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Crystallization and preliminary X-ray crystallographic studies of the mesophilic xylanase A from *Bacillus subtilis* 1A1

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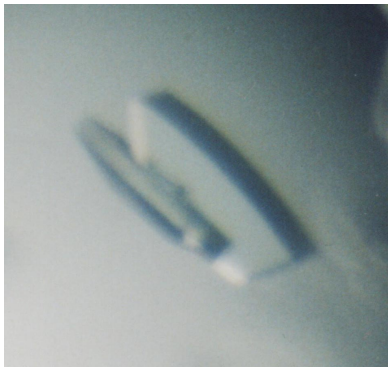


Xylanases have been the focus of research owing to their industrial potential in animal feed production, food processing and pulp and paper processes. In order to obtain insight into the structural stability of family 11 xylanases, the mesophilic family 11 xylanase (β-1,4-xylan xylanohydrolase; EC 3.2.1.8) from *Bacillus subtilis* 1A1 has been crystallized and diffraction data have been collected to 1.7 Å. The crystals belong to the orthorhombic space group $P2_12_12$, with unit-cell parameters $a = 50.93$, $b = 70.50$, $c = 40.05$ Å. The structure has been determined by molecular replacement, resulting in a crystallographic residual of 36.4% after rigid-body refinement.

1. Introduction

Xylanases (β-1,4-xylan xylanohydrolases; EC 3.2.1.8) cleave the β-1,4-xylosidic linkages of xylans and are of interest to the animal-feed, food-processing, and pulp and paper industries, where the use of xylanase has been found to be effective in reducing chlorine-dosage requirements in the Kraft pulp-bleaching process. Because of the industrial potential of these enzymes, a large number of bacterial and fungal xylanase genes have been isolated, sequenced and expressed as heterologous proteins.

On the basis of amino-acid sequence similarities, xylanases are classified into glycosyl hydrolase families 10 and 11 (Henrissat, 1991). The family 10 enzymes have an $(\alpha/\beta)_8$-barrel fold with a molecular weight of approximately 35 kDa. Family 11 xylanases are somewhat smaller, approximately 20 kDa, and their fold contains an α-helix and two β-sheets packed against each other, forming a so-called β-sandwich. Family 11 xylanases have been well studied because of their direct use in bio-bleaching in the paper industry (Gilkes *et al.*, 1991; Henrissat & Bairoch, 1993; Wakarchuk, Sung *et al.*, 1994; Törrönen *et al.*, 1994; Gruber *et al.*, 1998; McCarthy *et al.*, 2000; Wouters *et al.*, 2001; Oakley *et al.*, 2003; Moiseeva & Allaire, 2004), especially the mesophilic xylanases expressed by *Bacillus circulans* (PDB code 1xnb) and *Trichoderma longibrachiatum* (PDB codes 1enx and 1xyn). These enzymes are gaining importance since they serve as model enzymes for investigating protein folding, thermal stability, pH stability, enantiospecificity and stereospecificity (Wakarchuk, Campbell *et al.*, 1994; Törrönen & Rouvinen, 1995; Krengel & Dijkstra, 1996; Törrönen & Rouvinen, 1997; Fushinobu *et al.*, 1998; Sabini *et al.*, 1999). We present the results of the crystallization, data collection to 1.7 Å and initial structure determination of the mesophilic family 11 xylanase from *B. subtilis*. Comparative studies of this data and thermostable mutants generated by *in vitro* molecular evolution will be employed to obtain insight as to the structural basis of the thermostability of family 11 xylanases.

2. Methods

2.1. Dynamic light scattering and crystallization

Recombinant family 11 xylanase from *B. subtilis* strain 1A1 was expressed in *Escherichia coli* DH5α and purified from culture supernatants by cation-exchange chromatography as described elsewhere (Ruller *et al.*, 2005). Dynamic light-scattering experiments were carried out at 293 K using a DynaPro 810 (Protein Solutions Co.) equipped with a temperature stabilizer. A 3.4 mg ml^{-1} protein solution was prepared in 20 m*M* HEPES buffer pH 7.5. Standard

curves of bovine serum albumin were used for calibration and the experiments were conducted at 291 K. For crystallization, the protein was dialyzed and concentrated to 4.5 mg ml^{-1} against 20 m*M* HEPES pH 7.5. The protein was crystallized by the hanging-drop vapour-diffusion method at 291 K. HR2-110 and HR2-112 screens (Hampton Research) were used initially. Irregular and highly twinned crystals were obtained after 3 d in 2 µl droplets (1 µl protein solution and 1 µl reservoir solution) which contained 0.9 *M* potassium sodium tartrate tetrahydrate (Fig. 1*a*). Crystals suitable for X-ray diffraction were obtained using the same conditions upon addition of 1%(*v*/*v*) dioxane, a reagent commonly used to overcome twinning (Bergfors, 1999) (Fig. 1*b*).

