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Crystallization and preliminary X-ray analysis of endopolygalacturonase SE1 from *Trichosporon penicillatum*

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Endopolygalacturonase SE1 from *Trichosporon penicillatum* has been crystallized by the hanging-drop method of vapour diffusion using ammonium sulfate as a precipitant. The crystals belong to the hexagonal space group $P6_1$ or $P6_5$, with unit-cell parameters $a = b = 135.0$, $c = 70.7$ Å, $\gamma = 120^\circ$. The calculated V_M based on one molecule per asymmetric unit is 3.09 Å^3 Da^{-1}. A native data set from a crystal has been collected to 2.0 Å resolution on a Cu $K\alpha$ rotating-anode X-ray source.

1. Introduction

Polygalacturonases catalyze the hydrolysis of α-1,4-glycosidic linkages in polygalacturonic acid chains, which are a major component of pectic substances of plant cell walls. Depending on the target site of the attack, the enzymes can be divided into two types: the endo-type (E.C. 3.2.1.15), which has a random attack mechanism, and the exo-type (E.C. 3.2.1.67), which has a terminal attack mechanism. Pectolytic enzymes such as polygalacturonases are produced in a wide variety of organisms (Rombouts & Pilnik, 1980). Microbial pectolytic enzymes have been extensively studied in order to elucidate the mechanism of plant pathogenesis. These enzymes initiate soft-rot diseases in plants by degrading the pectic substances of the middle lamellae. Plant enzymes also play an important role in fruit ripening. The synthesis of pectic substances is performed in young enlarging cell walls during the early stages of growth. Compared with young actively growing tissues, lignified tissues have a low concentration of pectic substances. One of the most characteristic changes during the ripening of fleshy fruits is their softening. This change is attributed to enzymatic degradation and solubilization of the pectic substances (Soda *et al.*, 1986; Dick & Labavitch, 1989).

In most plant tissues, pectin is in a water-insoluble form called protopectin. Protopectin yields water-soluble pectins of various molecular weights upon limited hydrolysis. The decomposition of protopectin was originally attributed to the action of a specific protopectinase (Brinton *et al.*, 1927). Further research on pectolytic enzymes revealed that protopectin is decomposed by the action of a series of enzymes including endopolygalacturonase, pectin esterase, endopectate lyase and pectin lyase on a polygalacturonic acid chain. Pectin has characteristics such as gelation and emulsion stabilization, which make it useful in the manufacture of food, cosmetics and medicine. In pectin production, a process of acid extraction is involved. However, this process has several disadvantages: maceration of the pulp, difficulty in the filtration of the residue and corrosion of equipment. Thus, the enzymatic degradation of pectic substances has industrially invaluable advantages.

Sakai *et al.* (1993) have found many microorganisms to produce protopectinases during their studies to develop alternative methods for pectin production using specific enzymes. They isolated three protopectinases, SE1, SE2 and SE3, with endopolygalacturonase activity from a fungus-like yeast *Trichosporon penicillatum* B2 (Iguchi *et al.*, 1996). These enzymes produce polygalacturonic acid by specific hydrolysis of the polygalacturonic acid region of protopectin and by random hydrolysis of water-soluble polygalacturonic acid chains. The former reaction (a protopectinase activity) is superior to the latter reaction (an endopolygalacturonase activity). In addition, the protopectinase activity of each of the enzymes does not parallel their endopolygalacturonase activity. These results raise the question of how enzymes develop a high affinity for the polygalacturonic acid region of protopectin.

Recently, crystal structures of endopolygalacturonases from the bacterium *Erwinia carotovora* (Pickersgill *et al.*, 1998) and the fungus *Aspergillus niger* (Santen *et al.*, 1999) have been identified. They have a common unique structure consisting of a right-handed parallel β-helix with ten complete turns. The enzymes SE1, SE2 and SE3 have a substrate specificity similar to those of *E. carotovora* and *A. niger* and have amino-acid sequences of about 50% identity with *A. niger* and only about 20% identity with *E. carotovora*. These findings gave us further interest in elucidating the structure–function relationships of SE1, SE2 and SE3 in comparison with the enzymes

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of *E. carotovora* and *A. niger*. As the first step toward structural elucidation of the enzymes, we report here the crystallization and preliminary X-ray analysis of the endopolygalacturonase SE1 (289 amino acids), which has the highest affinity for protopectin of the three enzymes described above.

