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Purification, crystallization and preliminary crystallographic analysis of a GTP-binding protein from the hyperthermophilic archaeon *Sulfolobus solfataricus*

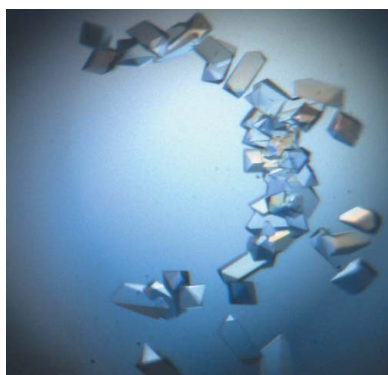
A predicted GTP-binding protein from the hyperthermophilic archaeon *Sulfolobus solfataricus*, termed SsGBP, has been cloned and overexpressed in *Escherichia coli*. The purified protein was crystallized using the hanging-drop vapour-diffusion technique in the presence of 0.05 M cadmium sulfate and 0.8 M sodium acetate pH 7.5. A single-wavelength anomalous dispersion data set was collected to a maximum resolution of 2.0 Å using a single cadmium-incorporated crystal. The crystal form belongs to space group $P2_12_12_1$, with approximate unit-cell parameters $a = 65.0$, $b = 72.6$, $c = 95.9$ Å and with a monomer in the asymmetric unit.

1. Introduction

Guanosine triphosphate (GTP) binding proteins are widely distributed across the three domains of life and constitute the GTPase superclass (Leipe *et al.*, 2002). A common feature of these proteins is the presence of a well conserved GTPase domain. The GTPase superclass is subdivided into several superfamilies and families, as the GTPase domains are often associated with different classes of (predicted) RNA-binding and/or protein-binding domains (Leipe *et al.*, 2002). This variable domain architecture allows GTP-binding proteins to act as molecular switches in a wide range of biological processes, including protein synthesis, signal transduction and protein trafficking (Bourne *et al.*, 1990). Biochemical and structural analysis of poorly characterized GTPase subfamilies is expected to provide insight into the control of numerous relevant biological processes.

Sulfolobus solfataricus is a model organism of the hyperthermophilic archaea that grows optimally at 353 K. Its complete genome sequence, genetic systems and functional genomics tools have been established (She *et al.*, 2001). A putative GTP-binding protein (SsGBP) has recently been identified in the genome of *S. solfataricus*. The C-terminal half of SsGBP (residues 179–357) corresponds to a classical 'GTPase domain' [COG2262, as classified in the Clusters of Orthologous Groups (COGs) database; Tatusov *et al.*, 1997]; the N-terminal domain has been described as a 'glycine-rich segment' (Leipe *et al.*, 2003). Homologues of SsGBP are present in several archaea, bacteria and eukaryotes (Caldon & March, 2003). The best characterized SsGBP homologue is a GTPase from *Escherichia coli* named HflX (Brown, 2005); a BLAST search on SsGBP at NCBI (<http://www.ncbi.nlm.nih.gov/BLAST/>) reveals significant homology to *E. coli* HflX (Z score = 123, E value = 10^{-26}) spanning the entire sequence. HflX is the prototype of a family within the Obg-HflX-like superfamily of GTPases (Leipe *et al.*, 2002). The *E. coli* hflX gene is present in a locus that governs the lysis-lysogeny decision and has been proposed to be involved in controlling the proteolysis of the λ phage cII repressor (Noble *et al.*, 1993). The molecular mechanism of the action of HflX is unknown and no three-dimensional structures of any members of the HflX subfamily are available.

In this communication, we report the cloning, purification, crystallization and preliminary X-ray analysis of SsGBP as an initial step



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towards the structural and functional characterization of this relatively unknown class of GTPases.

2. Cloning, overexpression and purification

The Sso0269 gene (gene ID 1455417) was PCR-amplified from genomic DNA using oligonucleotide primers BG1861 (5'-GCG-CGCTCATGAAAACAGCTGCTCTTTTTGTATC-3') and BG1837 (5'-CGCGCCTCGAGACTCAACTGAGTTGCTAGCTGG-3'). The PCR product of 1069 base pairs was purified using the Qiagen kit and digested with the restriction enzymes *Bsp*HI and *Xho*I. The restriction product was purified from agarose gel and ligated into an *Nco*I-*Xho*I pre-digested pET24d vector (Novagen), resulting in a 3' gene fusion to a six-histidine-tag encoding sequence. After transformation of the ligation mixture to *E. coli* HB101, a positive clone (pWUR335) was identified by PCR and restriction-fragment analysis. The sequence of pWUR335 has been verified by sequencing (AuGCT Biotechnology, Beijing, People's Republic of China).

The pWUR335 construct was transformed into *E. coli* BL21(DE3) and a single colony was used to inoculate an overnight culture in a rotary shaker at 310 K in 100 ml LB medium containing kanamycin (50 µg ml⁻¹). This 100 ml culture was used to inoculate two 1 l batches of selective LB medium. When these cultures reached an OD₆₀₀ of approximately 0.5, isopropyl β-D-thiogalactopyranoside (IPTG) was added to a final concentration of 1 mM, after which the cultures were incubated under the same conditions for 5 h. Cells were harvested by centrifugation at 5000g for 30 min at 277 K. The pellets were frozen immediately in liquid nitrogen and stored at 253 K.

