Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary X-ray study of endopolygalacturonase from the pathogenic fungus *Stereum purpureum*

Tetsuya Shimizu,[a,b] Toru Nakatsu,[a] Kazuo Miyairi,[b] Toshikatsu Okuno[b] and Hiroaki Kato[a]*

[a]Kinetic Crystallography Research Team, Membrane Dynamics Research Group, RIKEN, Harima Institute at SPring-8, 1-1-1 Kouto, Mikazuki-cho, Sayo-gun, Hyogo 679-5148, Japan, and [b]Department of Biochemistry and Biotechnology, Faculty of Agriculture and Life Sciences, Hirosaki University, 3 Bunkyo-cho, Hirosaki, Aomori 036-8561, Japan

Correspondence e-mail: katohiro@spring8.or.jp

Received 6 February 2001
Accepted 8 June 2001

Crystals of endopolygalacturonase I from *Stereum purpureum* have been obtained by the vapour-diffusion method. Prior to crystallization work, endopolygalacturonase I was deglycosylated with *endo*-β-*N*-acetylglucosaminidase H. The crystal diffracts to ultrahigh (0.96 Å) resolution using synchrotron radiation and belongs to space group *P*1, with unit-cell parameters a = 37.26, b = 46.34, c = 52.05 Å, α = 67.17, β = 72.44, γ = 68.90°.

1. Introduction

Pectin is a major component of plant cell walls and its degradation is an important process in infection by plant pathogenic organisms, plant senescence and fruit ripening (Collmer, 1986). Pectin consists of so-called 'smooth' and 'hairy' regions. The smooth region, or homogalacturonan part, consists of a backbone of α-1,4-linked galacturonic acid residues which are in part methylated and can be acetylated. The hairy regions, known as rhamnogalacturonan, are characterized by stretches of 1,2-α-L-rhamnose-1,4-α-D-galacturonic acid dimers. To the rhamnose residues, L-arabinose and D-galactose can be attached.

Endopolygalacturonases (E.C. 3.2.1.15) are involved in pectin degradation. The enzymes belong to family 28 of glycosyl hydrolases and catalyze the hydrolysis of the α-1,4 glycosidic bonds between adjacent α-D-galacturonic acid residues present in the pectin main chain (Henrissat, 1991). Since endopolygalacturonases are found in extracellular metabolites of many plant pathogenic microbials, investigation of their catalytic mechanism is necessary to understand their mode of action in plant pathology. Recently, crystal structures of endopolygalacturonases from the bacterium *Erwinia carotovora* (Pickersgill *et al.*, 1998) and the fungus *Aspergillus niger* (van Santen *et al.*, 1999) have been solved at 1.9 and 1.68 Å resolutions, respectively. These structures are right-handed parallel β-helices, which were also found in the other pectin-degrading enzymes rhamnogalacturonase A (Petersen *et al.*, 1997) and pectate lyase (Yoder *et al.*, 1993, Lietzke *et al.*, 1996). Three conserved aspartate residues critical for catalysis (Kester *et al.*, 1996) were assigned in those structures. However, other structural information for the active-site architecture is still unclear. To understand the functional mechanism precisely, a more accurate structure and details of the protonation states of these residues are necessary. This information seems to be provided by atomic resolution structures (Kuhn *et al.*, 1998; Berisio *et al.*, 1999).

In this study, we have crystallized endopolygalacturonase I (endoPG I) from *S. purpureum* at an ultrahigh resolution, 0.96 Å. *S. purpureum* is a pathogenic fungus that causes silver-leaf disease in apple trees and produces a great amount of several endopolygalacturonases into the culture medium (Miyairi *et al.*, 1977, 1985). These endopolygalacturonases degrade pectin in leaves and cause silver-leaf symptoms. EndoPG I is one of these endopolygalacturonases and is a glycoprotein of 335 amino-acid residues (Miyairi *et al.*, 1997). EndoPG I consists of three glycoforms differing in the number of glycosylation sites (Ia, two sugar chains; Ib, three sugar chains; Ic, four sugar chains). Each glycoform of endoPG I can be separated using cation-exchange column chromatography (Hasui *et al.*, 1998) and their glycosylation sites and sugar-chain structures have been well defined in a previous study (Shimizu *et al.*, 2000). To avoid heterogeneity of the sugar-chain structures, deglycosylated endoPG Ia was used in this crystallization study. The crystals obtained diffract to 0.96 Å resolution using synchrotron radiation.

