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

Crystallization and preliminary X-ray crystallographic analysis of the probable tRNA-modification GTPase (TrmE) from *Staphylococcus aureus*

Amit Priyadarshi,[a] Ki Hyun Nam,[b] Eunice EunKyeong Kim[a] and Kwang Yeon Hwang[b]*

[a]Biomedical Research Center, Life Science Division, Korea Institute of Science and Technology, 39-1 Hawolgok-dong, Seongbuk-gu, Seoul 136-791, Republic of Korea, and [b]Division of Biotechnology, College of Life Sciences, Korea University, Seoul 136-701, Republic of Korea

Correspondence e-mail: chahong@korea.ac.kr

Received 17 October 2008
Accepted 7 November 2008

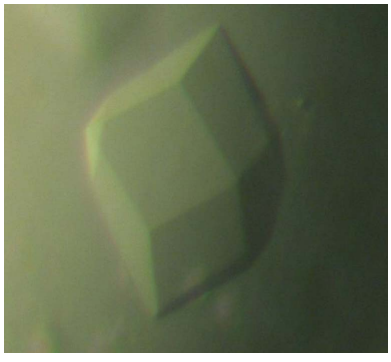
Probable tRNA-modification GTPase (TrmE) is a guanine nucleotide-binding protein that is conserved between bacteria and humans. GTPase hydrolyzes GTP and plays a pivotal role in signalling pathways. In this study, TrmE from *Staphylococcus aureus* was overexpressed in *Escherichia coli*. The enzyme was found to crystallize at 295 K when ammonium sulfate was used as a precipitant. X-ray diffraction data were collected to 2.9 Å resolution from the crystallized enzyme using synchrotron radiation. The crystal was found to belong to the cubic space group $I23$, with unit-cell parameters $a = b = c = 229.47$ Å, $\alpha = \beta = \gamma = 90^\circ$. The crystal is likely to contain four monomers in the asymmetric unit, with a corresponding V_M of 2.4 Å^3 Da^{-1} and a solvent content of 50%.

1. Introduction

Proteins associated with the GTPase superfamily are critical components of many signalling pathways in which conformational changes are associated with GTP binding. As a result, GTP hydrolysis plays an important role in processes such as cell division, cell cycling, signal transduction, mRNA translation and hormone signalling (Yamanaka *et al.*, 2000). A structural comparison of various GTPases from this superfamily has shown that they share a common fold, as well as strong sequence identity in five motifs (G1–G5) that are involved in nucleotide and metal binding (Hugo *et al.*, 1999). The common property shared by these proteins is the presence of a structural module, the G-domain, which is involved in switching the protein between a GTP-bound and a GDP-bound conformation. This conformational switch is essential to the function of all GTPases (Yamanaka *et al.*, 2000).

Probable tRNA-modification GTPase (TrmE) is a 52 kDa guanine nucleotide-binding protein that binds and hydrolyzes GTP. TrmE contains a canonical G-domain and is conserved in all three kingdoms of life. The GTP-bound state in G-domain proteins is known as the active state of the protein, whereas the protein is inactive when it is in the GDP-bound state (Martínez-Vicente *et al.*, 2005). It is believed that TrmE is directly involved in an enzymatic reaction that involves the modification of uridine bases (U34) at the first anticodon (wobble) position of tRNAs that decode two-family box triplets. TrmE-assisted modification of U34 at position 5 of the uridine base leads to 5-methylaminomethyluridine (mnm5U) in bacteria, 5-carboxymethylaminomethyluridine in yeast and 5-taurinomethyluridine in humans (Scrima & Wittinghofer, 2006).

TrmE consists of three regions: an N-terminal region of approximately 220 amino acids, a central G-domain of approximately 160 residues and a C-terminal region of 75 amino acids. The C-terminal end contains the C*x*GK motif, which is highly conserved across the TrmE family. In this motif, the C-terminal Cys may function as a catalytic residue and may be involved in the membrane-association tRNA-modifying activity of this protein, which plays an important role in membrane association and cell signalling (Yim *et al.*, 2003). To date, one crystal structure of a TrmE, that from *Thermotoga maritima*, has been described (Scrima *et al.*, 2005). The structure revealed a three-domain protein comprising an N-terminal α/β domain, a



central helical domain and a G-domain, which is responsible for GTP binding and hydrolysis. The N-terminal domain induces dimerization and binds formyltetrahydrofolate. To provide further structural data regarding this important class of GTPase, we have started to evaluate the structure of TrmE from *Staphylococcus aureus* (*Sa*TrmE), which is composed of 459 amino-acid residues (M_r = 52 000). In this study, we have overexpressed, purified and crystallized *Sa*TrmE and conducted a preliminary X-ray crystallographic analysis of the protein. The crystallization conditions and preliminary X-ray crystallographic data are reported here.

