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Crystallization and preliminary crystallographic analysis of tRNA (m^7G46) methyltransferase from *Escherichia coli*

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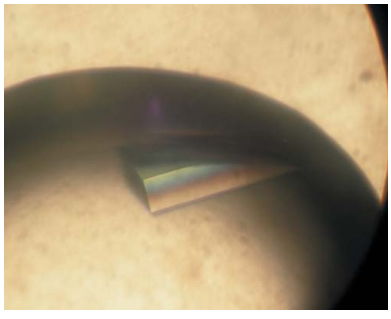

Transfer RNA (tRNA) (m^7G46) methyltransferase (TrmB) belongs to the Rossmann-fold methyltransferase (RFM) family and uses *S*-adenosyl-L-methionine (SAM) as the methyl-group donor to catalyze the formation of N^7-methylguanosine (m^7G) at position 46 in the variable loop of tRNAs. After attempts to crystallize full-length *Escherichia coli* TrmB (EcTrmB) failed, a truncated protein lacking the first 32 residues of the N-terminus but with an additional His_6 tag at the C-terminus was crystallized by the hanging-drop vapour-diffusion method using polyethylene glycol 3350 (PEG 3350) as precipitant at 283 K. An X-ray diffraction data set was collected using a single flash-cooled crystal that belonged to space group $P2_1$.

1. Introduction

Initial tRNA transcripts are processed to a standard length from tRNA precursors and then subsequently modified at various positions, such as the D-loop, anticodon loop and variable loop, by a series of modification enzymes. More than 80 different modifications have been identified for tRNAs (http://library.med.utah.edu/RNAmods/; Rozenski *et al.*, 1999; McCloskey & Crain, 1998); they are important for the correct function of tRNAs and result in the production of mature tRNA (Bjork *et al.*, 1999). Of these modifications, SAM-dependent methylation is believed to be the most prevalent and fundamental. tRNA (m^7G46) methyltransferase (TrmB) belongs to the Rossmann-fold methyltransferase (RFM) family and catalyzes methyl-group transfer from SAM to the N^7 atom of guanosine at position 46 in the variable loop of tRNA.

For a long time, studies of m^7G methylation enzymes were restricted to preliminary analyses of enzyme activities of proteins purified from natural organisms and knowledge of the genes and protein sequences of these enzymes was limited. Recently, the genes for m^7G methylation enzymes from several organisms have been identified. In 2002, the m^7G methylation machine of *Saccharomyces cerevisiae* was characterized as being a complex composed of two proteins encoded by *trm8* and *trm82* genes (Alexandrov *et al.*, 2002). Later, the *yggH* gene of *Escherichia coli* was found to encode a 27 kDa m^7G methylation monomeric enzyme which was named TrmB (De Bie *et al.*, 2003). Sequence analysis suggests that *trm8*, which encodes the 33 kDa Trm8p small subunit of the yeast m^7G methylation machine, is the orthologue of *yggH* in eukaryotes, while *trm82*, which encodes the 50 kDa Trm82p large subunit, does not share any similarity with *trm8* or *yggH*. *S. cerevisiae* Trm8p (ScTrm8p) was identified to be the catalytic subunit and, although the details were still unclear, Trm82p was suggested to maintain the level of active Trm8p and participate in tRNA recognition (Muneyoshi *et al.*, 2007; Alexandrov *et al.*, 2005). More recently, the gene encoding the 25 kDa *Bacillus subtilis* TrmB (BsTrmB) was cloned and BsTrmB was found to form a homodimer both in solution and in crystals (Zegers *et al.*, 2006). The homodimer was thought to be the active form when BsTrmB catalyzes the methylation of tRNA, although the docking model of homodimeric BsTrmB in complex with substrate tRNA did not suggest that the two BsTrmB molecules in a dimer cooperate to capture one tRNA (Zegers *et al.*, 2006).

Table 1
X-ray data-collection and processing statistics.

Values in parentheses are for the last shell.

