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X-ray analysis of crystals of polygalacturonase A from *Pseudomonas solanacearum*.

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Abstract

Crystals of the pectolytic protein, polygalacturonase A, have been obtained from polyethylene glycol 8000 using vapor-diffusion methods. The 52.4 kDa protein is secreted by the plant pathogenic bacteria *Pseudomonas solanacearum*, and is important in the virulence of this plant pathogen. The protein crystallizes in space group $P2_1$ and has unit-cell parameters of a = 101.9, b = 124.6, c = 48.1 Å, and β = 105°50′. The crystal has two molecules in the asymmetric unit, and diffracts maximally to a resolution of 2.1 Å.

Polygalacturonases (PG's) are one of a host of enzymes secreted by microbial plant pathogens capable of attacking plant cell components. Pectate-degrading enzymes have been shown to play an important role in pathogenesis by numerous bacterial plant pathogens (Collmer & Keen, 1986). *Pseudomonas solanacearum* is a soil-borne plant pathogen that causes wilting diseases in many crop plants worldwide (Hayward, 1991). Schell, Roberts & Denny (1988) and Denny, Carney & Schell (1990) were able to demonstrate that a major secreted PG encoded by the *pglA* gene plays a significant role in disease, but is not absolutely necessary for pathogenesis. *P. solanacearum* produces at least two other PG's besides PglA, both are apparently exopolygalacturonases (Schell, Denny & Huang, 1994). To facilitate purification and increase yields of PglA for crystallization experiments, the protein was purified from culture supernatants of a strain engineered to overproduce PglA and to have reduced production of other extracellular enzymes. Based on its deduced amino-acid sequence (Huang & Schell, 1990), mature PglA has 508 amino acids, a p*I* of 8.8, and a molecular mass of 52.4 kDa. The sequence of PglA between residues 309 and 371 has 55% sequence similarity with PG's from tomato and *E. carotovora* (Schell *et al.*, 1994). The two proteins may not be closely related though, due to differences in the restriction maps of the genes for the PG's from *P. solanacearum* and *E. carotovora*, and to differences in the molecular weights of the proteins (Hinton, Gill, Lalo, Plastow & Salmond, 1990).

There are two major families of pectolytic enzymes, pectate lyases and polygalacturonases. Although the substrates cleaved by both families of enzymes are similar, their enzymatic mechanisms differ. While pectate lyases cleave by a β elimination, PG's cleave by hydrolysis. One of the first reports of crystallization of a pectolytic enzyme was by Uchino, Kurono & Shinji (1966) who reported obtaining microcrystals of a fungal PG from ammonium sulfate during purification. Recently a second report of the crystallization of a fungal polygalacturonase from *Aspergillus niger* has appeared, and includes a preliminary X-ray crystallographic characterization of the protein (Schröter, Arkema, Kester, Visser & Dijkstra, 1994). Currently there are three high-resolution structures of pectolytic enzymes reported, all of which are pectate lyases (Yoder, Keen & Jurnak, 1992; Lietzke, Keen, Yoder & Jurnak, 1994; Pickersgill, Jenkins, Harris, Nasser & Robert-Baudouy, 1994). All three structures are composed of three parallel β-sheets in a parallel β-helix motif. Structural analysis by Goodenough *et al.* (1991) of a fungal PG from *Clostridium thermocellum* based on far UV–CD spectra suggests that PG will also contain predominately β structure, with very little α structure.

In this study, PG was purified from *P. solanacearum* AR containing pDR340, a broad host range plasmid construct overexpressing the *pglA* gene from a *lac* promoter (Schell *et al.*, 1988). To simplify purification *P. solanacearum* strain AR was used as host because it harbors a *phcA* mutation that simultaneously increases *pglA* expression and blocks expression of many other extracellular proteins and viscous extracellular polysaccharides (Schell *et al.*, 1994; Huang, Denny, & Schell, 1993). The protein was purified based on the procedure described by Schell *et al.* (1988). To a culture medium composed of 20 m*M* sodium-potassium phosphate, pH 7.0, 0.1% ammonium sulfate, 0.2% glucose, 0.1% yeast extract, 0.1% casamino acids, and 20 μg ml^{-1} tetracycline, the *P. solanacearum* AR pDR340 cells were inoculated and incubated at 305 K for 48 h. Cells were removed by centrifugation and the culture supernatant, containing the secreted PG protein, was concentrated by stir-cell ultrafiltration (Amicon, YM10 membrane). The concentrate was dialyzed against 5 m*M* Tris(hydroxymethyl)aminomethane–HCl (Tris–HCl), pH 7.0 and loaded onto a carboxymethyl cellulose column. After

