Glycosyl rotation and distortion by key residues in Endocellulase Cel6A from *Theromobifida fusca*

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Endocellulases are one kind of the important biodegrading cellulose enzymes. Experimental results show that a rotated and distorted preactivated structure exists before the substrate entering the transition state. The molecular dynamic simulation of endocellulase Cel6A models revealed a correlation between the rotation and distortion of pyranoside ring in −1 glycosyl unit of the substrate. The two key residues, Tyr73 and Ser189, in Cal6A cooperate to rotate and distort the pyranoside ring in the cellulose hydrolysis.

**Keywords:** cellobiohydrolase / cellulose / endocellulase / hydrolysis mechanism / molecular dynamic simulations

**Introduction**

Cellulose, a polymer of β-1,4 linked glucosyl units, very similar to the α-1,4 linkages in starch, is an abundant renewable biomass. Native cellulose is difficult to degrade because of their strong β-1,4 linkages with the k values on the order of 10⁻¹⁵/s at room temperature. On the other hand, some microbes produce cellulases that can degrade cellulose. Cellulase is not a solo enzyme, substrate structural transformations.

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The Cel6A hydrolyzes the β-1,4 linkage by a single-displacement mechanism (Knowles et al. 1988). Crystallography studies of the Cel6A ligand complex structures showed that the pyranoside ring distortion in the −1 glycosyl unit (GU) is a key component of the hydrolysis mechanism (Knowles et al. 1988; Damude et al. 1996; Varrot et al. 1999; Larsson et al. 2005).

Conformational analyses of the reaction coordinate showed that the best substrate preactivation in β-d-glucopyranose is a skew-boat, 2S₀ or 1S₃ (JCBN 1980), which were identified by both experimental and simulation studies (Knowles et al. 1988; Damude et al. 1996; Zou et al. 1999). The hydrolysis transition states (TS) conformation is a preactivated B₂,₅, which appears in the 2S₀ ↔ B₂,₅ transformation of −1 GU (Davies et al. 2012). The pyranoside ring of −1 GU in TS is distorted away from its lowest-energy conformation to one that favors orbital overlap (Davies et al. 2012). The preactivated substrate formation is a key process in the catalytic pathway of cellobiohydrolase, because, with the plane rotation and distortion, the −1 GU is pushed to a high energy level and easy to trap in the catalytic process (Davies et al. 2012). The conformational free-energy change in the preactivated conformation transformation is ~5-6 kcal/mol (Davies et al. 2012). Barnett et al. (2011) found that the free-energy barrier is 17 kcal/mol to break the cellulose chain of Cel6A.

A crystallography study of the Cel6A showed that to form the distortion structure of the pyranoside ring, −1 GU first rotates to a nearly perpendicular position to +1 GU (Zou et al. 1999). The conserved tyrosine that binds to the −1 GU determines the pyranoside ring distortion (Koivula et al. 2002b; Varrot et al. 2003). Moreover, the mutation of the Tyr73 causes the ring distortion to disappear, and this mutation even reduces the rotation angle of −1 GU (Koivula et al. 2002b; Larsson et al. 2005). However, when all the key tyrosines are mutated, crystal structures still present more or less some pyranoside ring distortion at the −1 GU, though the enzyme activity is reduced (Barr et al. 1998, Zou et al. 1999; Larsson et al. 2005). Therefore, there may exist other residues that affect the −1 GU distortion.

