J. Mol. Biol. (1993) 232, 995–996

Crystallization of a Chitosanase from *Streptomyces* N174

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(Received 10 March 1993; accepted 2 April 1993)

Chitosanases are produced by many soil fungi and bacteria to degrade chitosan present in fungal cell walls. Here, we report the crystallization of a 29,500 dalton protein with chitosan endo-hydrolase activity isolated from *Streptomyces* N174. The crystals were grown by vapor diffusion. They are mechanically strong and diffract to at least 1·9 Å resolution. The crystals belong to the monoclinic space group $P2_1$ with unit cell parameters $a = 56{\cdot}4$ Å, $b = 59{\cdot}6$ Å, $c = 86{\cdot}1$ Å and $\beta = 96{\cdot}6°$. Cell parameters and crystal density are consistent with two chitosanase molecules per asymmetric unit.

Keywords: crystallization; X-ray analysis; chitosanase; *Streptomyces*

Chitosan is a biopolymer of β-(1,4)-linked D-glucosamine residues with varying degrees of *N*-acetylated residues. The polymer has many diverse commercial applications, ranging from bioerodeable suture material to metal removal from waste-streams (Sandford, 1989). Although manufactured industrially *via* deacetylation of chitin, chitosan is found naturally as a cell-wall component of medically significant fungi, such as *Zygomycetes*. An estimated 1 to 7% of heterotrophic soil bacteria, as well as certain plants and fungi, express protein chitosanases capable of degrading chitosan (Davis & Eveleigh, 1984). Multiple chitosanase forms have been detected in several plant species, where they are believed to play a role in defense against invading pathogens (El Ouakfaoui & Asselin, 1992). Both chitosanases and chitosan oligomers have been shown to exhibit anti-fungal activity against various species (Price & Storck, 1975; ElGhaouth *et al*, 1992). In addition, chitosan is able to induce disease resistance response genes, including endo-β-glucanase and endochitinase, in many plant species, such as pea, tomato and wheat (Hadwiger *et al*, 1984). Chitosan heptamer appears to be optimal for both activities (Kendra & Hadwiger, 1984).

Chitosanases hydrolyze chitosan predominantly in an endo fashion, producing chitosan oligosaccharides. The *Streptomyces* N174 chitosanase is a 29,500 dalton protein (as observed from SDS-PAGE) that degrades chitosans with 1 to 60% acetylation. It shows no hydrolysis of chitin or cellulose, characteristics that vary considerably between chitosanases (Boucher *et al.*, 1992). The N174 chitosanase gene has been cloned and expressed to high levels in *Streptomyces lividans*, making it amenable to biochemical study (Fink *et al.*, 1991).

A knowledge of the structure of chitosanase should help in understanding the mechanism of substrate binding and catalysis, and may aid in redesigning the enzyme for specific commercial needs. Our laboratory has recently reported the structure of a non-homologous endochitinase (Hart *et al.*, 1993); apart from the intrinsic interest in the chitosanase structure, a comparison of the two structures should prove rewarding. Here, we report the crystallization of the N174 chitosanase as a preliminary step in its X-ray analysis.

Gram quantities of the N174 chitosanase were isolated as described (Boucher *et al.*, 1992) with the following modifications: the producing strain was a recombinant *S. lividans* harboring the pRL270 plasmid (Boucher & Brzezinski, unpublished results), and S-Sepharose Fast Flow (Pharmacia LKB) equilibrated with 25 mM sodium acetate buffer (pH 4), was used in the ion-exchange chromatography step. The protein was dialyzed to a

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0022-2836/93/150995-02 $08.00/0

final concentration of 30 mg/ml in 20 mM sodium acetate (pH 5·5) and mixed with 0·1 volume of a 0·5 M potassium phosphate, 20% (w/v) PEG 8000 solution. This precipitant was initially identified using the Crystal Screen macromolecular crystallization kit (Hampton Research). The protein mixture was suspended over the same precipitant solution using the hanging drop protocol. Crystals appeared within two days and grew to 0·5 mm on the longest side within one week. Crystals can be grown to over 0·7 mm on the longest side, and even small crystals diffract to nearly 2 Å resolution. The crystals form in the space group $P2_1$ with a = 56·4 Å, b = 59·6 Å, c = 86·1 Å and β = 96·6°.

The density of the crystals was measured as 1·19 g/ml, using a xylene/bromobenzene non-linear density gradient (Low & Richards, 1952). Leucine (ρ = 1·165 g/ml), valine (ρ = 1·230 g/ml) and methionine (ρ = 1·292 g/ml) were used as density standards. Assuming a standard protein partial specific volume of 0·74 ml/g, the chitosanase crystal density implies the presence of 4·3 molecules per unit cell. Since the actual number of molecules must be integral, there are most likely four molecules per unit cell and, therefore, two molecules per asymmetric unit. The Matthews parameter, assuming two monomers per asymmetric unit, is 2·44 Å^3/Da. This is within the normal range for protein crystals (V_m = 1·7 to 3·5 Å^3/Da; Matthews, 1968) and consistent with the high diffraction quality.

Native data are being collected on a SDMS multiwire system, and a search is in progress for isomorphous heavy-atom derivatives of the crystals.

This work was supported by grant GM30048 from the National Institutes of Health and by grants from the Foundation for Research and the Welch Foundation. Work at Sherbrooke University was supported by grants from the Natural Sciences and Engineering Council of Canada and from Les Fonds pour la Formation de Chercheurs at l'Aide a la Recherche du Gouvernement du Québec.

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Edited by A. Klug