For purification of SsGBP, approximately 5 g cell paste was resuspended in 40 ml buffer A [20 mM Tris-HCl pH 8.0, 0.5 M NaCl, 20 mM imidazole, 10%(v/v) glycerol] and the cells were lysed by three 15 s pulses of ultrasonication at 10 µm amplitude. After spinning down the cell debris, the resulting cell-free extract was incubated at 338 K for 25 min and centrifuged at 70 000g for 30 min at 277 K to effectively remove the majority of the contaminating *E. coli* proteins. The heat-stable supernatant was applied onto a Ni²⁺-chelating column packed with 2 ml Ni-NTA His-Bind Resin (Novagen) and equilibrated with buffer A. SsGBP eluted in a linear gradient of imidazole in buffer B [20 mM Tris-HCl pH 8.0, 0.5 M NaCl, 1.0 M

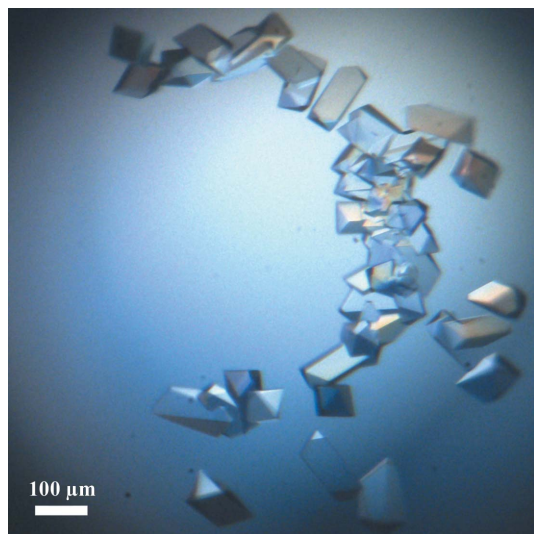


Figure 1 Crystals of *S. solfataricus* GBP grown and analyzed as described in the text.

Table 1

X-ray data-collection statistics for the cadmium-incorporated *S. solfataricus* GTPase crystal.

Values in parentheses are for the highest resolution shell.

Space group	<i>P</i> 2 ₁ 2 ₁ 2 ₁
Unit-cell parameters (Å)	<i>a</i> = 65.0, <i>b</i> = 72.6, <i>c</i> = 95.9
Resolution range (Å)	50–2.0 (2.07–2.00)
No. of unique reflections	30618 (2794)
Data completeness (%)	97.3 (89.6)
<i>R</i> _{merge} † (%)	7.6 (25.6)
Redundancy	13.8 (14.0)
Average <i>I</i> /σ(<i>I</i>)	29.9 (9.5)

† $R_{\text{merge}} = \frac{\sum_{\mathbf{h}} \sum_l |I_l - \langle I_{\mathbf{h}} \rangle|}{\sum_{\mathbf{h}} \sum_l I_l}$, where I_l is the l th observation of reflection \mathbf{h} and $\langle I_{\mathbf{h}} \rangle$ is the weighted average intensity for all observations l of reflection \mathbf{h} .

imidazole, 10%(v/v) glycerol] at an imidazole concentration of approximately 500 mM. Fractions containing SsGBP were combined and concentrated to a volume of 1.0 ml using an Amicon Ultra-15 centrifugal filter with a 10 kDa molecular-weight cutoff (Millipore). The concentrated sample was applied onto a 10/300 GL Superdex 200 column (Amersham Biosciences) equilibrated with buffer C (20 mM HEPES pH 7.0 and 150 mM NaCl). SsGBP eluted as a single peak at an apparent molecular weight of 38 kDa, suggesting that SsGBP is a monomer in solution. Analytical ultracentrifugation (ProteomeLab XL-1) confirmed a monomeric state under the conditions used (38 ± 2 kDa; data not shown).

3. Crystallization and preliminary X-ray analysis

A preliminary crystallization screen was carried out by the hanging-drop vapour-diffusion technique (290 K) using Hampton Research Crystal Screen with a protein concentration of approximately 10 mg ml⁻¹ in buffer C. For crystallization screening, 16-well tissue-culture plates were used. Typically, 1 µl protein solution was mixed with 1 µl precipitant solution in the drop and equilibrated over 200 µl precipitant solution. After one week, crystals of SsGBP appeared in 0.05 M cadmium sulfate, 0.1 M HEPES pH 7.5 and 1.0 M sodium acetate (condition No. 34). Optimization revealed that crystals of SsGBP grew optimally in 0.05 M cadmium sulfate, 0.1 M HEPES pH 7.5 and 0.8 M sodium acetate (Fig. 1). Crystals with typical dimensions of 0.06 × 0.07 × 0.18 mm were immersed in cryoprotectant (paraffin oil, Hampton Research) for 1 min, mounted into a nylon cryo-loop and flash-cooled to 100 K in a stream of nitrogen gas. Data were collected at 100 K using an in-house Rigaku MM007 rotating-anode Cu Kα X-ray generator operating at 45 kV and 45 mA (λ = 1.5418 Å) with an R-Axis IV⁺⁺ image-plate detector. The beam was focused using Osmic mirrors. Single-wavelength anomalous dispersive (SAD) X-ray data were collected to a maximum resolution of 2.0 Å. The diffraction data were indexed, integrated and scaled using the *HKL*-2000 program package (Otwinowski & Minor, 1997). The diffraction data were processed smoothly and the positions of three cadmium ions were located in the asymmetric unit. There is one protein monomer per asymmetric unit, with a *V*_M of 2.8 Å³ Da⁻¹ and 55% solvent content (Matthews, 1968). Data-collection statistics are summarized in Table 1.

Structure determination is currently in progress. Combined with biochemical analyses, we expect that this study will provide insights into the function of this relatively unknown subfamily of the GTPase superfamily in general and of the GBP of *S. solfataricus* in particular.

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