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Preliminary crystallographic studies of the double-stranded DNA-binding protein Sso10b from *Sulfolobus solfataricus*

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Crystals of Sso10b from the hyperthermophilic archaeon *Sulfolobus solfataricus* have been grown that diffract to 2.6 Å resolution. The protein is a highly abundant non-specific double-stranded DNA-binding protein, conserved throughout the archaea, that has been implicated in playing a role in the architecture of archaeal chromatin.

1. Introduction

S. solfataricus is a thermoacidophilic archaeon that grows optimally at 353–358 K and pH 2–4 (Zillig *et al.*, 1980). This organism has three classes of small abundant DNA-binding proteins, grouped according to their size (7, 8 and 10 kDa). Sso7d, the most abundant of the three classes, has been widely studied both biochemically and structurally (Baumann *et al.*, 1994; Knapp *et al.*, 1996; Agback *et al.*, 1998; Gao *et al.*, 1998; Lundbäck *et al.*, 1998; Robinson *et al.*, 1998) and has provided detailed insights into non-specific DNA binding in *Sulfolobus*. However, Sso7d is unique to the genus *Sulfolobus.* Unlike Sso7d, Sso10b is conserved in all archaeal organisms, in both the euryarchaeota (which possess histone proteins) and the non-nucleosome-containing crenarchaeota (Forterre *et al.*, 1999). Sso10b comprises 4% of the total soluble cellular protein (Xue *et al.*, 2000) and binds duplex DNA with high affinity, but shows little sequence specificity. These are hallmarks of a role in the architecture of archaeal chromatin. The protein has a molecular mass of 10 kDa per monomer and is dimeric in solution. Sso10b is capable of constraining negative DNA supercoils in a temperature-dependent fashion, suggesting that the overall topology of chromosomal DNA may be altered by this protein (Xue *et al.*, 2000). Electron-micrographic studies have previously shown Sso10b to bind cooperatively to DNA and to form numerous different complexes depending on protein concentrations (Lurz *et al.*, 1986).

The precise role of Sso10b is unknown, but its occurrence in all archaeal genomes sequenced to date suggests a universally conserved function, perhaps in influencing the architecture of DNA. Indeed, Sso10b may be the only conserved archaeal double-stranded DNA-binding protein, with a function both in the presence and absence of archaeal histones. Knowledge of the three-dimensional structure of Sso10b will provide insight into the precise role that this highly abundant and evolutionary conserved protein plays in affecting the topology of DNA.

2. Expression and purification

The Sso10b gene from *S. solfataricus* (EMBL accession number CAC23286) was amplified from *S. solfataricus* chromosomal DNA using the following primers (forward primer, 5′-CGGATCCCCATATGAGCAGCGGAACC-CCAACTCCAAG; reverse primer, 5′-GGG-AAATCCTCGAGTTACTTTTCCTTATGG-CAATCTC). The PCR product was digested with *Nde*I and *Xho*I and cloned into the PET30 (Novagen) to allow expression of recombinant protein with the native N-terminal sequence MSSGTPTPS—. Protein expression was carried out in *Escherichia coli* BL21Star CodonPlus (DE3) RIL cells (Stratagene). Expression was induced by addition of 1 m*M* IPTG at 310 K for 3 h, after which cells were pelleted and frozen until required. Cell lysis, centrifugation and chromatography steps were carried out at 277 K. 20 g cells were thawed in 50 ml lysis buffer (50 m*M* Tris–HCl pH 7.5, 100 m*M* NaCl, 1 m*M* EDTA, 1 m*M* DTT, 1 m*M* benzamidine, 1 m*M* AEBSF) and immediately sonicated for 5 × 1 min with cooling. The lysate was then centrifuged at 40 000*g*. The supernatant was heated to 338 K for 30 min in a water bath and denatured proteins were precipitated by centrifugation at 40 000*g*. The supernatant was analysed by SDS–PAGE and shown to contain a strong band of approximately 10 kDa, corresponding to the mass expected for recombinant Sso10b. The supernatant was diluted fourfold with buffer *A* (50 m*M* Tris–HCl pH 7.5, 1 m*M* EDTA, 1 m*M* DTT) and applied to an SP-Sepharose High Performance 26/10 column (Hi-Load, Amersham-Pharmacia). A gradient of 0–1 *M* NaCl in buffer *A* was used to elute

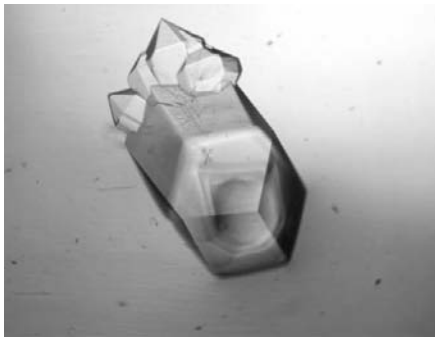

Figure 1
A picture of a crystal of Sso10b which is 1 mm in the longest dimension.

cationic proteins. Fractions corresponding to a distinct absorbance peak were analysed by SDS–PAGE, pooled, concentrated, loaded onto a gel-filtration column (Superdex 200 HiLoad, Amersham-Pharmacia) and developed with buffer *A* containing 300 m*M* NaCl. Peak fractions were pooled and shown to contain essentially homogenous Sso10b protein. This protein was then used for all crystallization trails. Approximately 10 mg of pure protein was obtained from a 1 l culture.

