Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary X-ray analysis of a novel pectolytic enzyme, polymethoxygalacturonase SX1 from *Trichosporon penicillatum*

Toshiji Tada,[a]* Chao-Tsang Lu,[a] Yoshihiro Nakamura,[a] Kei Wada,[a] Ikuko Miyahara,[b] Ken Hirotsu,[b] Yoshio Katsuya,[c] Masahiko Sawada,[d] Makoto Takao,[e] Takuo Sakai[e] and Keiichiro Nishimura[a]

[a]Research Institute for Advanced Science and Technology, Osaka Prefecture University, Sakai, Osaka 599-8570, Japan, [b]Department of Chemistry, Osaka City University, Sumiyoshi-ku, Osaka 558-8585, Japan, [c]Hyogo Prefectural Institute of Industrial Research, Suma-ku, Kobe, Hyogo 654-0037, Japan, [d]Central Research Laboratory, Godo Shusei Co. Ltd, Matsudo, Chiba 271-0064, Japan, and [e]Department of Food Science, Kinki University, Nakamachi, Nara 631-8505, Japan

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

Received 26 September 2000
Accepted 23 January 2001

A novel pectolytic enzyme, polymethoxygalacturonase SX1 from *Trichosporon penicillatum*, with a molcular weight of 36 kDa was crystallized by the hanging-drop vapour-diffusion method using polyethylene glycol 1000 as a precipitant. The crystals belonged to the monoclinic space group $C2$, with unit-cell parameters a = 165.6, b = 61.0, c = 48.7 Å, β = 93.1°. The calculated V_M based on one molecule per asymmetric unit was 3.40 Å^3 Da^{-1}. A native data set was collected to 2.08 Å resolution from a crystal on a Cu $K\alpha$ rotating-anode X-ray source. A molecular-replacement solution was obtained using the program *AMoRe* and the structure of endopolygalacturonase II from *Aspergillus niger* as a model.

1. Introduction

Pectolytic enzymes are widely distributed in microorganisms and higher plants (Rombouts & Pilnik, 1980; Whitaker, 1990). The enzymes from microorganisms have been extensively studied to elucidate the mechanism of plant pathogenesis, in which the enzymes initiate soft-rot diseases in plants by degrading the pectic substances of the primary cell wall and middle lamellae (Collmer & Keen, 1986; Cervone *et al.*, 1987). This type of plant enzyme also plays an important role in enlarging cell walls during growth and in softening certain plant tissues during maturation and storage (Soda *et al.*, 1986; Dick & Labavitch, 1989).

There are many studies investigating polymethoxygalacturonases in various species, but the existence of these enzymes has been doubted (Whitaker, 1990; Sakai *et al.*, 1993). Recently, Sakai *et al.* (1997) isolated an interesting gene from *T. penicillatum* B2 which encodes a novel pectolytic enzyme SX1. The SX1 gene codes for a 360 amino-acid protein. However, the N-terminal amino-acid sequence of the open reading frame corresponds to a signal peptide and the propeptide is processed by a Kex2-like protease to form the mature SX1 composed of 334 amino acids with a molecular weight of 36 kDa. SX1 is a polymethoxygalacturonase; it hydrolyzes an α-1,4-glycosidic linkage in the methoxylated polygalacturonate to give a series of galacturonate oligomers having various molecular weights. The enzyme also possesses an activity to release water-soluble pectins from protopectin, which is a water-insoluble parent pectic substance. Therefore, SX1 was considered to be a type of protopectinase.

Crystal structures of pectolytic enzymes including pectate lyases (Yoder *et al.*, 1993; Pickersgill *et al.*, 1994; Lietzke *et al.*, 1996), pectin lyases (Mayans *et al.*, 1997; Vitali *et al.*, 1998), an endopolygalacturonase from *Erwinia carotovora* (Pickersgill *et al.*, 1998) and an endopolygalacturonase II from *Aspergillus niger* (van Santen *et al.*, 1999) have been reported. All of these enzymes have a common unique motif of a parallel β-helix that is generated by coiling β-strands into a large right-handed cylinder. The amino-acid residues responsible for substrate binding of the endopolygalacturonases have been identified and a reaction mechanism has been proposed (Pickersgill *et al.*, 1998; van Santen *et al.*, 1999). These residues are conserved in SX1, which has 53.2% homology with the endopolygalacturonase II of *A. niger* and only 23.5% homology with the endopolygalacturonase of *E. carotovora*. These findings provided information elucidating the three-dimensional structure of SX1 for further study of the structure–function relationship of this class of pectolytic enzymes. As the first step towards the structure analysis, we report here the crystallization and preliminary X-ray analysis of SX1.

