Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary X-ray analysis of a thermostable pectate lyase PL 47 from *Bacillus* sp. TS 47

Tetsuko Nakaniwa,[a]* Toshiji Tada,[a] Keiko Ishii,[a] Makoto Takao,[b] Takuo Sakai[b] and Keiichiro Nishimura[a]

[a]Research Institute for Advanced Science and Technology, Osaka Prefecture University, Sakai, Osaka 599-8570, Japan, and [b]Department of Food Science, Kinki University, Nakamachi, Nara 631-8505, Japan

Correspondence e-mail: tetsuko@riast.osakafu-u.ac.jp

Received 5 August 2002
Accepted 11 November 2002

The thermostable pectate lyase PL 47 from *Bacillus* sp. TS 47, with a molecular weight of 50 kDa, was crystallized by the hanging-drop vapour-diffusion method using 2-propanol and polyethylene glycol 4000 as precipitants. The crystals belong to the trigonal space group $P3_121$, with unit-cell parameters $a = b = 58.8$, $c = 229.7$ Å, $\gamma = 120^\circ$. The calculated V_M based on one molecule per asymmetric unit is 2.30 Å^3 Da^{-1}. A native data set from a frozen crystal was collected to 1.8 Å resolution using synchrotron radiation at SPring-8. A molecular-replacement solution was obtained using the structure of pectate lyase from *B. subtilis* as a model.

1. Introduction

Pectate lyases (PLs; E.C. 4.2.2.2) are produced by a wide range of organisms (Rombouts & Pilnik, 1980). PLs cleave α-1,4-glycosidic linkages in polygalacturonic acid chains, which are a major component of the pectic substances of plant cell walls. The cleavage of the glycosidic linkage occurs through a β-elimination mechanism to generate products with a 4,5-unsaturated bond at the non-reducing end.

Pectolytic enzymes such as PLs play an important role in fruit ripening that is attributed to degradation and solubilization of the pectic substances responsible for cell cohesion. The enzymes are used effectively in several industrial processes, including the retting of flax, the extraction and clarification of fruit juices and the maceration of vegetables. In addition, Sakai (1995) has described the practical use of the enzymes in the scouring of cotton fabric. Biochemical studies have also been extensively conducted. Recently, the gene encoding a thermostable pectate lyase, PL 47, was isolated from the thermophilic *Bacillus* sp. TS 47 (Takao *et al.*, 2001). PL 47 showed optimal activity at 343 K and pH 8.0 and its thermostability was characterized by a half-life of 13 h at 338 K (Takao *et al.*, 2000). However, the enzyme does not possess sufficient activity to be used for the scouring of cotton fabric.

Three PLs have been isolated from mesophilic bacteria and their crystal structures determined: PelC and PelE from *Erwinia chrysanthemi* (Yoder *et al.*, 1993; Yoder & Jurnak, 1995; Lietzke *et al.*, 1996) and BsPel from *B. subtilis* (Pickersgill *et al.*, 1994). These enzymes have a common unique motif consisting of a parallel β-helix that is generated by coiling β-strands into a large right-handed helix. The amino-acid sequence of PL 47 (416 residues) showed 52% similarity to BsPel from mesophilic *B. subtilis*.

Knowledge of the three-dimensional structure of PL 47 should provide important information on the structure–stability relationships of enzymes of this class and on strategies to improve their stability. Information derived from the three-dimensional structure of PL 47 might potentially lead to the design of new examples of PLs that have higher activity and stability. Here, we report the crystallization and preliminary X-ray analysis of PL 47 from *Bacillus* sp. TS 47.

