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Crystallization and preliminary X-ray crystallographic analysis of tRNA(m^1G37)methyltransferase from *Haemophilus influenzae*

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The enzyme tRNA(m^1G37)methyltransferase (TrmD) catalyzes the transfer of a methyl group from *S*-adenosyl-L-methionine (AdoMet) specifically to guanosine at position 37 within a subset of tRNA species in bacteria. The modified guanosine is next to the anticodon and is important for the maintenance of the correct reading frame during translation. TrmD from *Haemophilus influenzae* with both N- and C-terminal tags was overexpressed in *Escherichia coli* and crystallized at 297 K using sodium acetate as a precipitant. Native X-ray diffraction data were collected to 1.85 Å resolution. The crystals are rhombohedral, belonging to the space group *R*32, with unit-cell parameters $a = b = 98.05$, $c = 176.79$ Å, $\alpha = \beta = 90$, $\gamma = 120°$. The presence of one monomer of recombinant TrmD in the crystallographic asymmetric unit gives a V_M of 3.07 $Å^3\,Da^{-1}$ and a solvent content of 59.9%.

1. Introduction

Modified nucleosides are present in different positions of the tRNA molecules in *Escherichia coli* and *Salmonella typhimurium* and there are a variety of modified nucleosides, especially in the anticodon region (Björk, 1996). Considerable cellular resources are devoted to these post-transcriptional modifications. In the case of *E. coli*, about 1.0% of the genome is devoted to encoding over 40 modification enzymes (Holmes *et al.*, 1995). One such enzyme is *E. coli* tRNA(m^1G37)methyltransferase (TrmD), which catalyzes the transfer of a methyl group from *S*-adenosyl-L-methionine (AdoMet) specifically to guanosine at position 37, which is next to and 3′ to the anticodon, in seven of the 46 tRNA species in *E. coli*. One specific function of m^1G37 is to prevent frameshifting and thereby to maintain the correct reading frame during translation (Hagervall *et al.*, 1993). It was shown that deficiency of 1-methylguanosine at position 37 induced strong pleiotropic effects such as reduction in growth rate and polypeptide-chain elongation rate *in vivo* (Li & Björk, 1995; Persson *et al.*, 1995). Genetic studies have indicated that the TrmD protein might have a second function in the cell (Persson *et al.*, 1995).

The G residue of the anticodon at position 36 is crucial for the optimal activity of TrmD; the dinucleotide GpG and the polynucleotide polyG were found to be both potent and specific inhibitors of the enzyme (Holmes *et al.*, 1995). However, it appears that TrmD recognizes and binds the general tRNA structure even in the absence of the GpG structural motif (Qian & Björk, 1997). The GpG sequence may be involved in stabilizing the enzyme–tRNA complex by binding in the active site of the enzyme. It was demonstrated that AdoMet is not required for tRNA binding (Holmes *et al.*, 1995). A molecular understanding of how TrmD recognizes the substrate tRNA and its anticodon-loop architecture requires the three-dimensional structure of TrmD. However, no structural information is presently available for TrmD from any source. Therefore, we have initiated the structure determination of TrmD from *Haemophilus influenzae*, a 246-residue protein (M_r = 27 542) which shows a high level of amino-acid sequence identity (83%) to *E. coli* TrmD. In this study, we overexpressed *H. influenzae* TrmD with both N- and C-terminal tags in *E. coli* and crystallized it. We report its crystallization conditions and preliminary X-ray crystallographic data.

2. Experimental

2.1. Protein expression and purification

The *trmD* gene encoding tRNA(m^1G37)-methyltransferase was amplified by the polymerase chain reaction using the *H. influenzae* genomic DNA as template. The forward and reverse oligonucleotide primers designed using the published genome sequence (Fleischmann *et al.*, 1995) were 5′-GGA ATT CCA TAT GTG GAT CGG GGT AAT TTC ATT ATT-3′ and 5′-CCG CCG CTC GAG ACT GTT ATG CTC GGC TTG CGC-3′, respectively. The amplified DNA was inserted into the *Nde*I/*Xho*I-digested expression vector pET-28b(+) (Novagen). This vector construction adds an eight-residue tag (LEHHHHHH) to the C-terminus and a 20-residue tag

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(MGSSHHHHHHSSGLVPRGSH) to the N-terminus of the *trmD* gene product to facilitate protein purification. The protein was overexpressed in *E. coli* B834(DE3) cells. Cells were grown at 310 K to an OD_{600} of 0.5 in Luria–Bertani medium containing 50 μg ml^{-1} ampicillin and protein expression was induced by 1.0 m*M* isopropyl-β-D-thiogalactopyranoside (IPTG). Cell growth continued at 303 K for 8 h after IPTG induction and cells were harvested by centrifugation at 4200*g* (6000 rev min^{-1}; Sorvall GSA rotor) for 10 min at 277 K. The cell pellet was resuspended in ice-cold lysis buffer (20 m*M* Tris–HCl pH 7.9, 500 m*M* NaCl, 5 m*M* imidazole and 1 m*M* phenylmethylsulfonyl fluoride) and was then homogenized with an ultrasonic processor. The crude cell extract was centrifuged at 36 000*g* (18 000 rev min^{-1}; Hanil Supra 21K rotor) for 30 min at 277 K and the recombinant protein in the supernatant fraction was purified by two chromatographic steps. The first step utilized the hexahistidine tags by metal-chelate chromatography on Ni–NTA resin (Qiagen). Next, gel filtration was performed on a HiLoad XK 16 Superdex 200 prep-grade column (Amersham-Pharmacia), which was previously equilibrated with buffer *A* (50 m*M* Tris–HCl pH 8.0) containing 200 m*M* sodium chloride. Homogeneity of the purified protein was assessed by SDS–PAGE (Laemmli, 1970). The protein solution was concentrated using a YM10 ultrafiltration membrane (Amicon). The protein concentration was estimated by measuring the absorbance at 280 nm, employing the calculated extinction coefficient of 34 850 M^{-1} cm^{-1} (SWISS-PROT; http://www.expasy.ch/).

