Acta Crystallographica Section F
Structural Biology and Crystallization Communications
ISSN 1744-3091

Crystallization and preliminary X-ray analysis of Psu, an inhibitor of the bacterial transcription terminator Rho

Susmita Khamrui,[a] Amitabh Ranjan,[b] Bibhusita Pani,[b] Ranjan Sen[b] and Udayaditya Sen[a,c]*

[a]Crystallography and Molecular Biology Division, Saha Institute of Nuclear Physics, 1/AF Bidhan Nagar, Kolkata 700 064, India, [b]Laboratory of Transcription Biology, Center for DNA Fingerprinting and Diagnostics, Tuljaguda Complex, 4-1-714 Mozamjahi Road, Nampally, Hyderabad 500 001, India, and [c]Structural Genomics Section, Saha Institute of Nuclear Physics, 1/AF Bidhan Nagar, Kolkata 700 064, India

Correspondence e-mail: udayaditya.sen@saha.ac.in

Received 13 October 2009
Accepted 14 December 2009

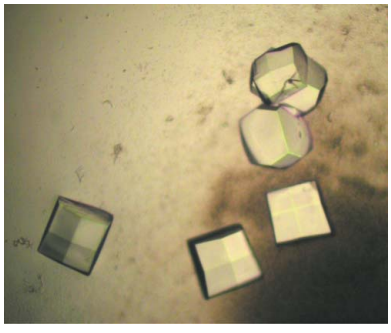
Psu, a coat protein from bacteriophage P4, inhibits Rho-dependent transcription termination both *in vivo* and *in vitro*. The Psu protein is α-helical in nature and appeared to be a dimer in solution. It interacts with Rho and affects the ATP binding and RNA-dependent ATPase activity of Rho, which in turn reduces the rate of RNA release from the elongation complex. Crystals of Psu were grown in space group *I*422 in the presence of PEG, with unit-cell parameters $a = b = 148.76$, $c = 63.38$ Å and a calculated Matthews coefficient of 2.1 Å^3 Da^{-1} (41.5% solvent content), assuming the presence of two molecules in the asymmetric unit. A native data set was collected to 2.3 Å resolution.

1. Introduction

The *Escherichia coli* protein Rho is required for Rho-dependent transcription termination by an RNA polymerase, which is a highly conserved process in bacteria (Richardson, 2002; Ciampi, 2006). Rho is a homohexameric molecular motor which dissociates the transcription elongation complex (EC) using its RNA-dependent ATPase activity (Banerjee *et al.*, 2006). Rho binds to the Rho-utilization (rut) site, an unstructured region of the exiting mRNA, and dislodges the EC, probably by the force exerted by the motor action resulting from its translocase activity along the RNA (Dutta *et al.*, 2008). Owing to its conserved nature and essentiality, this transcription-termination machinery may be a potential target for the design of inhibitors with bacteriocidal activity.

Rho-dependent transcription termination leads to polarity, an event characterized by the reduced expression of downstream genes in an operon. Polarity suppression can occur if the function of Rho is inhibited (Das *et al.*, 1976). Psu (**p**olarity **su**ppression), a unique 21 kDa protein, is a late gene product of bacteriophage P4 (Sauer *et al.*, 1981). Psu is a non-essential capsid-decoration protein (Sunshine & Six, 1976; Isaksen *et al.*, 1992) that inhibits Rho-dependent termination specifically and efficiently both *in vivo* (Sauer *et al.*, 1981; Isaksen *et al.*, 1992; Dokland *et al.*, 1993; Linderoth & Calendar, 1991; Linderoth *et al.*, 1997) and *in vitro* (Pani *et al.*, 2006). Psu interacts with Rho and affects the ATP binding and RNA-dependent ATPase activity of Rho, which in turn reduces the rate of RNA release from the elongation complex (Pani *et al.*, 2006). Co-overexpression of Psu and Rho led to a loss of viability of the cells as a consequence of the anti-Rho activity of Psu. The anti-termination property of Psu can be abolished by either the deletion of ten or 20 amino acids from its C-terminus or by a mutation, Y80C, in Rho. All these experiments indicate probable interactions between Rho and Psu. Co-purification of Rho and wild-type Psu on an affinity matrix and their co-elution in Superose-6 gel filtration suggests direct association of these proteins, whereas a C-terminal ten-amino-acid deletion derivative of Psu failed to be pulled down in this assay (Pani *et al.*, 2006). This indicates that the loss of the function of the mutants is correlated with their inability to interact with each other.