2. Experimental procedure

2.1. Purification and deglycosylation

EndoPG Ia was purified from culture filtrate of *S. purpureum* as described by Hasui *et al.* (1998). The purified enzyme was deglycosylated with *endo*-β-*N*-acetylglucosaminidase H (Endo H; BioLabs, New England, USA) as follows. 10 mg of the purified enzyme was desalted and dissolved in 400 µl 50 m*M* acetate–pyridine buffer pH 5.6 and 500 units of Endo H was then added. The reaction mixture

was incubated overnight at 310 K. After incubation, the reaction mixture was filtrated with an Ultrafree-MC 5000 FMWL filter unit (Millipore, Massachusetts, USA) to exclude sugar chains. The high molecular weight fraction was collected and lyophilized as the deglycosylated enzyme.

2.2. Crystallization

Crystals of deglycosylated endoPG Ia were obtained by the hanging-drop vapour-diffusion method. Crystallization experiments were carried out at 293 K. Lyophilized powder of the deglycosylated endoPG Ia was dissolved in 50 m*M* sodium acetate buffer pH 5.0 to a concentration of 10 mg ml^{-1}. Initially, the crystallization conditions search was performed using the Crystal Screen kit (Hampton Research, California, USA); crystals were obtained in several conditions containing PEGs. Finally, 50 m*M* sodium acetate buffer pH 5.0 containing 0.2 *M* NaCl and 12–17.5% PEG 4000 was used as reservoir solution. Each experiment utilizes a 4 µl drop containing equal volumes of the protein solution and reservoir solution equilibrated against 800 µl of reservoir solution.

2.3. X-ray diffraction analysis

Native data extending to a resolution of 0.96 Å were collected at 100 K from a single crystal of dimensions 0.5 × 0.2 × 0.2 mm. Prior to the measurement, the crystal was soaked in a cryoprotectant solution containing 50 m*M* acetate buffer pH 5.0, 20% PEG 4000, 0.1 *M* NaCl, 20% glycerol and 10% 2-propanol. Data collection was performed at beamline BL44B2, SPring-8 using a MAR CCD165 detector. The wavelength of the X-ray beam was 0.7 Å. Since low-resolution diffraction was saturated owing to the narrow dynamic range of the CCD detector, three data sets were collected at different resolutions with appropriate exposure times. Intensities were integrated with *MOSFLM* (Leslie, 1999) and scaled with *SCALA* (Collaborative Computational Project, Number 4, 1994).

3. Results and discussion

EndoPG Ia containing two sugar chains at specific N-glycosylation sites (Asn92, Asn161) was purified from *S. purpureum* culture filtrate. This purified endoPG Ia was deglycosyalated with *endo*-β-*N*-acetylglucosaminidase H before crystallization. In this deglycosylated endoPG Ia, one acetylglucosamine residue remains on each glycosyalation sites.

The crystals (Fig. 1) obtained belonged to the triclinic space group *P*1, with unit-cell parameters a = 37.26 (0.01), b = 46.34 (0.01), c = 52.05 (0.01) Å, α = 67.17 (0.01), β = 72.44 (0.01), γ = 68.90 (02)°. Assuming one monomer per asymmetric unit, a Matthews coefficient V_M of 2.17 Å^3 Da^{-1} was obtained; this is in the range normally found for proteins (Matthews, 1968).

Fig. 2 shows a diffraction pattern of the deglycosylated endoPG Ia crystal collected at the closest possible crystal-to-detector distance (85 mm) on beamline BL44B2, SPring-8. Diffraction spots were detected to the edge of the detector (0.96 Å). Crystallographic and data-collection parameters are given in Table 1. The data set was 89.4% complete even in the outermost shell (1.01–0.96 Å) and the mean $I/\sigma(I)$ value in this shell was 3.5. These observations correspond to the criteria of atomic resolution defined by Sheldrick (1990).

Table 1
Summary of data collection and statistics.

Data collection.

Resolution	High	Medium	Low
Crystal-to-detector distance (mm)	85	100	340
Minimum resolution (Å)	11.9	14.0	41.5
Maximum resolution (Å)	0.96	1.07	2.69
Exposure time (s)	15	15	5
Oscillation angle (°)	1.0	1.0	3.0
No. of images	360	360	120

Statistics of merged data. Values in parentheses are for the outer resolution shell (1.01–0.96 Å).

No. of unique reflections	171534
No. of total reflections	1186116
R_{merge} (%)	3.7 (20.6)
Completeness (%)	94.8 (89.4)
$I/\sigma(I)$, outer shell	3.5

Atomic resolution data will give highly accurate and anisotropic atomic description parameters. Furthermore, this allows the modelling of normally unidentified structure such as multiple conformation regions or H-atom positions. This information will make it possible to discuss the catalytic mechanism more accurately and to characterize the active-site architecture. Hg- and Pt-derivative crystals have been obtained. The structure will be solved by the isomorphous replacement method.

