Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Expression, purification, crystallization and preliminary crystallographic analysis of osmotically inducible protein C

Peter H. Rehse, Yuichi Nodake, Chizu Kuroishi and Tahir H. Tahirov*

Highthroughput Factory, RIKEN Harima Institute, 1-1-1 Kouto, Mikazuki-cho, Sayo-gun, Hyogo 679-5148, Japan

Correspondence e-mail: tahir@spring8.or.jp

Received 10 October 2003
Accepted 1 December 2003

Selenium-incorporated osmotically inducible protein C from the thermophilic bacterium *Thermus thermophilus* was overexpressed, purified and crystallized. The crystals belong to space group *P*1, with unit-cell parameters a = 37.58, b = 40.95, c = 48.14 Å, α = 76.93, β = 74.04, γ = 64.05°. Five data sets were collected from a single crystal to 1.6 Å using synchrotron radiation for MAD phasing. Self-rotation functions and the Matthews coefficient are consistent with two molecules in the asymmetric unit.

1. Introduction

Non-sporulating bacteria have evolved a number of mechanisms to deal with environmental stress, including heat, oxidative agents and osmotic shock (Hengge-Aronis, 1996; Kolter *et al.*, 1993; McCann *et al.*, 1991). The response to different types of stress is complex, involving the overlapping expression of multiple genes. Expression of the *osmC* gene was first demonstrated in *Escherichia coli* (Gutierrez & Devedjian, 1991) in response to osmotic shock. The resultant protein is therefore referred to as osmotically inducible protein C (OsmC). The distribution of the gene was examined by Völker *et al.* (1998) and was found in a number of bacterial species as one or two copies. The gene was found in the *Bacillus*, *Mycoplasma*, *Acinetobacter*, *Xanthomonas*, *Pseudomonas* and *Deinococcus* species examined, but not in the *Haemophilus*, *Heliocobacter* or *Synechocystis* species examined. Although the function of the protein remains unknown, the gene was highly conserved when found. Regulation of OsmC has been shown to be very complex in both *E. coli* (Bouvier *et al.*, 1998; Toesca *et al.*, 2001; Davalos-Garcia *et al.*, 2001) and *B. subtilus* (Völker *et al.*, 1998). In both cases, expression of OsmC appears to respond to a variety of stimuli in addition to osmotic shock. Conter *et al.* (2001) demonstrated that an OsmC mutant strain of *E. coli* was sensitive to H_2O_2 and *t*-butyl hydroperoxide.

Here, we present the expression, purification and preliminary X-ray crystallographic study of osmotically inducible protein C from *Thermus thermophilus* HB8. This is the first crystal of a member of the OsmC family to be reported.

2. Methods and results

2.1. Expression

The polymerase chain reaction (PCR) was used for gene amplification of *T. thermophilus* HB8 genomic DNA. The PCR product was ligated with pT7blue (Novagen) and digested with *Nde*I and *Bgl*II. The fragment was inserted into the expression vector pET-11a, made linear by digestion with *Nde*I and *Bam*HI and transformed into *E. coli* strain B834 (DE3) pLysS to produce SeMet protein. The cells were grown for 4 h at 310 K in 4.5 l medium containing SeMet and 50 µg ml^{-1} ampicillin, after which protein expression was induced by addition of 1 m*M* IPTG and cultivation was continued for a further 20 h. Cells (15.3 g) were harvested by centrifugation at 6500 rev min^{-1} for 5 min and were suspended in 29 ml of 20 m*M* Tris–HCl pH 8.0, 500 m*M* NaCl, 5 m*M* 2-mercaptoethanol. The cells were disrupted by sonication followed by heat treatment at 343 K for 11.5 min. *T. thermophilus* proteins are not expected to denature at this temperature. The cell debris and denatured proteins were removed by centrifugation (14 000 rev min^{-1}, 30 min, 277 K).

