

CRYSTALLIZATION NOTES

Crystallization of Recombinant Chitinase from the Cloned *chiA* Gene of *Serratia marcescens*

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(Received 16 January 1992; accepted 26 March 1992)

The *chiA* gene encoding for the chitinase enzyme from *Serratia marcescens* was efficiently overexpressed under the pL promoter and the enzyme was secreted into the growth medium. The chitinase was purified to homogeneity using affinity chromatography on a Phenyl-Sepharose column and the protein was successfully crystallized. The crystals are presently in the form of small needles in space group $C222_1$ and have unit cell dimensions $a = 204(\pm 0.5)$ Å, $b = 134(\pm 0.5)$ Å, $c = 60(\pm 0.5)$ Å. The crystals diffract X-rays to about 3 Å resolution and are suitable for three-dimensional structural analysis.

Keywords: chitinase; recombinant; crystallization; *Serratia marcescens*; X-ray

Serratia marcescens is a Gram-negative bacterium found to be an effective biocontrol agent of *Sclerotium rolfsii* under greenhouse conditions (Ordentlich *et al.*, 1988). A particular feature of this bacterium is its capability to secrete several chitinolytic enzymes. The major enzyme is encoded by the *chiA* gene (Jones *et al.*, 1986).

The *chiA* gene has been isolated and cloned into the pBR322 plasmid under the control of the λ oLpL promoter, producing the expression plasmid ppLCHIA (Shapira *et al.*, 1989). The plasmid has been introduced into *Escherichia coli* strain A2097, carrying the defective prophage λ cI857 Δ HIA Δ BamHI, to yield the A5745 clone. Chitinase expression is achieved by heating to 42°C for a short time (30 min), followed by continuous incubation at 40°C for several hours. Under these conditions, the cells continuously overexpress the recombinant chitinase, which is secreted into the growth medium as a major protein product (Oppenheim *et al.*, 1990). The mechanism of chitinase secretion into the growth medium is not yet known. Presumably a

leader sequence at the amino terminal end of the protein directs the synthesized protein to the periplasm where the leader sequence is removed by an enzymatic activity. Preliminary observations suggest that during the thermal induction, the outer bacterial membrane undergoes certain modifications that lead to secretion of the accumulated chitinase into the growth medium.

The chitinase was isolated from the growth medium by ammonium sulphate precipitation to 80% saturation and the precipitated protein was recovered by centrifugation. The precipitated proteins were dissolved in buffer A (20 mM-Tris·HCl (pH 8.0), 1 mM-EDTA, 0.1 mM-phenylmethylsulphonyl fluoride, 1 M-ammonium sulphate) and directly applied to a Phenyl-Sepharose CL-6B column (Pharmacia) equilibrated in the same buffer. The bound chitinase was eluted at the very end of a descending ammonium sulphate gradient from 1.0 to 0.0 M. The chitinase was about 75% pure as judged by SDS-PAGE. The final purification was achieved using a Mono-Q FPLC column at pH 8.0, where the protein was found in the flowthrough to be nearly homogeneous. Figure 1(a) shows a 12.5% SDS-PAGE protein profile of purified chitinase (lane 1). The purified protein was further concentrated using a Centricon, dialysed against buffer B

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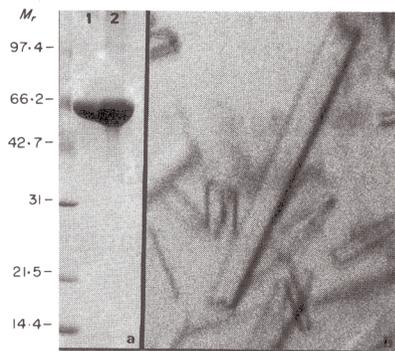


Figure 1. (a) A 12.5% SDS-PAGE of the purified chitinase (lane 1), and chitinase crystals dissolved in Laemmli-buffer (Laemmli, 1970; lane 2). (b) Crystals of chitinase protein obtained at 16°C under the conditions described in the text. The long axis of the large crystal is about 0.5 mm.

