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Preliminary crystallography confirms that the archaeal DNA-binding and tryptophan-sensing regulator TrpY is a dimer

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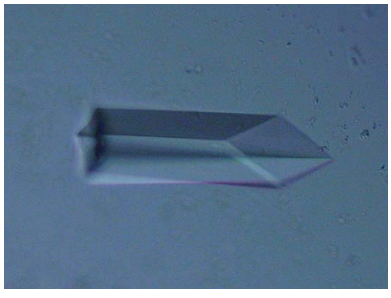

TrpY regulates the transcription of the metabolically expensive tryptophan-biosynthetic operon in the thermophilic archaeon *Methanothermobacter thermautotrophicus*. TrpY was crystallized using the hanging-drop method with ammonium sulfate as the precipitant. The crystals belonged to the tetragonal space group $P4_32_12$ or $P4_12_12$, with unit-cell parameters $a = b = 87$, $c = 147$ Å, and diffracted to 2.9 Å resolution. The possible packing of molecules within the cell based on the values of the Matthews coefficient (V_M) and analysis of the self-rotation function are consistent with the asymmetric unit being a dimer. Determining the structure of TrpY in detail will provide insight into the mechanisms of DNA binding, tryptophan sensing and transcription regulation at high temperature by this novel archaeal protein.

1. Introduction

The presence of bacterial-like transcription regulators in archaea is intriguing given the eukaryotic-like mechanism of transcription initiation in archaea (Kyrpides & Ouzounis, 1995; Aravind & Koonin, 1999; Krüger *et al.*, 1998). In the thermophilic archaeon *Methanothermobacter thermautotrophicus*, the *trpEGCFBAD* operon encodes the enzymes that catalyze the synthesis of tryptophan *via* an energetically very expensive pathway. With tryptophan available, transcription of this operon is repressed by TrpY (Xie & Reeve, 2005). Bioinformatics predicts that TrpY has an N-terminal helix–turn–helix (HTH) DNA-binding motif and a C-terminal ACT domain that is likely to bind tryptophan as an allosteric effector. In the absence of tryptophan, TrpY does not repress transcription of the *trpEGCFBAD* operon but auto-represses *trpY* transcription, facilitating the synthesis of tryptophan (Xie & Reeve, 2005; Cubonova *et al.*, 2007).

In *Escherichia coli*, transcription of the *trp* operon is similarly regulated by a tryptophan-sensing repressor designated TrpR and with an attenuation mechanism based on the translation of a short leader peptide (Merino *et al.*, 2008). The structure of TrpR has been determined by crystallographic techniques to a resolution of 1.8 Å (Zhang *et al.*, 1987; Lawson & Sigler, 1988). The sequences of the tryptophan- and DNA-binding domains of TrpR have no recognizable similarity to the domains that are predicted to embody these activities in TrpY. There is therefore no evidence for a common ancestry or for an evolutionarily related mechanism of transcription regulation. The functions of HTH domains in transcription regulation in eukaryotes and bacteria have been well established, but there is very little corresponding structural information about transcription regulators in the Archaea (Aravind & Koonin, 1999). To address this, TrpY crystals have been generated with the goal of gaining insight into the mechanics of TrpY regulation and the evolution of transcription regulation in the Archaea. Here, we report the preliminary data obtained from these crystals.

Table 1
Data-collection statistics.

Values in parentheses are for the highest resolution shell.

Space group	$P4_32_12$ or $P4_12_12$
Unit-cell parameters (Å)	$a = b = 87.5$, $c = 147.5$
Resolution range (Å)	20–2.9
Wavelength (Å)	1.0809
Total reflections	137052
Unique reflections	10714
Completeness (%)	91
$R_{\rm merge}$ (%)	7.1 (45.4)
$I/\sigma(I)$	32 (2)

2. Materials and methods

2.1. Protein purification and crystallization

TrpY was purified from *E. coli* as described previously (Cubonova *et al.*, 2007). Fractions containing the eluted protein were pooled in 100 m*M* Tris–HCl pH 8, 500 m*M* NaCl and concentrated to 6.8 mg ml^{-1} for crystallization. Conditions for crystallization were initially screened by the hanging-drop vapour-diffusion method with sparse-matrix crystallization kits (Hampton Research, California, USA) in 24-well plates. Conditions that resulted in crystals were then optimized, at a protein concentration of 6.8 mg ml^{-1}, by varying the concentrations of ammonium sulfate and sodium acetate. Droplets consisting of 2 µl protein solution and 2 µl reservoir solution were equilibrated against 700 µl reservoir solution at room temperature. Crystals were grown in 1.6 *M* ammonium sulfate plus 0.1 *M* sodium acetate (Fig. 1).

