Acta Crystallographica Section F
Structural Biology and Crystallization Communications
ISSN 1744-3091

Crystallization and preliminary X-ray analysis of a 1,3–1,4-β-glucanase from *Paecilomyces thermophila*

Shaoqing Yang, Yaxi Wang, Zhengqiang Jiang* and Chengwei Hua

Department of Biotechnology, College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, People's Republic of China

Correspondence e-mail: zhqjiang@cau.edu.cn

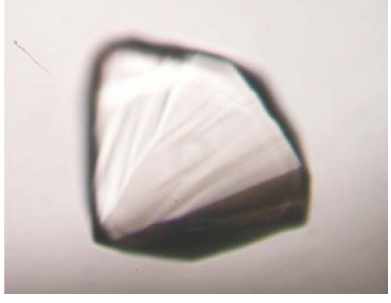
Received 25 May 2008
Accepted 8 July 2008

In this study, the crystallization and preliminary X-ray analysis of a thermostable 1,3–1,4-β-glucanase produced by *Paecilomyces thermophila* is described. The purified 1,3–1,4-β-glucanase was crystallized using the hanging-drop vapour-diffusion method. The crystal belongs to the hexagonal space group $P6_322$, with unit-cell parameters $a = b = 154.54$, $c = 87.62$ Å. X-ray diffraction data were collected to a resolution of 2.54 Å and gave a data set with an overall $R_{\rm merge}$ of 7.3% and a completeness of 94.6%. The Matthews coefficient ($V_{\rm M}$) and the solvent content are 2.38 Å^3 Da^{-1} and 48%, respectively.

1. Introduction

1,3–1,4-β-Glucanases (β-1,3–1,4-D-glucan-4-glucanohydrolases or lichenases; EC 3.2.1.73) hydrolyze 1,3–1,4-β-glucans, which are the polysaccharide components of the cell walls of higher plants or lichenan from Icelandic moss (Anderson & Stone, 1975). A number of 1,3–1,4-β-glucanases have been found in *Bacillus* species (Planas, 2000), including *Bacillus subtilis* (Murphy *et al.*, 1984) and *B. brevis* (Louw *et al.*, 1993). Several 1,3–1,4-β-glucanases from other bacteria have also been identified, such as those from *Fibrobacter succinogenes* (Teather & Erfle, 1990) and *Ruminococcus flavefaciens* (Flint *et al.*, 1993). In addition, 1,3–1,4-β-glucanases have been found in some plants, including barley (Fincher *et al.*, 1986) and tobacco (Loose *et al.*, 1988). So far, little is known about fungal β-1,3–1,4-glucanases. Extracellular β-1,3–1,4-glucanases have been purified from several fungi such as *Orpinomyces* sp. (Chen *et al.*, 1997), *Talaromyces emersonii* (Murray *et al.*, 2001), *Rhizopus microsporus* var. *microsporus* (Celestino *et al.*, 2006) and *Trichoderma koningii* (Wang *et al.*, 2007). The first fungal 1,3–1,4-glucanase to be reported was LicA from the anaerobic fungus *Orpinomyces* strain PC-2 (Chen *et al.*, 1997). Another aerobic fungus, *Talaromyces emersonii*, can secrete a 1,3–1,4-β-glucanase (Murray *et al.*, 2001). This type of enzyme displays a strict substrate specificity for the hydrolysis of β-1,4 glycosidic bonds in 3-*O*-substituted glucopyranose units (Planas, 2000). The main final hydrolysis products from barley β-glucan are trisaccharides and tetrasaccharides.

The microbial β-1,3–1,4-glucanases belong to glycosyl hydrolase family 16 and have a jelly-roll β-sandwich structure, whereas the plant enzymes are classified as members of family 17 with a $(\beta/\alpha)_8$-barrel three-dimensional structure (Henrissat & Bairoch, 1993; Planas, 2000). The newly isolated thermophilic fungus *Paecilomyces thermophila* J18 is known to be a good source of xylanases (Yang *et al.*, 2006). It also secretes an extracellular 1,3–1,4-β-glucanase (data not shown). In order to compare the catalytic mechanisms of β-glucanases from different sources, the crystallization and preliminary crystallographic studies of the 1,3–1,4-β-glucanase from *P. thermophila* J18 are reported.



