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Crystallization and preliminary X-ray diffraction crystallographic study of tRNA m^{1}A58 methyltransferase from *Saccharomyces cerevisiae*

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In *Saccharomyces cerevisiae*, TRM6 and TRM61 compose a tRNA methyltransferase which catalyzes the methylation of the N1 of adenine at position 58 in tRNAs, especially initiator methionine tRNA. TRM61 is the subunit that binds *S*-adenosyl-L-methionine and both subunits contribute to target tRNA binding. In order to elucidate the catalytic mechanism of TRM6–TRM61 and the mode of interaction between the two subunits, expression, purification, crystallization and X-ray diffraction analysis of the TRM6–TRM61 complex were performed in this study. The crystals diffracted to 2.80 Å resolution and belonged to the trigonal space group $P3_121$ or $P3_221$, with unit-cell parameters $a = b = 139.14$, $c = 101.62$ Å.

1. Introduction

Post-transcriptional modifications of tRNAs are essential for stabilization of their secondary structure (Anderson *et al.*, 1998; Helm *et al.*, 1998). These modifications also influence translation accuracy, reading-frame maintenance and recognition by aminoacyl-tRNA synthetases (Madore *et al.*, 1999; Urbonavičius *et al.*, 2001; Yarian *et al.*, 2002). Methylation is common amongst these modifications. Methyltransferases possess various fold patterns and catalyze the methylation of different positions of tRNAs using *S*-adenosyl-L-methionine (SAM) as a methyl donor.

Adenine at position 58 (A58) is the most conserved nucleoside in tRNA and is located in the TψC loop. The occurrence of methylation of the N1 position of A58 (m^{1}A58) in tRNA is widespread, being found in most eukaryotic and archaeal tRNAs (Ozanick *et al.*, 2007), but is not universal, occurring less frequently in eubacteria (Bujnicki, 2001), and is not found in all sequenced tRNAs in *Saccharomyces cerevisiae* (Sprinzl *et al.*, 1998). This methylation of initiator methionine tRNA (tRNA$_i^{Met}$) is essential for cell growth and stability of the initiator in *S. cerevisiae* (Anderson *et al.*, 1998). Bacterial and archaeal tRNA m^{1}A58 methyltransferases are composed of one subunit that forms a methyltransferase domain and function as homotetramers, while the known eukaryotic tRNA m^{1}A58 methyltransferases consist of two subunits and are believed to function as heterotetramers (Ozanick *et al.*, 2007).

In *S. cerevisiae*, the genes GCD10 and GCD14 encode TRM6 and TRM61, respectively, which compose a tRNA m^{1}A58 methyltransferase that methylates m^{1}A58 in tRNAs, especially tRNA$_i^{Met}$ (Anderson *et al.*, 2000). TRM61 is the subunit that binds SAM and both subunits contribute to target tRNA binding *via* their N-terminal domains in addition to the methyltransferase domain (Ozanick *et al.*, 2007). TRM61 shows obvious and extensive sequence similarity to bacterial and archaeal tRNA m^{1}A58 methyltransferases. TRM6 only has close homologues in eukaryotic organisms and does not share evident sequence similarity with any proteins other than its orthologues (Bujnicki, 2001), and no structures of its homologues have been reported. The TRM6–TRM61 complex localizes in the nucleus and is required specifically in the initiation step of translation because of a strong requirement for m^{1}A58 in the processing and accumulation of tRNA$_i^{Met}$ (Anderson *et al.*, 1998; Calvo *et al.*, 1999). It is also required for repression of translation of GCN4 (a gene encoding a transcriptional activator of amino-acid biosynthetic enzymes) mRNA (Cuesta *et al.*, 1998).

The structures of several tRNA m^{1}A58 methyltransferases from bacteria and archaea have been determined in complex with SAM and the product *S*-adenosyl-L-homocysteine (Gupta *et al.*, 2001; Barraud *et al.*, 2008; Guelorget *et al.*, 2010). These studies revealed the catalytic mechanism of tRNA m^{1}A58 methyltransferases composed of one subunit. However, no structural information on eukaryotic tRNA m^{1}A58 methyltransferases has been reported and the requirement for two subunits in the mechanism is poorly understood. In order to elucidate the catalytic mechanism of yeast tRNA m^{1}A58 methyltransferase and the mode of interaction of its two subunits, the TRM6–TRM61 complex was expressed, purified and crystallized and X-ray diffraction data were collected.

