176 were charged in the following molecular dynamics (MD) simulations. According to the experimental condition, the protonation states of other residues were checked carefully using the VMD program [27], and the missing hydrogen atoms were added via the HBUILD facility in the CHARMM package [28]. Then, the system was solvated with 6944 water molecules that formed a water sphere of 40 Å radius centered on E176 residue. Finally, a number of 9 Cl⁻ ions were used to neutralize the system at random positions. A neutral system of 28,347 atoms was then generated. To equilibrate the prepared system, a series of minimizations and a 500 ps MD simulation were performed with the CHARMM2227 force field [29]. The obtained structure is shown in Fig. 3a, which is further optimized by QM/MM method.

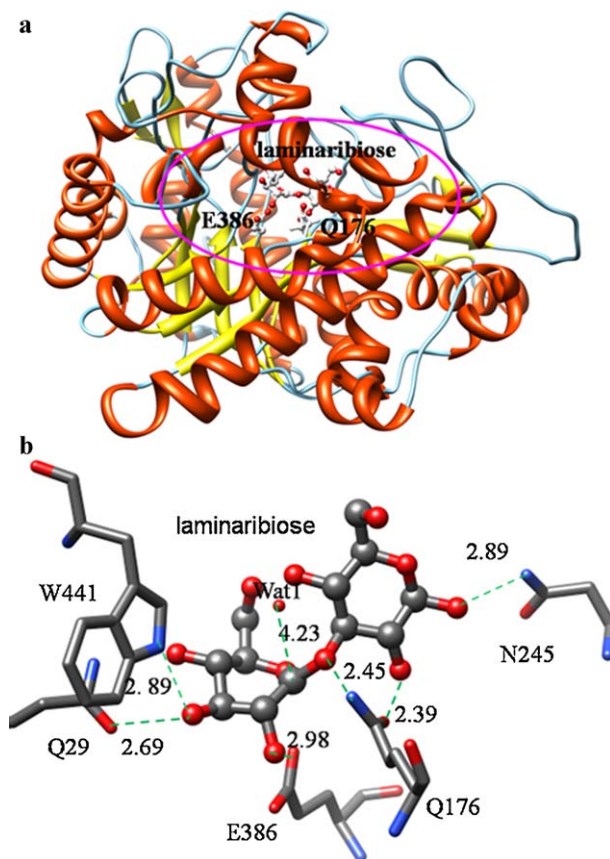


Fig. 2. (a) The crystal of rice BGLu1 β -glucosidases E176Q mutant in complex with laminaribiose (PDB code: 3F5L) and (b) the corresponding crucial residues that interact with laminaribiose in the active site.

2.2. QM/MM calculations

In the QM/MM calculations, the QM region (shown in Fig. 4) contains 61 atoms in the active site, including a substrate laminaribiose and a crystal water molecule Wat1 as well as residues E176 and E386. The remaining 28,286 atoms of the enzyme and

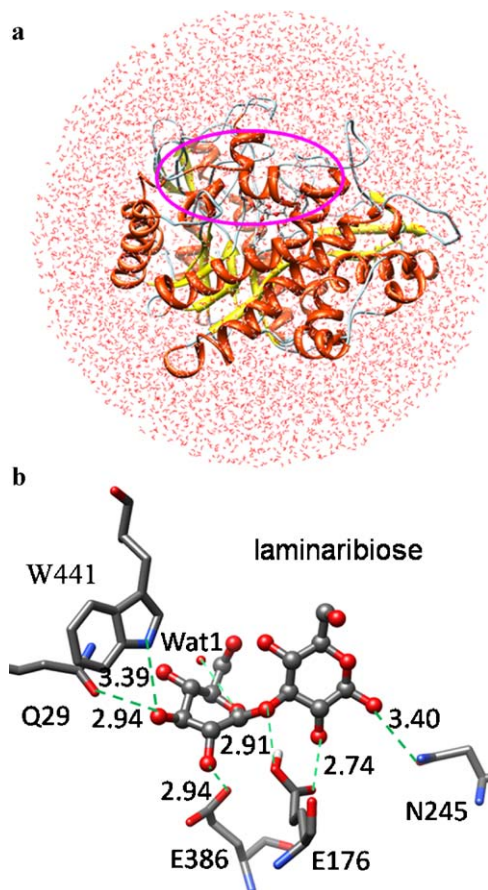


Fig. 3. (a) The structure of model system treated by CHARMM package and (b) the corresponding crucial residues that interact with laminaribiose in the active site.