2. Materials and methods

2.1. Purification of the β-1,3–1,4-glucanase

P. thermophila J18 was used in this investigation (Yang *et al.*, 2006). The purification of 1,3–1,4-β-glucanase from the extracellular culture

filtrate was carried out by fractional ammonium sulfate precipitation followed by two steps of ion-exchange chromatography on a DEAE Sephadex column and a Q-Sepharose Fast Flow column. The crude supernatant was subjected to 40–60% ammonium sulfate saturation. The precipitated protein was collected by centrifugation (10 000*g*) and dissolved in 20 m*M* phosphate buffer pH 7.2. The crude enzyme was then dialyzed against 20 m*M* phosphate buffer pH 7.2. 50 ml of solution was applied onto a DEAE 52 column (8 × 1.0 cm) pre-equilibrated with 20 m*M* phosphate buffer pH 7.2. The bound β-glucanase was eluted with a gradient of 100–200 m*M* NaCl at a flow rate of 0.8 ml min^{-1}. The active fractions were combined and concentrated to 0.6 ml by ultrafiltration using a 10 kDa membrane (Stirred Cell Model 8050, Millipore). The concentrated solution was applied onto a Q-Sepharose Fast Flow column (8 × 1.0 cm) equilibrated with 25 m*M* phosphate buffer pH 6.5. The unbound fractions with high β-glucanase activity were pooled and their homogeneity was checked by SDS–PAGE, which was performed using 12.5%(*w*/*v*) acrylamide in gels as described by Laemmli (1970). Protein bands were visualized by Coomassie Brilliant Blue R-250 staining. The location of 1,3–1,4-β-glucanase activity in SDS–PAGE was determined from a zymogram using 0.2% barley β-glucan as substrate. Finally, the β-glucanase was purified 122.5-fold to apparent homogeneity with a recovery yield of 8.9% (Fig. 1).

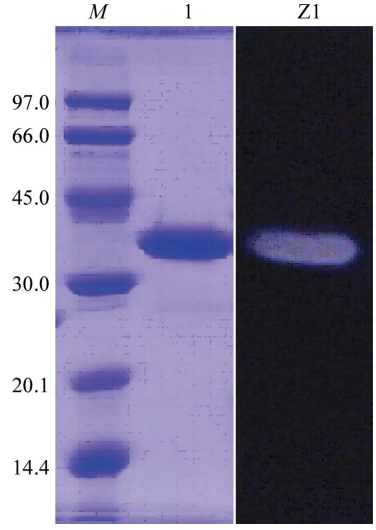


Figure 1
SDS–PAGE and zymogram analysis of purified 1,3–1,4-β-glucanase. Lane *M*, low molecular-weight calibration kit (kDa); lane 1, purified 1,3–1,4-β-glucanase; lane Z1, zymogram of purified 1,3–1,4-β-glucanase.

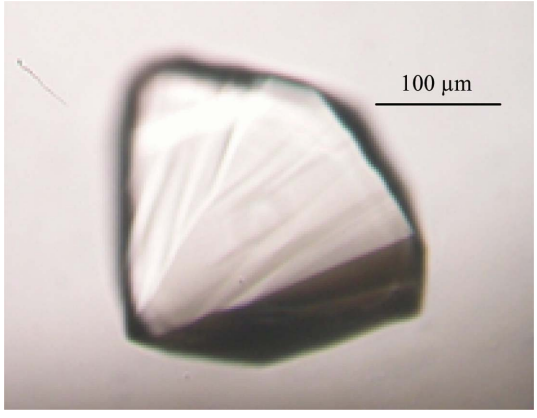


Figure 2
Single crystal of native 1,3–1,4-β-glucanase from *P. thermophila*. Crystals were grown in Hampton Crystal Screen 1 condition No. 35. The protein concentration is 9 mg ml^{-1}.