2. Materials and methods

2.1. Crystallization

PL 47 from *Bacillus* sp. TS 47 was prepared from the culture filtrate of *B. subtilis* MI112 harbouring the plasmid pPL 47 (a plasmid containing the PL 47 gene) and purified to homogeneity as reported previously (Takao *et al.*, 2001). For crystallization, the purified protein was dialysed against 20 m*M* Tris–HCl buffer pH 7.5. Crystallization trials were performed by the hanging-drop vapour-diffusion method at 277 K (McPherson, 1999). Conditions for crystallization were initially investigated using the commercially available sparse-matrix screening kit Crystal Screen I from Hampton Research (Jancarik & Kim, 1991). Each drop was prepared by mixing 1 µl of 10 mg ml^{-1} PL 47 solution with 1 µl of reservoir solution and was equilibrated against 500 µl of the respective reservoir solution. The initial conditions found to be the best were optimized by varying the pH and the concentrations of polyethylene glycol (PEG) and protein, as small crystals were obtained from several drops containing PEG as a precipitant.

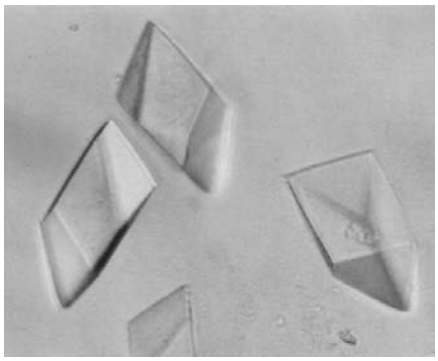

Figure 1
Typical crystal of pectate lyase PL 47 from *Bacillus* sp. TS 47. The crystal dimensions are approximately 0.05 × 0.1 × 0.05 mm.

2.2. X-ray diffraction analysis

Crystals of PL 47 were transferred to a cryoprotectant solution containing 30.0%(*v*/*v*) glycerol, 20.0%(*v*/*v*) 2-propanol, 20.0%(*w*/*v*) PEG 4000, 0.1 *M* Tris–HCl buffer pH 7.7 and flash-cooled in the gas stream from a liquid-nitrogen cryostat. A complete data set was collected at 100 K on a MAR CCD 165 detector using synchrotron radiation of wavelength 1.0 Å at the BL41XU station of SPring-8, Japan. The crystal-to-detector distance was 170 mm and 120 images were recorded at 1° intervals with an exposure time of 3 s per image. The intensity data were processed with the program *MOSFLM* (Steller *et al.*, 1997) and scaled using the program *SCALA* (Collaborative Computational Project, Number 4, 1994).

3. Results and discussion

In the initial crystallization trials, small crystals were obtained from condition No. 41 of Hampton Research Crystal Screen I containing 2-propanol and PEG 4000 as precipitants. Crystals also were obtained with condition No. 22 containing sodium acetate and PEG 4000, but they diffracted weakly to a maximum resolution of 3.5 Å and decayed quickly after a few images because of radiation damage. Thus, the crystallization conditions finally established based on condition No. 41 consisted of mixing 1 µl of PL 47 (6.5 mg ml^{-1}) in 20 m*M* Tris–HCl buffer pH 7.5 with the same volume of reservoir solution containing 20.0%(*v*/*v*) 2-propanol and 15.0%(*w*/*v*) PEG 4000 in 0.1 *M* Tris–HCl buffer pH 7.7. The crystals grew to maximal dimensions of approximately 0.05 × 0.1 × 0.05 mm in two weeks (Fig. 1).

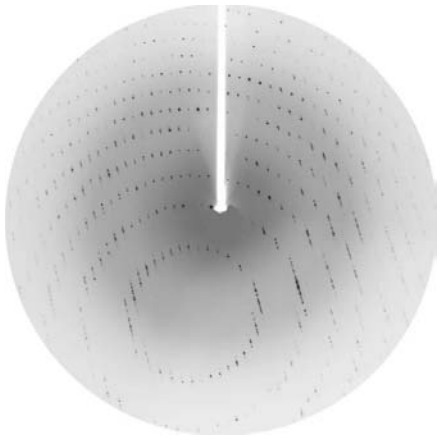

Figure 2
A diffraction image of pectate lyase PL 47 from *Bacillus* sp. TS 47. The diffraction image was taken using a MAR CCD detector on beamline BL41XU at SPring-8.

