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Expression, purification, crystallization and preliminary X-ray analysis of a DNA-binding protein from *Methanococcus jannaschii*

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A small DNA-binding protein of 87 amino-acid residues from the hyperthermophilic archaeon *Methanococcus jannaschii* (Mja10b) was cloned and overexpressed in *Escherichia coli*. The protein was crystallized and the crystals belong to the space group $P6_122/P6_522$, with unit-cell parameters $a = b = 50.85$, $c = 124.02$ Å, $\alpha = \beta = 90$, $\gamma = 120^\circ$. The crystals diffracted to a maximum resolution of 2.2 Å at 100 K using Cu $K\alpha$ radiation. The presence of one molecule per asymmetric unit gives a crystal volume per protein mass (V_M) of 2.4 Å^3 Da^{-1} and a solvent content of 49% by volume. A full set of X-ray diffraction data was collected to 2.2 Å from the native crystal.

1. Introduction

Members of the Sac10b DNA-binding protein family occur in all thermophilic archaea whose genomes have so far been sequenced. Given their ubiquity, these proteins may play an important physiological role in thermophilic archaea (Forterre *et al.*, 1999; Xue *et al.*, 2000). Biochemical studies have been performed on two proteins of the Sac10b family: Sac10b from *Sulfolobus acidocaldarius* and Ssh10b from *S. shibatae*. Sac10b exists as a dimer in solution and binds cooperatively to DNA (Dick & Reinhardt, 1986). This protein does not compact DNA, but is capable of enveloping two strands of duplex DNA into a helix protein structure (Lurz *et al.*, 1986). Ssh10b displays the ability to constrain negative DNA supercoils in a temperature-dependent fashion, suggesting that the Sac10b proteins are involved in the chromosomal organization in archaea (Xue *et al.*, 2000).

A number of small DNA-binding proteins have been isolated from both the euryarchaeotal and crenarchaeotal branches of the archaea (Choli *et al.*, 1988; Musgrave *et al.*, 1991; McAfee *et al.*, 1995). True histone-like proteins, such as HMf, were found only in euryarchaeota. Structural studies have shown that the archaeal histones share a similar fold to their eukaryal counterparts (Grayling *et al.*, 1996; Tarich *et al.*, 1996; Luger *et al.*, 1997; Decanniere *et al.*, 2000). No histones have been identified in crenarchaeota. Instead, a group of small abundant DNA-binding proteins (*e.g.* Sac7, Sso7 and Ssh7) have been isolated from *Sulfolobus*, a genus of the crenarchaeota (Choli *et al.*, 1988; Mai *et al.*, 1998). Based on the crystal structures of Sso7d and Sac7d, a second type of chromatin structure in archaea has been suggested (Robinson *et al.*, 1998; Gao *et al.*, 1998). It seemed that crenarchaea and euryarchaea employ different mechanisms in chromosomal organization. Since members of the Sac10b family are conserved among all thermophilic archaea, there is an opportunity to investigate the common chromosomal structure of the two archaeal branches. So far, only the crystallization of the Sso10b protein from the crenarchaeon *S. solfataricus* has been reported (Wardleworth *et al.*, 2001) and no crystallographic structure of Sac10b proteins is yet available.

Mja10b, the smallest Sac10b protein, is from the euryarchaeon *Methanococcus jannaschii*. Here, we report the overexpression, purification and preliminary X-ray crystallographic data at 2.2 Å of Mja10b from *M. jannaschii*.

2. Materials and methods

2.1. Plasmid construction

The *mja10b* gene was amplified by the polymerase chain reaction (PCR) using the genomic DNA from *M. jannaschii* as the template and cloned into the expression vector pET11a (Novagen). Two PCR primers, 5′-CCGGAATT**CATATG**GATAATGTAGTGT-TG-3′ and 5′-CGC**GGATCC**TTATTTAGC-TAAAACAATTT-3′, were designed to incorporate an *Nde*I site upstream of the initiator ATG position and a unique *Bam*HI site downstream from the stop codon. The amplification protocol consisted of 5 min denaturation at 367 K followed by 30 cycles of denaturation at 367 K for 1 min, annealing at 318 K for 2 min and extension at 345 K for 2 min. The PCR products and the pET11a DNA were purified, cleaved with *Nde*I and *Bam*HI and ligated using T4 DNA ligase. The ligation mixture was transformed into *E. coli*

DH5α cells and plasmids with an insert of the right size were selected by double digestion with *Nde*I and *Bam*HI. The sequence of the insert was verified by dideoxynucleotide sequencing. The positive clones harbouring the recombinant plasmid were used for protein expression.