2.2. Crystallization

Purified 1,3–1,4-β-glucanase was concentrated to 9 mg ml^{-1} in 20 m*M* MES pH 7.0 using a Microcon centrifugal filter device (Millipore, Billerica, Massachusetts, USA). Protein concentrations were measured by the Lowry method (Lowry *et al.*, 1951) using bovine serum albumin (BSA) as the standard. All crystallization experiments were set up manually and performed at 291 K using the hanging-drop vapour-diffusion method (Unge, 1999). Initial crystallization conditions were screened using the commercially available Crystal Screen 1 and 2 kits (Hampton Research, California, USA) with drops formed by mixing equal volumes (1 µl) of protein and precipitant solutions; the drops were equilibrated against 200 µl precipitant solution. Crystals of good quality were grown from drops containing Hampton Crystal Screen 1 condition No. 35 (0.1 *M* sodium HEPES pH 7.5, 0.8 *M* sodium phosphate monobasic monohydrate and 0.8 *M* potassium phosphate monobasic).

2.3. Data collection

X-ray diffraction experiments were performed on a Microstar-H X-ray generator (Bruker, Germany) with a Smart 6000 CCD operating at 45 kV and 60 mA (λ = 1.5418 Å). Data were collected at 100 K with a crystal-to-detector distance of 60 mm, $\Delta\varphi = 0.2^\circ$ and an exposure time of 3 min per frame. Prior to data collection, the crystals were transferred briefly into an anti-icing fluid (100% paraffin oil) and flash-cooled in liquid nitrogen using a cryoloop at 100 K. All diffraction data were processed using the program *Proteum*.

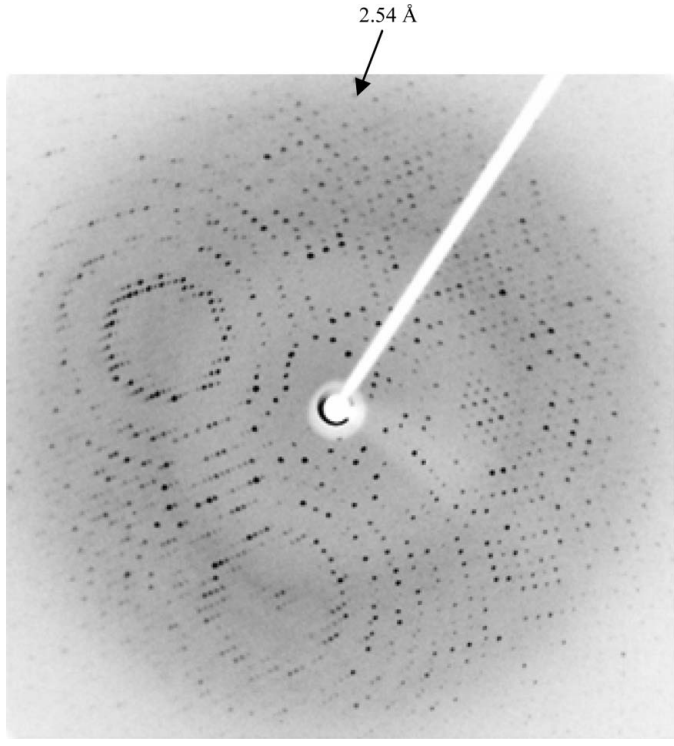


Figure 3
A typical diffraction pattern of a crystal of 1,3–1,4-β-glucanase from *P. thermophila*.

Table 1
X-ray diffraction data-collection statistics.

Values in parentheses are for the highest resolution shell.

