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Crystallization and preliminary crystallographic analysis of the catalytic domain cellobiohydrolase I from *Talaromyces emersonii*

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Cellobiohydrolase IB is the first native enzyme from the filamentous fungus *Talaromyces emersonii* to be crystallized. It is a highly thermostable exo-acting enzyme. The native enzyme (MW = 56 kDa) was crystallized using the hanging-drop vapour-diffusion method with ammonium phosphate (dibasic) as a precipitant at pH 8.5. The crystal belongs to the tetragonal space group $P4_12_12$, with unit-cell parameters $a = b = 74.43$, $c = 176.92$ Å, and diffracted to 1.77 Å resolution at room temperature.

1. Introduction

Cellulose, a β-1,4-glucan polysaccharide and the most abundant component of plant biomass, is found in nature predominately in cell walls, where it constitutes ~40% of the dry weight of these walls (Lynd *et al.*, 2002; Delmer & Amor, 1995). It exists as a crystalline array of numerous parallel oriented chains called microfibrils which are themselves made up of approximately 36 cellulose/β-1,4-glucan chains known as elementary fibrils (Brown *et al.*, 1996). The highly organized crystalline structure of cellulose contains both crystalline and less crystalline or amorphous regions. Therefore, microorganisms must produce a complete battery of enzymes in order to degrade this complex structure effectively (Levy *et al.*, 2002). Extracellular cellulases, either free or cell-associated, are required to hydrolyse and metabolize insoluble cellulose (Parry *et al.*, 2002). There are three major types of enzymatic activities in a complete cellulase system: (i) endoglucanases (EC 3.2.1.4), (ii) exoglucanases (EC 3.2.1.74 and EC 3.2.1.91) and (iii) β-glucosidases (EC 3.2.1.21). Endoglucanases cleave β-1,4-linked glycosyl bonds randomly at internal amorphous regions in the cellulose polysaccharide chain, generating oligosaccharides of various lengths and consequentially new chain ends, thus reducing the degree of polymerization (DP) of the substrate (Kleywegt *et al.*, 1997). Exoglucanases act in a processive manner on the reducing or non-reducing ends of the cellulose polysaccharide chains, liberating either glucose or cellobiose as major products (Divne *et al.*, 1998). Cellobiohydrolases (EC 3.2.1.91) can also act to decrease the DP of the cellulose (Cao & Tan, 2002). β-Glucosidases hydrolyse the soluble cellobiose to glucose, relieving end-product inhibition of cellobiohydrolases.

Cellobiohydrolase (CBH) occurs widely in fungal sources, for example, *Aspergillus ficum* (Hayashida *et al.*, 1988), *Trichoderma viride* (Berghem *et al.*, 1975), *T. reesei* (Fägarstam & Pettersson, 1980), *Bacillus* sp. (West *et al.*, 1989), *Humicola grisea* (Takashima *et al.*, 1998) and *Phanerochaete chrysosporium* (Covert *et al.*, 1992). Many CBH proteins consist of a catalytic domain and a cellulose-binding domain (CBD) linked by a Pro/Ser/Thr-rich linker peptide; however, the CBHs from *H. grisea*, *P. chrysosporium* and *A. niger* (Gielkens *et al.*, 1999) have been shown to contain only a catalytic domain. Fungal CBHs have been classified as members of glycosyl hydrolase (GH) families 6 and 7 (Henrissat, 1991).

Members of GH family 7 are thought to follow a retaining mechanism of action whereby the configuration at the anomeric carbon is retained after hydrolysis. The most characterized member of this family is Cel7A from *T. reesei*. This enzyme has been shown to consist of two β-sheets that pack face-to-face to form a β-sandwich. Long loops on one face of the sandwich form an ~50 Å cellulose-binding tunnel, while the shorter loops form an extended open substrate-binding cleft (Divne *et al.*, 1998). The catalytic residues have been shown to be Glu212 and Glu217, which are located on opposite sides of the active site with a separation suitable for a double-displacement retaining mechanism (Kleywegt *et al.*, 1997).

2. Materials and methods

The catalytic domain CBH IB was purified from *Talaromyces emersonii* as described elsewhere (Walsh, 1997). CBH IB was concentrated to 20 mg ml^{-1} in 20 m*M* Tris buffer pH 7.5 and used for hanging-drop vapour-diffusion (McPherson, 1982) crystallization. Initial trials were conducted using sparse-matrix crystallization screens from Hampton Research. 2 µl of protein solution was mixed with 2 µl of precipitant solution.

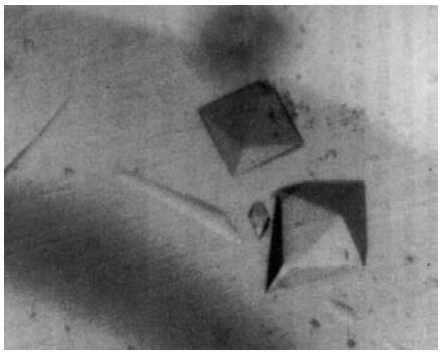

Figure 1
Bipyramidal crystals of CBH IB. The dimensions of the crystals were approximately 0.2 × 0.2 × 0.4 mm.