Circular-dichroism and *in vitro* cross-linking studies revealed that Psu exists as a dimer in solution and is predominantly α-helical in nature; it is likely that its N-terminus forms a compact globular fold while its C-terminus forms a solvent-exposed tail-like structure (Pani



et al., 2006, 2009). Extensive mutational and cross-linking studies also indicated that the C-terminal part of Psu is the likely interaction surface with Rho, while its N-terminal domain helps to maintain the conformational integrity of the C-terminal tail (Pani *et al.*, 2009).

Psu is a unique protein that has no sequence homologue. Although we have some understanding of the domain organization of this protein, structural information at the atomic level is essential in order to understand the molecular basis of its inhibition of the function of Rho. An understanding of this will aid in the design of a minimal peptide fragment of Psu that is capable of inhibiting Rho function. Here, we report the crystallization and preliminary crystallographic analysis of the Psu protein.

2. Materials and methods

2.1. Purification of Psu

Non-His-tagged wild-type Psu was cloned in a pET21b vector, expressed and purified essentially following the procedure described previously (Pani *et al.*, 2006). In brief, the full-length *psu* gene was amplified from the plasmid pNLM150 which bears the wild-type *psu* gene (a gift from Richard Calendar) using the following primers: forward primer RS123, gCGCGCGC*CATATG*GAAAGCACAGCCTTACGCAGGCC, containing an *Nde*I site (shown in italics), and reverse primer RS124a, GCGCGC*CTCGAG***TTA**CACTGACTGACGTGATGCCAGTTGC, containing an *Xho*I site (shown in italics) and a stop codon (shown in bold).

This PCR fragment was cloned at the *Nde*I/*Xho*I sites of the pET21b vector, which adds a His-tag sequence at the C-terminus of the protein. To obtain a non-His-tagged derivative of the protein, we introduced a stop codon into the reverse primer when amplifying the *psu* gene. This cloning process did not introduce any non-native amino acids into the protein.

E. coli BL21 (DE3) cells freshly transformed with the pET21b plasmid bearing the *psu* gene were grown until the mid-log stage and induced with 0.1 m*M* IPTG for 3 h. Cells were then harvested and lysed by sonication in TGED buffer [10 m*M* Tris–HCl pH 7.9, 0.1 m*M* EDTA, 0.1 m*M* DTT and 5%(*v*/*v*) glycerol]. After precipitating the lysate with 0.5% polymin P (Sigma), the supernatant was subjected to 25% ammonium sulfate fractionation. The precipitate was resuspended in TGED buffer and further dialyzed against TGED buffer to remove excess ammonium sulfate. Subsequent protein-purification steps were carried out using an ÄKTA protein purifier (GE Healthcare). The protein was first loaded onto a Q-Sepharose column (GE Healthcare). Psu protein was collected from the flow-through fractions and was further loaded onto a CM Sepharose column (GE Healthcare). Psu was eluted between 50 and 150 m*M* NaCl and the eluted fractions were stored in 20 m*M* Tris–HCl pH 7.9, 0.1 m*M* EDTA, 0.1 m*M* dithiothreitol and 100 m*M* NaCl. This procedure yielded about 95% pure protein.