2.2. X-ray data collection, processing and analysis

Crystals were initially tested for diffraction under cryogenic conditions on beamline X3A of the National Synchrotron Light Source (NSLS), Brookhaven National Laboratory, Upton, New York, USA. No diffraction was observed when native crystals were flash-cooled directly from the drop. Optimization of cryoprotectants improved the diffraction, with the best diffraction being obtained using a cryoprotectant solution consisting of the appropriate mother liquor plus 20% ethylene glycol. Crystals were placed in the cryoprotectant for ∼10 s before mounting in a cryoloop and flash-cooling in a stream of liquid nitrogen. Diffraction data from native crystals were measured on beamline X29 of the NSLS and a data set was collected from one crystal. Data were processed, integrated and scaled using the *HKL*-2000 suite of programs (Otwinowski, 1993; Otwinowski & Minor, 1997). The crystals diffracted anisotropically and the data-collection statistics are shown in Table 1. Unit-cell contents were analyzed using the Matthews coefficient (Matthews, 1968) and the self-rotation function was calculated using *POLARRFN* (Collaborative Computational Project, Number 4, 1994).

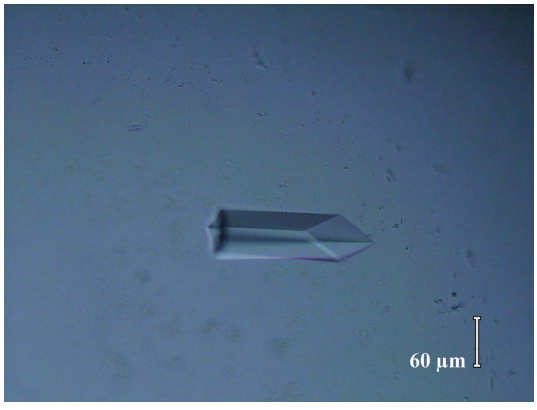

Figure 1
Representative native crystal of TrpY.

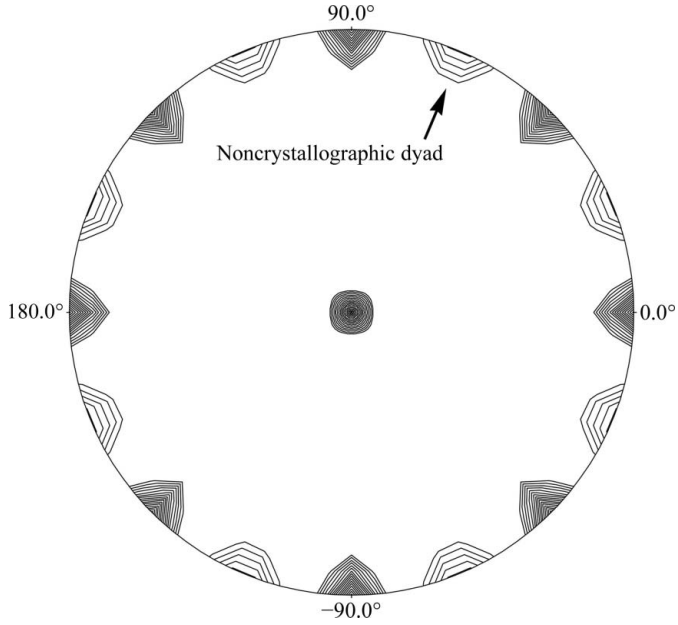

Figure 2
Stereographic plot of the $\kappa = 180^\circ$ section of the self-rotation function for the TrpY native data. The arrow indicates one position of the noncrystallographic dyad symmetrically placed between the crystallographic twofold axis and perpendicular to the tetragonal axis.

3. Results and discussion

The TrpY crystals belonged to the tetragonal space group $P4_32_12$ or $P4_12_12$ and diffracted to 2.9 Å resolution. Assuming the presence of two TrpY molecules of molecular mass 16.4 kDa (146 residues) in the asymmetric unit, the Matthews coefficient ($V_{\rm M}$) was 3.62 Å^3 Da^{-1}, corresponding to a solvent content of ∼66%. The highest peaks in the $\kappa = 180^\circ$ section of the Patterson self-rotation function belonged to 4/*mmm* symmetry arising from a set of crystallographic twofold axes perpendicular to the fourfold axis (Fig. 2). Additional peaks appeared at $\varphi = 22.5^\circ$ with ∼55% of the intensity of the highest peaks and corresponded to the noncrystallographic dyad (shown in Fig. 2) located symmetrically between the crystallographic twofold axes ($\varphi = 45^\circ$) and perpendicular to the fourfold (tetragonal) axis ($\varphi = 90^\circ$). This orientation creates an apparent eightfold symmetry. A peak with the same height (∼55% of the main intensity) as observed for the noncrystallographic dyad in the $\kappa = 180^\circ$ section is also observed in the $\kappa = 45^\circ$ section. This observation clearly suggests the presence of a noncrystallographic twofold axis relating two TrpY molecules in the asymmetric unit. As TrpY has been shown to be a dimer in solution (Xie & Reeve, 2005) and appears to initially bind to DNA as a dimer (Karr *et al.*, 2008), it seems very unlikely that the dimers present in the crystals are an artifact of crystal packing.

We have now generated crystals of selenomethionine-labelled TrpY in order to determine a detailed structure of TrpY by the MAD phasing method. These crystals diffracted to ∼7 Å resolution and we are therefore currently pursuing crystallization optimization. We anticipate that an informative TrpY structure will then be readily solved using carefully measured MAD data from selenomethionine-labelled TrpY crystals.

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