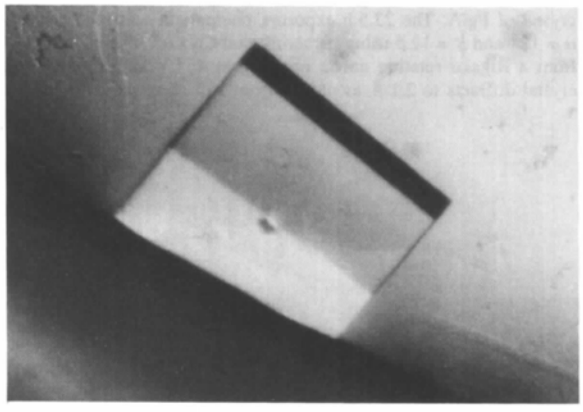

Fig. 1. Typical crystal of polygalacturonase from *Pseudomonas solanacearum*. Crystals grow to dimensions of approximately 0.75 × 0.5 × 0.2 mm.

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washing with the above Tris buffer, the protein was eluted with a linear gradient from 0.0 to 0.25 *M* KCl. The protein fractions were precipitated by addition of ammonium sulfate to 80%, and the centrifuged pellet resuspended in 5 m*M* Tris–HCl, pH 7.0. The protein was deemed pure by analysis with sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis.

Crystals were grown from polyethylene glycol (PEG) using the sitting-drop vapor-phase equilibration method (McPherson, 1982). A solution containing 5 OD_{280} ml^{-1} of PglA, 15% PEG 8000, 0.075 *M* $MgCl_2$, 0.05 *M* HEPES, pH 6.5, was placed in the depression of the post of a 24-well Cryschem plate (Supper Co.). The solution was equilibrated over a reservoir of 30% PEG 8000, 0.15 *M* $MgCl_2$, and 0.1 *M* HEPES, pH 6.5 and sealed with clear tape. Crystals grew at room temperature in about two weeks. A sample crystal is shown in Fig. 1.

Crystals were mounted in glass capillaries with mother liquor and sealed. Preliminary X-ray diffraction data were collected by precession-photography techniques at room temperature using Ni-filtered Cu $K\alpha$ radiation from a rotating anode X-ray source operated at 45 kV and 170 mA. Based on the precession photographs (Fig. 2), the space group is assigned to $P2_1$, and the unit-cell dimensions calculated are a = 101.9, b = 124.6, c = 48.1 Å, $\beta = 105°50'$, giving a unit-cell volume of 5.9×10^5 Å^3.

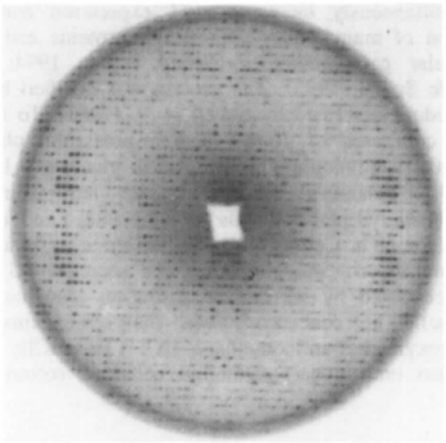

Fig. 2. Precession photograph of the 0*kl* reciprocal lattice zone from a crystal of PglA. The 23.5 h exposure photograph was recorded with $\mu = 12°$ and R = 12.5 using nickel-filtered Cu $K\alpha$ radiation generated from a Rigaku rotating anode operated at 45 kV and 170 mA. The crystal diffracts to 2.1 Å as judged from still photographs.

The number of molecules per asymmetric unit was estimated based on a comparison of V_m, the ratio of unit cell volume to protein mass, and V_s, the fractional volume occupied by solvent. Assuming either one or two molecules per asymmetric unit, V_m is calculated to be 5.6 or 2.8 Å^3 Da^{-1}, respectively. The fractional solvent content was estimated by $V_s = 1 - (\rho/V_m)$, where ρ is the crystal density. An average value of 1.23 g cm^{-3} was assumed for ρ. Then, V_s values calculated with either one or two molecules per asymmetric unit, are 78.0 and 56.1%, respectively. Typical V_m values for protein crystals range from 1.68 to 3.53 Å^3Da^{-1}, while solvent content values typically fall between 27 and 65% (Matthews, 1968). Therefore, the crystals are presumed to contain two molecules per asymmetric unit. It is unknown if the protein forms dimers, or if the protein exists in the crystal as isolated monomers.

The crystals diffract to at least 2.1 Å and are adequate for the X-ray crystallographic structure analysis of the protein. The structure solution will be solved using multiple isomorphous replacement techniques.

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