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Purification, crystallization and preliminary X-ray crystallographic study of the tRNA pseudouridine synthase TruB from *Streptococcus pneumoniae*

The RNA pseudouridine synthase TruB catalyses the isomerization of uridine to pseudouridine (Ψ) at residue 55 of elongator tRNAs. In order to better elucidate the functions of TruB in the formation of pseudouridine, the three-dimensional structure of full-length TruB was determined by X-ray crystallography. Here, the expression, purification, crystallization and preliminary crystallographic analysis of TruB from *Streptococcus pneumoniae* are reported. The crystal belonged to space group *P*2₁, with unit-cell parameters $a = 37.65$, $b = 78.09$, $c = 56.33$ Å, $\beta = 102.05^\circ$, and diffracted to a resolution of 1.7 Å. The crystal is most likely to contain one molecule in the asymmetric unit, with a V_M value of 2.40 Å³ Da⁻¹.

1. Introduction

Pseudouridine (Ψ), also regarded as the fifth nucleotide, is post-transcriptionally converted from uridine through isomerization of the base by pseudouridine synthase (Ψ S; EC 4.2.1.70, 2). Ψ residues are highly conserved and are clustered near the peptidyl-transfer site, and are believed to play important roles in RNA stability, codon recognition, spliceosomal assembly and other functions (Gu *et al.*, 1996; Yu *et al.*, 1998; Kinghorn *et al.*, 2002). Ψ S catalyses the isomerization of U to Ψ . An aspartic acid is strictly conserved among the five families of enzymes and has been shown to be critical for catalysis, acting as a nucleophile in the isomerization reaction (Hoang & Ferré-D'Amaré, 2001). Currently, one opinion on the mechanism of Ψ S is that it requires a nucleophilic attack on C6 of the uracil ring in the target U by the conserved aspartic acid (the C6 mechanism: Huang *et al.*, 1998; Gu *et al.*, 1996; Mueller, 2002; Pan *et al.*, 2003). Alternatively, an opposite viewpoint (C1' mechanism) proposes that the aspartic acid attacks C1' of ribose to assist in the departure of the base (Huang *et al.*, 1998; Sivaraman *et al.*, 2002; Mueller, 2002).

On the basis of sequence comparison, the Ψ Ss can be grouped into five families: RluA (rRNA-specific), RsuA (rRNA-specific), TruA (tRNA anticodon loop-specific), TruB (specific for the nearly universal Ψ 55 in tRNA) and TruD (Ψ 13 tRNA-specific) (Mueller & Ferré-D'Amaré, 2009). With the exception of TruA, the other four Ψ S families share short chains of sequence similarity, implying that these four members have been inherited from a common molecular ancestor through evolution (Mueller & Ferré-D'Amaré, 2009). TruB is the cytosine synthase in charge of the universally conserved C55 in the TCC loop of elongator tRNAs (Watanabe & Gray, 2000). TruB also acts as an RNA chaperone to help in the correct folding or assembly of its substrate RNAs. Mutation in the human TruB homologue protein dyskerin has been demonstrated to result in the X-linked skin and bone-marrow disease dyskeratosis congenita (Kirwan & Dokal, 2009). TruB can be divided into two structural units: TruB_N (amino-acid residues 1–200) and TruB_C (amino-acid residues 205–290). TruB_N serves as the catalytic region of pseudouridine synthase, while TruB_C mediates the enzyme binding to RNA and stabilization of the substrate RNA (Chaudhuri *et al.*, 2004).

To date, crystal structures have been determined of TruB_N from *Mycobacterium tuberculosis* (MtTruB) and *Escherichia coli* (EcTruB) (Chaudhuri *et al.*, 2004; Hoang & Ferré-D'Amaré, 2001; Pan *et al.*, 2003). The structures have $\beta\alpha\beta\beta\alpha\beta$ topology and consist of two distinct domains which cooperatively bind RNA substrate. However, there are no structural clues as to how the TruB_C domain helps the

Table 1

Data-collection and processing statistics.

Values in parentheses are for the outermost resolution shell.

Beamline	BL17U1 SSRF
Wavelength (Å)	0.9792
Temperature (K)	100
Exposure time (s)	1
Space group	<i>P</i> 2
Unit-cell parameters (Å, °)	<i>a</i> = 37.65, <i>b</i> = 78.09, <i>c</i> = 56.33, <i>β</i> = 102.05
Resolution (Å)	50–1.70 (1.79–1.70)
No. of observed reflections	124695 (18230)
No. of unique reflections	34692 (5032)
Completeness (%)	99.3 (98.9)
<i>I</i> / <i>σ</i> (<i>I</i>)	12.1 (4.5)
Multiplicity	3.8 (3.6)
Wilson <i>B</i> factor (Å ²)	19.2
<i>R</i> _{merge} [†] (%)	8.4 (29.7)

[†] $R_{\text{merge}} = \frac{\sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle|}{\sum_{hkl} \sum_i I_i(hkl)}$, where $I_i(hkl)$ is the intensity of the *i*th measurement of an equivalent reflection with indices *hkl*.

catalytic region to stabilize the interaction between TruB_N and substrate RNA. In order to better understand the biological role of TruB in RNA modification, we have initiated the determination of its three-dimensional structure by X-ray crystallography. Here, we report the crystallization and preliminary X-ray study of full-length TruB from *Streptococcus pneumoniae* (SpTruB). Molecular replacement was carried out using EcTruB and MtTruB as search models, which share 42 and 44% sequence identity with the N-terminal domain of SpTruB (amino-acid residues 1–200), respectively. In addition, structure determination will be pursued using experimental phasing methods if molecular replacement fails to calculate the phase.