2.2. Crystallization

For crystallization, the Psu protein was dialyzed against 20 m*M* Tris–HCl pH 8.0, 0.1 m*M* EDTA, 0.5 m*M* dithiothreitol (DTT) and 300 m*M* NaCl and concentrated to 5 mg ml^{-1} using Amicon Centriprep centrifugal filtration units (5000 Da molecular-weight cutoff). Crystallization trials were set up using Crystal Screens I and II, Grid Screen Ammonium Sulfate, Grid Screen PEG and PEG/Ion Screen (Hampton Research, USA). Crystallizations were performed at 277 and 293 K using the hanging-drop vapour-diffusion method in 24-well tissue-culture plates. Typically, 2 µl protein solution (5 mg ml^{-1}) was

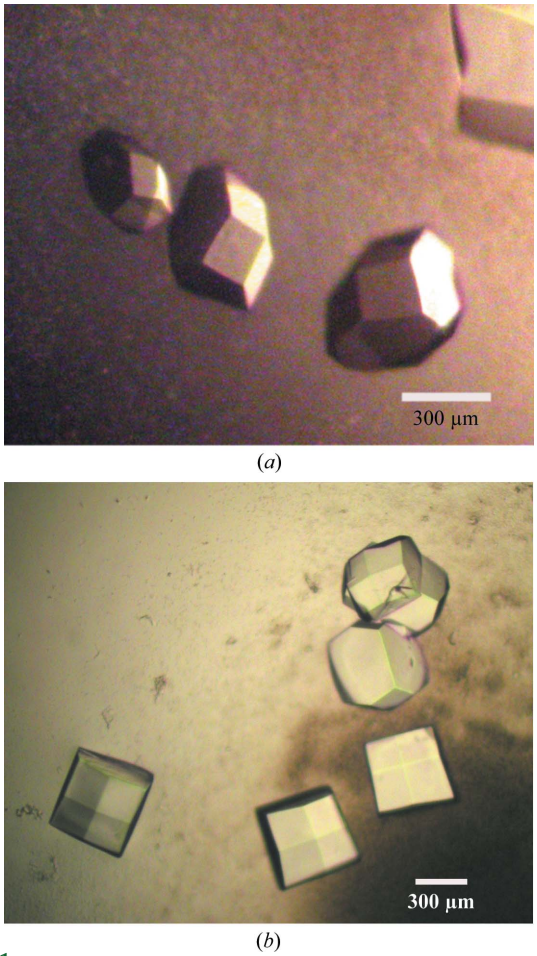


Figure 1
Crystals of Psu. (*a*) Crystals grown in the presence of PEG 6000, 5%(*v*/*v*) glycerol and 300 m*M* NaCl (0.3 × 0.3 × 0.25 mm). (*b*) Crystals grown in the presence of 7.5% PEG 6000, 5%(*v*/*v*) glycerol, 300 m*M* NaCl and 0.2 *M* iodoacetamide (0.4 × 0.4 × 0.25 mm).

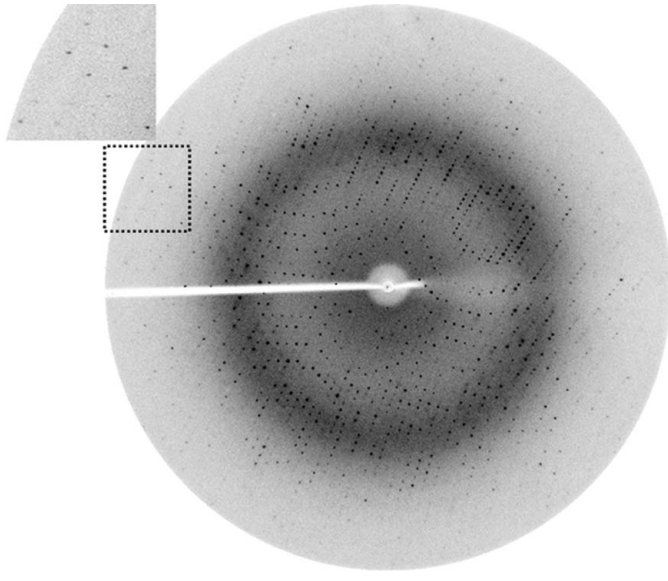


Figure 2
X-ray diffraction image of a Psu crystal. The region marked with a square at the edge of the detector, which corresponds to a resolution of 2.3 Å, is enlarged to show the quality of the reflections in the highest bin.