2. Materials and methods

2.1. Cloning, expression and purification

The cDNAs of both full-length TRM6 and TRM61 were obtained *via* PCR from the *S. cerevisiae* genome. The cDNA of TRM6 was cloned downstream of the first T7 promoter of pETDuet-1 (Novagen, USA) using the *Bam*HI and *Not*I sites to create recombinant TRM6 with an N-hexahistidine tag (MGSSHHHHHHSNDP). The cDNA of TRM61 was cloned downstream of the second T7 promoter of pETDuet-1 cleaved with *Nde*I and *Xho*I to create recombinant TRM61 without any additional residues. The sequences of the two cDNAs were confirmed by DNA sequencing. A single colony of *Escherichia coli* Rosetta (DE3) (Novagen, USA) bacteria harbouring the expression vector was cultured in 8 ml Luria–Bertani broth overnight and was then used to inoculate 0.8 l medium containing 50 µg ml^{-1} ampicillin. Cells were grown at 310 K for 2.5 h until the $OD_{600\,nm}$ reached 0.5–0.8 and protein expression was then induced for 20 h with 0.25 m*M* isopropyl β-D-1-thiogalactopyranoside (IPTG) at 289 K. The bacteria were collected and resuspended in 50 ml binding buffer (20 m*M* Tris–HCl pH 8.0, 500 m*M* NaCl). The cells were disrupted by sonication and then centrifuged at 15 200*g* for 0.5 h. The recovered clean supernatant lysate was loaded onto Ni–NTA agarose (GE Healthcare, USA) resin pre-equilibrated with binding buffer. Most of the TRM61 associated with tagged TRM6 was eluted with 30 ml binding buffer including 100 m*M* imidazole. The target protein complex was then concentrated to 1 ml for further purification using Superdex 200 (GE Healthcare, USA) gel-filtration chromatography eluted with binding buffer containing 5 m*M* dithiothreitol. The retention volume corresponding to the target protein complex indicates that it is a heterodimer in solution. The fractions corresponding to the peak were pooled and concentrated to 34 mg ml^{-1} using a 10 kDa cutoff Amicon centrifugal ultrafilter concentrator (Millipore, USA). All purification steps were performed at 287 K. Examination of the purified protein complex by SDS–PAGE revealed two bands that corresponded to the expected molecular weight (Fig. 1). The protein concentration was measured with the BCA Protein Assay Kit (Pierce, USA) using BSA as a standard.

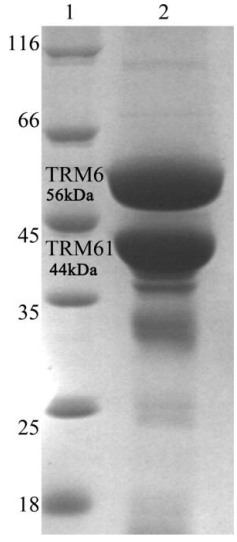

Figure 1
SDS–PAGE of yeast tRNA m^{1}A58 methyltransferase. The protein was analyzed on 12% SDS–PAGE and stained with Coomassie Blue. Lane 1, molecular-weight markers (kDa); lane 2, yeast tRNA m^{1}A58 methyltransferase after two purification steps; the two subunits are indicated. The molecular weights of recombinant TRM6 and TRM61 are 56 and 44 kDa, respectively.

2.2. Crystallization

Preliminary screening for initial crystallization conditions was performed by the hanging-drop vapour-diffusion method using ProPlex and ProPlex 2 (Molecular Dimensions, UK) at 287 K by mixing 1 µl of 34 or 17 mg ml^{-1} protein solution with an equal volume of reservoir solution and equilibrating against 100 µl reservoir solution in 24-well plates. Small block crystals were obtained from the condition 0.1 *M* KCl, 0.1 *M* Tris–HCl pH 8.5, 15%(*w*/*v*) PEG 2000 MME. The condition was further optimized using various concentrations of PEG 2000 MME *versus* a pH range of 7.5–8.5 in the presence or absence of 0.1 *M* KCl. Crystals of good diffraction quality (Fig. 2) appeared in about 2 d from 0.1 *M* KCl, 0.1 *M* Tris–HCl pH 8.0, 25%(*w*/*v*) PEG 2000 MME.

2.3. X-ray diffraction data collection and processing

Before data collection, the crystals were quick-soaked in a cryoprotectant solution consisting of 25%(*v*/*v*) glycerol, 0.1 *M* KCl, 0.1 *M* Tris–HCl pH 8.0, 25%(*w*/*v*) PEG 2000 MME and flash-cooled in a nitrogen stream at 100 K. X-ray diffraction data were collected on beamline 17U1 of Shanghai Synchrotron Radiation Facility (SSRF) using an MX-225 CCD detector (MAR Research). The crystal-to-detector distance was maintained at 250 mm and the crystal was rotated through a total of 180° with 1° rotation per frame over 1.2 s.

