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Crystallization and preliminary X-ray crystallographic analysis of β-xylosidase from *Thermoanaerobacterium saccharolyticum*, a thermophilic anaerobe

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β-Xylosidases are involved in the breakdown of xylans into xylose and belong to either family 39 or 43 of the glycosyl hydrolases. At present, no structural information is available for any member of these families. β-Xylosidase from the thermophilic anaerobe *Thermoanaerobacterium saccharolyticum*, a member of glycosyl hydrolase family 39, has been crystallized at 296 K using the hanging-drop vapour-diffusion method. The crystal diffracts to 2.4 Å resolution with synchrotron X-rays and belongs to space group 4_12_12 (or $P4_32_12$), with unit-cell parameters $a = b = 92.75$, $c = 241.37$ Å. The asymmetric unit contains two monomers of the recombinant enzyme, giving a corresponding V_M of 2.21 Å^3 Da^{-1} and a solvent content of 44.3%.

1. Introduction

1,4-β-D-Xylans represent the most abundant polysaccharides constituting the plant cell-wall hemicelluloses. Xylans are composed of a backbone of β-1,4-linked D-xylopyranosyl units harboring arabinofuranose, glucuronic acid, methylglucuronic acid and acetyl side-groups. Complete breakdown of xylan requires the action of several hydrolytic enzymes as follows: (i) α-*N*-arabinofuranosidase, α-glucuronidase and acetylesterase cleave the lateral substituents, (ii) endo-1,4-β-xylanase randomly cleaves the β-1,4 bonds in the xylan backbone, producing xylo-oligosaccharides, xylobiose and xylose and (iii) β-xylosidase (or 1,4-β-D-xylan xylohydrolase; EC 3.2.1.37) hydrolyzes the resulting xylo-oligosaccharides at their non-reducing end, producing xylose (Saha & Bothast, 1999).

Glycosyl hydrolases have been grouped into a number of families based on general amino-acid sequence similarities. Some of these families have been grouped into 'clans' based also on sequence similarities or on structure comparisons (Henrissat, 1991; Henrissat & Bairoch, 1993). Inside a glycosyl hydrolase family and inside a clan, enzymes typically share a similar three-dimensional fold and catalytic mechanism. Two catalytic mechanisms have been described for glycosyl hydrolases depending on the stereochemical outcome of catalysis. In the retaining mechanism, the product retains the same anomeric stereochemistry as the substrate. In the inverting mechanism, the product has the inverse anomeric stereochemistry.

β-Xylosidases have been grouped into glycosyl hydrolase families 39 and 43, which belong to the GH-A and GH-F clans, respectively. While the hydrolytic mechanism of family 43 β-xylosidases results in inversion of the anomeric configuration (Braun *et al.*, 1993), family 39 β-xylosidases operate *via* a retaining mechanism (Armand *et al.*, 1996). No crystal structures have been reported for any β-xylosidases of either family. Low sequence identity among families of the GH-A clan makes it difficult to compare the sequences by simple alignment methods. However, hydrophobic cluster analysis of the protein sequences of the GH-A clan suggests that family 39 members may share a similar catalytic domain consisting of a $(\beta/\alpha)_8$ barrel fold (Durand *et al.*, 1997).

β-Xylosidase from the thermophilic anaerobe *T. saccharolyticum*, a 500-residue protein with a calculated molecular mass of 58 606 Da, is one of the best characterized members of the family 39 glycosyl hydrolases. The *T. saccharolyticum* β-xylosidase gene (*xynB*) has been expressed at high levels in *Escherichia coli* and the recombinant enzyme was shown to be optimally active at 343 K (Lee & Zeikus, 1993). *T. saccharolyticum* β-xylosidase displays a significant transglycosylating activity, a property often found among glycosyl hydrolases operating *via* a retaining mechanism. The transglycosylation activity of *T. saccharolyticum* β-xylosidase shows little regiospecificity, since this enzyme can synthesize β-1,2-, β-1,3- and β-1,4-linked xylo-oligosaccharides (Armand *et al.*, 1996). The residue acting as the catalytic nucleophile in *T. saccharolyticum* β-xylosidase was identified as Glu277 (Vocadlo *et al.*, 1998), while Glu160 in the sequence Asn-Glu-Pro was identified as the possible acid/base catalyst. The same Asn-Glu-Pro sequence contains the acid/base catalyst in family 1 *Agrobacterium* sp. β-glucosidase and in family 10 xylanases. Both families belong to the GH-A clan (Vocadlo *et*

al., 1998) like family 39 β-xylosidases. Crystallization of a fungal β-xylosidase from *Trichoderma reesei* has been reported (Golubev *et al.*, 2000). In order to provide the missing structural information and to better understand the catalytic mechanism of family 39 β-xylosidases, we have initiated the structure determination of *T. saccharolyticum* β-xylosidase. Here, we report its crystallization and preliminary X-ray crystallographic data.

