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Crystallization and preliminary X-ray diffraction analysis of *Mycobacterium smegmatis* Dps

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Three crystal forms of a DNA-binding protein from stationary phase cells (Dps) of *Mycobacterium smegmatis* have been grown. They are hexagonal, tetragonal and cubic. The structure of the cubic form, which has one subunit in the asymmetric unit, has been solved using the molecular-replacement method. The application of the crystallographic symmetry operations on the subunit leads to eight dodecamers with 23 symmetry in the unit cell.

1. Introduction

The integrity of genetic information, stored in the form of DNA, is of critical importance for the endurance of any living cell. Thus, microorganisms have evolved a number of strategies to protect their DNA under various kinds of stress conditions. For all aerobic forms of life, oxidative stress is a major threat to survival. Highly specific and regulated systems are thus present which prevent damage to DNA from reactive oxygen species (ROS). The DNA-binding protein from stationary phase cells (Dps) constitutes a family of such proteins, which protect DNA in starved bacterial cells from oxidative damage (Almiron *et al.*, 1992). This protection is afforded either by direct physical nonspecific binding to DNA (Grant *et al.*, 1998; Martinez & Kolter, 1997; Wolf *et al.*, 1999; Chen & Helmann, 1995) or *via* its ability to sequester iron ions (Gupta & Chatterji, 2003; Bozzi *et al.*, 1997). Dps has also been shown to have ferroxidation and iron-loading capacity in some organisms (Ilari *et al.*, 2002).

The X-ray structure analysis of the *Escherichia coli* protein showed that Dps dodecamer has a shell-like structure of 23 symmetry with an external diameter of ~90 Å and a central cavity diameter of ~45 Å (Grant *et al.*, 1998). The dodecamer has a significant net negative charge inside the hollow core, providing an ideal microenvironment for iron mineralization (Grant *et al.*, 1998; Pena & Bullerjahn, 1995). The Dps monomer has essentially the same four-helix bundle fold as that of a ferritin monomer. In fact, Dps and ferritin have been considered to be structural homologues and are thought to be evolutionarily related (Ilari *et al.*, 2002), although ferritin is a 24-mer with 432 symmetry. The subsequently determined structures of Dps from other bacterial species (Papinutto *et al.*, 2002; Ceci *et al.*, 2003; Ren *et al.*, 2003) confirm these conclusions.

A mycobacterial Dps, that from *Mycobacterium smegmatis* (Ms-Dps), has been identified recently (Gupta *et al.*, 2002). Each subunit of the protein is 183 residues long with a molecular weight of 20.27 kDa. Fast-growing non-pathogenic *M. smegmatis* is often used as a model for the pathogenic slow-growing *M. tuberculosis*, the causative agent of tuberculosis. Ms-Dps has 35% sequence identity with *E. coli* Dps. Homology-modelling studies have indicated that Ms-Dps could also exist as a dodecamer (Gupta *et al.*, 2002). Subsequent electron-micrographic and electrophoretic studies have shown that the protein does have a dodecameric quaternary arrangement (Gupta & Chatterji, 2003). However, unlike other known members of the Dps family, Ms-Dps also has a stable trimeric state. This trimeric form, although devoid of the DNA-binding property, can afford protection to DNA *via* its ferroxidase activity (Gupta & Chatterji, 2003). Here, we report the crystallization and preliminary X-ray studies of the dodecameric form of Ms-Dps, the first mycobacterial Dps to be studied crystallographically.

2. Materials and methods

2.1. Cloning, expression and purification

The cDNA of Ms-Dps was cloned and the recombinant protein was purified as described previously (Gupta *et al.*, 2002). Briefly, the PCR product was cloned into the pGEM-T Easy vector (Promega). The insert was then subcloned into pET21b to produce pET-DPS and transfected into *E. coli* (BL21DE3). The cells were grown at 310 K in LB medium and the overexpression of the gene was induced by IPTG. The cells were then lysed and Ms-Dps containing an N-terminal His_6 tag was purified

using Ni-NTA chromatography according to the manufacturer's protocol (Qiagen).

2.2. Crystallization

Hampton Crystal Screens I and II (Jancarik & Kim, 1991) were used to screen crystallization conditions employing the hanging-drop vapour-diffusion technique at 293 K using Linbro multiwell plates. Each well was filled with 500 µl of reservoir solution. Drops consisting of 4 µl of a 20 mg ml^{-1} protein solution in 0.1 *M* Tris-HCl buffer pH 7.9 and 1 µl of reservoir solution were placed on cover slips and equilibrated against the reservoir solutions. Three conditions yielded crystals.

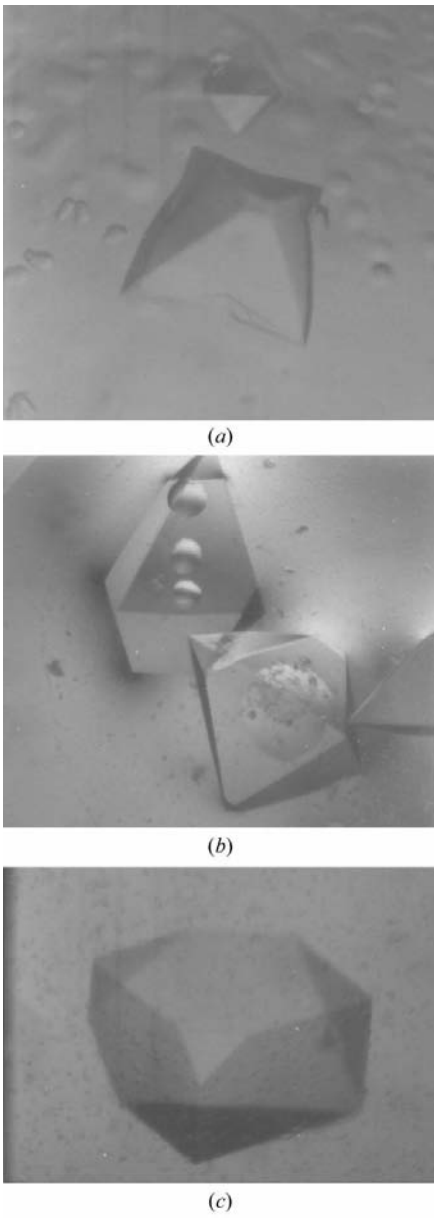

Figure 1
Crystals of (*a*) the hexagonal, (*b*) the tetragonal and (*c*) the cubic forms of Ms-Dps.

