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Crystallization and preliminary X-ray diffraction analysis of XAC1151, a small heat-shock protein from *Xanthomonas axonopodis* pv. *citri* belonging to the α-crystallin family

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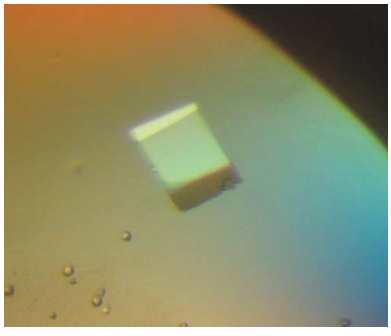

The *hspA* gene (XAC1151) from *Xanthomonas axonopodis* pv. *citri* encodes a protein of 158 amino acids that belongs to the small heat-shock protein (sHSP) family of proteins. These proteins function as molecular chaperones by preventing protein aggregation. The protein was crystallized using the sitting-drop vapour-diffusion method in the presence of ammonium phosphate. X-ray diffraction data were collected to 1.65 Å resolution using a synchrotron-radiation source. The crystal belongs to the rhombohedral space group *R*3, with unit-cell parameters $a = b = 128.7$, $c = 55.3$ Å. The crystal structure was solved by molecular-replacement methods. Structure refinement is in progress.

1. Introduction

Xanthomonas axonopodis pv. *citri* (*Xac*) is a phytopathogenic bacterium that causes citrus canker disease in most commercial citrus cultivars, resulting in significant losses worldwide. Symptoms of infected plants include canker lesions on fruits and leaves associated with progressive tree decline. Sequencing and annotation of the *Xac* genome (da Silva *et al.*, 2002) allowed us to initiate structural and functional studies of genes and proteins in order to elucidate the mechanisms involved in plant–pathogen interaction.

In *X. axonopodis* pv. *citri*, the *hspA* gene (XAC1151) encodes a small heat-shock protein (sHSP) with 158 amino-acid residues. This protein shows high identity to sHSPs from various species from the genus *Xanthomonas* and also to another citrus pathogen *Xylella fastidiosa* (strain 9a5c).

Heat-shock proteins (HSPs), also called stress proteins or molecular chaperones, are a superfamily of proteins that are present in various organisms. They are essential for normal cell function and confer stability on the cell proteome by protecting a diverse group of proteins engaged in signal transduction, metabolism, translation and other activities, improving the resistance of the cell under stress conditions. However, HSPs are also present under normal cell conditions (Sun & MacRae, 2005). HSP synthesis is stimulated in response to environmental challenges when organisms are exposed to abnormal or extreme environmental stress conditions such as heat shock, desiccation, humidity, light, starvation, chemical stress caused by organic compounds or strong oxidants, oxygen deprivation, exposure to heavy metals or oxidative stress (Laksanalamai & Robb, 2004).

Protein folding and refolding are mediated by ATP-dependent molecular-chaperones, including HSP60 (chaperonins), HSP70, HSP90, HSP104/C1pb and HSP110. The sHSPs appear to prevent irreversible protein aggregation and insolubilization of unfolded proteins, without chaperone activity, in an ATP-independent process. They have a high capacity to bind unfolded proteins and deliver them to the ATP-dependent chaperone systems (Sun & MacRae, 2005). Members of this protein family have a central conserved domain of approximately 90 amino-acid residues called the α-crystallin domain (Kappe *et al.*, 2002). Furthermore, the sHSP family members share common features such as small molecular weight (12–30 kDa) and the formation of large oligomers (9–30 subunits), resulting in a dynamic quaternary structure (Stromer *et al.*, 2003).

The amino-acid sequences of the N- and C-termini are not conserved and contribute to the structural diversity among sHSPs.

Table 1
Data-collection statistics.

Values in parentheses are for the last resolution shell.