2.2. Expression and purification of Mja10b

E. coli cells containing the recombinant plasmid were grown to an OD_{600} of 0.6 at 310 K with shaking in 1 l of LB medium containing 0.1 mg ml^{-1} ampicillin. IPTG was added to 1 m*M*. Incubation continued for 4–6 h. Cells were harvested by centrifugation, resuspended in lysis buffer [20 m*M* Tris–HCl pH 7.5, 2 *M* KCl, 0.1 m*M* EDTA, 1 m*M* dithiothreitol (DTT)] and sonicated at 277 K. The lysed material was clarified by centrifugation and heated at 358 K for 20 min. The sample was centrifuged and the supernatant dialyzed against buffer *A* (20 m*M* potassium phosphate buffer pH 6.6, 0.1 m*M* EDTA, 1 m*M* DTT). The dialyzed sample was treated with RNase A (0.1 mg ml^{-1}) overnight at room temperature to reduce the level of contaminating RNA. This sample was subsequently applied to a SP-Sepharose column (Pharmacia) and bound proteins were eluted with a KCl gradient from 0 to 1 *M* in buffer *A*. Further purification was accomplished by gel-filtration chromatography on Superdex 75 (Pharmacia) in buffer *A* containing 150 m*M* KCl. Mja10b fractions were pooled and shown to be homogenous. This protein was then used for all crystallization trails.

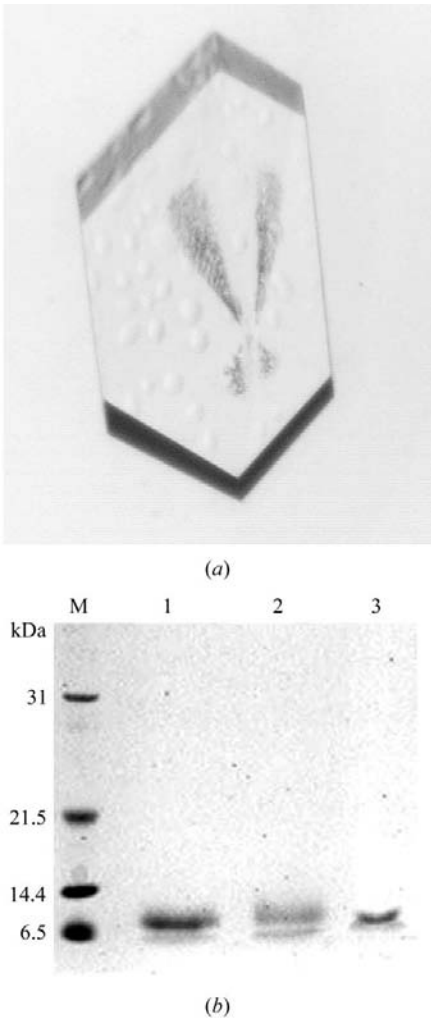

Figure 1
(*a*) A typical crystal of Mja10b grown in 40% MPD, 0.1 *M* Tris–HCl pH 8.25. The dimensions of the crystal are approximately 0.5 × 0.35 × 0.05 mm. (*b*) SDS–PAGE analysis of the protein recovered from Mja10b crystals. Lane M, marker protein with the given molecular mass; lane 1, fresh Mja10b protein; lanes 2 and 3, recovered Mja10b protein.

2.3. Crystallization and preliminary X-ray analysis

Crystallization experiments were carried out at 291 K using the hanging-drop vapour-diffusion method. The concentration of the purified protein was adjusted to 10 mg ml^{-1} in water. Hampton Research Crystal Screen kits were used to investigate crystallization conditions and then fine-tuned. Drops were prepared by mixing 2 µl of protein solution with 2 µl of reservoir solution and were allowed to equilibrate against 0.5 ml of reservoir solution. The conditions yielding small crystals were further optimized by variation of precipitant and protein concentration and buffer pH. The best crystals were obtained from a reservoir consisting of 40% MPD, 0.1 *M* Tris–HCl pH 8.25. Crystals grew to dimensions of 0.5 × 0.35 × 0.05 mm within six months (Fig. 1*a*). The protein recovered from the Mja10b crystals was analyzed by SDS–PAGE in the same way as the fresh preparation of Mja10b (Fig. 1*b*).

