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Crystallization and preliminary crystallographic analysis of endo-1,3-β-glucanase from *Arthrobacter* sp.

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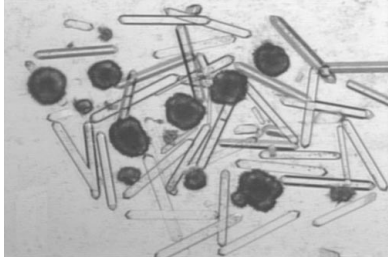

Endo-1,3-β-glucanases hydrolyze internal 1,3-β-glucosyl linkages. The endo-1,3-β-glucanase from *Arthrobacter* sp. was crystallized by the hanging-drop vapour-diffusion method. The crystals belonged to space group $P4_1$, with unit-cell parameters a = 71.31, c = 60.07 Å, and contained one molecule per asymmetric unit. The Matthews coefficient (V_M) and the solvent content were 2.35 Å^3 Da^{-1} and 47.63%, respectively. Diffraction data were collected to a resolution of 1.66 Å at SPring-8 using a MAR CCD area detector and gave a data set with an overall R_{merge} of 5.4% and a completeness of 99.4%.

1. Introduction

1,3-β-Glucanases are widely distributed among bacteria, fungi and higher plants. Based on the hydrolysis reactions catalyzed by the glucanase, 1,3-β-glucanases are classified into exo-1,3-β-glucanases (EC 3.2.1.58) and endo-1,3-β-glucanases (EC 3.2.1.6 and EC 3.2.1.39). 1,3-β-Glucanases are thought to be important in the protection of plants against fungal invasion through their capacity to hydrolyze 1,3-β-glucan, a major cell-wall component (Chen *et al.*, 1993). In bacteria, a metabolic function has been reported for endo-1,3-β-glucanases and endo-1,3-1,4-β-glucanases (EC 3.2.1.73; Watanabe *et al.*, 1992; Gueguen *et al.*, 1997; Fuchs *et al.*, 2003). Endo-1,3-β-glucanases hydrolyze internal 1,3-β-glucosyl linkages, while endo-1,3-1,4-β-glucanases only hydrolyze internal 1,4-β-glucosyl linkages when the glucosyl residue itself is linked at the O-3 position. Despite these functional differences, bacterial endo-1,3-β-glucanases share sequence similarity with endo-1,3-1,4-β-glucanases (Gueguen *et al.*, 1997) and belong to glycosyl hydrolase family GH16 (Henrissat & Bairoch, 1996; http://afmb.cnrs-mrs.fr/CAZY/).

The application of 1,3-β-glucanases is also well established for the preparation of protoplasts and for use in biotechnological processes such as cell fusion, transformation and extraction of protein products (Ballou, 1982; Parrado *et al.*, 1996). Additionally, 1,3-β-glucanases are of increasing importance in the modification of β-glucan for pharmaceutical purposes (Ooi & Liu, 2000; Wasser, 2002). We recently purified and characterized an endo-1,3-β-glucanase from *Arthrobacter* sp. (Pang *et al.*, 2004) with a molecular weight of 32.5 kDa. This enzyme has the unique characteristic of hydrolyzing 1,3-β-glucans with 1,4-β- and 1,6-β-linkages, such as lichenan and laminarin, more effectively than the other endo-1,3-β-glucanases reported previously (Sakellaris *et al.*, 1990; Gueguen *et al.*, 1997; Fuchs *et al.*, 2003; Nobe *et al.*, 2003). In addition, we found that the β-glucan oligomer (average degree of polymerization = 13; average ratio of 1,3-β- to 1,6-β-linkages = 1.3) digested by this enzyme from laminarin exhibited a strong immunostimulating activity towards human monocytes (Pang *et al.*, unpublished results).

While the crystal structures of the endo-1,3-1,4-β-glucanases from *Bacillus* and *Pyrococcus furiosus* have been determined (Keitel *et al.*, 1993; Hahn *et al.*, 1995; Tsai *et al.*, 2003), the structure of a bacterial endo-1,3-β-glucanase has not yet been reported. Although the crystal structures of barley endo-1,3-β-glucanase and endo-1,3-1,4-β-glucanases have been reported, there is neither sequential nor tertiary structural homology between the plant and bacterial enzymes (Varghese *et al.*, 1994; Müller *et al.*, 1998). As no three-dimensional structural information is available for endo-1,3-β-glucanase from

bacteria, the crystal structure of the enzyme from *Arthrobacter* sp. will help in the understanding of the structure–function relationship of not only this enzyme but also the bacterial type in general. Here, we report the crystallization and preliminary crystallographic analysis of endo-1,3-β-glucanase from *Arthrobacter* sp.

