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Crystallization and preliminary X-ray analysis of a cold-active endo-β-1,4-D-xylanase from glycoside hydrolase family 8

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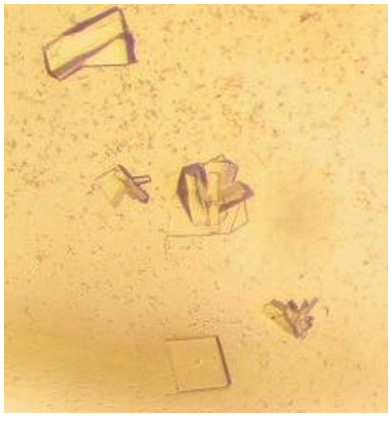

Endo-β-1,4-D-xylanases are used in a multitude of industrial applications. Native crystals of a cold-adapted xylanase from glycoside hydrolase family 8 were obtained by the vapour-diffusion technique. The crystals belonged to space group $I222$, with unit-cell parameters a = 46.6, b = 110.8, c = 150.2 Å at 100 K, and diffracted to 2.7 Å resolution at a synchrotron source. The asymmetric unit is likely to contain one molecule, with a V_M of 2.07 Å^3 Da^{-1}, corresponding to a solvent content of ∼40%.

1. Introduction

The highly complex polymer xylan is the main component of hemicellulose. Endo-β-1,4-D-xylanases (xylanases; EC 3.2.1.8) play a crucial role in degrading this macromolecule by cleaving its internal xylosidic bonds. These enzymes are used in a large number of industrial processes. For instance, in the pulp and paper industry xylanases can be used as an effective substitute for the polluting chemicals that are used in the prebleaching of kraft pulp (Bajpai, 1999; Beg *et al.*, 2001). Moreover, xylanases are used as food additives to poultry (Bedford & Classen, 1992) or in wheat flour to facilitate dough handling and to improve the quality of bakery products (Maat *et al.*, 1992). They are also used for the extraction of coffee, plant oils and starch (Wong & Saddler, 1992), for the improvement of the nutritional properties of agricultural silage and grain feed (Kuhad & Singh, 1993) and, together with pectinase and cellulase, for the clarification of fruit juices (Biely, 1985). Xylanases are also used in the degumming of plant fibre sources such as flax, ramie and jute (Sharma, 1987; Puchart *et al.*, 1999; Kapoor *et al.*, 2001). The commercial bottleneck in the conversion of cellulosic biomass to biofuels such as propanol or especially ethanol is the degradation of the polymers to small sugar molecules (Lynd *et al.*, 2008). Here, xylanases could also help in the production of inexpensive biofuels in order to achieve competitive marketability. Thus, a deeper understanding of the properties and function of xylanases is of great value for the design of better enzymes, in particular for industrial processes.

Because of their stability, thermophilic enzymes are generally preferable for commercial purposes (Lee & Lim, 2004). In some cases, however, psychrophilic enzymes can also be advantageous (Gerday *et al.*, 2000) since low temperature can preserve heat-labile products, enzymes can be deactivated by heat and the use of a lower temperature could also help by cutting energy costs (Lee *et al.*, 2006).

Glycoside hydrolase family (GH) 8 includes cellulases, lichenases, chitosanases and various other xylanases which all share an $(\alpha/\alpha)_6$ fold (Collins, Gerday *et al.*, 2005). The psychrophilic Xyn8 was discovered using a metagenomic approach by screening a genomic DNA library from a population of microorganisms from the waste lagoon of a dairy farm. According to its predicted catalytical domain, Xyn8 belongs to the GH8 family (Lee *et al.*, 2006; Cantarel *et al.*, 2009; http://www.cazy.org). In comparison with the well studied and industrially important GH10 and GH11 xylanases, the GH8 xylanases have so far shown very different substrate specificities. In particular, Xyn8 preferably releases xylotrioses from its substrate. Moreover, Xyn8 favours substrates with a low degree of polymerization. It is believed that only a few amino-acid substitutions may cause the

differences in substrate specificity between Xyn8 and other GH8 members (Pollet *et al.*, 2010). Structural information gained by crystallography could strengthen this hypothesis and streamline subsequent attempts to engineer these biotechnologically interesting enzymes. In this paper, we describe the crystallization and preliminary X-ray analysis of Xyn8.

2. Materials and methods

2.1. Protein expression and purification

The plasmid DNA for Xyn8 was kindly provided by Dr Charles C. Lee (Agricultural Research Service, Albany, California, USA; Lee *et al.*, 2006). The recombinant Xyn8 is C-terminally His_6-tagged and contains 406 residues. The protein was recombinantly expressed in *Escherichia coli* and purified as described elsewhere (Pollet *et al.*, 2010). Xyn8 was rebuffered in 20 m*M* Na HEPES pH 7.5 by dialysis and concentrated to 12.5 mg ml^{-1} in an Amicon ultrafiltration device (Millipore, USA) using the same buffer. The protein concentration was determined from the absorbance at 280 nm and the purity of the protein was judged by nonreducing SDS–PAGE. Electrospray ionization mass spectrometry using a Q-ToF2 (Micromass, England) was used to determine the exact mass of Xyn8.