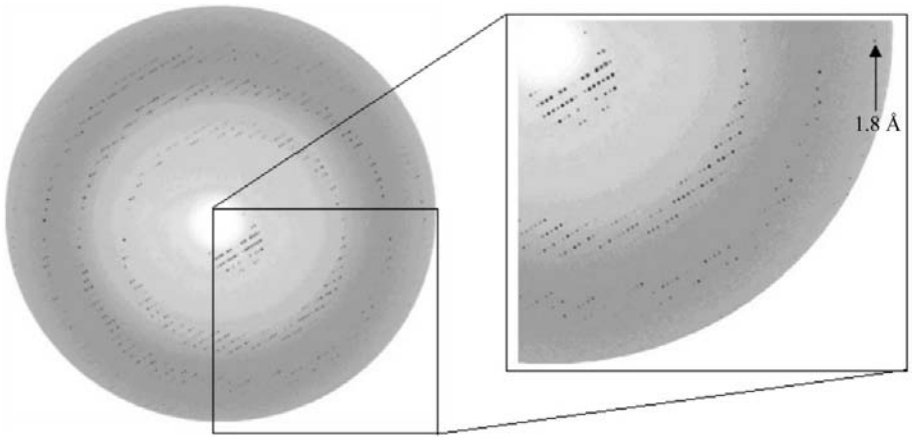

Figure 2
A typical diffraction pattern of CBH IB crystals. The detector edge corresponds to 1.8 Å resolution and an enlarged image of the indicated area is shown. The exposure time was 120 s.

Small crystals appeared in 2.0 *M* ammonium phosphate, 100 m*M* Tris–HCl pH 8.5 after 40 d at room temperature. After optimization, a small number of bipyramidal shaped crystals of approximate dimensions 0.2 × 0.2 × 0.4 mm (Fig. 1) were obtained in 2.5 *M* ammonium phosphate, 100 m*M* Tris–HCl pH 8.5 after 5 d at 291 K.

Data were collected at room temperature on the multipolar wiggler beamline BW7B at the DORIS storage ring, EMBL Hamburg Outstation using a FAST MAR 345 area detector. Aluminium foil was used to attenuate the beam and to prevent significant radiation damage to the crystal during data collection. The crystal-to-detector distance was set at 289.90 mm and 0.8384 Å radiation was used with a sweep per image of 1.5° and an exposure time of 120 s. A data set 94% complete to 2.30 Å was collected at room temperature using a single crystal (Fig. 2, Table 1). The data were processed and scaled using the programs *DENZO* and *SCALEPACK* from the *HKL* suite (Otwinowski & Minor, 1997).

Table 1
Data-collection statistics.

Values in parentheses refer to the last resolution shell.

Space group	$P4_12_12$
Unit-cell parameters	
$a = b$ (Å)	74.42
c (Å)	176.92
$\alpha = \beta = \gamma$ (°)	90.00
Resolution range (Å)	100–2.30 (2.38–2.30)
Total reflections	65121
Unique reflections	21616
$R_{\rm merge}(I)$† (%)	7.8 (36.6)
Mean $I > 2\sigma(I)$ (%)	78.8 (56.8)
Completeness (%)	94.3 (94.3)
Redundancy	4.2 (2.6)

† $R_{\rm merge}(I) = \sum_h \sum_i |I_i(h) - \langle I(h)\rangle| / \sum_h \sum_i I_i(h)$, where $I_i(h)$ is the intensity of the ith observation of reflection h.

3. Results and discussion

CBH IB crystallized in the tetragonal space group $P4_12_12$, with unit-cell parameters $a = b = 74.4$, $c = 176.9$ Å, and initially diffracted to a maximum of 1.77 Å resolution; however, owing to radiation damage over the course of the experiment only data to 2.30 Å were useable. The solvent content, estimated according to the method of Matthews (1968), was 43.2%, with one molecule (MW = 56 kDa) in the asymmetric unit and a Matthews coefficient of 2.2 Å^3 Da^{-1}. Molecular replacement was performed using the *CCP*4 (Collaborative Computational Project, Number 4, 1994) program *AMoRe* (Navaza, 1994). The coordinates of the catalytic domain (434 residues) of Cel7A from *T. reesei* (PDB code 1cel; Divne *et al.*, 1994), which has a 64% sequence identity with the CBH IB from *Tal. emersonii*, was used as the search model. A single solution was obtained with a correlation coefficient of 0.611 and an *R* factor of 39.6%. Further experiments are under way to determine cryoconditions so that a complete data set can be collected to the diffraction limit of these crystals. Since the crystallization of this protein, the complete genomic and coding sequence of CBH IB has been determined (GENBANK accession number AF439935).

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