Space group	$P2_1$
Unit-cell parameters (Å, °)	a = 46.42, b = 47.01, c = 58.64, β = 90.01
Resolution (Å)	46.42–2.04 (2.15–2.04)
Unique reflections	15183 (1925)
Redundancy	7.6 (7.6)
Completeness (%)	92.7 (82.0)
Average $I/\sigma(I)$	15.1 (4.5)
$R_{\rm merge}$† (%)	4.4 (17.1)
No. of molecules in unit cell (Z)	2
$V_{\rm M}$ (Å^3 Da^{-1})	2.6
Solvent content (%)	52.6

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the ith observation of reflection hkl and $\langle I(hkl)\rangle$ is the weighted average intensity for all observations i of reflection hkl.

Two crystal structures of bacterial TrmB proteins have been reported: those from *B. subtilis* (Zegers *et al.*, 2006) and *Streptococcus pneumonia* (PDB code 1yzh; Y. Kim, H. Li, F. Collart & A. Joachimiak, unpublished work). Recently, the crystal structures of the yeast m^{7}G46 methylation complex and of its catalytic subunit Trm8p in complex with SAM have also been published (Leulliot *et al.*, 2008). These three enzymes/subunits are the most similar structurally characterized proteins to EcTrmB. EcTrmB, BsTrmB, SpTrmB and ScTrm8p contain 239, 213, 211 and 286 amino-acid residues, respectively. The sequence identity/homology between EcTrmB and BsTrmB, SpTrmB and ScTrm8p are 26%/48% for 176 comparable residues, 32%/50% for 131 comparable residues and 26%/43% for 149 comparable residues, respectively. As the representative of monomeric TrmBs, the structure of EcTrmB is still unknown. In the present study, we report the crystallization and preliminary crystallographic analysis of EcTrmB in order to understand the function and properties of TrmB proteins more clearly.

2. Materials and methods

2.1. Protein expression and purification

The gene (gene ID 947448) encoding the 27 kDa full-length EcTrmB protein (residues 1–239) was extracted from the *Escherichia coli* K12 genome and amplified using the polymerase chain reaction (PCR) method with primer *A* (5′-CAG**CCATGG**GATCCAAAAA-CGACGTCATTTC-3′; forward) and primer *B* (5′-AGT**CTCGAG**-TTTCACCCTCTCGAAC-3′; reverse). The PCR product was then digested and inserted into the p28 vector [derived from pET28a (Novagen) and described previously; Lv *et al.*, 2006] with *Nco*I and *Xho*I sites. Using the plasmid containing the full-length *trmb* gene as the template, the gene fragment encoding residues 33–239 of EcTrmB was amplified with primer *C* (5′-ATT**CCATGG**GATCCGGCCAG-GAACATG-3′; forward) and primer *B* (reverse) and then inserted into the p28 vector using the same two restriction sites. The p28 vector adds an –LEHHHHHH tag at the C-terminus of the target protein for purification. Successful cloning was confirmed by DNA sequencing. The plasmid containing the correct gene sequence encoding residues 33–239 of EcTrmB was used to transform *E. coli* BL21 (DE3) cells (Stratagene). The cells containing the target plasmid were grown in Luria–Bertani medium supplemented with 50 µg ml^{-1} kanamycin to an OD$_{600}$ of 0.6 and then induced with 1 m*M* isopropyl β-D-1-thiogalactopyranoside (IPTG) for a further 4 h at 310 K. The cells were harvested by centrifugation at 8000*g* for 10 min. The cells were then resuspended in buffer *A* (20 m*M* Tris–HCl pH 8.0, 500 m*M* NaCl) and lysed by sonication. The cell lysates were centrifuged at 20 000*g* for 30 min and the supernatant was loaded onto a nickel-chelating column (GE Healthcare) pre-equilibrated with buffer *A*. The column was first washed with five column volumes of buffer *A* and then with more than 20 column volumes of buffer *A* supplemented with 25 m*M* imidazole to remove remaining impurities. The target protein was then eluted with buffer *B* (20 m*M* Tris–HCl pH 8.0, 500 m*M* NaCl and 200 m*M* imidazole). The fraction containing the target protein was desalted and concentrated by ultrafiltration (Millipore, 10 kDa cutoff). The final concentration of the target protein as estimated using the BCA Protein Assay Kit (Pierce) was 22 mg ml^{-1}. According to SDS–PAGE, the purity of the protein was about 95%. Finally, the purified protein was stored at 193 K in buffer *C* (5 m*M* Tris–HCl pH 8.0, 50 m*M* NaCl).

