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Structural analysis of biomass degrading enzymes from a white rot fungus

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Introduction

The white-rot fungus (basidiomycete) *Phanerochaete chrysosporium* can effectively degrade cellulose with the synergistic action of extracellular endo-glucanases, cellobiohydrolases, and cellobiose dehydrogenase. The genome of *P. crysosporium* contains 166 glycoside hydrolases (GHs) [1]. In this study, we focused on structural determination of GH enzymes from *P. crysosporium.*

Results and Discussion

Recently, two intracellular GH family 1 BGLs of *P. chrysosporium* (BGL1A and BGL1B) have been cloned and characterized [2]. The BGL1B enzyme can effectively hydrolyze cellobiose and cellobionolactone, and its gene is expressed in cellobiose culture but repressed in glucose culture, suggesting that BGL1B contributes to cellobiose metabolism. In contrast, the BGL1A enzyme barely hydrolyzes cellobiose and cellobionolactone, and its gene is expressed constitutively, making its biological role unclear. We have determined the crystal structure of BGL1A in substrate-free and gluconolactone complexed forms at 1.5 and 1.9 Å, respectively [3]. This was the first 3D structure of a fungal GH1 enzyme.

The BGL1A crystal was found to contain two chains per asymmetric unit. The final model contained 459 residues of each chain. The overall structure of BGL1A was almost the same as those of known GH1 enzymes, which have classical $(\beta/\alpha)_8$ barrel folds. Gluconolactone was bound in the ring-form. The substrate-free and gluconolactone complex structures were very similar, with RMSD of Cα being less than 0.24 Å. The only significant difference in these structures was observed at the side chain of Glu422. The movement is prerequisite for the recognition of O-6 hydroxyl group of the substrate. The residues involved in the interactions at subsite -1 (glycone site) are almost conserved by every GH1 enzymes.

On the other hand, the aglycone binding site (subsite +1) of GH1 enzymes is less conserved, and this site is the basis of diversity in their substrate specificity. In the $(\beta/\alpha)_8$ barrel structure, the entrance of the substrate binding pocket is formed primarily by the four extended loops connecting strands and helices at the C terminal side of the barrel. BGL1A has a tunnel-shaped active site pocket, whereas the pocket is a flattened crater or slot-like in plant BGLs. BGL1A has unique aglycone specificity compared with other structurally known GH1 enzymes, because of the unique structure at this site. BGL1A shows relatively high sequence identity (50 – 60%) to biochemically characterized GH1 BGLs from cellulolytic fungi. Therefore, this structure will provide a good template for modeling of fungal cellulolytic BGLs and can be used to determine structure–function relationships.

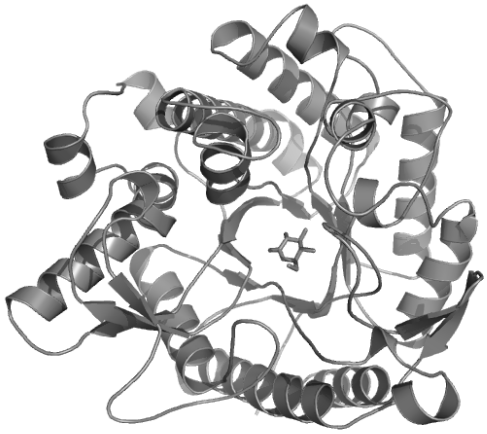

Figure 1 The crystal structure of BGL1A.

References

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