(10 mM-sodium phosphate (pH 7.0), 1 mM-EDTA, 100 mM-NaCl) and used for crystallization experiments.

The crystallization of chitinase was screened using the "50 different conditions system" at 4°C and 16°C (Jancarik & Kim, 1991). The protein was at a concentration of 8 to 14 mg/ml in buffer B. Using hanging drop vapour diffusion 2.5 to 5 μ l of protein solution were mixed with an equal volume of precipitating solution and equilibrated against 0.75 to 1.0 ml precipitating solution (McPherson, 1982). Useful crystals were obtained in the pH range 7.4 to 8.0 with 1.8 to 1.9 M-ammonium sulphate in the presence of 0.75 to 1% isopropanol. The crystals appeared in an interesting manner. After mixing the drop with isopropanol, a precipitate appeared after 24 hours. The crystals grow from the precipitate in the following 24 hours.

The crystals were mounted in a glass capillary and diffraction data measured using the EMBL X31 beam line in HASYLAB on the DORIS storage ring. Data were collected using a MAR research imaging plate scanner with a crystal to plate distance of 420 mm and radiation of wavelength 1.009 Å (1 Å = 0.1 nm).

The unit cell dimensions, $a = 204(\pm 0.5)$ Å, $b = 134(\pm 0.5)$ Å, $c = 60(\pm 0.5)$ Å, were determined from the diffraction images. The molecular mass of recombinant chitinase, 60,800 Da, and the unit cell volume, 1.6×10^6 Å³, give a $V_m = 3.4$ Å³/Da. This value suggests that there are one or two chitinase protein molecules in the asymmetric unit. Although the crystals were quite small, the diffraction extended to about 3 Å. We are currently working on improving the crystallization procedure.

In summary, the overexpression of recombinant chitinase in *E. coli* and the simple and fast purification scheme made crystallization trials possible. In a relatively short time we obtained crystals that



Figure 2. 1° rotation image from a chitinase crystal. The diffraction pattern extends to about 3.0 Å resolution.

diffract to medium resolution. Preliminary X-ray diffraction data are reported for the chitinase crystals and we will start to use the diffraction data for the structural determination of the protein.

We are grateful to M. Dauter for excellent technical assistance. This research is a part of an ongoing joint research program between A.B.O. and Dr I. Chet, Otto Warburg Center, The Hebrew University, Faculty of Agriculture, Rehovot, Israel.

References

- Jancarik, J. & Kim, S. H. (1991). Sparse matrix sampling: a screening method for crystallization of proteins. *J. Appl. Crystallogr.* **24**, 409–411.
- Jones, J. D. G., Grady, K. L., Suslow, T. V. & Bedbrook, J. R. (1986). Isolation and characterization of genes encoding two chitinase enzymes from *Serratia marcescens*. *EMBO J.* **5**, 467–473.
- Laemmli, U. K. (1970). Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature (London)*, **227**, 680–685.
- McPherson, A. (1982). *Preparation and Analysis of Protein Crystals*. J. Wiley and Sons, New York.
- Oppenheim, B. A., Hirsch, Y., Koby, S. & Chet, I. (1990). Protein secretion in biotechnology. In *Biologicals from Recombinant Microorganisms and Animal Cells* (White, M. D., Reuveny, S. & Shafferman, A., eds), Proceedings of the 34th Ohoto Conference, Eliat, Israel.
- Ordentlich, A., Elad, Y. & Chet, I. (1988). The role of chitinase of *Serratia marcescens* in biocontrol of *Sclerotium rolfsii*. *Soil Biol. Biochem.* **49**, 747–751.
- Shapira, R., Ordentlich, A., Chet, I. & Oppenheim, A. B. (1989). Control of plant diseases by chitinase expressed from cloned DNA in *Escherichia coli*. *Phytopathology*, **79**, 1246–1249.