Crystallization and preliminary X-ray analysis of TBP-interacting protein from the hyperthermophilic archaeon *Thermococcus kodakaraensis* strain KOD1

The 26 kDa TBP (TATA-binding protein) interacting protein from the hyperthermophilic archaeon *Thermococcus kodakaraensis* KOD1 (Tk-TIP26) is a possible transcriptional regulatory protein in *Thermococcales*. Here, the crystallization of both the native and selenomethionine-substituted proteins and data collection are described. The native crystals belong to the tetragonal space group \( P4_2_12 \) or \( P4_2_2_2 \), with unit-cell parameters \( a = 73.83 \), \( c = 86.41 \, \text{Å} \), and diffract to 2.2 Å using synchrotron radiation. MAD data was collected and a Bijvoet difference Patterson map showed strong peaks sufficient to determine the positions of the Se atoms.

1. Introduction

The Archaea are the third primary kingdom of life, distinct from the Bacteria and the Eukarya (Woese *et al.*, 1990). Archaeal cells are evolutionarily more closely related to eukaryotic cells than to bacterial cells, especially in their transcription mechanisms (Keeling & Doolittle, 1995; Thomm, 1996; Reeve *et al.*, 1997; Bell & Jackson, 2001).

In bacterial cells, transcription is initiated by a single RNA polymerase whose holoenzyme structure is composed of only five subunits: \( \alpha, \beta, \beta', \omega \), and \( \sigma \) (Darst, 2001). In eukaryotic cells, the transcription of nuclear genes is initiated by RNA polymerase II, which has 14 subunits and six general transcription factors: TFIIA, TFIIB, TFIID, TFIIE, TFIIF and TFIIF (Roeder, 1996). TFIID is a multi-protein complex containing TATA-binding protein (TBP) and TBP-associated factors (TAFs).

The basal transcription machinery in the archaeal cells is strikingly similar to the core components of the eukaryotic RNA polymerase II transcription system (Qureshi *et al.*, 1997). Archaeal promoters contain a TATA-like element referred to as an 'A-box' which is located 25 bp upstream of the transcriptional initiation site. Archaeal genomes contain genes encoding a TBP homologue and a TFIIH homologue (TFB), but do not contain genes encoding homologues of other eukaryotic basal transcription factors and TAFs. These results suggest that there are only three factors (TBP, TFB and RNA polymerase) required for archaeal basal transcription and that the archaeal transcriptional initiation machinery is simpler than that in eukaryotes. In fact, TBP, TFB and RNA polymerase have been shown to be the only factors required for promoter-specific transcription in archaeal cells (Qureshi *et al.*, 1997). However, this raises the question as to whether archaeal cells contain unique factors that interact with TBP and regulate transcription.

*Thermococcus kodakaraensis* strain KOD1 was isolated from a solfatara at a wharf on Kodakara Island, Kagoshima, Japan. The growth temperature of this strain ranges from 338 to 373 K; its optimal growth temperature is 368 K (Morikawa *et al.*, 1994; Fujiwara *et al.*, 1998). TBP-interacting protein (Tk-TIP26) was isolated from cell lysates of this strain by affinity chromatography with TBP-agarose (Matsuda *et al.*, 1999). Cloning of the gene encoding this protein showed that Tk-TIP26 is composed of 224 amino-acid residues (molecular weight 25 558 Da) and exists in a dimeric form. Database analyses indicate that orthologous genes are only present in the genomes of two genera, *Pyrococcus* and *Thermococcus*. Recombinant Tk-TIP26 and Tk-TBP interact with each other with an equilibrium dissociation constant \( K_D \) of 1.24–1.46 \( \mu \text{M} \). In the presence of Tk-TIP26, Tk-TBP and a DNA fragment (TATA-DNA), Tk-TIP26 prevents Tk-TBP from binding to TATA-DNA (Matsuda *et al.*, 1999). On the other hand, in the presence of Tk-TIP26, Tk-TFB, Tk-TBP and TATA-DNA, Tk-TIP26 does not inhibit the formation of the TFB–TBP–DNA ternary complex, but interacts with this complex to form the TIP26–TFB–TBP–DNA quaternary complex (Matsuda *et al.*, 2001). These results suggest that TIP26 plays an important role in transcriptional regulation in *Thermococcales*.