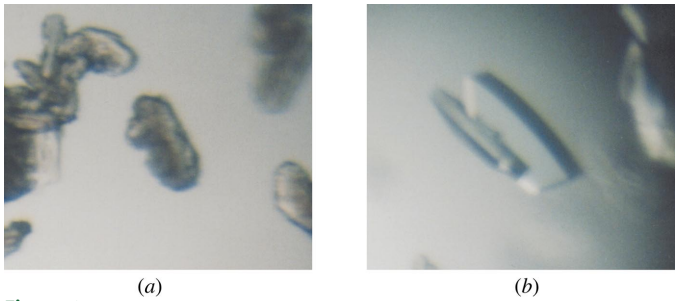
2.2. X-ray diffraction analysis

X-ray diffraction data were collected at the CPr beamline of the synchrotron source at the Laboratório Nacional de Luz Síncrotron, Brazil. A single crystal with a maximum dimension of 0.3 mm was soaked in mother liquor containing 25% glycerol and flash-frozen at 100 K. The diffraction data were processed with *DENZO* and scaled with *SCALEPACK* (Otwinowski & Minor, 1997) to a resolution of 1.7 Å. Molecular replacement was carried out using the program *AMoRe* (Navaza, 1994) and a model based on the atomic coordinates of the xylanase from *B. circulans* (PDB code 1hv0).

3. Results

The structural homogeneity of the xylanase from *B. subtilis* in solution was verified by dynamic light scattering, which demonstrated a monomodal distribution with a hydrodynamic radius of 1.7 nm, corresponding to a molecular weight of 22 kDa. Suitable crystals for X-ray diffraction analysis with a maximum dimension of 0.3 mm (Fig. 1*b*) were obtained in the presence of 1%(*v*/*v*) dioxane and diffraction data were collected to 1.7 Å at cryogenic temperature (100 K). The diffraction data were indexed in the orthorhombic space group $P2_12_12$, with unit-cell parameters a = 50.93, b = 70.50, c = 40.05 Å. Processing the 21 487 measured reflections to 1.7 Å resulted in a data set containing 14 449 unique reflections, with an $R_{\rm merge}$ of 9.2% (26.9% in the last shell) and a completeness of 83.5% (81.3% in the last shell). The Merohedral Crystal Twinning Server (Yeates, 1997) clearly indicated that the data were not twinned ($\langle I^2\rangle/\langle I\rangle^2 > 1.95$ in all resolution shells). Data-processing statistics are summarized in Table 1.

Assuming a molecular weight of 20.38 kDa, calculation of the Matthews coefficient resulted in a $V_{\rm M}$ value of 1.7 Å^3 Da^{-1} for the presence of one molecule in the asymmetric unit, corresponding to a solvent content of 27.7% (Matthews, 1968). Molecular replacement was carried out using the program *AMoRe* (Navaza, 1994) and the atomic coordinates of a xylanase from *B. circulans* (PDB code 1hv0). A unique rotation and translation solution was obtained for the single molecule in the asymmetric unit. Rigid-body refinement of the solution using data in the resolution range 41.9–1.7 Å resulted in a correlation coefficient of 65.4% and an R factor of 36.4% (Table 1). The structure of the xylanase A from *B. subtilis* 1A1 contains an α-helix and two β-sheets packed against each other, indicating that it belongs to the β-sandwich class of proteins.



(*a*) (*b*)

Figure 1
Photomicrographs of crystals of mesophilic xylanase A from *B. subtilis* (*a*) in the absence and (*b*) in the presence of 1%(*v*/*v*) dioxane.

Table 1
Data-collection and structure-determination statistics for *B. subtilis* xylanase A.

Values in parentheses are for the high-resolution bin.

Data collection	
Synchrotron-radiation source	LNLS Brazil, beamline CPr
Temperature (K)	100
Wavelength used (Å)	1.427
Detector	MAR CCD
Space group	$P2_12_12$
Unit-cell parameters (Å)	a = 50.93, b = 70.50, c = 40.05
Resolution range (Å)	41.9–1.7
No. observed reflections	21487 (1850)
Data completeness (%)	83.5 (81.3)
No. unique reflections	14449 (1364)
$I/\sigma(I)$	7.16 (2.5)
$R_{\rm merge}$† (%)	9.2 (26.9)
Molecules per asymmetric unit	1
Molecular replacement	
Correlation coefficients (%)	
Rotation	33.7
Translation	52.3
Rigid-body refinement	65.4
Rigid-body refinement R factor (%)	36.4

† $R_{\rm merge} = \sum|I(h)_i - \langle I(h)\rangle|/\sum\langle I(h)\rangle$, where $I(h)_i$ is the observed intensity of the ith measurement of reflection h and $\langle I(h)\rangle$ is the mean intensity of reflection h calculated after scaling.

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