Temperature (K)	100
Crystal-to-detector distance (mm)	60
Oscillation range (°)	0.2
R_{merge}† (%)	7.3 (27.4)
Resolution range (Å)	50.0–2.40 (2.54–2.40)
Space group	$P6_322$
Unit-cell parameters (Å)	$a = b = 154.54$, $c = 87.62$
Completeness (%)	94.6 (76.4)
Redundancy	5.7 (2.3)
Total no. of reflections	133366
Unique reflections	23312
Mean $I/\sigma(I)$	7.86 (1.81)
No. of molecules per ASU	2
Matthews coefficient (Å^3 Da^{-1})	2.38
Solvent content (%)	48

† $R_{merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of the ith observation and $\langle I(hkl)\rangle$ is the mean intensity of reflections.

3. Results and discussion

SDS–PAGE and zymogram analysis showed a single band with β-glucanase activity (Fig. 1). The molecular weight of the purified enzyme was 31 692.9 Da as detected by mass spectrometry (data not shown). After four weeks, well shaped crystals appeared in Hampton Crystal Screen 1 condition No. 35 (Fig. 2). The crystal showed good diffraction to 2.54 Å resolution. A diffraction pattern is shown in Fig. 3. The parameters for X-ray diffraction data collection are shown in Table 1. The crystal belongs to space group $P6_322$, with unit-cell parameters $a = b = 154.54$, $c = 87.62$ Å. Assuming the presence of two molecules (each with a molecular weight of 31 692.9 Da) per asymmetric unit, the calculated Matthews coefficient (V_M) is 2.38 Å^3 Da^{-1} and 48% of the crystal volume is occupied by solvent. This V_M value is well within the normal range of V_M values observed for soluble protein crystals (1.68–3.5 Å^3 Da^{-1}; Matthews, 1968).

Crystal structures of bacterial β-1,3–1,4-glucanases have been reported from several *Bacillus* species and *F. succinogenes*. The first 1,3–1,4-β-glucanase to have its three-dimensional structure determined by X-ray crystallography was the hybrid *Bacillus* H(A16-M) (2.0 Å resolution; Keitel *et al.*, 1993). Subsequently, the crystal structures of 1,3–1,4-β-glucanases from *B. licheniformis* (Hahn, Pons *et al.*, 1995) and *B. macerans* (Hahn, Olsen *et al.*, 1995) have been solved by X-ray crystallography. The crystal structure and catalytic activity of 1,3–1,4-β-glucanase from *F. succinogenes* have been studied in detail (Chen *et al.*, 2001; Tsai *et al.*, 2003, 2005). The three-dimensional structures of two plant 1,3–1,4-β-glucanases with distinct substrate specificities have been determined by X-ray crystallography at 2.2–2.3 Å resolution (Varghese *et al.*, 1994). By comparison of the β-glucanases from *Bacillus* and barley, it has been demonstrated that all *Bacillus* enzymes have a similar 'jelly-roll' β-barrel structure containing two seven-stranded antiparallel β-sheets and that the active site is located at the cleft on the concave side of the β-sheet. In contrast, the enzymes from barley are folded into a $(\beta/\alpha)_8$-barrel structure (Muller *et al.*, 1998; Varghese *et al.*, 1994). To date, no crystal structure of a fungal β-1,3–1,4-glucanase has been reported.

Comparison of the crystal structure of this 1,3–1,4-β-glucanase with other known enzyme structures should provide useful information to better understand the protein folding and molecular evolution of β-glucanases. Trials to solve the structure *via* molecular replacement are ongoing.

This work was financially supported by the Foundation for the Author of National Excellent Doctoral Dissertation of China (Project No. 200555) and the Program for New Century Excellent Talents in University (NCET-05-0130). We are grateful to Dr Xiaodong Su, Dr Yuhe Liang and Dr Xiang Liu for useful discussions and X-ray data collection at the College of Life Science, Peking University, Beijing 100871, China.