2.2. Crystallization

Initial crystallization trials were performed using the hanging-drop vapour-diffusion method with Crystal Screens I and II and PEG/Ion Screen (Hampton Research) at 277 K. Hanging drops, each consisting of 1 µl reservoir solution and 1 µl protein solution, were equilibrated against 200 µl reservoir solution. Small crystals were observed in condition No. 32 of PEG/Ion Screen after 2 d. After several rounds of optimization of protein concentration, precipitant concentration, temperature, buffer pH, ionic strength, additives and cationic compounds, well diffracting crystals was grown at 283 K with an optimized protein concentration of 6 mg ml^{-1} (stored and diluted in buffer *C*) using a reservoir solution comprising 15%(*w*/*v*) PEG 3350 and 150 m*M* $MgSO_4$. After 3 d, the well diffracting crystals had grown to typical dimensions of 0.4 × 0.2 × 0.1 mm (Fig. 1).

2.3. Data collection

Initial X-ray diffraction experiments were performed using in-house Cu $K\alpha$ X-rays generated by a RA-Micro007 rotating-anode X-ray source (Rigaku) and the diffraction images were collected using a MAR 345dtb imaging-plate detector (MAR Research). Crystals were quickly passed through a cryoprotectant buffer comprising 15%(*w*/*v*) PEG 3350, 150 m*M* $MgCl_2$ and 15%(*v*/*v*) glycerol and flash-cooled to 100 K in a stream of cold nitrogen gas produced by an Oxford Cryosystems cooling device (Oxford Cryosystems Ltd). A complete diffraction data set consisting of 358 images was collected using a single EcTrmB crystal and the above in-house

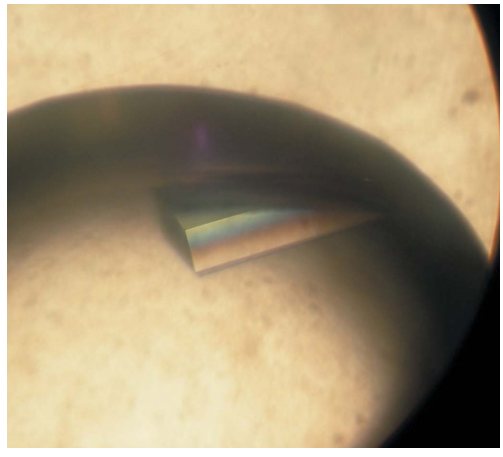

Figure 1
Photomicrograph of an EcTrmB crystal. The dimensions of this single crystal are about 0.4 × 0.2 × 0.1 mm.

equipment with an oscillation angle of 1° per image at 100 K. Diffraction data were processed and scaled using *iMosflm* (Leslie, 1994) and programs from *CCP*4 (Collaborative Computational Project, Number 4, 1994). Data-collection and processing statistics are listed in Table 1.