2.2. Crystallization

Crystallization was performed at 297 K by the hanging-drop vapour-diffusion method using 24-well tissue-culture plates. Each hanging drop was prepared on a siliconized cover slip by mixing equal volumes (2 μl each) of the protein solution (at 15 mg ml^{-1} protein concentration) and the reservoir solution. Each hanging drop was placed over a 1.0 ml reservoir solution. Initial crystallization conditions were established using screening kits from Hampton Research (Crystal Screens I, II and MembFac) and from Emerald BioStructures Inc. (Wizard I and II).

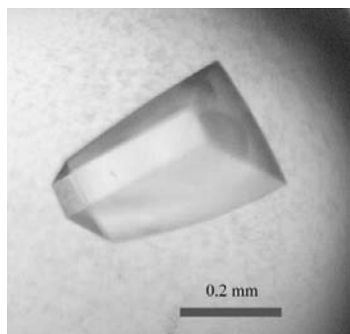

Figure 1
A crystal of tRNA(m[1]G37)methyltransferase from *H. influenzae*. Its approximate dimensions are 0.4 × 0.25 × 0.25 mm.

Table 1
Data-collection statistics.

Values in parentheses refer to the highest resolution shell (1.95–1.85 Å).

X-ray wavelength (Å)	0.9500
Temperature (K)	100
Space group	*R*32
Unit-cell parameters (Å)	*a* = 98.05, *b* = 98.05, *c* = 176.79
Resolution range (Å)	30.0–1.85
Total/unique reflections	415570/28096
R_{merge}† (%)	6.0 (35.6)
Data completeness (%)	99.7 (99.7)
Average $I/\sigma(I)$	9.3 (2.2)

† $R_{merge} = \sum_h \sum_i |I(h)_i - \langle I(h)\rangle| / \sum_h \sum_i I(h)_i$, where $I(h)$ is the intensity of reflection h, $\sum_h$ is the sum over all reflections and $\sum_i$ is the sum over i measurements of reflection h.

2.3. X-ray diffraction experiment

The crystal was flash-frozen using the cryoprotectant solution, which consisted of 100 m*M* sodium cacodylate pH 6.5, 1.4 *M* sodium acetate and 30%(*v*/*v*) glycerol. Crystals were soaked in 5 μl of the cryoprotectant solution for 10 s before being flash-frozen in liquid nitrogen. X-ray diffraction data were collected at 100 K with an ADSC Quantum 4R CCD detector at the BL-18B experimental station of the Photon Factory, Tsukuba, Japan (Watanabe *et al.*, 1995), using 0.9500 Å X-rays. The crystal was rotated for a total of 128°, with 1.0° oscillation range per frame. The data set was processed and scaled using the programs *MOSFLM* (Leslie, 1994) and *SCALA* (Collaborative Computational Project, Number 4, 1994).

3. Results

When the recombinant TrmD from *H. influenzae* was expressed as a fusion with the C-terminal eight-residue tag (LEHHHHHH), the expression level was very low. However, when the 20-residue tag (MGSSHHHHHHSSGLVPRGSH) was also fused at the N-terminus of the protein, it was overexpressed in a soluble form with a much higher yield of ~11 mg of the purified enzyme per litre of culture. Despite the presence of both the N-terminal eight-residue tag and the C-terminal 20-residue tag, the recombinant enzyme readily formed well diffracting crystals. The best crystals were obtained with a reservoir solution comprising 100 m*M* sodium cacodylate pH 6.5 and 1.4 *M* sodium acetate. Rectangular crystals grew to dimensions of 0.4 × 0.25 × 0.25 mm within a week (Fig. 1). The flash-frozen crystal diffracted to ~1.8 Å. A total of 28 096 unique reflections were measured with a redundancy of 14.8, with a completeness of 99.7% to 1.85 Å and an R_{merge} of 6.0%. The space group was determined to be *R*32 on the basis of systematic absences and the unit-cell parameters were $a = b = 98.05$ (24), $c = 176.79$ (87) Å, $\alpha = \beta = 90$, $\gamma = 120°$, where the estimated standard deviations are given in parentheses. Table 1 summarizes the data-collection statistics. The presence of one monomer of recombinant TrmD in the crystallographic asymmetric unit gives a crystal volume per protein mass (V_M) of 3.07 $Å^3$ Da^{-1} and a solvent content of 59.9% (Matthews, 1968). Since structural models for homologous proteins are not available for molecular-replacement trials, the structure will be solved by the multiwavelength anomalous diffraction method.

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