2.2. Purification

The supernatant was applied to a HiPrep 26/10 desalting column (53 ml, Amersham Biosciences) using 20 m*M* Tris–HCl pH 8.0 (buffer *A*). The elutant was applied to a SuperQ Toyopearl 650M column (30 ml, Tosoh) equilibrated in buffer *A* and eluted with a 0–0.3 *M* NaCl linear gradient. The main protein peak was desalted using a HiPrep 26/10 column with buffer *A*, applied to a Resource Q column (6 ml, Amersham Biosciences) equili-

brated in buffer *A* and eluted with a 0–0.2 *M* NaCl gradient. The main protein fraction was desalted with a HiPrep 26/10 desalting column equilibrated in 10 m*M* sodium phosphate H 7.0 (buffer *B*), applied to a CHT10-I (10 ml, Bio-Rad) column and eluted with a 0.01–0.15 *M* sodium phosphate gradient in buffer *B*. The main protein peak was then concentrated and applied to a HiLoad 16/60 Superdex 75 (120 ml, Amersham Biosciences) column equilibrated with buffer *A,* 50 m*M* NaCl. The purified protein was homogenous on SDS–PAGE. The protein was concentrated to 44 mg ml^{-1} using ultrafiltration (Amicon, 5 kDa cutoff).

2.3. Crystallization

Crystals were grown using the sitting-drop method, mixing 1 µl of protein solution (prepared as above) with 1 µl of a reservoir solution containing 100 m*M* Tris–HCl pH 8.4, 30% PEG 4K. Initially, a number of small crystals appeared over the course of several days using conditions obtained from Hampton Research Crystal Screen I (Jancarik & Kim, 1991; 100 m*M* Tris–HCl pH 8.5, 30% PEG 4K, 0.2 *M* sodium acetate trihydrate), but they remained very small over an extended period of time. Further conditions were explored by varying the buffer type and pH, the precipitant type and concentration and using various salts and additives, but the problem of small crystals persisted. The final conditions described above suffered from the same problem, but seven months after being laid down the small crystals were replaced by two or three parallelepiped-shaped crystals 0.1 × 0.2 × 0.2 mm in size. The crystallization temperature was 295 K.

2.4. Collection and processing of X-ray diffraction data

The buffer surrounding the crystals was changed to a cryoprotectant buffer consisting of 100 m*M* Tris–HCl pH 8.4, 30% PEG 4K, 30% PEG 400 by initial addition of reservoir solution (8 µl) followed by increasing amounts of cryoprotectant buffer (2–5 µl) coupled with removal of excess solution. The crystals were mounted in cryoloops and flash-frozen under an N_2 stream at 100 K.

Multiwavelength anomalous diffraction (MAD) data were collected at the synchrotron beamline BL-26a at SPring-8 (Harima, Japan) using an ADSC Quantum 4 CCD detector. Five data sets were collected to 1.6 Å from a single crystal using wavelengths determined from the selenium-absorption spectrum: peak (0.97894 Å), two inflection points (0.97910 and 0.97920 Å), a high-energy remote (0.97500 Å) and a low-energy remote (1.0000 Å). Data were collected as a series of 2° oscillation images covering a rotation range of 340°. A full data set for each wavelength was collected before moving on to the next. The crystal-to-image plate distance was 150 mm, with 10 s exposures. The crystal mosaicity was determined to be 1.1°. Intensity data were processed and scaled using *d*TREK* (Pflugrath, 1999) and are summarized in Table 1. The program *SOLVE* (Terwilliger & Berendzen, 1999) was used to find four of the possible six selenium sites and obtain initial protein phases with an overall figure of merit of 0.61.

Table 1
Data collection and processing of triclinic crystals of OsmC.

Unit-cell parameters a = 37.58, b = 40.95, c = 48.14 Å, α = 76.93, β = 74.04, γ = 64.05°; space group $P1$. Values in parentheses refer to the highest resolution shell (1.66–1.60 Å).

	Peak	Inflection point I	Inflection point II	Low-energy remote	High-energy remote
Resolution (Å)	20.0–1.6	20.0–1.6	19.7–1.6	19.7–1.6	19.7–1.6
Wavelength (Å)	0.97894	0.97920	0.97910	1.00000	0.97500
$R_{\rm merge}$† (%)	3.3 (16.2)	2.9 (18.5)	3.1 (32.6)	2.9 (19.6)	3.1 (29.1)
$\langle I/\sigma(I)\rangle$	15.0 (4.1)	16.5 (3.7)	13.3 (1.9)	15.7 (3.0)	13.8 (2.1)
Completeness (%)	90.4 (79.3)	91.0 (79.9)	91.8 (80.3)	89.3 (63.1)	91.6 (81.6)
Multiplicity	1.85 (1.76)	1.85 (1.75)	1.81 (1.66)	1.84 (1.72)	1.84 (1.73)

† $R_{\rm merge} = \sum|I_j - \langle I_j\rangle|/\sum\langle I_j\rangle$, where I_j is the intensity of reflection j and $\langle I_j\rangle$ is the average intensity for reflection j.

