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Crystallization and preliminary X-ray analysis of high-alkaline pectate lyase

Masatake Akita,[a] Atsuo Suzuki,[a]* Tohru Kobayashi,[b] Susumu Ito[b] and Takashi Yamane[a]

[a]Department of Biotechnology and Biomaterial Chemistry, Graduate School of Engineering, Nagoya University, Chikusa-ku, Nagoya 464-8603, Japan, and [b]Tochigi Research Laboratories, Kao Corporation, 2606 Akabane, Ichikai, Haga, Tochigi 321-3497, Japan

Correspondence e-mail: a41114a@nucc.cc.nagoya-u.ac.jp

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Pel-15, a high-alkaline pectate lyase (pectate transeliminase; E.C. 4.2.2.2) from *Bacillus* sp. strain KSM-P15, has been crystallized using the hanging-drop vapour-diffusion method at 277 K. Two different crystal forms were obtained and preliminary X-ray diffraction data were collected from each crystal form at 100 K. Both forms belong to the orthorhombic space group $P2_12_12_1$ and contain one molecule per asymmetric unit. The unit-cell parameters of form I are a = 43.2 (2), b = 60.2 (2), c = 82.2 (2) Å and those of form II are a = 42.9 (1), b = 43.4 (1), c = 105.9 (3) Å. Diffraction data to a resolution of 1.5 Å were collected from form II crystals using a synchrotron-radiation source.

1. Introduction

Pectate lyases (Pel; pectate transeliminase; E.C. 4.2.2.2) degrade the components of the middle lamella and cell wall of higher plants and cause soft-rot disease. Pels catalyse the degradation of polygalacturonic acid (PGA) through a *trans*-elimination mechanism; the degradation requires Ca^{2+} ions in order for enzymatic activity to occur. Crystal structures of PelC and PelE from *Erwinia chrysanthemi* (Yoder, Keen *et al.*, 1993; Yoder, Lietske *et al.*, 1993; Lietzke *et al.*, 1996) and BsPel from *Bacillus subtilis* (Pickersgill *et al.*, 1994) have been determined. These Pel structures have a charactaristic domain motif, the parallel β-helix. The parallel β-helix consists of predominantly parallel β-strands that are coiled into a helix. Within the core of the helix, amino acids form linear stacks, including an asparagine ladder (Yoder, Keen *et al.*, 1993).

Bacillus sp. strain KSM-P15 produces a low molecular-weight high-alkaline Pel (Pel-15) in alkaline culture (Kobayashi *et al.*, 1999). The molecular weight of Pel-15 was estimated to be 20 924 Da; it consists of 197 amino-acid residues. The molecular weight of Pels from other species range from 20 to 74 kDa and Pel-15 belongs to one of the low molecular-weight Pel groups. Pel-15 has the highest optimal pH (10.5) for activity of the Pels that have been reported thus far. Generally, Pels from other strains have an optimal pH in the range 8–10. Tsuchiya *et al.* (1997) suggest that the thermostability of 3-isopropylmalate dehydrogenase increases on the removal of redundant parts. Pel-15 may similarly adapt itself to the high-alkaline conditions by removing redundant fractions such as surface loops, leading to its drastic downsizing. The sequence identities of Pel-15 to PelC (37 676 Da; Yoder, Keen *et al.*, 1993; Yoder, Lietske *et al.*, 1993), PelE (38 069 Da; Lietzke *et al.*, 1996) and BsPel (43 505 Da; Pickersgill *et al.*, 1994) are 13.2, 16.7 and 11.8%, respectively. On the other hand, sequence identities between PelC (Yoder, Keen *et al.*, 1993; Yoder, Lietske *et al.*, 1993), PelE (Lietzke *et al.*, 1996) and BsPel (Pickersgill *et al.*, 1994) are almost 30%. In fact, the N-terminal amino-acid sequence of the intact Pel-15 and its lysyl endopeptidase-cleaved polypeptides were different to those of other Pels that have been reported thus far (Kobayashi *et al.*, 1999). Pel-15 more closely resembles PelD from the fungus *F. solani* f. sp. *Pisi* (Guo *et al.*, 1996) than other Pels from bacteria, suggesting that the Pel-15 may belong to a new family of Pels.

2. Materials and methods

Isolation and purification of pectate lyase were performed as described previously (Kobayashi *et al.*, 1999).

The initial crystal screening was carried out using Crystal Screen I (Hampton Research). Crystals were prepared by the hanging-drop vapour-diffusion method at 277 K. 2 µl protein solution was mixed with an equal volume of reservoir solution. The protein solution consisted of 2.5%(*w*/*v*) Pel-15 and 1 m*M* $CaCl_2$ in 50 m*M* Tris–HCl buffer pH 7.5. The volume of the reservoir solution was 1 ml. Crystals of maximum dimensions 75 × 50 × 150 µm were grown using a reservoir solution containing 28%(*w*/*v*) polyethylene glycol 8000 (PEG 8000) in 100 m*M* MES–NaOH pH 6.7 (Fig. 1).