water molecules belong to the MM region. During the subsequent QM/MM calculations, the QM region and 742 MM atoms within 10 Å of E176 were loosened, while the remaining part was frozen. In the process of geometry optimization, the QM part was considered by quantum mechanics at the B3LYP/6-31G(d,p) level by Turbomole module [30], while the MM region was treated with molecular mechanics under the CHARMM22/73 force field by DL-POLY program [31]. The convergence criterion of TURBOMOLE for the SCF calculations was set to 3×10^{-6} a.u. for energy gradient and 1.35×10^{-3} a.u. for geometry gradient. The calculations of frequency for the BGLu1-laminaribiose reactant, transition state (TS) and product were computed at the same level. All the optimized geometries correspond to a local minimum that has no imaginary frequency mode or a saddle point that has only one imaginary frequency mode. To avoid hyperpolarization of the QM wave function, the electronic embedding scheme [32] was used to perform one-electron Hamiltonian of the QM calculations with the

MM charges [33]. Hydrogen link atoms with charge shift model for the QM/MM boundary were adopted in the QM/MM treatment [34]. The ChemShell package [35] integrating Turbomole and DL-POLY programs was used to carry out the QM/MM calculations.

3. Results and discussion

The proposed catalytic mechanism for rice BGLu1 β -glucosidase consists of double displacement mechanisms involving a covalent glycosyl-enzyme intermediate. In the glycosylation step, one carboxyl group of two glutamates acts as a general acid/base catalyst (here E176), protonating the glycosidic oxygen followed by the cleavage of glycosidic bond. The other carboxyl group of E386 functions as the nucleophile, forming a covalent glycosyl-enzyme intermediate (Fig. 1). In the deglycosylation step, one incoming water molecule attacks on the anomeric center (C_1) with the help of the deprotonated acid/base residue. Both transition states of the two steps have substantial oxocarbenium ion character.

As the first step is the rate-determining step in the whole catalytic reaction, therefore, in the present study, we only focus on the glycosylation step, in which the formation of the covalent glycosyl-enzyme intermediate occurs.

3.1. Comparison of substrate binding in crystal and CHARMM treated structures

Fig. 2b gives the important residues that interact with laminaribiose in the pocket from crystal. It shows that the side chain of the acid/base residue Q176 forms strong hydrogen bonds (HBs) with glycosidic oxygen and C_2 -hydroxyl group of laminaribiose with distances of 2.45 and 2.39 Å, respectively. Besides, the side chains of residues W441 and Q29 are hydrogen bonded to C_3 -hydroxyl and N245 to C_1 -hydroxyl group with distances of 2.89, 2.69 and 2.89 Å, respectively. Furthermore, the nucleophile residue E386 interacts with the C_2 -hydroxyl group of the sugar ring with a distance of 2.98 Å, and a water molecule (Wat1) lies above C_1 atom, interacting weakly with laminaribiose.

The structure treated by CHARMM package is given in Fig. 3a and the corresponding active site is shown in Fig. 3b. Because the backbones of Q176 and E176 are similar, after mutation (Fig. 3b), the glycosidic oxygen and C_2 -hydroxyl group of the substrate still form two HBs with the protonated E176 with distances of 2.91 and 2.74 Å, respectively. The interaction modes of residues W441, Q29 and N245 with the substrate are almost the same as in Fig. 2b. We believe these HB interactions play an important role for the glycosylation reaction.

3.2. Analyses of the glycosylation reaction

The structures of the BGLu1-laminaribiose reactant, TS, and product were optimized by using QM/MM method, as shown in Fig. 5. Frequency calculations give the unique imaginary frequency of 400.9i for the TS, and no imaginary frequency mode in reactant and product.