Space group	*R*3
Unit-cell parameters	$a = b = 128.7$, $c = 55.3$
Resolution range	50.0–1.65 (1.71–1.65)
Total No. of reflections	207262
No. of unique reflections	40597
R_{sym} (%)	4.3 (47.6)
Completeness (%)	98.9 (89.2)
$\langle I/\sigma(I)\rangle$	24.5 (2.2)
Multiplicity	5.1 (3.5)
Solvent content (%)	43.6
Subunits per ASU	2

Both ends are essential for multimerization and molecular-chaperone activity (Sun & MacRae, 2005; Ganea, 2001). αA-Crystallin, a classical member of the sHSP protein family, is a large protein composed of 30–40 identical subunits. Deletion of the first 19 N-terminal amino-acid residues showed little effect on the quaternary structure, while removal of the first 56 or more N-terminal residues converts αA-crystallin into a predominantly small multimeric complex with 3–4 subunits, suggesting an essential function for the N-terminus in oligomerization (Bova *et al.*, 2000). The C-terminal extension is flexible and is enriched in polar and charged amino-acid residues that contribute to the solubility of the sHSP (Ganea, 2001). Loss of chaperone activity was observed when the last 11–12 residues from the rat α-crystallin C-terminus were removed with calpain II (Kelley *et al.*, 1993). The importance of the C-terminal end to the chaperone activity was confirmed by using *Escherichia coli* IbpB, another member of the sHSP protein family. A truncated protein lacking the last 11 residues was produced in *E. coli*, which showed loss of chaperone activity, although the protein was able to dimerize (Jiao *et al.*, 2005).

In this report, we describe the cloning, purification, crystallization, data collection and preliminary X-ray diffraction analysis of a recombinant sHSP encoded by the gene *hspA* from *X. axonopodis* pv. *citri*.

2. Cloning, expression and purification

The full-length *hspA* gene (XAC1151) was amplified by PCR from the genomic DNA of *X. axonopodis* pv. *citri* strain 306 using the primers XAC1151F (5′-CG**GAATTC**ATGAACATCGTTCGTTAT-3′) and XAC1151R (5′-CCC**AAGCTT**TTACTGCACCGTGCTGCC-3′), which were designed based on the genome sequence (da Silva *et al.*, 2002). The sequences in bold correspond to *Eco*RI and *Hin*dIII sites, respectively. The PCR product was subcloned into the pMOS-Blue PCR cloning kit (Amersham Bioscience) and the whole sequence was confirmed by DNA sequencing. The insert was then transferred to the pET28a expression vector (Novagen) using *Eco*RI/*Hin*dIII restriction sites and the plasmid was used to transform *E. coli* strain BL21 (DE3) competent cells. A single clone of BL21 (DE3) cells harbouring the *hspA* gene was grown overnight in 2YT medium containing 0.2% glucose and 30 µg ml^{-1} kanamycin at 303 K and 250 rev min^{-1}. Cells were transferred to 2 l fresh medium and cultivated under the same conditions until the OD_{600} reached 0.6. Protein production was induced with 0.1 m*M* IPTG and cells were harvested after 4 h induction. Cells were resuspended in 50 ml lysis buffer containing 50 m*M* Tris–HCl pH 8.0, 500 m*M* NaCl, 30 m*M* imidazole and 1 m*M* benzamidine and lysed by sonication (ten cycles of 30 s sonication followed by 30 s on ice). Cell debris and nucleic acids was separated by centrifugation at 30 000*g* for 30 min and the supernatant was applied onto a nickel-affinity column in an Äkta Prime Purification System (Amersham Bioscience). Recombinant protein was eluted in a linear elution gradient of 0–500 m*M* imidazole and the fractions were analyzed by SDS–PAGE. Fractions containing high amounts of the pure protein were pooled and dialyzed against 5 m*M* Tris–HCl buffer pH 8.0. Recombinant sHSP from *X. axonopodis* pv. *citri* was overexpressed in *E. coli* in a soluble form with a yield of ∼15 mg purified protein per litre of culture. Single-step purification by immobilized metal-affinity chromatography was sufficient to produce crystallization-quality protein.