Table 1
Data-collection and data-processing parameters for the Psu crystal.

Values in parentheses are for the outermost resolution shell.

Space group	*I*422
Unit-cell parameters (Å)	$a = b = 148.76$, $c = 63.38$
Oscillation range (°)	1
Maximum resolution (Å)	2.3 (2.38–2.3)
No. of molecules per ASU	2
Mathews coefficient (V_M; Å^3 Da^{-1})	2.19
Solvent content (%)	43.9
No. of observations	118776 (10757)
No. of unique reflections	16080 (1550)
Mosaicity (°)	0.28
Completeness (%)	99.6 (98.4)
R_{merge}† (%)	6.3 (29.7)
Average $I/\sigma(I)$	5.9 (2.1)

† $R_{merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the observed intensity of the ith measurement of reflection hkl and $\langle I(hkl)\rangle$ is the mean intensity of reflection hkl calculated after scaling.

mixed with an equal volume of screening solution and equilibrated over 700 µl of the latter as reservoir solution. Small crystals appeared within 7 d at 277 K using 5–10%(*w*/*v*) PEG 6000 in both 0.1 *M* MES pH 6.0 and 0.1 *M* HEPES pH 7.0. Both conditions were further optimized and larger crystals were obtained using 5–10%(*w*/*v*) PEG 6000, 5%(*v*/*v*) glycerol, 0.5 m*M* DTT and 300 m*M* NaCl in 0.1 *M* MES pH 6.0 at 277 K (Fig. 1*a*). To further optimize the condition, iodoacetamide was added to the protein solution to a final concentration of 0.3 m*M* and incubated for about 1 h at 277 K prior to setting up the experiment. The best crystals appeared when 3 µl of this protein solution was mixed with 2 µl reservoir solution containing 7.5%(*w*/*v*) PEG 6000, 5%(*v*/*v*) glycerol, 0.5 m*M* DTT and 300 m*M* NaCl in 0.1 *M* MES pH 6.0 at 277 K (Fig. 1*b*).

2.3. Data collection and processing

Crystals of Psu were fished out from the crystallization drops using a 10 µm nylon loop (Hampton Research, Laguna Niguel, California, USA), briefly soaked in a cryoprotectant solution consisting of 7%(*w*/*v*) PEG 6000, 20%(*v*/*v*) glycerol and 300 m*M* NaCl in 0.1 *M* MES pH 6.0 and flash-frozen in a stream of nitrogen (Oxford Cryosystems) at 100 K. A native diffraction data set was collected to 2.3 Å resolution (Fig. 2) using an in-house MAR Research image-plate detector of diameter 345 mm and Cu $K\alpha$ radiation generated by a Bruker–Nonius FR591 rotating-anode generator equipped with Osmic MaxFlux confocal optics and running at 50 kV and 90 mA. A total of 94 frames were collected with a crystal-to-detector distance of 210 mm. The exposure time for each image was 4 min and the oscillation range was maintained at 1°. Data were processed and scaled using *AUTOMAR* (http://www.marresearch.com/automar/run/htm). Data-collection and processing statistics are given in Table 1.