As the first step towards understanding the regulation mechanism in archaeal transcription, we report here the crystallization and preliminary X-ray diffraction studies of Tk-TIP26.
2. Materials and methods

2.1. Overproduction, purification and crystallization of native Tk-TIP26

The gene encoding Tk-TIP26 was cloned into pET-28a (Novagen). The resultant plasmid was used to transform *Escherichia coli* HMS174(DE3)pLysS (Novagen). Cells were grown in NZCYM medium containing 100 µg ml⁻¹ kanamycin at 310 K. At an OD₆₆₀ of 0.6, 1 mM IPTG was added to induce gene expression and cultivation was continued for an additional 8 h. Cells were then harvested by centrifugation at 15 000 g for 20 min at 277 K. The following procedures were carried out at 277 K.

Cells were suspended in 50 mM NaH₂PO₄ pH 8.0 containing 500 mM NaCl, 50 mM imidazole and 1 mM 2-mercaptoethanol (2-Me), disrupted by sonication and centrifuged at 15 000 g for 30 min. The supernatant was applied onto a HiTrap Chelating HP column (Amersham Pharmacia Biotech) equilibrated with the same buffer as that for sonication lysis. The sample was eluted with a 100–250 mM imidazole gradient. The active fractions were combined and dialyzed sequentially against the following buffers with a 1 h interval: (i) 50 mM NaH₂PO₄ pH 8.0 containing 500 mM NaCl, 100 mM imidazole, 1 mM 2-Me and 1 mM PMSF; (ii) 50 mM NaH₂PO₄ pH 8.0 containing 500 mM NaCl, 50 mM imidazole, 1 mM 2-Me and 1 mM PMSF (twice); (iii) 20 mM NaH₂PO₄ pH 7.2 containing 500 mM NaCl, 50 mM imidazole and 1 mM DTT and (iv) 20 mM NaH₂PO₄ pH 7.0 containing 500 mM NaCl and 1 mM DTT (twice). After dialysis, the sample was concentrated with Centricon YM-10 (Millipore) and applied onto a HiLoad 26/60 Superdex 75 prep-grade column (Amersham Pharmacia Biotech) equilibrated with 20 mM NaH₂PO₄ pH 7.0 containing 500 mM NaCl and 1 mM DTT. The active fractions were pooled, concentrated with Centriprep YM-10 (Millipore) and used for crystallization without thrombin cleavage.

Crystals of Tk-TIP26 were obtained by the hanging-drop vapour-diffusion method. Initial conditions were found using Crystal Screen II (Hampton Research). After some improvement steps, optimized crystallization was performed under the following conditions. The drop solution (4 µl) contained 5 mg ml⁻¹ Tk-TIP26, 10 mM NaH₂PO₄ pH 7.0, 250 mM NaCl, 0.5 mM DTT, 50 mM MES pH 5.8, 6% (w/v) PEG 8000, 5% (v/v) MPD and 5% (v/v) glycerol. The well solution (500 µl) contained 100 mM MES pH 5.8, 7.5% (w/v) PEG 8000, 10% (v/v) MPD and 10% (v/v) glycerol. Crystals of Tk-TIP26, shown in Fig. 1, grew to maximum dimensions of 0.15 × 0.15 × 0.2 mm in a week.