In reactant, the length of glycosidic bond is 1.45 Å. The glycosidic oxygen establishes a hydrogen bridge (1.76 Å) with the carboxylate group of catalytic acid/base E176, and the bond distance of O–H in E176 is 1.0 Å. In TS, the length of glycosidic bond elongates to 1.70 Å, meaning this bond is weakened at this stage. The proton in E176 has almost been transferred to the glycosidic oxygen, because the distance between the two atoms is approximately a covalent bond (1.08 Å). The carboxyl group of the nucleophilic E386 approaches to the anomeric C_1 of the sugar ring, and the distance between oxygen and C_1 shortens to 2.73 Å from 3.10 Å. For product, the length of glycosidic bond is 3.25 Å, indicating the cleavage of this bond

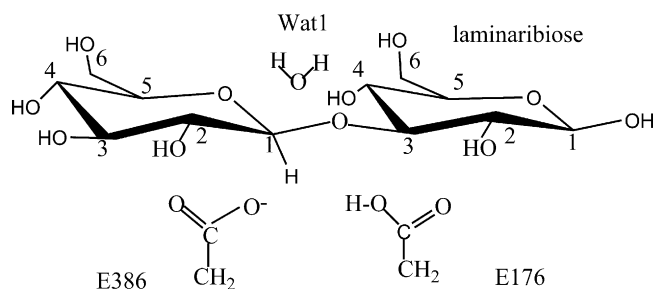


Fig. 4. The selected quantum mechanics (QM) region in QM/MM calculations.

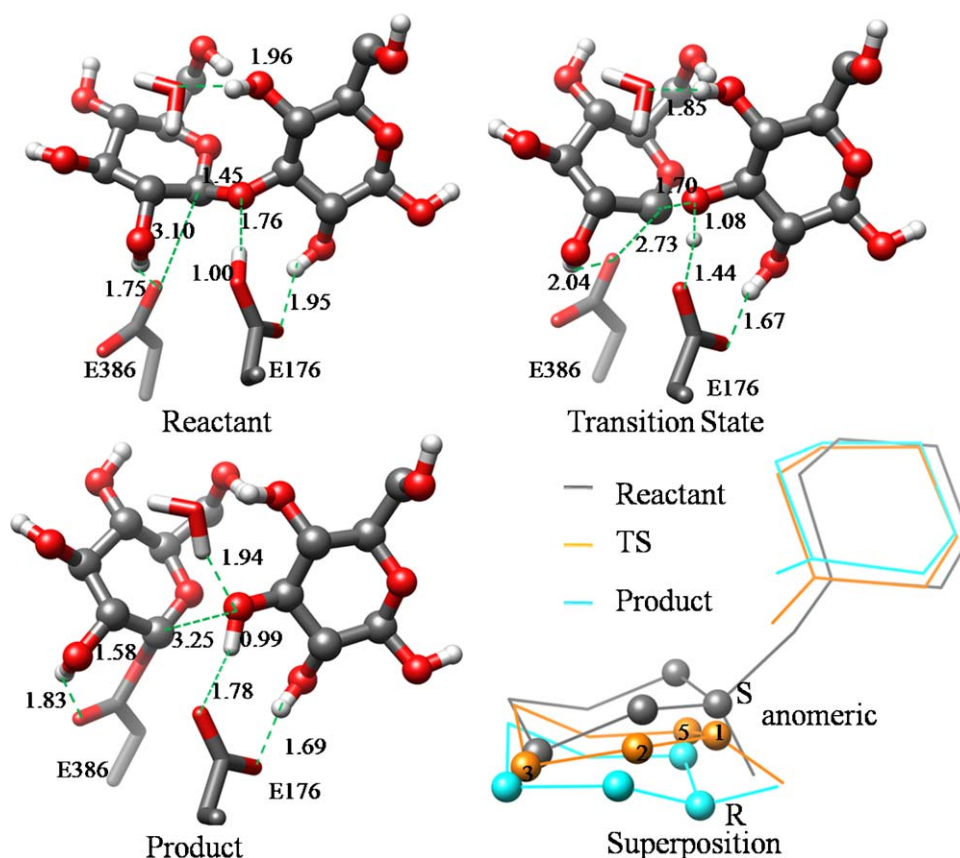


Fig. 5. Optimized geometries of reactant, transition state and product. The superposition of laminaribiose in the three states is also shown for comparison.

is completed. Meanwhile, the carboxylate oxygen of nucleophilic E386 has been attached to the anomeric carbon with a covalent bond length of 1.58 Å. So, a covalent intermediate is formed as soon as the leaving group departs.

