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Crystallization and preliminary crystallographic analysis of a thermostable family 52 β-D-xylosidase from *Geobacillus stearothermophilus* T-6

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β-D-Xylosidases (EC 3.2.1.37) are hemicellulases that hydrolyze short xylooligosaccharides into single xylose units. In this study, the first crystallization and preliminary X-ray analysis of a family 52 glycoside hydrolase, the β-D-xylosidase (XynB2) from *Geobacillus stearothermophilus* T-6, is described. XynB2 is a dimeric protein consisting of two identical subunits of 705 amino acids with a calculated molecular weight of 79 894 Da. XynB2 was crystallized by the hanging-drop vapour-diffusion method and the crystals were found to belong to space group *P*1, with unit-cell parameters a = 80.6, b = 97.5, c = 107.2 Å, α = 107.4, β = 98.2, γ = 106.6°. The native crystals diffracted X-rays to a resolution of 2.0 Å.

1. Introduction

β-D-Xylosidases (EC 3.2.1.37) are hemicellulases that hydrolyze short xylooligosaccharides into single xylose units. These enzymes are part of an array of glycoside hydrolases responsible for the complete degradation of xylan (Shallom & Shoham, 2003). Xylan is the major hemicellulosic polysaccharide in the plant cell wall, representing up to 30–35% of the total dry mass (Beg *et al.*, 2001). The polymer is composed of a β-1,4-linked xylopyranosyl backbone substituted with different side chains such as arabinofuranose, methyl glucuronic acid and acetyl. Together with cellulases, hemicellulases play a key role in the carbon cycle in nature, since they are responsible for the complete degradation of plant biomass to soluble saccharides. These in turn are used as carbon and energy sources for microorganisms and higher animals. Hemicellulases gained much attention owing to their capability to reduce costs and environmental impact in various industrial and biotechnological processes such as biobleaching in the paper and pulp industry (Beg *et al.*, 2001; Suurnakki *et al.*, 1997), the degradation of lignocellulosic material for the production of bio-ethanol (Galbe & Zacchi, 2002; Mielenz, 2001) and recently in the field of oligosaccharide and thioglycoside synthesis (Jahn *et al.*, 2003, 2004; Mackenzie *et al.*, 1998). The glycosidic bond is one of the most stable bonds in nature, with a half-life of over 5×10^6 y (Wolfenden *et al.*, 1998). Glycoside hydrolases can accelerate the hydrolysis of the glycosidic bond by more than 10^{17}-fold, making them one of the most proficient catalysts in nature. The enzymatic hydrolysis of the glycosidic bonds occurs *via* two major mechanisms, in which the anomeric configuration of the substrate is either retained or inverted. In both mechanisms, the hydrolysis usually requires two carboxylic acids, which are conserved within each glycoside hydrolase family, and proceeds through oxocarbenium-ion-like transition states. Inverting glycosidases use a single displacement mechanism with the assistance of a general acid and a general base catalytic residues. Retaining glycosidases follow a two-step double-displacement mechanism involving two catalytic residues, one functioning as a nucleophile and the other as an acid–base (Davies *et al.*, 1998).

Based on amino-acid sequence similarities, β-D-xylosidases are currently divided into families 3, 39, 43, 52 and 54 of the glycoside hydrolases (Henrissat & Bairoch, 1996; Henrissat & Davies, 1997). These families, together with all other glycoside hydrolase families, can be readily accessed at the website http://afmb.cnrs-mrs.fr/CAZY. Most of the β-D-xylosidase families have been extensively studied and a large body of kinetic and mechanistic data has been collected. The enzymes of family 43 were shown to cleave the glycosidic bond with inversion of the anomeric configuration (Braun *et al.*, 1993) and, very recently, the crystal structure of a member of this family was determined, showing a unique topology (Nurizzo *et al.*, 2002). Members of family 39 were found to proceed *via* retention of the anomeric configuration (Armand *et al.*, 1996) and their catalytic residues have been determined (Bravman, Mechaly *et al.*, 2001; Vocadlo *et al.*, 1998; Vocadlo *et al.*, 2002*a*,*b*). Three β-xylosidases from families 3 and 39 have been crystallized previously (Yang *et al.*,

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2002; Golubev *et al.*, 2000; Czjzek *et al.*, 2004); recently, the crystal structure of a member of family 39 has been reported (Yang *et al.*, 2004).

Previously, we reported the cloning and sequencing of a 23.5 kbp chromosomal segment from *Geobacillus stearothermophilus* that contains a cluster of xylan-utilization genes. This segment includes an open reading frame of a β-xylosidase (*xynB2*) gene showing homology to family 52 glycoside hydrolases (Shulami *et al.*, 1999). The *xynB2* gene encodes a 705-amino-acid protein, XynB2, with a calculated molecular weight of 79 894 Da. Based on gel filtration, the enzyme is a homodimer in solution. The *xynB2* gene can be easily overexpressed and purified and thus can serve as an excellent candidate for structure–function studies and as a representative of family 52 glycoside hydrolases. Recently, we determined the stereochemical course of hydrolysis of XynB2 and described a detailed kinetic analysis of the enzyme together with the identification of its catalytic pair (the acid–base and the nucleophile residues; Bravman, Belakhov *et al.*, 2003; Bravman, Zolotnitsky *et al.*, 2001, 2003).