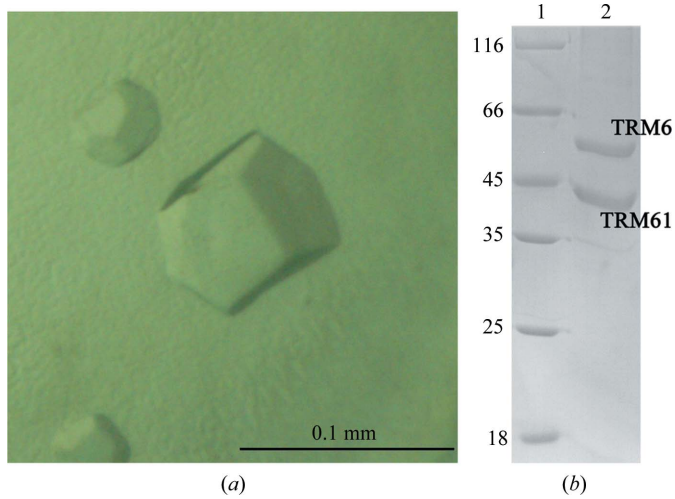

Figure 2
(*a*) Crystals of yeast tRNA m^{1}A58 methyltransferase grown in 0.1 *M* KCl, 0.1 *M* Tris–HCl pH 8.0, 25%(*w*/*v*) PEG 2000 MME. (*b*) 12% SDS–PAGE of the contents of a crystal of yeast tRNA m^{1}A58 methyltransferase stained with Coomassie Blue. Lane 1, molecular-weight markers (labelled in kDa); lane 2, the contents of a crystal of yeast tRNA m^{1}A58 methyltransferase.

Table 1
Data-collection statistics for yeast tRNA m^{1}A58 methyltransferase.

Values in parentheses are for the highest resolution shell.

Space group	$P3_121$ or $P3_221$
Wavelength (Å)	0.9999
Unit-cell parameters (Å)	$a = b = 139.14$, $c = 101.62$
Resolution (Å)	45.00–2.80 (2.95–2.80)
Total No. of observations	306651 (44539)
No. of unique reflections	28353 (4092)
Completeness (%)	100.0 (100.0)
Average $I/\sigma(I)$	13.1 (3.3)
$R_{\rm merge}$†	0.163 (0.756)

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

The crystal diffracted to a maximum resolution of 2.80 Å. All data were integrated with *iMOSFLM* (Battye *et al.*, 2011) and scaled using *SCALA* (Evans, 2006) from the *CCP*4 suite (Winn *et al.*, 2011). Analysis of the systematic absences in the diffraction data narrowed the possible space-group choice to either $P3_121$ or $P3_221$. The final statistics of data collection and processing are tabulated in Table 1.

3. Results and discussion

TRM6 and TRM61, the two subunits of tRNA m^{1}A58 methyltransferase from *S. cerevisiae*, were successfully cloned in *E. coli* and the complex was purified to homogeneity. The complex was crystallized from 0.1 *M* KCl, 0.1 *M* Tris–HCl pH 8.0, 25%(*w*/*v*) PEG 2000 MME. The crystals of the TRM6–TRM61 complex diffracted to a maximum resolution of 2.80 Å and belonged to the trigonal space group $P3_121$ or $P3_221$, with unit-cell parameters $a = b = 139.14$, $c = 101.62$ Å. The resulting $R_{\rm merge}$ was 16.3% overall and the average mosaicity was 1.00°. A Matthews coefficient (Matthews, 1968) of 2.85 Å^3 Da^{-1} and a solvent content of 56.81% were calculated assuming one complex per asymmetric unit.

Because only TRM61 has a homologue for which the structure is known (PDB entry 1o54; Joint Center for Structural Genomics, unpublished work), we attempted structure determination *via* molecular replacement using 1o54 as a search model. This effort failed, perhaps owing in part to the rather low sequence identity (26% for 265 aligned residues) between TRM61 (383 residues) and 1o54 (277 residues) and the fact that the content of recombinant TRM6 (with 492 residues) in the complex is greater than that of TRM61. SeMet-derivative TRM6–TRM61 crystals have already been obtained and are undergoing optimization for anomalous dispersion data collection.

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