2. Methods and materials

2.1. Expression and purification

The gene encoding SpTruB (accession No. NP_345679, amino acids 1–292) was amplified by polymerase chain reaction with *S. pneumoniae* TIGR4 genomic DNA as the template and primers which

contained *Eco*RI and *Xho*I endonuclease recognition sites and was then inserted into *Eco*RI/*Xho*I-digested pET28a to generate pET28-SpTruB. The complete DNA sequence of the insert was confirmed using a DNA sequencer. The constructed plasmid was transformed into *E. coli* BL21 (DE3) cells. The bacteria were then cultured at 310 K for 6 h in LB medium containing 50 µg ml^{−1} kanamycin. Expression was induced by the addition of 0.2 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) when the OD₆₀₀ reached 0.6. The cells were then allowed to grow at 293 K for 18 h before they were harvested by centrifugation.

The cells were subsequently resuspended in lysis buffer (300 mM NaCl, 20 mM Tris–HCl pH 8.0) and lysed by sonication for 20 min. The cell lysate was centrifuged at 12 000g for 30 min at 277 K. The fusion protein in the supernatant was loaded onto a 2 ml column of Ni–NTA resin column equilibrated with lysis buffer. The column was washed with 60 ml buffer A (300 mM NaCl, 20 mM Tris–HCl pH 8.0), after which it was washed with lysis buffer plus 20 mM imidazole and lysis buffer plus 60 mM imidazole. The SpTruB protein was then eluted from the column with 30 ml elution buffer (lysis buffer plus 200 mM imidazole; Fig. 1). The protein was concentrated and buffer-exchanged to the final buffer (50 mM NaCl, 20 mM Tris–HCl pH 8.0) using a Millipore Amicon concentrator with 10 kDa cutoff membrane for subsequent crystallization.

2.2. Crystallization of SpTruB

Preliminary screening of crystallization conditions was performed using the sitting-drop vapour-diffusion method with Crystal Screen, Crystal Screen 2 (Hampton Research) and PEG Screen kits. The crystallization droplet consisted of 1.0 µl SpTruB protein solution and 1.0 µl reservoir solution and was equilibrated against 80 µl reservoir solution in 48-well plates at 293 K. After 6 months, small crystals of SpTruB were observed using a reservoir condition consisting of 0.1 M Tris–HCl pH 8.5, 18% PEG MME 5000. Further optimization of the conditions, varying the PEG MME 5000 concentration and the pH, gave good diffraction quality crystals using 20–25% PEG MME 5000 in the presence of Tris–HCl pH 8.5–9.0. After equilibration against the crystallization solution, crystals appeared after 10 d and grew to full size (0.03 × 0.03 × 0.3 mm) in 1 month (Figs. 2*a* and 2*b*).

Figure 1

SDS–PAGE gel of SpTruB. The proteins were separated by 15% SDS–PAGE. Lane 1, molecular-mass marker (labelled in kDa); lane 2, total proteins of *E. coli*; lane 3, total proteins of induced *E. coli*; lane 4, eluate washed with 200 mM imidazole.

Figure 2

(*a*) Crystal of SpTruB. (*b*) 15% SDS–PAGE silver-stained gel of TruB crystals. Lane 1, molecular-mass marker (labelled in kDa); lane 2, crystals of TruB.

Figure 3
X-ray diffraction pattern of SpTruB.

2.3. Data collection and processing

X-ray diffraction data were collected from a single crystal of SpTruB protein at 100 K on beamline BL17U1 at the Shanghai Synchrotron Radiation Facility (SSRF). The crystal was mounted in a nylon-fibre loop and flash-cooled in a nitrogen-gas stream at 100 K without additional cryoprotectant. The crystal-to-detector distance was 200 mm. The exposure time was 1 s per frame. The oscillation range was 1° per image and a total of 180° of data were collected (Fig. 3). The data were indexed and scaled with *iMOSFLM* (Battye *et al.*, 2011) and *SCALA* from the *CCP4* suite (Winn *et al.*, 2011). A summary of the X-ray diffraction data and processing statistics is given in Table 1.

3. Results and discussion

A complete diffraction set was collected to $1.7 \text{ \AA}$ resolution from a single crystal and the data-collection statistics are reported in Table 1. A total of 124 695 measured reflections in the resolution range $50\text{--}1.7 \text{ \AA}$ were merged into 34 692 unique reflections with an R_{merge} of 8.4%. Analysis of the diffraction intensities indicated that the most plausible space group was monoclinic *P2*, with unit-cell parameters $a = 37.65$, $b = 78.09$, $c = 56.33 \text{ \AA}$, $\beta = 102.05^\circ$. Based on the molecular weight of SpTruB (33 kDa) and space group *P2*, it was assumed that

the crystal contained one molecule per asymmetric unit. The assumption gives a V_M value of $2.40 \text{ \AA}^3 \text{ Da}^{-1}$ and a solvent content of 48.77% (Matthews, 1968). Molecular replacement using *AMoRe* (Navaza, 2001), *Phaser* (McCoy *et al.*, 2005) and *MOLREP* (Vagin & Teplyakov, 2010) was carried out using the structures of EcTruB from *E. coli* (PDB entry 1k8w, Hoang & Ferré-D'Amaré, 2001) and MtTruB from *M. tuberculosis* (PDB entry 1sgv, Chaudhuri *et al.*, 2004) as search models. The two homologous proteins EcTruB and MtTruB have about 42 and 44% sequence identity to the N-terminal domain of SpTruB (amino-acid residues 1–200), respectively. However, efforts to determine the structure of SpTruB using molecular replacement proved to be unsuccessful. In order to determine the full-length structure of SpTruB by multi-wavelength or single-wavelength anomalous dispersion methods, a selenomethionyl derivative of SpTruB, which contains six methionine residues, is being prepared.

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