Table 1
Crystallographic parameters and data-collection statistics.

Values in parentheses are for the outer resolution shell.

Space group	$P6_122/P6_522$
Unit-cell parameters (Å, °)	$a = b = 50.85$, $c = 124.02$, $\gamma = 120$
Matthews coefficient (Å^3 Da^{-1})	2.4 (1 mol/a.u.)
Solvent content (%)	49
Resolution (Å)	50–2.20 (2.28–2.20)
Total observations	62566
Unique reflections	5315
Redundancy	11.8
Average $I/\sigma(I)$	20.0 (5.0)
$R_{\rm merge}$† (%)	4.4 (32.0)
Completeness (%)	99.5 (96.8)

† $R_{\rm merge} = \sum |I - \langle I \rangle| / \sum I$.

The diffraction data were collected in-house on a Rigaku rotating-copper-anode X-ray generator at 48 kV and 98 mA (λ = 1.5418 Å) with a MAR 345 image-plate detector. The crystals were picked up with a nylon loop and flash-cooled in a stream of nitrogen gas cooled to 100 K. MPD in the reservoir solution was used as cryoprotectant. A Cryostream (Oxford Cryosystem, Oxford, UK) was used to keep the crystal at 100 K during data collection. The crystal-to-detector distance was 137.0 mm. A total of 120 rotation images were collected with an oscillation angle of 1° and an exposure time of 90 s for each image. All intensity data were indexed, integrated and scaled with the *HKL* programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997).

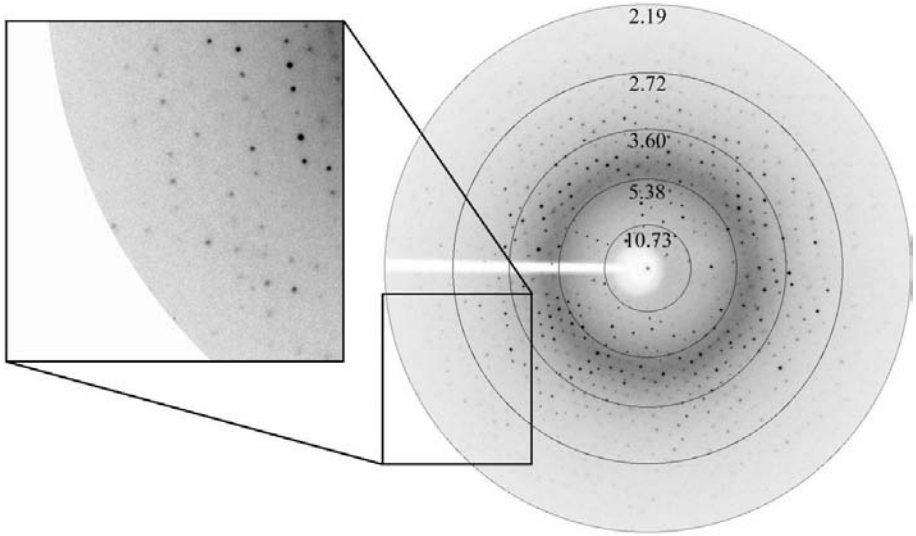

Figure 2
A 1.0° oscillation image of an Mja10b crystal. The crystal diffracted beyond 2.2 Å resolution.

3. Results

When the crystals were exposed to X-rays, diffraction spots were observed to at least 2.2 Å Bragg spacing (Fig. 2). Unit-cell parameters were determined to be $a = b = 50.85$, $c = 124.02$ Å, $\alpha = \beta = 90$, $\gamma = 120^\circ$ in space group $P6_122/P6_522$. Processing and scaling of the crystallographic data resulted in an overall $R_{\rm merge}$ of 4.4% and an $R_{\rm merge}$ in the highest resolution shell (2.28–2.20 Å) of 32.0%. Complete data-collection statistics are shown in Table 1. The value of the Matthews coefficient (Matthews, 1968) is 2.4 $\text{Å}^3\,\text{Da}^{-1}$ for one molecule in the asymmetric unit, which corresponds to a solvent content of 49%.

Since there are no protein structures available that show significant sequence homology with Mja10b, molecular replacement is ruled out as a possible method for structure determination. Therefore, our efforts are currently focused on the acquisition of phase information through heavy-atom derivatives and selenomethionine-substituted protein.

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