Table 1
Crystal data and data-collection statistics.

Values in parentheses are for the highest resolution shell.

	Form I	Form II	Form III
Space group	*P*6 or screw equivalent	$P4_12_12$ or $P4_32_12$	*F*432
Unit-cell parameters			
a (Å)	194.67	123.64	179.97
c (Å)	120.61	262.33	
Packing density, V_M (Å^3 Da^{-1})	2.7	2.1	3.0
Solvent content (%)	54.3	39.9	58.6
No. of subunits in the asymmetric unit	12	12	1
Resolution range (Å)	20.0–4.0 (4.14–4.0)	30.0–3.0 (3.11–3.0)	30.0–2.85 (2.95–2.85)
Observed reflections	44121	136243	91017
Unique reflections	20375 (1961)	38007 (2800)	6271 (604)
Completeness (%)	91.6 (89.3)	90.6 (68.1)	99.8 (99.5)
Multiplicity	2.2	3.6	14.5
$I/\sigma(I)$	4.6	7.6	21.6
R_{merge}† (%)	0.205 (0.478)	0.201 (0.514)	0.097 (0.557)

† $R_{merge} = \sum_h \sum_i |I(h,i) - \langle I(h)\rangle| / \sum_h \sum_i I(h,i)$, where $I(h,i)$ is the intensity of the *i*th measurement of reflection *h* and $\langle I(h)\rangle$ is the average value over multiple measurements.

Crystal form I was grown using a reservoir solution consisting of 0.2 *M* $CaCl_2.2H_2O$, 28%(*v*/*v*) PEG 400 and 0.1 *M* Na HEPES buffer pH 7.5. Crystals suitable for X-ray diffraction experiments appeared after two weeks (Fig. 1*a*) and reached maximum dimensions of about 0.3 × 0.3 × 0.2 mm. A reservoir solution consisting of 0.1 *M* $CdCl_2$, 30% PEG 400 and 0.1 *M* sodium acetate buffer pH 4.6 yielded crystal form II. A large number of small crystals appeared within 1 d. Diffraction-quality crystals were obtained using 12.5% PEG 400 and 0.02 *M* $CdSO_4$ in the same buffer. After 5 d, bipyramidal crystals (Fig. 1*b*) of dimensions 1.0 × 0.4 × 0.4 mm appeared. Crystal form III was produced with a reservoir solution consisting of 0.2 *M* potassium sodium tartrate tetrahydrate, 2 *M* ammonium sulfate and 0.1 *M* trisodium citrate dihydrate pH 5.6. Crystals suitable for X-ray data collection appeared (Fig. 1*c*) after six months with maximum dimensions of 0.6 × 0.5 × 0.4 mm.

2.3. X-ray data collection and processing

Diffraction data for crystal form II were collected using a MAR 345 imaging plate mounted on a Rigaku Ultrax-18 rotating-anode X-ray generator. A MAR 300 imaging plate and a Rigaku RU-200 rotating-anode X-ray generator were used for forms I and III. Form III was very stable in the X-ray beam, while forms I and II were radiation-sensitive. All data were collected at room temperature (293 K). The crystal-to-plate distance varied between 180 mm for form III and 300 mm for form II. The data were processed using *DENZO* and scaled using *SCALEPACK* from the *HKL* program package (Otwinowski & Minor, 1997).

3. Results

Crystal data and data-collection statistics are given in Table 1. From all considerations, form III was recognized as the most suitable for structure analysis. In addition to the better quality of the X-ray data obtained from it, form III has only one subunit in the asymmetric unit. In fact, this is so far the only form among the crystals of Dps from different species with only one crystallographically independent subunit. The structure of form III was readily solved using the molecular-replacement program *AMoRe* (Navaza, 1994) with a 155-residue-long polyalanine model constructed using the *E. coli* Dps structure (PDB code 1dps) as the search model. Calculations using data in the 7.5−3.5 Å resolution range yielded a correlation coefficient (CC) of 0.30 and an *R* value of 0.44 for the best solution, which was acceptable from packing considerations. The next best solution had a much lower CC of 0.21 and a higher *R* value of 0.48. Refinement of the structure using *CNS* (Brünger *et al.*, 1998) and further model building using *FRODO* (Jones, 1978), including the identification of side chains, are in progress. The current *R* and R_{free} values are 0.28 and 0.33, respectively. The application of crystallographic symmetry on the subunit in the asymmetric unit leads to eight dodecamers with 23 symmetry situated at special positions.

The X-ray analysis, when completed, is expected to reveal the structural basis of the unique properties exhibited by Ms-Dps such as the presence of a trimeric species with ferroxidase activity. A major difference between the sequences of Dps molecules of known three-dimensional structure from other sources and that of Ms-Dps is the presence of an additional C-terminal stretch

containing 20 residues. In contrast, the Dps from *E. coli* has an additional N-terminal stretch. The structure of Ms-Dps might also help to elucidate the significance of this difference.

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