2. Materials and methods

2.1. Crystallization

The polymethoxygalacturonase SX1 of *T. penicillatum* was prepared from a culture filtrate of *Saccharomyces cerevisiae* harbouring the plasmid pMKPSX1 (a plasmid containing the SX1 gene) and purified to homogeneity as reported previously (Sakai *et al.*, 1997). Crystallization trials were performed by the hanging-drop vapour-diffusion method (McPherson, 1998). Conditions for crystallization were searched initially using the

© 2001 International Union of Crystallography
Printed in Denmark – all rights reserved

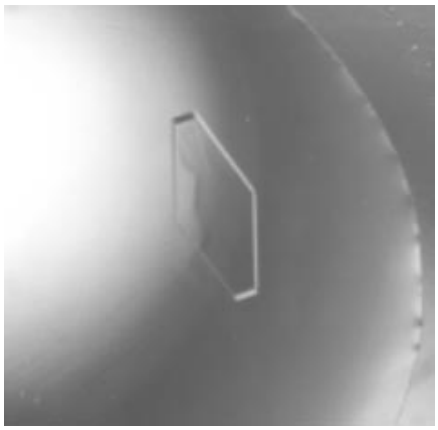

Figure 1
Typical crystal of polymethoxygalacturonase SX1 from *T. penicillatum*. The dimensions of the crystal are approximately 0.7 × 0.3 × 0.08 mm.

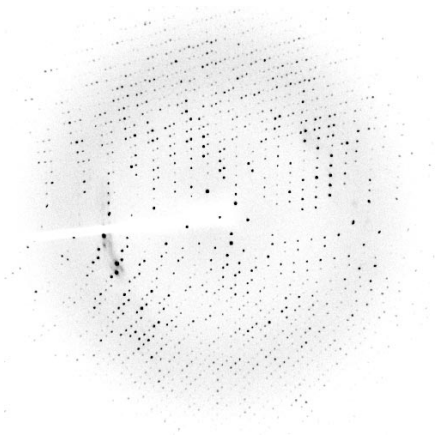

Figure 2
A diffraction image of polymethoxygalacturonase SX1 from *T. penicillatum*.

commercially available sparse-matrix screening kit Crystal Screen I from Hampton Research (Jancarik & Kim, 1991), using drops consisting of 1 µl of 10 mg ml^{-1} protein solution and an equal volume of reservoir solution and equilibrating against 500 µl of the reservoir solution at 277 K. The crystallization conditions were refined by varying the pH, the molecular weight and concentrations of polyethylene glycol (PEG) and the concentration of the protein, as small crystals were obtained from several drops containing PEG as a precipitant.

2.2. X-ray diffraction analysis

The SX1 crystals were mounted in quartz capillaries and subjected to X-ray diffraction. A complete data set was collected at room temperature on a Rigaku R-AXIS IIc imaging-plate system using Cu $K\alpha$ radiation from a Rigaku RU-200 rotating-anode generator operated at 40 kV and 100 mA. The crystal-to-detector distance was 100 mm and the oscillation range was 2°. Data were reduced using the Rigaku *PROCESS* crystallographic data-reduction package (Higashi, 1989; Sato *et al.*, 1992).

3. Results and discussion

Well formed crystals were obtained using 0.1 *M* sodium acetate pH 5.5 and 7.5%(*w*/*v*) PEG 6000. However, the crystals only diffracted to a maximal resolution of 3.0 Å. Crystals with a much higher quality were obtained using PEG 1000 instead of PEG 6000 as a precipitant. As a result, the crystallization conditions were established as mixing 2 µl of a 5 mg ml^{-1} solution of SX1 in 20 m*M* acetate buffer pH 5.0 with an equal volume of reservoir solution containing 15.0%(*v*/*v*) PEG 1000 in 0.1 *M* acetate buffer pH 5.5. The crystals grew to maximum dimensions of approximately 0.7 × 0.3 × 0.08 mm in three weeks (Fig. 1).