3. Result and discussion

As all crystallization trials using wild-type EcTrmB protein failed, the protein sequence of EcTrmB was modified in order to improve its crystallizability. The program *RONN* (Yang *et al.*, 2005) predicted that about 30 residues at the N-terminus of EcTrmB were probably disordered and structureless. In addition, the fact that the N-terminus of EcTrmB possesses about 20 additional amino acids compared with most prokaryotic TrmBs was revealed by multiple sequence alignment of the TrmB family and suggested that these N-terminal residues were not essential to the function of TrmB. Therefore, three truncated forms of EcTrmB were designed for crystallization experiments that lacked 18, 25 and 32 residues from the N-terminus. The form which lacked 32 N-terminal residues was crystallized successfully and diffracted to high resolution. The strategy of truncating N-terminal residues has also been reported recently in the crystallization of yeast Trm8p, a eukaryotic orthologue of EcTrmB, and its complex with the noncatalytic subunit Trm82p (Leulliot *et al.*, 2008). In this study, the first 46 N-terminal residues of Trm8p were removed at the cDNA level to help crystallization.

The crystal of EcTrmB belongs to space group $P2_1$ and both Matthews coefficient analysis and the self-rotation function suggested the presence of one molecule per asymmetric unit in the EcTrmB crystals. De Bie *et al.* (2003) had characterized that EcTrmB is a monomer in solution and our gel-filtration data supported their observations (data not shown).

Furthermore, because the β angle is close to 90° and the *a* and *b* unit-cell parameters have nearly the same length, this indicates that EcTrmB crystals may have higher symmetry. Therefore, the X-ray data were indexed and processed in different Laue groups and then scaled in an appropriate higher symmetry space group. However, the $R_{\rm merge}$ values for these space groups were always very high in all resolution shells. For example, the overall $R_{\rm merge}$ was 48% for space group *P*222 and 62% for *P*4. In addition, SDS–PAGE of the dissolved crystals revealed that the protein molecules in the crystals had a similar size to intact EcTrmB and no obvious degradation was observed. Therefore, this additionally confirmed the exclusion of the higher symmetry space groups as their asymmetric units are too small to accommodate even one EcTrmB molecule. Ultimately, the recent solution of the EcTrmB crystal structure and progress in refinement confirmed the $P2_1$ space group in this case.

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References

Alexandrov, A., Grayhack, E. J. & Phizicky, E. M. (2005). *RNA*, **11**, 821–830.
Alexandrov, A., Martzen, M. R. & Phizicky, E. M. (2002). *RNA*, **8**, 1253–1266.
Bjork, G. R., Durand, J. M., Hagervall, T. G., Leipuviene, R., Lundgren, H. K., Nilsson, K., Chen, P., Qian, Q. & Urbonavicius, J. (1999). *FEBS Lett.* **452**, 47–51.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
De Bie, L. G., Roovers, M., Oudjama, Y., Wattiez, R., Tricot, C., Stalon, V., Droogmans, L. & Bujnicki, J. M. (2003). *J. Bacteriol.* **185**, 3238–3243.
Leslie, A. G. W. (1994). *MOSFLM User Guide.* MRC–LMB, Cambridge, England.
Leulliot, N., Chaillet, M., Durand, D., Ulryck, N., Blondeau, K. & van Tilbeurgh, H. (2008). *Structure*, **16**, 52–61.
Lv, X., Fan, J., Ge, H., Gao, Y., Zhang, X., Teng, M. & Niu, L. (2006). *Acta Cryst.* F**62**, 483–485.
McCloskey, J. A. & Crain, P. F. (1998). *Nucleic Acids Res.* **26**, 196–197.
Muneyoshi, Y., Matsumoto, K., Tomikawa, C., Toyooka, T., Ochi, A., Masaoka, T., Endo, Y. & Hori, H. (2007). *Nucleic Acids Symp. Ser. (Oxf.)*, **51**, 359–360.
Rozenski, J., Crain, P. F. & McCloskey, J. A. (1999). *Nucleic Acids Res.* **27**, 196–197.
Yang, Z. R., Thomson, R., McNeil, P. & Esnouf, R. M. (2005). *Bioinformatics*, **21**, 3369–3376.
Zegers, I., Gigot, D., van Vliet, F., Tricot, C., Aymerich, S., Bujnicki, J. M., Kosinski, J. & Droogmans, L. (2006). *Nucleic Acids Res.* **34**, 1925–1934.