**Results and discussion**

In order to study the −1 GU rotation and distortion in the catalytic pathway, we conducted molecular dynamic (MD) simulations to a wild-type (WT) Cel6A-ligand complex structure and a series of its mutants. In the simulation trajectory, the relationship between the rotation and distortion of pyranoside ring in −1 GU was studied. The −1 GU plane rotation of each conformation in the trajectory was measured by the angle between the normal vectors of two pyranoside ring planes on −1 and +1 GUs. The planes of two pyranoside rings were obtained by a
least-square-fitting to six atoms, and the rotation angle from 0 to 90° means that the pyranoside ring in −1 GU rotates from the parallel position with the pyranoside ring in +1 GU to the perpendicular position. The distortion to $^{2}S_0$ is measured by the angle between the normal vectors of two planes: The least-square fitted plane of \( \text{C}_\text{B1}-\text{C}_\text{B2}-\text{C}_\text{B3}-\text{C}_\text{B4}-\text{O} \) and the triangle plane of \( \text{C}_\text{B4}-\text{C}_\text{B5}-\text{O} \) in −1 GU ring (Figure 1). The distortion angle changes from 20 to 70°, which corresponds to a change from no distortion to full distortion, and Supplementary data, Figure S4 shows that different structures with the same distortion angle have different standard Cremer–Pople ring pucker parameters (Cremer and Pople 1975). The distribution of pyranoside ring conformations with different rotation and distortion angles is shown in Figure 2. A correlation between the rotation and distortion of pyranoside ring in −1 GU is revealed. The more perpendicular a conformation of −1 GU pyranoside ring is to the +1 GU pyranoside ring, the more its distortion. This correlation indicates that the rotation of pyranoside ring in −1 GU is a necessary step for its distortion. From quantum mechanical/molecular mechanical (QM/MM) trajectories, the change in potential energy from the normal $^{2}S_0$ conformation to \( B_{2,5} \) distorted conformation is $\sim 8.66$ kcal/mol, and the TS structure, the \( B_{2,5} \) conformation, is shown in Supplementary data, Figure S3. The potential energy surface (PES) was scanned along the \( \text{C}_\text{B2}-\text{C}_\text{B1}-\text{C}_\text{A4}-\text{C}_\text{A3} \) dihedral angle in cellobiose (Glu2), and it showed that the pyranoside ring distortion in −1 GU needs less energy than the ring plane rotation (Supplementary data, Figure S2). This discovery also supports the same conclusion that rotation of pyranoside ring in −1 GU is a necessary step for its distortion.

According to the complex structures, there are only five residues within close proximity of −1 GU, defined as having closest atoms within 4 Å distance from each other, and they are Tyr73, Ser189, Asp117, Lys259 and Asp265. The distances between these residues and −1 GU were collected, and different behaviors were uncovered. The residues Asp117, Lys259 and Asp265 have relatively constant distances from −1 GU. For example, there is an H-bond between oxygen on carboxyl of Asp and hydroxyl on C3 of −1 GU, and the distance between these two atoms does not have significant change (Figure 3). Therefore, these residues do not directly contribute to the rotation of the pyranoside ring.

The distances between Tyr73/Ser189 and −1 GU vary with time (Figure 3). The oxygen atom of the hydroxyl in Tyr73 and the oxygen atom of the hydroxyl in Ser189 were collected, and different behaviors were uncovered. The residues Asp117, Lys259 and Asp265 have relatively constant distances from −1 GU. For example, there is an H-bond between oxygen on carboxyl of Asp and hydroxyl on C3 of −1 GU, and the distance between these two atoms does not have significant change (Figure 3). Therefore, these residues do not directly contribute to the rotation of the pyranoside ring.

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Fig. 1. The catalytic cleft of Cel6A and two glycosyl units of cellulose.
and Ser189 are opposite. When two rings on $-1$ and $+1$ GU have parallel positions, Tyr73 has the shortest distance to $-1$ GU, whereas Ser189 has the shortest distance to $+1$ GU. Since pyranoside ring in $-1$ GU is distorted at the perpendicular position with $+1$ GU, one can conclude that Tyr73 contributes to the rotation of pyranoside ring in $-1$ GU. An MD simulation on a mutant structure of Cel6A (Y73S) was conducted to study the rotation of the pyranoside ring in $-1$ GU (details of the simulation setup are in the Supplementary data). In the simulation trajectory, there are only a few conformations that have large rotation angles of pyranoside ring (Supplementary data, Figure S5). This study agrees with previous works (Koivula et al. 2002a; Larsson et al. 2005) and further supports the statement that Tyr73 is a key residue for the rotation of the pyranoside ring in $-1$ GU.