2. Experimental procedures

2.1. Protein expression and purification

The full-length TrmE gene (NC_002952) was amplified *via* PCR using *S. aureus* subsp. *aureus* MRSA genomic DNA as a template. The sequences of the forward and reverse oligonucleotide primers, which were designed from the published genome sequence (Holden *et al.*, 2004), were as follows: 5′-ATAAGAAT**GCGGCCGCCATG**-GATTTAGATACAATTACGA-3′ and 5′-CCG**CTCGAG**CTATTT-ACCTAAGCAGAATTG-3′ (the bases in bold indicate the *Not*I and *Xho*I digestion sites, respectively). The amplified DNA was then inserted into the *Not*I/*Xho*I-digested expression vector pET-28a (Novagen) to produce recombinant *Sa*TrmE with a hexahistidine tag and a thrombin cleavage site at the N-terminus (MGSSHHHHHH-SSGLVPRGSHMASMTGGQQMGRDPNSSSVDKLAAA).

*Sa*TrmE was then overexpressed in *Escherichia coli* BL21 (DE3) cells. To accomplish this, the cells were grown at 310 K to an OD_{600} of 0.7 in Luria–Bertani medium containing 50 µg ml^{-1} kanamycin. Protein expression was then induced by the addition of 1.0 m*M* isopropyl *β*-D-1-thiogalactopyranoside (IPTG), after which cell growth was continued at 295 K overnight. The cells were harvested by a 30 min centrifugation at 4200*g* and 277 K. Next, the cell pellet was resuspended in ice-cold lysis buffer [50 m*M* Tris–HCl pH 8.2, 200 m*M* NaCl, 5%(*v*/*v*) glycerol] and homogenized using a sonicator (Sonics, USA). The crude cell extract was then centrifuged for 40 min at 12 000 rev min^{-1} (Hanil Supra 21K rotor) and 277 K. The recombinant protein in the supernatant fraction was purified *via* three chromatographic steps. The first step employed the N-terminal histidine tag by using metal-chelate chromatography on Ni^{2+}–NTA resin (Qiagen). Ion-exchange chromatography was then conducted using a Q column (GE Healthcare), followed by chromatography on a Superdex 200 26/60 (GE Healthcare) prep-grade column that had previously been equilibrated with buffer *A* (25 m*M* Tris–HCl pH 8.2, 200 m*M* NaCl, 2 m*M* DTT). Preparation of SeMet-labelled protein was carried out using B834 cells and using the same protocol with one exception: 2 m*M* DTT was added to all buffer solutions. The homogeneity of the purified protein was assessed by SDS–PAGE, after which the protein was concentrated to a final concentration of 10 mg ml^{-1} using a Centri-Prep centrifugal filter (Millipore) with buffer *A*.

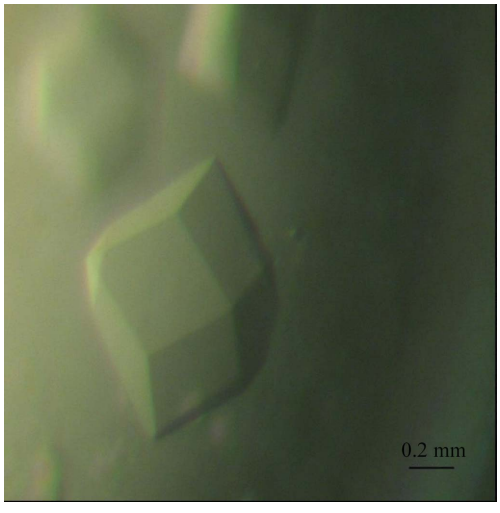


Figure 1
Crystals of *Sa*TrmE grown in 0.1 *M* MES pH 5.6, 1.4 *M* ammonium sulfate.