The crystals diffracted to 1.8 Å resolution at SPring-8 (Fig. 2). The diffraction pattern indicated trigonal 3*m* Laue symmetry and the systematic absences for 00*l* (l = $\bar{3}n$) reflections indicated the space group $P3_121$ or its enantiomorph $P3_221$, with unit-cell parameters $a = b = 58.8$, $c = 229.7$ Å, $\gamma = 120°$. Detailed crystal parameters and data-collection statistics are shown in Table 1. Assuming one molecule of PL 47 (50 kDa) per asymmetric unit, the V_M value was calculated to be 2.30 Å^3 Da^{-1}, corresponding to a solvent content of 46.0%. This is within the range of values tabulated by Matthews (1968).

Molecular-replacement calculations were carried out with the program *AMoRe* (Navaza, 1994) implemented in the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994) using BsPel from *B. subtilis* (PDB code 1bn8; Pickersgill *et al.*, 1994) as a starting model. A clear peak was found with a correlation coefficient of 32.5 and an *R* factor of 49.7% (8–4 Å) after translation-function calculations in the space group $P3_121$, whereas no significant peak was observed in the space group $P3_221$. There were no unfavourable molecular contacts observed in the crystal packing. Model building by manual fitting to the electron-density map using the program *O* (Jones *et al.*, 1991) is now under way.

Table 1
Summary of crystal parameters and data-collection statistics.

Values in parentheses are for the highest resolution shell (1.91–1.81 Å).

Resolution (Å)	1.81
Space group	$P3_121$
Unit-cell parameters (Å, °)	$a = b = 58.8$, $c = 229.7$, $\gamma = 120$
R_{merge} (%)	6.5
$I/\sigma(I)$	7.3 (4.2)
No. of reflections	248222
Unique reflections	75722
Completeness (%)	64.8 (93.8)
Mosaicity (°)	0.253

The synchrotron-radiation experiments were performed at SPring-8 with the approval of the Japan Synchrotron Radiation Reseach Institute (JASRI; Proposal No. 2001B0316-NL-np). We thank Dr M. Kawamoto for his help in data collection at station BL41XU.

References

Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.
Jones, T. A., Zou, J.-Y., Cowan, S. W. & Kjeldgaard, M. (1991). *Acta Cryst.* A**47**, 110–119.
Lietzke, S. E., Scavetta, R. D., Yoder, M. D. & Jurnak, F. (1996). *Plant Physiol.* **111**, 73–92.
McPherson, A. (1999). *Crystallization of Biological Macromolecules*, pp. 188–191. New York: Cold Spring Harbor Laboratory Press.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Navaza, J. (1994). *Acta Cryst.* A**50**, 157–163.
Pickersgill, R., Jenkins, J., Harris, G., Nasser, W. & Robert-Baudouy, J. (1994). *Nature Struct. Biol.* **1**, 717–723.
Rombouts, F. M. & Pilnik, W. (1980). *Econ. Microbiol.* **5**, 227–282.
Sakai, T. (1995). *Senshoku Kogyo*, **43**, 162–173. In Japanese.
Steller, I., Bolotovsky, R. & Rossmann, M. G. (1997). *J. Appl. Cryst.* **30**, 1036–1040.
Takao, M., Nakaniwa, T., Yoshikawa, K., Terashita, T. & Sakai, T. (2000). *Biosci. Biotechnol. Biochem.* **64**, 2360–2367.
Takao, M., Nakaniwa, T., Yoshikawa, K., Terashita, T. & Sakai, T. (2001). *Biosci. Biotechnol. Biochem.* **65**, 322–329.
Yoder, M. D. & Jurnak, F. (1995). *Plant Physiol.* **107**, 349–364.
Yoder, M. D., Keen, N. T. & Jurnak, F. (1993). *Science*, **260**, 1503–1507.