The MD simulation on the WT structure shows that, when the $-1$ GU pyranoside ring is distorted, some interactions are formed between Ser189 and the $-1$ GU, including an H-bond interaction and a Coulomb interaction. A theoretical study already showed that the positive charge accumulates on the pyranoside ring of TS substrate (Barnett et al. 2011). In the simulation model, Ser189 attacks the hydroxyl of C$_6$ on $-1$ GU, which is important for forming the ring distortion conformation. To further test the importance of Ser189 for ring distortion, in our study MD simulations were conducted for three mutants of Ser189: S189A, S189C and S189N, with the same setup as the simulation for the WT (see the Supplementary data for more details about simulation). The distribution of distortion angles in each simulation was studied. The same method was used to calculate the rotation and distortion angles. For three mutated models of Ser189, the distributions of the distorted conformations of pyranoside ring with a rotation angle of $>45^\circ$ were calculated, and the histogram is shown in Figure 4. When Ser189 is mutated to alanine, the distortion angles have a narrow peak of $\approx 10^\circ$, while the WT has a peak $\approx 75^\circ$. This indicates that the pyranoside ring is not distorted in the S189A mutated model. The reason is that alanine cannot form an H-bond with the hydrogen or oxygen of the hydroxyl on C$_6$ in $-1$ GU, and it also does not have negatively charged atoms to have Coulomb interaction with the pyranoside ring in $-1$ GU. Therefore, the pyranoside ring could not be distorted. When Ser189 is mutated to cysteine, the conformations have a smaller peak around distortion angle $\approx 60^\circ$, which is similar to the mutation of S189A. The reason is that, although oxygen atom (serine) and sulfur atom (cysteine) have similar chemical properties, oxygen atom has a much higher electronegativity than sulfur atom, which causes cysteine to have less chance to form H-bonds and weaker Coulomb interactions.
with the pyranoside ring to distort it. However, compared with serine, asparagine has more electronegative atoms, nitrogen and oxygen. Therefore, the mutant, Ser189Asn, could distort the pyranoside ring in −1 GU more easily than the WT because it has higher chance to form H-bonds and stronger Coulomb interactions with the pyranoside ring in −1 GU. The MD simulation supported this hypothesis; the distribution of rotation angles has a higher peak ~65° than that for the WT at 75°. The distributions of rotation angles of the pyranoside ring in −1 GU for three mutants were collected as well. All mutated models have similar distributions as the WT; there is only one peak ~40° for rotation angles (Supplementary data, Figure S6). Therefore, one can conclude that Ser189 is a key residue for pyranoside ring distortion.

In summary, the rotation pyranoside ring in −1 GU is important for its distortion, the B2,5 preactivated conformation. The key residues Tyr73 and Ser189 in CalbA cooperate to rotate the pyranoside ring and distort it. The residue Tyr73 pushes the pyranoside ring to rotate it to a position close to Ser189, and Ser189 affects the pyranoside ring distortion of −1 GU with H-bond interactions and coulomb interactions.

Materials and methods

The three-dimensional structure of Cel6A was obtained from PDB (ID: 2BOD) (Larsson et al. 2005), and the initial complex structure was built by replacing the sulfur atom in the inhibitor with a carbon atom. The complex structures were put into a cubic water box (15 × 15 × 15 Å³) with TIP3P water (Jorgensen et al. 1983), and sodium ions were added to neutralize charges, after adding hydrogen atoms on both substrate and protein. The GROMACS MD package (Hess et al. 2008) with GLYCAM06 force field (Kirschner et al. 2008) and AMBER force field (Lindorf-Larsen et al. 2010) were used for cellulose and for protein, respectively, in the isothermal–isobaric ensemble. GROMACS, a widely used open source MD simulation package for biological molecules, supports various types of force fields, and can naturally conduct simulation with a combination of several different force fields. The GLYCAM06 force field is a force field specifically designed for carbohydrate molecule simulation and it is dependent on and compatible with AMBER force field to simulate the carbohydrate interaction with proteins. The trajectories of 10 ns were built with steps of 1 fs for each structure. In order to improve the simulation precision and to identify the TS structure of hydrolysis to compare our results with experimental data and previous theoretical studies, amino acid residues, Tyr73, Asp117 (protonated), Ser189, Lys259, +1 GU and −1 GU are treated with the QM/MM method. The density functional tight binding method (Elstner et al. 1998; Han et al. 2000), implemented in Gaussian 09 (Frisch et al. 2009), is used as the QM layer. A Glu2 structure model with lowest energy was established and the relaxed PES scan was performed using the B3LYP method, 6–31 + G (d,p) basis, and conductor-like polarizable continuum model implicit water solution model in Gaussian 09 package (Frisch et al. 2009), to assess the structural parameters and potential energy changes in the QM/MM model.

Supplementary data

Supplementary data for this article are available online at http://glycob.oxfordjournals.org/.

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Conflict of interest

None declared.

Abbreviations

CPCM, conductor-like polarizable continuum model; GU, glycosyl unit; Glu2, cellulobiose; MD, molecular dynamic; MM, molecular mechanical; PES, potential energy surface; QM, quantum mechanical; TS, transition states; 3D, three-dimensional.

References