Analyses of the TS indicate that the carboxyl oxygen of the nucleophile (E386) establishes one HB with the hydroxyl group of the second carbon of sugar ring, and the bond distance is 2.04 Å (Fig. 5). This kind of HB is also called hydrogen bridge by Brás et al. [20]. Another hydrogen bridge is formed between the carboxyl oxygen of E176 and C₂-hydroxyl group of laminaribiose with length of 1.67 Å. The role of these HB interactions is to lower the energy of TS, contributing considerably to the stabilization of this state in the glycosylation process. DFT calculations by Brás et al. revealed that the hydrogen bridge between the nucleophile and C₂-OH group could bring down the activation barrier by 5.1 kcal/mol [20].

The superposition of substrates in reactant, TS and product is also shown in Fig. 5. The superposition indicates that the initial conformation of anomeric center (C₁) is reversed after the glycosylation process, in which the conformation of C₁ changes from S to R type. In TS, the terminal saccharide ring planarizes toward the half-chair conformation. This can be seen from the changes of dihedral angle C₁-C₂-C₃-O₅. This angle is -20.2° in reactant, and changes to -2.8° in TS and -20.7° in product. It is supposed that the conformational change from the typical chair conformation to the half-chair form in TS is caused by the hydrogen bridges, which facilitates the stabilization of oxocarbenium ion-like TS [36].

As to our system, the total atoms of the QM region remain unchanged during the whole calculations and the number of wave functions remains the same from reactant to product. In addition, a bigger basis function is used. Therefore, the basis set superposition error (BSSE) is expected to be small and can be ignored for simplifying the calculations. After identifying the geometries

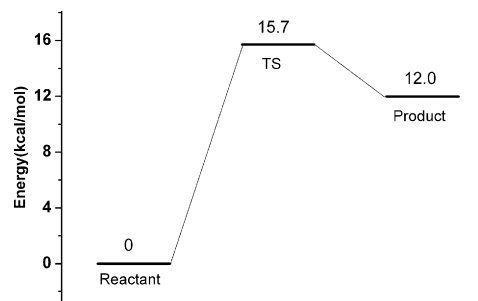


Fig. 6. Energy profile of the glycosylation step.

of reactant, TS and product, the energies were recalculated with single point (SP) calculations using 6-31++G(2d,2p) basis set. The obtained values are shown in Fig. 6. It shows that the activation barrier is only 15.7 kcal/mol, which is smaller than that of the DFT calculations [20]. Brás et al. calculated this barrier using small models under different functionals, such as B3LYP, B98 MPW1K, MPWB1K and BB1K. All the obtained values were bigger than 21 kcal/mol. Fig. 6 also reveals that the relative energy of the product is 12.0 kcal/mol higher than that of reactant, implying the glycosylation step is endothermic. To our knowledge, another computational study was conducted with the β-galactosidase using ONIOM method [22]. The obtained relative energies of the covalent galactosyl-enzyme intermediate were 6.7 and 23.0 kcal/mol with and without the presence of magnesium ion, respectively. Our value of the intermediate is just between these values, indicating our result is reasonable.

In the crystal, there is water molecule just above the anomeric center (C₁) with a distance of 4.23 Å (Fig. 2b). QM/MM results in

Fig. 5 reveal that this water plays an assistant role in the catalytic reaction. The direct evidence is that the water molecule forms a hydrogen bridge with the C₄-hydroxyl group of sugar ring with a distance of 1.96 Å in reactant, and this distance decreases to 1.85 Å in TS, which might be useful to stabilize this state. When the product is formed, this water molecule moves away from C₄-hydroxyl and closes to the glycosidic oxygen (1.94 Å). We suppose the movement of the water molecule facilitates the deglycosylation process in the next step.

4. Conclusions

In the present studies, QM/MM calculations were performed to understand the glycosylation step of wildtype rice BGLu1 β-glucosidase. Calculations reveal that the glycosylation process experiences an oxocarbenium cation-like TS with an energy barrier of 15.7 kcal/mol. The proton attack of E176 on glycosidic oxygen and nucleophile attack of E386 on anomeric carbon are a concerted process. In TS, the glycosidic bond is much weakened (1.70 Å) and the proton of acid/base E176 is almost transferred to the glycosidic oxygen (1.08 Å), and the sugar ring planarizes toward a half-chair conformation. In all the reaction species, both the nucleophilic glutamate and acid/base catalyst form strong hydrogen bridges with the substrate.

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