3. Crystallization and data collection

Crystallization trials were set up using the hanging-drop diffusion method in Linbro plates with Hampton Research Crystal Screen kits (Hampton Research, California, USA). Sso10b was concentrated to 15 mg ml^{-1} in 50 m*M* Tris pH 7.5 and 300 m*M* NaCl. Crystals with an elongated hexagonal habit appeared in approximately 30% of the initial screen conditions. No other crystal habit was ever observed. Optimal crystallization conditions were refined to 18% PEG 8000, 0.1 *M* sodium cacodylate pH 6.5, 0.2 *M* calcium chloride and 0.1 *M* 1,2,3-heptanetriol. The reservoir of the hanging drop was covered in 50:50 Al's oil and paraffin oil, which was important for slowing down the initial crystal nucleation. Crystals of dimensions 1 × 0.5 × 0.3 mm were routinely grown (Fig. 1). The crystals, however, were very fragile and transfer into a suitable cryoprotective solution required the initial transfer of the crystals into mother liquor containing 2.5% glycerol. The percentage of glycerol was then increased to 17.5% in steps of 2.5%. Data collection at 100 K exhibited an improvement in the maximal resolution of the observed diffraction from 4 to 2.6 Å compared with room-temperature data collection.

Table 1
Data-collection statistics.

Values in parentheses refer to the highest resolution shell (2.70–2.60 Å).

Space group	$P6_122/P6_522$
Unit-cell parameters (Å)	$a = b = 84.31$, $c = 162.22$
No. of measured reflections	127796
No. of unique reflections	11085
Redundancy	11.5 (3.2)
Resolution (Å)	30–2.6
$R_{\rm merge}$ (%)	10.9 (56.7)
Completeness (%)	99.1 (96.8)
$I/\sigma(I)$	14.1 (1.8)

† $R_{\rm merge} = \sum I(k) - \langle I \rangle | / \sum I(k)$, where $I(k)$ is the value of the kth measurement of the intensity of a reflection, $\langle I \rangle$ is the mean value of the intensity of that reflection and the summation is over all measurements.

The data for the hexagonal crystals were collected at 100 K on an in-house Cu rotating-anode X-ray source (operating at 50 kV, 100 mA) and an R-AXIS IV++ image-plate detector, with an oscillation angle of 1° and a crystal-to-detector distance of 200 mm. Data were processed using *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997) and the data-collection statistics are shown in Table 1. The crystals belong to space group $P6_122/P6_522$ and diffract to a resolution of 2.6 Å (Table 1).

Assuming a MW of 20 kDa for the functional dimer, the crystal is likely to contain either two, three or four monomers per asymmetric unit, with solvent contents of 70% ($V_{\rm M}$ = 4.16 Å^3 Da^{-1}), 56% ($V_{\rm M}$ = 2.77 Å^3 Da^{-1}) and 41% ($V_{\rm M}$ = 2.08 Å^3 Da^{-1}), respectively (Matthews, 1968).

We are attempting to solve the structure by traditional heavy-atom soaks and by selenomethionine labelling for multi-wavelength anomalous dispersion phasing.

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References

Agback, P., Baumann, H., Knapp, S., Ladenstein, R. & Hard, T. (1998). *Nature Struct. Biol.* **5**, 579–584.
Baumann, H., Knapp, S., Lundback, T., Ladenstein, R. & Hard, T. (1994). *Nature Struct. Biol.* **11**, 808–819.
Forterre, P., Confalonieri, F. & Knapp, S. (1999). *Mol. Microbiol.* **32**, 669–670.
Gao, Y. G., Su, S. Y., Robinson, H., Padmanabhan, S., Lim, L., McCrary, B. S., Edmondson, S. P., Shriver, J. W. & Wang, A. H. (1998). *Nature Struct. Biol* **5**, 782–786.
Knapp, S., Karshikoff, A., Berndt, K. D., Christova, P., Atanasov, B. & Ladenstein, R. (1996). *J. Mol. Biol.* **264**, 1132–1144.
Lundbäck, T., Hansson, H., Knapp, S., Ladenstein, R. & Hard, T. (1998). *J. Mol. Biol.* **276**, 775–786.
Lurz, R., Grote, M., Dijk, J., Reinhardt, R. & Dobrinski, B. (1986). *EMBO J.* **5**, 3715–3721.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–374.
Robinson, H., Gao, Y. G., McCrary, B. S., Edmondson, S. P., Shriver, J. W. & Wang, A. H. (1998). *Nature (London)*, **392**, 202–205.
Xue, H., Guo, R., Wen, Y., Liu, D. & Huang, L. (2000). *J. Bacteriol.* **182**, 3929–3933.
Zillig, W., Stetter, K., Wunderl, S., Schulz, W., Priess, H. & Scholz, L. (1980). *Arch. Microbiol.* **125**, 259–269.