References

Anderson, M. A. & Stone, B. A. (1975). *FEBS Lett.* **52**, 202–207.
Celestino, K. R., Cunha, R. B. & Felix, C. R. (2006). *BMC Biochem.* **7**, 23.
Chen, H. Z., Li, X. L. & Ljungdahl, L. G. (1997). *J. Bacteriol.* **179**, 6028–6034.
Chen, J. L., Tsai, L. C., Wen, T. N., Tang, J. B., Yuan, H. S. & Shyur, L. F. (2001). *J. Biol. Chem.* **276**, 17895–17901.
Fincher, G. B., Lock, P. A., Morgan, M. M., Ligelbach, K., Wettenhall, R. E. H., Mercer, J. F. B., Brandt, A. & Thomsen, K. K. (1986). *Proc. Natl Acad. Sci. USA*, **83**, 2081–2085.
Flint, H. J., Martin, J., McPherson, C. A., Daniel, A. S. & Zhang, J. X. (1993). *J. Bacteriol.* **175**, 2943–2951.
Hahn, M., Olsen, O., Politz, O., Borriss, R. & Heinemann, U. (1995). *J. Biol. Chem.* **270**, 3081–3088.
Hahn, M., Pons, J., Planas, A., Querol, E. & Heinemann, U. (1995). *FEBS Lett.* **374**, 221–224.
Henrissat, B. & Bairoch, A. (1993). *Biochem. J.* **293**, 781–788.
Keitel, T., Simon, O., Borriss, R. & Heinemann, U. (1993). *Proc. Natl Acad. Sci. USA*, **90**, 5287–5291.
Laemmli, U. K. (1970). *Nature (London)*, **227**, 680–685.
Loose, M. D., Alliotte, A., Gheysen, G., Genetello, C., Gielen, J., Soetaert, P., Montagu, M. V. & Inze, D. (1988). *Gene*, **70**, 13–23.
Louw, M. E., Reid, S. J. & Waston, T. G. (1993). *Appl. Microbiol. Biotechnol.* **38**, 507–513.
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. Biol. Chem.* **193**, 265–275.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Muller, J. J., Thomsen, K. K. & Heinemann, U. (1998). *J. Biol. Chem.* **273**, 3438–3446.
Murphy, N., McConnell, D. J. & Cantwell, B. A. (1984). *Nucleic Acids Res.* **12**, 5355–5367.
Murray, P. G., Grassick, A., Laffey, C. D., Cuffe, M. M., Higgins, T., Savage, A. V., Planas, A. & Tuohy, M. G. (2001). *Enzyme Microb. Technol.* **29**, 90–98.
Planas, A. (2000). *Biochim. Biophys. Acta*, **1543**, 361–382.
Teather, R. M. & Erfle, J. D. (1990). *J. Bacteriol.* **172**, 3837–3841.
Tsai, L. C., Shyur, L. F., Cheng, Y. S. & Lee, S. H. (2005). *J. Mol. Biol.* **354**, 642–651.
Tsai, L. C., Shyur, L. F., Lee, S. H., Lin, S. S. & Yuan, H. S. (2003). *J. Mol. Biol.* **330**, 607–620.
Unge, T. (1999). *Protein Crystallization: Techniques, Strategies and Tips*, edited by T. M. Bergfors, pp. 9–17. La Jolla, USA: International University Line.
Varghese, J. N., Garrett, T. P. J., Colman, P. M., Chen, L., Hoj, P. B. & Fincher, G. B. (1994). *Proc. Natl Acad. Sci. USA*, **91**, 2785–2789.
Wang, J. L., Ruan, H., Zhang, H. F., Zhang, Q., Zhang, H. B., He, G. Q. & Shen, S. R. (2007). *J. Food Sci.* **72**, C452–C456.
Yang, S. Q., Yan, Q. J., Jiang, Z. Q., Li, L. T., Tian, H. M. & Wang, Y. Z. (2006). *Bioresour. Technol.* **97**, 1794–1800.