The authors are grateful to Professor Kanzo Sakata for support and encourage-

Figure 1
Crystal of deglycosylated endopolygalacturonase I.

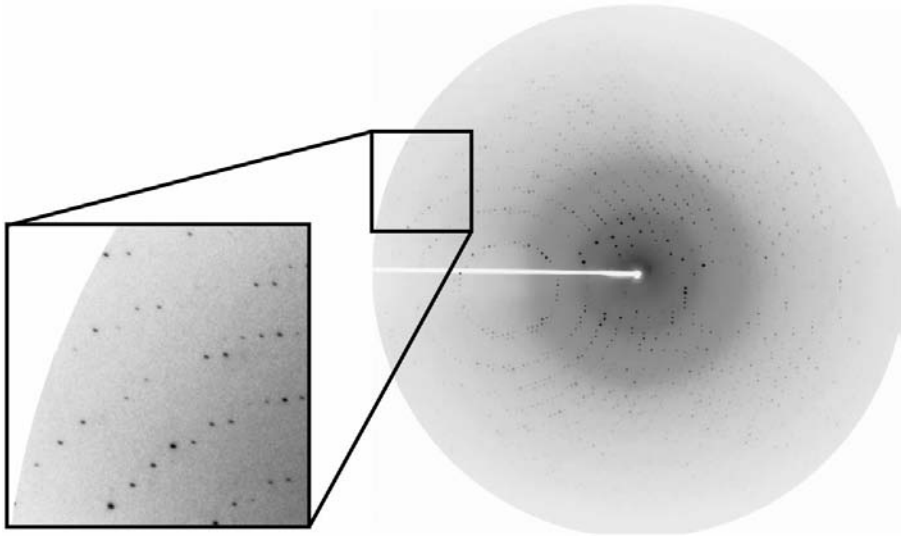

Figure 2
Detail from the diffraction pattern of deglycosylated endopolygalacturonase I with synchrotron radiation at cryogenic temperature (100 K). The detector edge corresponds to 0.96 Å resolution.

ment during the work at the Institute for Chemical Research, Kyoto University. This work was partially supported by a grant from the Ministry of Education, Science, Sports and Culture of Japan.

References

Berisio, R., Lamzin, V. S., Sica, F., Wilson, K. S., Zagari, A. & Mazzarella, L. (1999). *J. Mol. Biol.* **292**, 845–854.

Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.

Collmer, A. (1986). *Annu. Rev. Phytopathol.*, **24**, 385–409.

Hasui, Y., Fukui, Y., Kikuchi, J., Kato, N., Miyairi, K. & Okuno, T. (1998). *Biosci. Biotechnol. Biochem.* **62**, 852–857.

Henrissat, B. (1991). *Biochem. J.* **280**, 309–316.

Kester, H. C., Kusters-van Someren, M. A., Muller, Y. & Visser, J. (1996). *Eur. J. Biochem.* **240**, 738–746.

Kuhn, P., Knapp, M., Soltis, S. M., Ganshaw, G., Thoene, M. & Bott, R. (1998). *Biochemistry*, **37**, 13446–13452.

Leslie, A. G. W. (1999). *Acta Cryst.* D**55**, 1696–1702.

Lietzke, S. E., Scavetta, R. D., Yoder, M. D. & Jurnak, F. (1996). *Plant Physiol.* **111**, 73–92.

Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.

Miyairi, K., Fujita, T., Okuno, T. & Sawai, K. (1977). *Agric. Biol. Chem.* **41**, 1897–1902.

Miyairi, K., Okuno, T. & Sawai, K. (1985). *Agric. Biol. Chem.* **49**, 1111–1118.

Miyairi, K., Senda, M., Watanabe, M., Hasui, Y. & Okuno, T. (1997). *Biosci. Biotechnol. Biochem.* **61**, 655–659.

Petersen, T. N., Kauppinen, S. & Larsen, S. (1997). *Structure*, **5**, 533–544.

Pickersgill, R., Smith, D., Worboys, K. & Jenkins, J. (1998). *J. Biol. Chem.* **273**, 24660–24664.

Santen, Y. van, Benen, J. A., Schroter, K. H., Kalk, K. H., Armand, S., Visser, J. & Dijkstra, B. W. (1999). *J. Biol. Chem.* **274**, 30474–30480.

Sheldrick, G. M. (1990). *Acta Cryst.* A**46**, 467–473.

Shimizu, T., Miyairi, K. & Okuno, T. (2000). *Eur. J. Biochem.* **267**, 2380–2389.

Yoder, M. D., Keen, N. T. & Jurnak, F. (1993). *Science*, **260**, 1503–1507.