3. Results

After optimizing the initial crystallization conditions, single block-shaped crystals with dimensions of 0.3 × 0.3 × 0.25 mm were obtained after 10 d using 5–10% PEG 6000 containing 5%(*v*/*v*) glycerol, 0.5 m*M* DTT and 300 m*M* NaCl in 0.1 *M* MES pH 6.0 at 277 K (Fig. 1*a*). Larger crystals with maximum dimensions of 0.4 × 0.4 × 0.25 mm were obtained by using iodoacetamide (0.2 m*M*) as an additive with 5–10%(*w*/*v*) PEG 6000 containing 5%(*v*/*v*) glycerol, 0.5 m*M* DTT and 300 m*M* NaCl in 0.1 *M* MES pH 6.0 at 277 K (Fig. 1*b*). After soaking the crystals briefly in cryo-buffer [7%(*w*/*v*) PEG 6000, 20%(*v*/*v*) glycerol and 300 m*M* NaCl in 0.1 *M* MES pH 6.0], diffraction data were recorded to a resolution of 2.3 Å.

Crystals grown in either the presence or absence of iodoacetamide belonged to space group *I*422, with unit-cell parameters $a = b = 148.76$, $c = 63.38$ Å. Careful scrutiny of the systematic absences indicated that the space group was *I*422 and not $I4_122$. The collected native data set was 99.6% complete, with a redundancy of 7.3 and an $I/\sigma(I)$ of 5.8. Data-collection statistics are summarized in Table 1. Packing considerations indicated the presence of a dimer in the asymmetric unit, which corresponds to a Matthews coefficient (V_M) of 2.1 Å^3 Da^{-1} and a solvent content of 41.5% (Matthews, 1968); the corresponding values for one molecule (4.2 Å^3 Da^{-1} and 70.5% solvent content) or three molecules (1.39 Å^3 Da^{-1} and 11.6% solvent content) in the asymmetric unit are clearly outside the range normally observed for protein crystals.

Since a search for a homologous structure to this protein using *3D-JIGSAW* (Bates *et al.*, 2001) did not give any results, structure determination by means of molecular replacement was not attempted and the phases will have to be obtained experimentally in the future.

The laboratory of US is supported by the SINP core fund. The laboratory of RS is funded by the DBT-COE in Microbial Physiology, a DST Swarnajayanti Fellowship and CDFD core funds. SK is a senior research fellow of SINP. AR is an ICMR junior research fellow and BP was an UGC senior research fellow.

References

Banerjee, S., Chalissery, J., Bandey, I. & Sen, R. (2006). *J. Microbiol.* **44**, 11–22.
Bates, P. A., Kelley, L. A., MacCallum, R. M. & Sternberg, M. J. E. (2001). *Proteins*, **45**, *Suppl.* 5, 39–46.
Ciampi, M. S. (2006). *Microbiology*, **152**, 2515–2528.
Das, A., Court, D. & Adhya, S. (1976). *Proc. Natl Acad. Sci. USA*, **73**, 1959–1963.
Dokland, T., Isaksen, M. L., Fuller, S. D. & Lindqvist, B. H. (1993). *Virology*, **194**, 682–687.
Dutta, D., Chalissery, J. & Sen, R. (2008). *J. Biol. Chem.* **283**, 20243–20251.
Isaksen, M. L., Rishovd, S. T., Calendar, R. & Lindqvist, B. H. (1992). *Virology*, **188**, 831–839.
Linderoth, N. A. & Calendar, R. (1991). *J. Bacteriol.* **173**, 6722–6731.
Linderoth, N. A., Tang, G. & Calendar, R. (1997). *Virology*, **227**, 131–141.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Pani, B., Banerjee, S., Chalissery, J., Abishek, M., Loganathan, R. M., Suganthan, R. B. & Sen, R. (2006). *J. Biol. Chem.* **281**, 26491–26500.
Pani, B., Ranjan, A. & Sen, R. (2009). *J. Mol. Biol.* **389**, 647–660.
Richardson, J. P. (2002). *Biochim. Biophys. Acta*, **1577**, 251–260.
Sauer, B., Ow, D., Ling, L. & Calendar, R. (1981). *J. Mol. Biol.* **145**, 29–46.
Sunshine, M. & Six, E. (1976). *J. Mol. Biol.* **106**, 673–682.