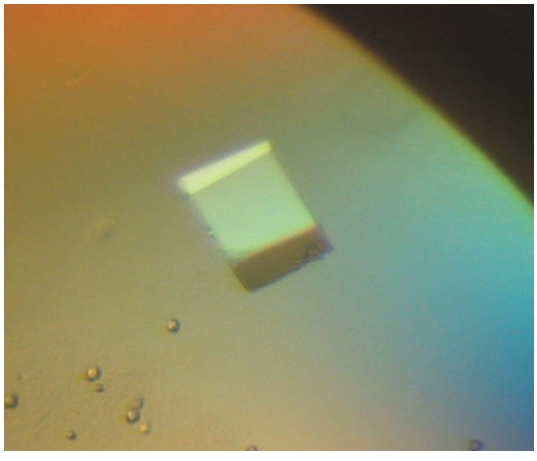

Figure 1
Crystal of the small heat-shock protein Xac1151 from *X. axonopodis* pv. *citri*. The approximate dimensions of the crystal are 0.40 × 0.35 × 0.20 mm.

3. Crystallization

Initial crystallization experiments were carried out at a constant temperature of 293 K by the sitting-drop vapour-diffusion method (McPherson, 1982) using the sparse-matrix crystallization screening protocols described by Jancarik & Kim (1991). All crystallization trials were performed in Chryschem multi-well plates (sitting drop) with 300 µl reservoir solution using Crystal Screen and Crystal Screen II from Hampton Research, Wizard I and II from Emerald and JB Screens 1–10 from Jena Biosciences. Drops consisted of 2 µl protein solution (7 mg ml^{-1} in 5 m*M* Tris–HCl buffer pH 8.0) and 2 µl reservoir solution. Small crystals were observed in solution Nos. 3, 12, 15, 21, 29 and 33 from Crystal Screen, solution Nos. 2, 7, 23 and 43 from Crystal Screen II, solution Nos. 32 and 43 from Wizard I and solution Nos. 25, 37 and 48 from Wizard II. New screenings varying the pH of the buffer and the concentration of the precipitant were performed and we obtained crystals suitable for X-ray data collection (Fig. 1) in 0.1 *M* Tris–HCl buffer pH 7.7 containing 1.2 *M* $(NH_4)_2HPO_4$. The crystals grew in 4–5 weeks.

4. Data collection

Crystals were mounted in nylon loops and flash-frozen in a nitrogen stream at 100 K in mother liquor containing 25% glycerol as a cryoprotectant. X-ray diffraction intensities were collected at the D03B-MX1 beamline, Laboratório Nacional de Luz Síncrotron (Campinas, Brazil) using a wavelength of 1.43 Å and a MAR CCD 165 detector (MAR Research) with 35 s exposures. Diffraction data were collected from 260 images using the oscillation method; individual frames consisted of 0.7° oscillation steps over a range of 182°.

Diffraction data were indexed, integrated, scaled and merged using the *HKL*2000 package (Otwinowski & Minor, 1997).

5. Results

The crystals of XAC1151 belong to the rhombohedral space group *R*3 (No. 146), with unit-cell parameters $a = b = 128.7$, $c = 55.3$ Å. The calculated packing parameter, based on a molecular weight of 21.7 kDa, indicates the presence of two monomers in the asymmetric unit. This corresponds to a typical Matthews coefficient (V_M) of 2.2 Å^3 Da^{-1}, which is within the expected range (Matthews, 1968). This V_M corresponds to a solvent content of approximately 43.6%. The data-collection statistics are shown in Table 1.

A molecular-replacement solution was found using the crystallographic structure of the small heat-shock protein sHSP from *Triticum aestivum* (PDB code 1gme; van Monfort *et al.*, 2001) as a search model. Molecular-replacement procedures were performed with the program *AMoRe* (Navaza, 1994) implemented in the *CCP*4 package (Collaborative Computational Project, Number 4, 1994). The best solution was obtained using the dimer with 45 residues from the amino-terminus and 15 residues from the carboxy-terminus removed. The solution has a correlation coefficient of 36.5% and an R_{cryst} of 48.6% after rigid-body refinement. Structure refinement is in progress.

In summary, we have obtained well diffracting crystals of a small heat-shock protein from the phytopathogenic bacterium *X. axonopodis* pv. *citri*. There are three structures of small heat-shock proteins deposited in the Protein Data Bank: two eukaryotic (*Tenia saginata* and *T. aestivum*) and one from a hyperthermophilic bacteria (*Methanococcus jannashii*). These structures have been solved at relatively poor resolution (2.5, 2.7 and 2.9 Å, respectively). We have collected data to a resolution of 1.65 Å; this higher resolution will help to better define the structure of this important family of proteins involved in the folding process of proteins.

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