2.2. Preparation of selenomethionine-substituted (SeMet) crystals

The plasmid for overproduction of Tk-TIP26 mentioned above was used to transform *E. coli* B834(DE3)pLysS cells (Novagen). Transformed cells were initially precultured in LB medium containing 100 µg ml⁻¹ kanamycin until OD₆₆₀ reached 0.6. Cells were grown at 310 K in a modified minimal medium comprising 0.5% (w/v) glucose, 1 mM MgSO₄, 40 µg ml⁻¹ each of all the common amino acids except methionine, 0.5 mg ml⁻¹ each of all nucleic acids, 25 µg ml⁻¹ selenomethionine and 100 µg ml⁻¹ kanamycin. When the OD₆₆₀ reached 0.6, 1 mM IPTG was added to induce gene expression and cultivation was continued at 298 K for an additional 12 h. Cells were then harvested and the SeMet protein was purified as described for the native protein.

Crystals of SeMet Tk-TIP26 were obtained by the hanging-drop vapour-diffusion method. Crystallization was performed under the following conditions. The drop solution (4 µl) contained 7.5 mg ml⁻¹ SeMet Tk-TIP26, 10 mM NaH₂PO₄ pH 7.0, 250 mM NaCl, 0.5 mM DTT, 50 mM MES pH 5.8 and 3% (w/v) PEG 8000. The well solution (500 µl) contained 100 mM MES pH 5.8 and 5% (w/v) PEG 8000.

2.3. Data collection

The native crystals were mounted directly from drops into the nitrogen stream at 100 K and the diffraction data were collected to 2.2 Å on SPring-8 beamline BL41XU with a MAR CCD.

A MAD experiment was performed on SeMet Tk-TIP26 on beamline BL40B2 at SPring-8. The crystals of SeMet Tk-TIP26 were soaked in 100 mM MES pH 5.8 containing 7% (w/v) PEG 8000 and 20% (v/v) glycerol for a few seconds and were flash-frozen in the nitrogen-gas stream at 100 K. Three different wavelengths were chosen from inspection of the fluorescence spectrum of the crystal, corresponding to the inflection point, peak and low-energy remote. The diffraction data were recorded on an ADSC Quantum 4 CCD detector.

All diffraction data were processed using MOSFLM (Leslie, 1997) and the CCP4 program suite (Collaborative Computational Project, Number 4, 1994).

3. Results and discussion

Native crystals diffracted to 2.2 Å and belonged to the tetragonal space group *P*₄₁₂₂ or *P*₂₁₂₂, with unit-cell parameters *a* = 73.83, *c* = 86.41 Å. The value of the Matthews coefficient is 2.17 Å³ Da⁻¹ for a

### Table 1

<table>
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<td></td>
<td><em>a</em> = 73.83, <em>c</em> = 86.41</td>
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<td>9.0 (3.5)</td>
<td>9.0 (3.5)</td>
<td>10.6 (2.4)</td>
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$p_{\text{SeMet}} = \sum |I - |I|/\sum |I|$, where $I$ is the intensity of observation $I$ and $|I|$ is the mean intensity of the reflection.
monomer in the asymmetric unit, corresponding to a solvent content of 43.0%, a typical value for protein crystals (Matthews, 1968).

SeMet-substituted crystals also belonged to the tetragonal space group $P4_12_12$ or $P4_32_12$, with unit-cell parameters $a = 74.34 \AA$, $c = 86.38 \AA$. The value of the Matthews coefficient is $2.14 \AA^3 \text{Da}^{-1}$ for a monomer in the asymmetric unit, corresponding to a solvent content of 42.0% (Matthews, 1968). The crystals diffracted to 3.0 Å (peak) and 2.8 Å (inflexion and remote), respectively. Details of the data processing and statistics are shown in Table 1.

A Bijvoet difference Patterson map very clearly showed the positions of two of the three Se atoms in the asymmetric unit at 4.0 Å resolution. Harker sections of the Bijvoet difference Patterson map of the peak data are shown in Fig. 2. Phasing and density modification are now in progress using the program CNS (Brünger et al., 1998).

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**References**