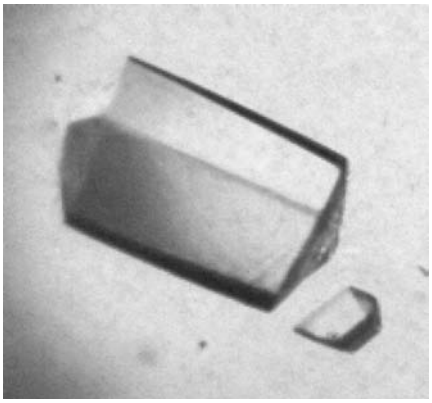

Figure 1
A typical crystal of polygalacturonase SE1 from *T. penicillatum*. The dimensions of the crystal are approximately 0.7 × 0.4 × 0.2 mm.

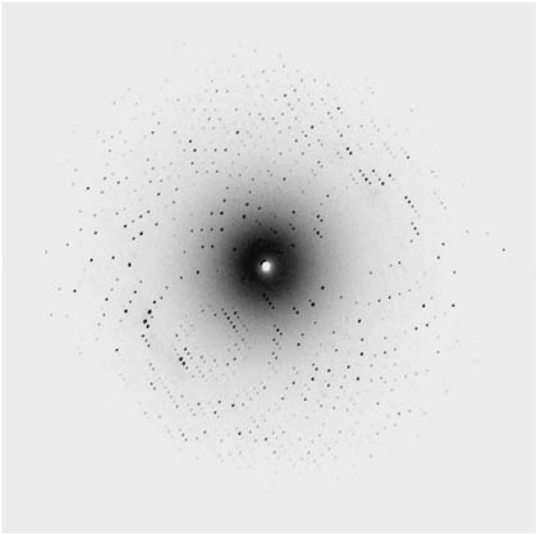

Figure 2
A diffraction image of polygalacturonase SE1 from *T. penicillatum*.

2. Methods and results

2.1. Crystallization

T. penicillatum B2, which is a γ-ray irradiation mutant induced from *T. penicillatum* SNO3 to increase the production of SE1, was cultured. Its endopolygalacturonase SE1 was collected from the culture medium and purified to homogeneity as reported previously (Iguchi *et al.*, 1996). Prior to crystallization, the enzyme was concentrated to approximately 9 mg ml^{-1} in 20 m*M* acetate buffer at pH 5.0 containing 0.02%(*w*/*v*) sodium azide. All crystallization trials were carried out using the hanging-drop method of vapour diffusion (McPherson, 1990). Since microcrystals of SE1 were obtained from the precipitated fraction with ammonium sulfate during purification (Sakai & Okushima, 1982), conditions for crystallization were optimized by the conventional method in which the buffer system and the concentration of ammonium sulfate were varied. Optimized crystallization conditions were established as mixing 2 µl of protein solution with an equal volume of reservoir solution containing 2.0 *M* ammonium sulfate and 0.02%(*w*/*v*) sodium azide in 0.1 *M* acetate buffer pH 5.5 at 277 K. Well formed crystals grew to maximal dimensions of approximately 0.7 × 0.4 × 0.2 mm in two weeks (Fig. 1).

2.2. Data collection

X-ray diffraction data were 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 110 mm and the oscillation range was 1°. A limited number of reflections were observed to 2.0 Å resolution (Fig. 2). Data were reduced using the Rigaku *PROCESS* crystallographic data-reduction package.

A total of 104 953 observed reflections were scaled and reduced to yield a data set containing 36 843 unique reflections with an $R_{\rm merge}$ of 6.8%. The data set was 93.8% complete to 2.5 Å resolution and 90.3% complete to 2.0 Å resolution, with data in the 2.2–2.0 Å resolution shell being 81.8% complete. The average $I/\sigma(I)$ value in this shell was 5.24.

The crystal class was determined to be hexagonal space group $P6_1$ (or its enantiomorph $P6_5$) based on the symmetry and the systematic absences of the reflections, with unit-cell parameters $a = b = 135.0$, $c = 70.7$ Å, $\gamma = 120°$. The $V_{\rm M}$ value of 3.09 Å^3 Da^{-1} is consistent with the presence of one molecule (30 kDa) per asymmetric unit and corresponds to a solvent content of 60.2%. This value is in the range of values tabulated by Matthews (1968). Structural analysis of the endopolygalacturonase SE1 is in progress.

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