3. Discussion

Osmotically inducible protein C crystallized in the triclinic ($P1$) form, with unit-cell parameters a = 37.58, b = 40.95, c = 48.14 Å, α = 76.93, β = 74.04, γ = 64.05°. Given a molecular weight of 15.3 kDa for the protein and assuming there to be two molecules in the asymmetric unit, the resultant Matthews coefficient is 2.1 Å^3 Da^{-1} (Matthews, 1968), corresponding to a solvent content of 40.3%. A self-rotation search using *MOLREP* (Collaborative Computational Project, Number 4, 1994; Vagin & Teplyakov, 1997) yielded two strong peaks (19.6 and 11.7σ) clearly indicating the presence of two molecules related by a twofold rotation. θ, φ, χ were 0, 0, 0° and 55.6, 74.2, 180.0°, respectively. The next eight strongest peaks ranged from 4.4 to 3.8σ. Attempts to obtain a molecular-replacement solution using the recently solved X-ray crystallographic structure of the hydroperoxide-resistance protein (Lesniak *et al.*, 2002) did not yield an obvious solution. Considering that the two proteins, although members of the same superfamily, are related by moderate (20%) levels of identity and are distinct with respect to function and expression (Atichartpongkul *et al.*, 2001), this is not too surprising. Using phases obtained from the MAD experiment, the structure was built, refined and the coordinates deposited with PDB code 1ukk. The structure will be published elsewhere.

We would like to acknowledge Yoko Ukita, Sayuri Ishijima and Yumi Tokunaga for technical assistance in the purification and Seiki Kuramitsu and Shigeyuki Yokoyama for the plasmid vector used in the protein expression. This work was supported by the National Project on Protein Structural and Functional Analysis funded by MEXT of Japan (project TT1149/HTPF00058).

References

Atichartpongkul, S., Loprasert, S., Vattanaviboon, P., Whangsuk, W., Helmann, J. D. & Mongkolsuk, S. (2001). *Microbiology*, **147**, 1775–1782.
Bouvier, J., Gordia, S., Kampmann, G., Lange, R., Hengge-Aronis, R. & Gutierrez, C. (1998). *Mol. Microbiol.* **28**, 971–980.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Conter, A., Gangneux, C., Suzanne, M. & Gutierrez, C. (2001). *Res. Microbiol.* **152**, 17–26.
Davalos-Garcia, M., Conter, A., Toesca, I., Gutierrez, C. & Cam, K. (2001). *J. Bacteriol.* **183**, 5870–5876.
Gutierrez, C. & Devedjian, J. C. (1991). *J. Mol. Biol.* **20**, 959–973.
Hengge-Aronis, R. (1996). *Mol. Microbiol.* **21**, 887–893.
Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.
Kolter, R., Siegele, D. A. & Tormo, A. (1993). *Annu. Rev. Microbiol.* **47**, 855–874.
Lesniak, J., Barton, W. A. & Nikolov, D. B. (2002). *EMBO J.* **21**, 6649–6659.
McCann, M. P., Kidwell, J. P. & Matin, A. (1991). *J. Bacteriol.* **173**, 4188–4194.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Pflugrath, J. W. (1999). *Acta Cryst.* D**55**, 1718–1725.
Terwilliger, T. C. & Berendzen, J. (1999). *Acta Cryst.* D**55**, 849–861.
Toesca, I., Perard, C., Bouvier, J., Gutierrez, C. & Conter, A. (2001). *Microbiology*, **147**, 2795–2803.
Vagin, A. & Teplyakov, A. (1997). *J. Appl. Cryst.* **30**, 1022–1025.
Völker, U., Andersen, K. K., Antelmann, H., Devine, K. M. & Hecker, M. (1998). *J. Bacteriol.* **180**, 4212–4218.