2. Experimental

2.1. Protein purification

Recombinant *T. saccharolyticum* β-xylosidase was overexpressed in *Escherichia coli* strain XA-90 (Amann *et al.*, 1983) harbouring the plasmid pXHP3 (Lee & Zeikus, 1993). Its purification was performed as described in Armand *et al.* (1996), except that two additional chromatography steps were added. After the ion-exchange chromatography described previously, the enzyme solution was supplemented with 0.5 *M* NaCl and 10%(*v*/*v*) glycerol. This solution was applied to a phenyl-Sepharose FF column (Amersham Pharmacia). β-xylosidase was recovered in the flow-through, whereas some of the contaminating proteins remained adsorbed on the column. Next, gel filtration was performed on a HiLoad XK 16 Superdex 200 prep-grade column (Amersham-Pharmacia), which was previously equilibrated with buffer *A* (50 m*M* Tris–HCl pH 7.0, 200 m*M* NaCl). Finally, the purified enzyme was concentrated to 10 mg ml^{-1} using a YM30 membrane (Amicon) and stored at 253 K. The protein concentration was estimated by measuring the absorbance at 280 nm, employing the calculated extinction coefficient of $1.01 \times 10^5\ M^{-1}\ \mathrm{cm}^{-1}$.

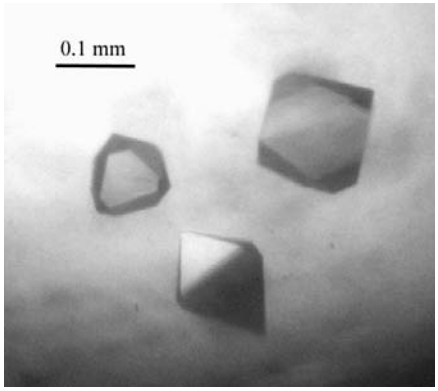

Figure 1
Crystals of *T. saccharolyticum* β-xylosidase. The largest crystals have approximate dimensions of 0.1 × 0.1 × 0.1 mm.

2.2. Crystallization

Crystallization was achieved by the hanging-drop vapour-diffusion method at 296 K using 24-well VDX plates (Hampton Research). Before preparing hanging drops, 100 µl of β-xylosidase solution at 10 mg ml^{-1} concentration was mixed with 2 µl of 1.0 *M* D-xylose. Each hanging drop, prepared by mixing equal volumes (2 µl each) of the protein solution containing D-xylose and the reservoir solution, was placed over a 1.0 ml reservoir solution. Initial crystallization conditions were established using screening kits from Hampton Research (Crystal Screen I, II and MembFac) and from Emerald BioStructures, Inc. (Wizard I and II).

2.3. Data collection

X-ray diffraction data were collected on a DIP-2030 image-plate detector (MacScience Co.) using 1.000 Å X-rays at beamline BL-6B of Pohang Light Source, South Korea. The crystal was mounted in a nylon loop (Hampton Research) after quickly soaking in a cryoprotectant solution consisting of the same reservoir solution, except that the concentration of polyethylene glycol 2000 monomethyl ether (PEG 2000 MME) was raised to 39%(*w*/*v*). The crystal was flash-frozen at 100 K during data collection and was rotated through a total of 90° with a 1.0° oscillation range per frame. The data set was processed and scaled using the *HKL* program suite (Otwinowski & Minor, 1997).

3. Results

Well diffracting crystals of *T. saccharolyticum* β-xylosidase were obtained in the presence of D-xylose with the optimized reservoir solution comprising 24%(*w*/*v*) PEG 2000 MME, 5 m*M* dithiothreitol and 0.1 *M* Tris–HCl pH 7.0. The crystals grew to maximum dimensions of 0.1 × 0.1 × 0.1 mm within one week (Fig. 1) and the crystallization required the presence of D-xylose.

The crystals diffracted to 2.4 Å resolution with synchrotron X-rays. A total of 37 528 unique reflections were measured with a redundancy of 4.6. The merged data set is 89.1% complete to 2.4 Å and gives an $R_{\rm merge}$ of 6.6%. The space group was determined to be $P4_12_12$ (or $P4_32_12$) on the basis of systematic absences, with unit-cell parameters $a = b = 92.75$, $c = 241.37$ Å. The asymmetric unit contains two monomers of β-xylosidase, giving a $V_{\rm M}$ of 2.21 Å^3 Da^{-1} and a solvent content of 44.3% (Matthews, 1968). Table 1 summarizes the statistics for data collection. As molecular replacement is not possible, the structure will be solved by the multiple-wavelength anomalous diffraction method.

Table 1
Data-collection statistics.

Values in parentheses refer to the highest resolution shell (2.49–2.40 Å).

X-ray wavelength (Å)	1.000 (Pohang Light Source BL-6B)
Space group	$P4_12_12$ (or $P4_32_12$)
Unit-cell parameters (Å)	$a = b = 92.75$, $c = 241.37$
Resolution (Å)	20.0–2.40
No. of measured reflections	172649
No. of unique reflections	37 528
$R_{\rm merge}$† (%)	6.6 (24.7)
Data completeness (%)	89.1 (76.6)
$\langle I/\sigma(I)\rangle$	15.1 (2.3)
Redundancy	4.6 (2.4)

† $R_{\rm merge} = \sum_h \sum_i |I(h)_i - \langle I(h)\rangle| / \sum_h \sum_i I(h)_i$, where $I(h)$ is the intensity of reflection h, $\sum_h$ is the sum over all reflections and $\sum_i$ is the sum over i measurements of reflection h.

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