2.2. Protein crystallization

Initial crystallization screening of Xyn8 was performed with a Mosquito Crystal robot (TTP LabTech, England) using commercial kits from Hampton Research (Crystal Screen, Crystal Screen 2 and Index), Emerald BioSystems (Wizard I and II) and Qiagen (JCSG+ and PACT) at 293 K. The sitting-drop method was used for optimization of the initially obtained crystals, manually mixing 5 µl protein sample and 5 µl reservoir in Cryschem plates (Hampton Research, USA) at 293 K.

2.3. Data collection and analysis

Prior to data collection, the crystal was soaked for a few seconds in cryoprotectant solution composed of 100 m*M* Na HEPES pH 7.5, 20%(*w*/*v*) PEG 3350, 10%(*v*/*v*) PEG 400. The crystal was harvested with a cryoloop and vitrified in liquid nitrogen. Diffraction data were collected on beamline X06DA of the Swiss Light Source, Villigen, Switzerland using a MAR Research 225 Mosaic charge-coupled device (CCD) detector. During the measurements, the crystal was cryocooled to approximately 100 K using a Cryojet cryostream (Oxford Cryosystems, Oxford, England). A total of 180 frames were collected at λ = 0.9798 Å with an oscillation range of 0.5° and an exposure time of 2 s per frame. The crystal-to-detector distance was 180 mm.

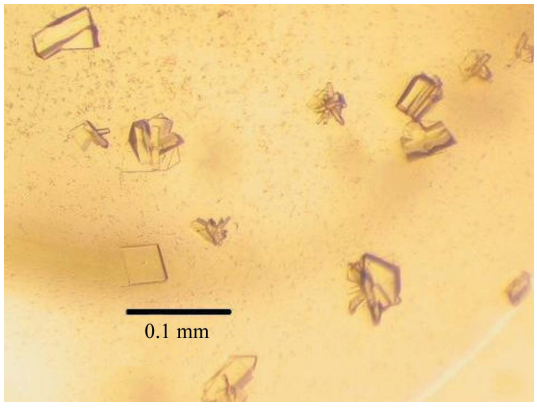

Figure 1
Crystals of Xyn8 belonging to space group *I*222.

Table 1
Data-collection statistics.

Values in parentheses are for the highest resolution shell.

Resolution range (Å)	89.16–2.70 (2.77–2.70)
Measured reflections	37459 (2402)
Unique reflections	11065 (775)
Multiplicity	3.4 (3.1)
Completeness (%)	99.7 (96.2)
Mean $I/\sigma(I)$	11.0 (3.7)
$R_{\rm merge}$† (%)	8.3 (27.4)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the *i*th intensity measurement of reflection *hkl* and $\langle I(hkl)\rangle$ is the weighted mean of all intensity measurements of *hkl*.

The obtained native data set was integrated using the program *iMOSFLM* (Leslie, 1992). Data reduction was performed with *SCALA* and *CTRUNCATE* from the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994). *phenix.xtriage* as implemented in *PHENIX* (Adams *et al.*, 2010) was used to detect potential twinning of the crystals.

3. Results and discussion

The purified Xyn8 was estimated to be >95% pure. Mass spectrometry precisely confirmed the mass calculated from the polypeptide chain (46 899 Da). This indicated that no post-translational modifications had occurred during the overexpression procedure. Initial crystallization conditions were obtained from the PACT screen [0.2 *M* sodium iodide, 0.1 *M* bis-tris propane pH 7.5, 20%(*w*/*v*) PEG 3350]. These conditions could be reproduced on a larger scale and were finally optimized to 20 m*M* Na HEPES pH 7.7, 120 m*M* sodium iodide, 15%(*w*/*v*) PEG 3350. The optimized crystals grew within a few days to a much larger size and and were less twinned (Fig. 1). Further optimization trials such as the use of additive screens or lowering the temperature to 277 K did not show any positive effect on crystal growth.

Cryoprotection was essential to prevent ice formation and hence damage to the crystal since no reflections could be observed when the crystals were cryoprotected in mother liquor.

The crystals belonged to the body-centred orthorhombic space group *I*222, with unit-cell parameters a = 46.6, b = 110.8, c = 150.2 Å at 100 K. Assuming one polypeptide chain per asymmetric unit leads to a likely $V_{\rm M}$ value (Matthews, 1968) of 2.07 Å^3 Da^{-1}, which corresponds to a solvent content of about 40%. Based on the Wilson plot, the average *B* factor is 54.4 Å^2. Additional statistics are given in Table 1. There were no indications of twinning.

The primary structure of Xyn8 shares about 43% sequence identity to a reducing-end xylose-releasing exo-oligoxylanase from *Bacillus halodurans* (PDB codes 1wu4, 1wu5, 1wu6, 3a3v, 2drr, 2drs, 2drq and 2dro; Fushinobu *et al.*, 2005; Hidaka *et al.*, 2009) and about 33% sequence identity to a cold-adapted GH8 xylanase from *Pseudoalteromonas haloplanktis* (PDB codes 1xw2, 1xwq, 1xwt, 2a8z, 2b4f, 1h12, 1h13 and 1h14; Collins, De Vos *et al.*, 2005; De Vos *et al.*, 2006; Van Petegem *et al.*, 2003). Trials to solve the structure of Xyn8 by molecular replacement using the known three-dimensional structures of the above-mentioned enzymes are in progress.

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