

Structural study of a cellulase, CcCel6C, from the basidiomycete, *Coprinopsis cinerea*

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Introduction

Cellulose is the predominant structural component of plant cell walls and is the most abundant biomass resource. Cellulases belonging to the glycoside hydrolase family 6 (GH6) are known as major cellulolytic enzymes. Several reports are available on the crystal structures of the GH6 enzymes from ascomycetes but no crystal structure of the basidiomycete-derived GH6 enzyme has been determined yet. Recently, we cloned five genes encoding GH6 enzymes from a basidiomycete *Coprinopsis cinerea*, and the enzymes have been designated CcCel6A, -6B, -6C, -6D, and -6E. The presence of cellobiose strongly induced transcription of the CcCel6A gene but weakly induced transcription of the CcCel6B, -6D, and -6E genes. Interestingly, the transcript level of CcCel6C was not influenced by either glucose or cellobiose. CcCel6C also exhibits unusual cellobiohydrolase activity; it hydrolyzes carboxymethyl cellulose, which is a poor substrate for typical cellobiohydrolases. Here, we present the crystal structure of CcCel6C, which is the first report of the crystal structure of a basidiomycete GH6 enzyme.

Materials and Methods

The enzyme was crystallized at 20°C using the hanging drop vapor diffusion method, where 1 μ L of CcCel6C was mixed with the same volume of well solution (100 mM HEPES-KOH pH7.0, 30% polyethylene glycol 8000, 150 mM magnesium acetate)[1]. The obtained crystal was transferred to a cryo-solution of 40% (wt/vol) polyethylene glycol 8000 in well solution and flash-frozen in a stream of nitrogen gas. The crystal of the complex of *p*-nitrophenyl β -D-cellobioside (*p*NPG3) or cellobiose was obtained by soaking in the same well solution containing 60 mM *p*NPG3 for 2 h or 220 mM cellobiose for 5 min, and the solution containing the ligand also acted as a cryoprotectant. The diffraction data were collected at beamline PF-AR NW12A. The data were processed and scaled with the program HKL2000. The structure of CcCel6C was solved by molecular replacement with the program MOLREP in the CCP4 suite, and a model of *Humicola insolens* Cel6A was employed as a probe model. The automated model building was performed with the program ARP/wARP. The refinement was carried out using the program REFMAC in the CCP4 suite.

Results and Discussion

The crystal structures of unliganded CcCel6C, and the enzyme-substrate complexes of CcCel6C-*p*NPG3 and CcCel6C-cellobiose were determined (Fig. 1)[2]. CcCel6C consists of a seven-stranded β/α barrel fold. Structural homology was researched using the DALI server, and CcCel6C was found to most resemble cellobiohydrolases from ascomycetes, *Humicola insolens* Cel6A and *Hypocrea jecorina* Cel6A. These ascomycete-derived cellobiohydrolases have been characterized by the conformational change of the enclosed tunnel, where the active site is located. However, the unliganded CcCel6C, CcCel6C-*p*NPG3 and CcCel6C-cellobiose are superposed well, appearing that the conformational change in the enclosed tunnel of CcCel6C is less favorable and the tunnel still has an open space near the binding sites of cellobiose or *p*NPG3. It is likely that this open tunnel is suitable for the hydrolysis of carboxymethyl cellulose.

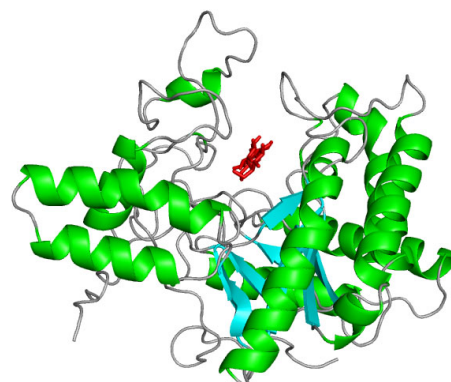


Fig. 1. Structure of CcCel6C. Cellobiose is indicated in red.

References

- [1] Y. Kurakata et al., Acta Crystallogr. Sect. F 65, 140 (2009).
- [2] Y. Liu et al., FEBS J. 277, 1532 (2010).

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