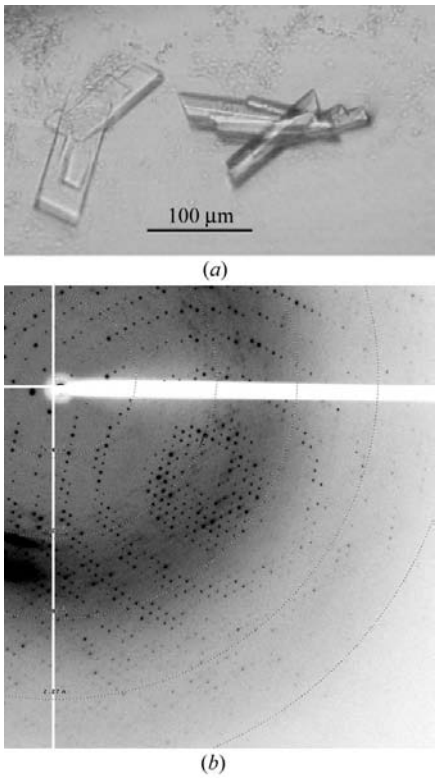

Figure 1
(*a*) A photograph of crystals of XynB2 from *G. stearothermophilus* T-6. (*b*) An extract from a typical X-ray diffraction image from a XynB2 crystal. The 1° oscillation image was taken at the ESRF (Grenoble, France) on an ADSC CCD detector (Quantum 4) at beamline ID14-EH4. The resolution is 2.0 Å at the edges of the plate.

In this study, we describe the crystallization and preliminary X-ray analysis of the family 52 β-D-xylosidase from *G. stearothermophilus* T-6. Its three-dimensional structure alone and in complex with with various substrates will provide meaningful insights into the binding properties and specificity of family 52 glycoside hydrolases.

2. Experimental

2.1. Overexpression and purification of XynB2

The *xynB2* gene (GenBank accession No. AJ305327) from *G. stearothermophilus* T-6 was cloned in the pET9d vector and overexpressed in *Escherichia coli* BL21(DE3). The purification procedure involved two steps, heat treatment at 333 K and gel filtration, as previously described (Bravman, Zolotnitsky *et al.*, 2001) and resulted in near-gram quantities of >99% purified enzyme.

2.2. Crystallization of XynB2

The protein concentration was 5 mg ml^{-1} in a solution containing 50 m*M* Tris buffer pH 7, 100 m*M* NaCl and 0.02% NaN_3. Initial crystallization trials were performed with MDL (Molecular Dimensions Limited) and Decode Genetics (Wizard 1 and 2, Emerald Biostructures) screens: a total of 192 trials were conducted in two 96-well crystallization plates (Greiner). These trials were set up using a Cartesian crystallization robot, mixing 200 nl of protein solution with 100 nl of reservoir solution. The most promising initial crystallization conditions were reproduced in 24-well Linbro plates using the hanging-drop vapour-diffusion method. 2 µl protein solution was mixed with 2 µl well solution and subsequently positioned over wells containing 0.5 ml well solution. In a second step, these conditions were then optimized by variation of the precipitant, additive and protein concentration, pH and drop volume. The best formed crystals were obtained from well solutions containing 2.3–2.4 *M* ammonium sulfate and 40–60 m*M* sodium citrate buffer pH 5.5.

2.3. Data collection and processing

X-ray diffraction data were collected from a native XynB2 crystal at 100 K on beamline ID14-EH4 at the European Synchrotron Radiation Facility (Grenoble, France) using an ADSC Quantum 4R CCD detector. All crystals were flash-cooled in a liquid-nitrogen stream with 15%(*v*/*v*) glycerol as a cryoprotectant. The wavelength of the synchrotron X-rays was 0.979 Å. The crystal was rotated through 550° with a 1° oscillation range per frame. All raw data were processed using the program *MOSFLM* (Leslie, 1990). The resultant data were merged and scaled using the program *SCALA* (Collaborative Computational Project, Number 4, 1994).

Table 1
Data-collection statistics.

Values in parentheses correspond to the highest resolution shell.

ESRF beamline	ID14-EH4
Wavelength (Å)	0.979
Space group	*P*1
Unit-cell parameters (Å, °)	a = 80.6, b = 97.5, c = 107.2, α = 107.4, β = 98.2, γ = 106.6
Resolution (Å)	24.9–2.0 (2.11–2.0)
No. observations	1103805
No. unique reflections	189655
R_{sym}† (%)	5.9 (25.3)
$\langle I/\sigma(I)\rangle$	19.1 (5.4)
Redundancy	5.8 (5.5)
Completeness (%)	97.1 (95.7)

† $R_{sym} = \sum|I - I_{av}|/\sum I$, where the summation is over all symmetry-equivalent reflections.

3. Results

XynB2 was overexpressed in soluble form in a sufficient quantity for crystallization. The optimized reservoir conditions were 2.3–2.4 *M* ammonium sulfate and 40–60 m*M* sodium citrate buffer pH 5.5. Crystals of native XynB2 grew to maximum dimensions of 0.15 × 0.15 × 0.05 mm within a few weeks (Fig. 1*a*).

The native crystals diffracted to at least 2.0 Å Bragg spacing (Fig. 1*b*) and the unit-cell parameters were a = 80.6, b = 97.5, c = 107.2 Å, α = 107.4, β = 98.2, γ = 106.6° in space group *P*1. Since the biochemical characterization of XynB2 has shown that the protein is a homodimer in solution (Bravman, Zolotnitsky *et al.*, 2001), the asymmetric unit most probably contains four molecules (two dimers), each with a weight of 79.9 kDa. This gives a crystal volume per protein weight (V_M) of 2.4 Å^3 Da^{-1} and a solvent content of 48% by volume (Matthews, 1968). The presence of two dimers per asymmetric unit is confirmed by the self-rotation function, which displays a peak corresponding to the presence of a non-crystallographic fourfold axis. A complete data set to 2.0 Å resolution was collected at the European Synchrotron Radiation Facility (Grenoble, France) beamline ID14-EH4 and the data-collection statistics are reported in Table 1.

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