2. Purification and crystallization

Endo-1,3-β-glucanase in crude enzyme extracts from *Arthrobacter* sp. was precipitated with ammonium sulfate and then purified by anion-exchange chromatography using a DEAE Toyopearl 650M column followed by gel-filtration chromatography usuing a Sephacryl S-200 HR column as described previously (Pang *et al.*, 2004).

Crystallization conditions were identified by the hanging-drop vapour-diffusion method (Jancarik & Kim, 1991) using Hampton Research Crystal Screen. Crystals were obtained from condition No. 11 of Hampton Research Crystal Screen (1.0 *M* monoammonium dihydrogen phosphate, 0.1 *M* trisodium citrate dehydrate pH 5.6). All drops were prepared by mixing 5 µl protein solution at 10 mg ml^{-1} with 5 µl reservoir solution. Droplets were placed on siliconized cover slips and equilibrated against 1 ml reservoir solution at a temperature of 277 K. The crystals appeared within a few weeks (Fig. 1).

3. Data collection

A crystal of the enzyme picked up from a droplet in a nylon loop (Hampton Research, Laguna Niguel, CA, USA) was transferred into a cryoprotectant solution (1.0 *M* monoammonium dihydrogen phosphate, 30% glycerol, 0.1 *M* trisodium citrate dehydrate pH 5.6) and then placed directly into a cold nitrogen-gas stream at 100 K. Preliminary X-ray diffraction data were collected at 100 K under a nitrogen-gas stream with a MAR CCD detector using synchrotron radiation of wavelength 0.9792 Å at BL41XU, SPring-8 (Hyogo, Japan). Data were collected by the standard oscillation method using a crystal-to-detector distance of 120 mm. Images were collected in 1.5° increments with an exposure time of 6 s per image. Diffraction data for the crystal were obtained in the resolution range 50–1.66 Å and were processed using the *HKL*2000 program package (*DENZO* and *SCALEPACK*; Otwinowski & Minor, 1997). X-ray data statistics are summarized in Table 1.

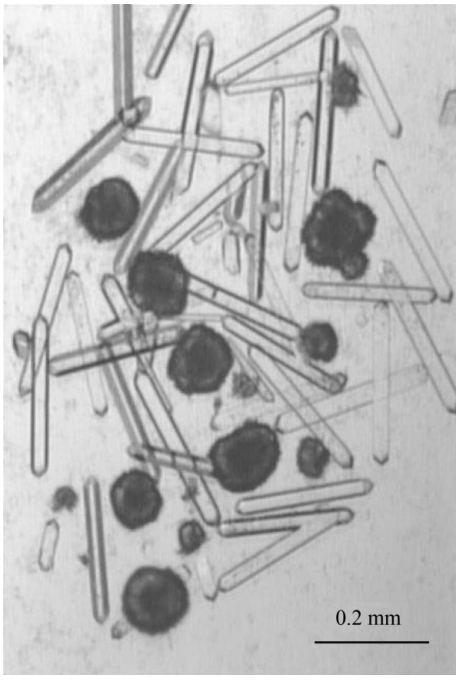

Figure 1
Crystals of endo-1,3-β-glucanase.

Table 1
Data-collection statistics for endo-1,3-β-glucanase.

Statistics for the highest resolution shell are given in parentheses.

X-ray source	BL41XU
Detector	MAR CCD
Wavelength (Å)	0.9792
Space group	$P4_1$
Unit-cell parameters (Å)	a = 71.31, c = 60.07
Resolution range (Å)	50.0–1.66 (1.72–1.66)
Measured reflections	336462 (21718)
Unique reflections	35478 (3469)
Completeness (%)	99.4 (98.8)
$R_{\rm merge}$ (%)	5.4 (13.0)

4. Results

The crystals of endo-1,3-β-glucanase belonged to space group $P4_1$ ($P4_3$), with unit-cell parameters $a = b = 71.31$, $c = 60.07$ Å. The $V_{\rm M}$ value (Matthews, 1968), *i.e.* the crystal volume per unit of protein molecular weight, was calculated to be 2.35 Å^3 Da^{-1} assuming the presence of one molecule in an asymmetric unit; the solvent content was 47.6%. The $V_{\rm M}$ value and solvent content are within the range usually observed in protein crystals.

A total of 336 462 reflections containing 35 478 unique data were collected with 99.4% completeness and an $R_{\rm merge}$ of 5.4% to 1.66 Å resolution. Structure determination of endo-1,3-β-glucanase by heavy-atom derivative and by molecular-replacement methods using the coordinates of *Bacillus macerans* 1,3-1,4-β-D-glucan 4-glucanohydrolase complexed with calcium (PDB code 1mac; Hahn *et al.*, 1995) as a search model is currently under way.

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