2.2. Crystallization and data collection

Initial crystallization trials were conducted at 295 K in 96-well sitting-drop Intelli-Plates (Hampton Research) using the Hydra II Plus One system (Matrix Technology) with approximately 1400 conditions and a ratio of 300 nl precipitant solution to 300 nl protein solution over 70 µl well solution. Crystallization trials were conducted using screening kits obtained from Hampton Research, Emerald Biostructures and Jena Biosciences. Initially, microcrystals were observed from Hampton Crystal Screen 2 condition No. 25 [0.1 *M* MES pH 6.5, 1.8 *M* ammonium sulfate, 0.01 *M* cobalt(II) chloride hexahydrate] and No. 32 (0.1 *M* HEPES pH 7.5, 1.6 *M* ammonium sulfate, 0.1 *M* sodium chloride). These crystallization conditions were further optimized by the hanging-drop vapour-diffusion method using 24-well VDX plates (Hampton Research). The drops used in the optimized crystallization conditions were prepared by mixing 1.0 µl protein solution with 1.0 µl reservoir solution (0.1 *M* MES pH 5.6, 1.4 *M* ammonium sulfate). Each hanging drop was positioned over 1 ml reservoir solution. Crystals of *Sa*TrmE were cryoprotected by soaking for 5 s in 20 µl cryoprotectant solution containing 0.1 *M* MES pH 5.6, 2.0 *M* ammonium sulfate and 20%(*v*/*v*) glycerol and were flash-frozen in liquid nitrogen. They were then mounted on the goniometer in a stream of cold nitrogen at 100 K. X-ray diffraction data were collected from the cooled crystals using an ADSC Quantum CCD 210 detector on beamline 4A MXW at Pohang Light Source (PLS), South Korea. The crystal was oscillated by 1.0° per frame over a total range of 110°; the wavelength was 1.0000 Å. X-ray diffraction data were collected to 2.9 Å resolution. Data were integrated and scaled using the *DENZO* and *SCALEPACK* crystallographic data-reduction routines (Otwinowski & Minor, 1997).

3. Results and discussion

3.1. Protein expression, purification and crystallization

*Sa*TrmE was overexpressed in *E. coli* in soluble form with a yield of ∼5 mg homogeneous protein per litre of culture. Based on the results of Superdex 200 26/60 gel filtration, the molecular weight of this protein was approximately 100 kDa, corresponding to a dimer. Initially, very irregular microcrystals were observed; however, after optimization cubic shaped crystals were observed over a period of 2–3 d when a reservoir solution containing 0.1 *M* MES pH 5.6 and 1.4 *M* ammonium sulfate was used (Fig. 1).

3.2. Data collection and preliminary X-ray diffraction analysis

Flash-cooled *Sa*TrmE crystals diffracted to 2.9 Å resolution using 20%(*v*/*v*) glycerol as a cryoprotectant. Although the diffraction pattern was initially poor, this problem was overcome by 30 s of annealing in cryosolution. Autoindexing was conducted using *DENZO* and the results indicated that the crystals belonged to the

Table 1
Data-collection statistics for *Sa*TrmE.

Values in parentheses are for the last resolution shell.

Space group	*I*23
Unit-cell parameters (Å)	$a = b = c = 229.47$
Resolution (Å)	50–2.90 (3.02–2.90)
No. of unique reflections	38523 (4192)
Redundancy	5.0 (3.2)
Completeness (%)	90 (86)
$R_{\rm merge}$† (%)	8.0 (33.5)
Average $I/\sigma(I)$	8.9 (2.0)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the observed intensity of an individual reflection and $\langle I(hkl)\rangle$ is the mean intensity of that reflection.

cubic space group *I*23 on the basis of systematic absences, with unit-cell parameters $a = b = c = 229.47$ Å, $\alpha = \beta = \gamma = 90^\circ$. These results indicate that four monomers are likely to be present in the asymmetric unit, which has a corresponding calculated Matthews coefficient ($V_{\rm M}$) of 2.4 Å^3 Da^{-1} and a solvent content of 50% (Matthews, 1968). Data-collection statistics are provided in Table 1. The molecular-replacement method was first used in an attempt to solve the crystal structure of *Sa*TrmE using the crystal structure of TrmE from *T. maritima*, which was solved at a resolution of 2.3 Å (PDB code 1xzp; Scrima *et al.*, 2005), as a model. However, none of our attempts provided a clear solution. Therefore, a crystal of SeMet-labelled *Sa*TrmE protein was obtained under the same crystallization conditions as used for the native protein and will be used to solve the phase problem. As a result, the structure will be determined by the MAD method (Hendrickson *et al.*, 1990) using selenium as an anomalous scatterer; the structural details will be described in a separate paper.

We thank Dr H. S. Lee and Dr K. H. Kim at beamline 4A, Pohang Light Source (PLS), South Korea for their assistance. AP and EEK were supported by the 21C Functional Proteomics Frontier R&D Program of the Korea Ministry of Science and Technology and KIST grants. This work was supported by a Korea Research Foundation Grant funded by the Korean Government (MOEHRD, Basic Research Promotion Fund; KRF-2007-C00445).

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