A limited number of reflections were observed to 2.08 Å resolution (Fig. 2). A total of 106 223 observed reflections were scaled and reduced to yield a data set containing 28 168 unique reflections with an $R_{\rm merge}$ of 7.6%. The data set was 97.0% complete to 2.50 Å resolution and 95.2% complete to 2.08 Å resolution, with the data in the 2.25–2.08 Å resolution shell being 91.5% complete. The average $I/\sigma(I)$ value in this shell was 2.94. The crystal class was determined to be monoclinic, space group *C*2, with unit-cell parameters a = 165.6, b = 61.0, c = 48.7 Å, β = 93.1°. Assuming one molecule of SX1 (36 kDa) in the asymmetric unit, the $V_{\rm M}$ value was calculated to be 3.40 Å^3 Da^{-1}, corresponding to a solvent content of 63.8%. This is in the range of values tabulated by Matthews (1968).

Molecular-replacement calculations were carried out using the program *AMoRe* (Navaza, 1994) implemented in the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994) using endopolygalacturonase II from *A. niger* (PDB code 1czf; van Santen *et al.*, 1999) as a starting model. A clear peak was found with a correlation coefficient of 59.9 and an *R* factor of 43.4% (15–4 Å) after translation-function calculations. Rigid-body refinement (8–4 Å) resulted in a correlation coefficient of 65.6 and an *R* factor of 40.1%. 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.

References

Cervone, F., De Lorenzo, G., Degra, L., Salvi, G. & Bergami, M. (1987). *Plant Physiol.* **85**, 631–637.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Collmer, A. & Keen, N. T. (1986). *Annu. Rev. Phytopathol.* **24**, 383–409.
Dick, A. J. & Labavitch, J. M. (1989). *Plant Physiol.* **89**, 1394–1400.
Higashi, T. (1989). *J. Appl. Cryst.* **22**, 9–18.
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. (1998). *Crystallization of Biological Macromolecules*, pp. 188–191. New York: Cold Spring Harbor Laboratory Press.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Mayans, O., Scott, M., Connerton, I., Gravesen, T., Benen, J., Visser, J., Pickersgill, R. & Jenkins, J. (1997). *Structure*, **5**, 677–689.
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.
Pickersgill, R., Smith, D., Worboys, K. & Jenkins, J. (1998). *J. Biol. Chem.* **273**, 24660–24664.
Rombouts, F. M. & Pilnik, W. (1980). *Econ. Microbiol.* **5**, 227–282.
Sakai, T., Sakamoto, T., Hallaert, J. & Vandamme, E. J. (1993). *Adv. Appl. Microbiol.* **39**, 213–294.
Sakai, T., Sirasaka, N., Hirano, H., Kishida, M. & Kawasaki, H. (1997). *FEBS Lett.* **414**, 439–443.
Santen, Y. van, Benen, J. A. E., Schroter, K.-H., Kalk, K. H., Armand, S., Visser, J. & Dijkstra, B. W. (1999). *J. Biol. Chem.* **274**, 30474–30480.
Sato, M., Yamamoto, M., Imada, K., Katsube, Y., Tanaka, N. & Higashi, T. (1992). *J. Appl. Cryst.* **25**, 348–357.
Soda, I., Hasegawa, T., Suzuki, T. & Ogura, N. (1986). *Agric. Biol. Chem.* **50**, 3191–3192.
Vitali, J., Schick, B., Kester, H. C. M., Visser, J. & Jurnak, F. (1998). *Plant Physiol.* **116**, 69–80.
Whitaker, J. R. (1990). *Microbial Enzymes and Biotechnology*, edited by W. M. Fogarty & C. T. Kelly, pp. 133–176. London/New York: Elsevier Applied Science.
Yoder, M. D., Keen, N. T. & Jurnak, F. (1993). *Science*, **260**, 1503–1507.