The crystals were scooped up in nylon loops and were cooled by plunging them into liquid nitrogen without cryoprotectant. Preliminary X-ray studies were performed using a Rigaku

Table 1
Data collection from form II crystal (at the Photon Factory).

Figures in parentheses refer to reflections with $I > 3\sigma(I)$.

Resolution (Å)	20–1.5
Total no. of reflections	66265
No. of independent reflections	30234 (26397)
$R_{\rm merge}$†	3.4
Completeness (%)	91.2 (79.6)
Completeness of final shell (1.55–1.5 Å) (%)	75.8 (51.6)

† $R_{\rm merge} = \sum|I - \langle I\rangle|/\sum I$.

R-AXIS IV detector system on a Rigaku RU-300 rotating-anode generator, using double focusing mirror monochromated Cu $K\alpha$ radiation. The crystals were placed in a cold nitrogen-gas stream which was maintained at 100 K (Oxford Cryosystems Cryostream). High-resolution diffraction data were collected using a screenless macromolecular Weissenberg camera (Sakabe, 1983) on the BL-6A station at the Photon Factory, the High Energy Accelarator Research Organization, Tsukuba, Japan, which was operated at 2.5 GeV. Incident X-rays were monochromated by a triangular single monochromator. X-ray diffraction patterns were recorded on a Fuji imaging plate using the Weissenberg method. Each diffraction image was digitized using a Fuji BAS2000 scanner and processed using the *DENZO* and *SCALEPACK* programs (Otwinowski, 1993; Minor, 1993).

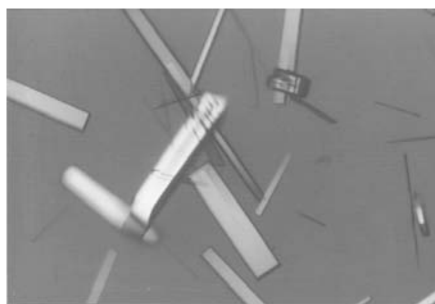

Figure 1
Crystals of Pel-15 grown in 28%(w/v) PEG 8000 in 100 mM MES–NaOH pH 6.7 buffer.

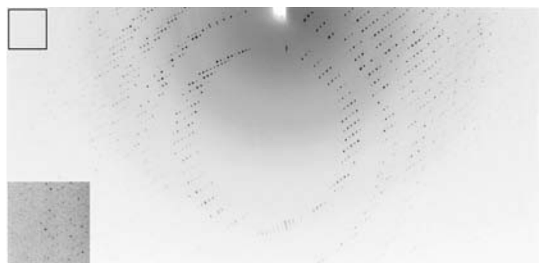

Figure 2
X-ray diffraction pattern from a Weissenberg image of Pel-15. A film-cassette radius of 286.5 mm and image plates of dimensions 400 × 200 mm were used. The oscillation range was 3°, the coupling constant was 0.6° mm^{-1} and the wavelength of the X-rays was 1.0 Å. The bottom-left inset shows detail of the image around 1.5 Å resolution, indicated by upper-left box.

3. Results and discussion

The Pel-15 crystals appeared using 8–28%(w/v) PEG 8000. When crystallization was performed below a concentration of 18%(w/v) PEG 8000, tiny needle-like crystals (5 × 5 × 50 µm) appeared in a week. However, these crystals did not grow further. When crystallization was performed in the range 18–24%(w/v) PEG 8000, needle-like crystals appeared in a few days and after one or two months many crystals appeared in one drop. These crystals tended to be aggregated together. When crystallization was performed in 28%(w/v) PEG 8000, thin plate-shaped crystals (of average dimensions 75 × 50 × 150 µm) were obtained in one month. These crystals were suitable for X-ray analysis. When crystals were grown in 28%(w/v) PEG 8000, the crystallization solution did not have to be replaced with cryoprotectant before cooling, as the PEG 8000 acts as a cryoprotectant.

X-ray analysis revealed that Pel-15 has two crystal forms: form I and form II. However, these forms cannot be differentiated based on the appearance of the crystals. Form I belongs to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 43.2 (2), b = 60.2 (2), c = 82.2 (2) Å. Form II also belongs to the orthorhombic space group $P2_12_12_1$; however, the unit-cell parameters of form II are a = 42.9 (1), b = 43.4 (1), c = 105.9 (3) Å. When the asymmetric unit is assumed to contain one Pel-15 molecule (M_w = 21 kDa), the V_m value (Matthews, 1968) for form I is 2.54 Å^3 Da^{-1} and that for form II is 2.34 Å^3 Da^{-1}. V_m values for both forms are within the accepted range.

Both forms of Pel-15 crystal can diffract to 2.5 Å resolution using a cryogenic mounting device on a rotating-anode X-ray generator. However, the form I crystal has a higher mosaicity than the form II crystal, so that high-resolution data from the form I crystal have a lower completeness than those from the form II crystal.

High-resolution data to 1.5 Å (Fig. 2) was collected from the form II crystal using synchrotron radiation at the Photon Factory. The crystal did not decay after a full data collection. The processed data is listed in Table 1. Heavy-atom derivatives of these crystals are now being prepared.

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