Crystallographic analysis of archaeal ribosomal protein L11

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Received 21 April 2015

Accepted 12 June 2015

Edited by I. Tanaka, Hokkaido University, Japan

Keywords: ribosome; archaea; L11; ribosomal stalk.

Ribosomal protein L11 is an important part of the GTPase-associated centre in ribosomes of all organisms. L11 is a highly conserved two-domain ribosomal protein. The C-terminal domain of L11 is an RNA-binding domain that binds to a fragment of 23S rRNA and stabilizes its structure. The complex between L11 and 23S rRNA is involved in the GTPase activity of the translation elongation and release factors. Bacterial and archaeal L11–rRNA complexes are targets for peptide antibiotics of the thiazole class. To date, there is no complete structure of archaeal L11 owing to the mobility of the N-terminal domain of the protein. Here, the crystallization and X-ray analysis of the ribosomal protein L11 from *Methanococcus jannaschii* are reported. Crystals of the native protein and its selenomethionine derivative belonged to the orthorhombic space group *I*222 and were suitable for structural studies. Native and single-wavelength anomalous dispersion data sets have been collected and determination of the structure is in progress.

1. Introduction

In all studied organisms, ribosomes contain a very flexible morphological element called the ‘stalk’. This lateral protuberance of the ribosome, together with the sarcin–ricin loop of 23S RNA, forms the ‘GTPase-associated centre’. The stalk is involved in binding translation factors and recruiting them to the ribosome (Uchiumi *et al.*, 2002). The shape and organization of the ribosomal stalk are similar in bacteria, archaea and eukarya (Lake, 1983). Biochemical experiments have shown that L11-like proteins (L11 in bacteria and archaea and L12e in eukarya), L10–L12-like protein complexes (L10–L12 in bacteria, P0–P1 in archaea and P0–P1/P2 in eukarya) and a conserved fragment of the large ribosomal RNA are components of the stalk (Rosendahl & Douthwaite, 1993).

The L11 protein is one of the most conserved ribosomal proteins (Liao & Dennis, 1994). Its homologues have been found in bacterial, archaeal and eukaryotic ribosomes. It stimulates factor-dependent GTP hydrolysis on the ribosome during protein biosynthesis (Schrier & Möller, 1975; Tate *et al.*, 1984). L11 is not essential for cell viability (Dabbs, 1979); cells lacking L11 grow less rapidly than wild-type cells (Cundliffe *et al.*, 1979; Dabbs, 1979). The polypeptide-synthesis activity of ribosomes of *Bacillus megaterium* lacking L11 decreases by around twofold in comparison with wild-type ribosomes (Cundliffe *et al.*, 1979).

L11 consists of two compact domains connected by a short linker region (Wimberly *et al.*, 1999). The C-terminal domain of the protein (L11CTD) is an RNA-binding domain that is involved in binding to a highly conserved 58-nucleotide fragment of domain II of 23S rRNA (nucleotides 1051–1108,

Escherichia coli numbering; Egebjerg *et al.*, 1990). L11 binds tightly to rRNA, and the affinity of L11 from *B. stearothermophilus* for rRNA reaches 1.2×10^{-9} M (Iben & Draper, 2008). L11CTD binds to the minor groove of helix H43 of 23S rRNA. Helix 5 of L11CTD is positioned lengthways in the minor groove, forming the largest contact between L11 and rRNA (Wimberly *et al.*, 1999). Biophysical experiments have shown that the structure of the rRNA fragment is stabilized in the presence of L11 (Xing & Draper, 1996). Also, interactions between L11 and rRNA are essential to form the rRNA native tertiary structure. It is worth noting that the L11 and L10 proteins are closely positioned in the ribosome. Their binding sites on rRNA are located adjacent to each other, and L11 enhances the affinity of the L10–L12 complex for rRNA by ~100-fold (Iben & Draper, 2008).

The N-terminal domain of L11 (L11NTD) is not an RNA-binding domain, but forms limited interactions with rRNA (Xing & Draper, 1996; Wimberly *et al.*, 1999; Ilin *et al.*, 2005). L11NTD interacts with translation factors and promotes their function. *E. coli* cells lacking the N-terminal domain of L11 grow twice as slowly as wild-type cells (Van Dyke & Murgola, 2003). Reconstitution of 70S (Δ L11) ribosomes with modified L11 (with the hinge region deleted) did not stimulate their polyphenylalanine synthetic activity or association of elongation factors (Jenvert & Schiavone, 2007). NMR and crystallographic data and direct observations using cryoelectron microscopy have shown that L11NTD occupies different positions during the ribosome cycle. The location of the domain differs upon the binding of elongation factors EF1 and EF2, release factors RF1 and RF2, and kirromycin to the ribosome (Ilin *et al.*, 2005). This indicates that the mobility of the N-terminal domain of L11 is important for its function during the ribosomal cycle.

The L11–rRNA complex is a functionally important region of the ribosome. It is a target of the thiazole class of antibiotics, for example thiostrepton and mycrococcin (Thompson *et al.*, 1979). Thiostrepton interacts with large ribosomal RNAs with low affinity. However, the presence of L11NTD greatly stimulates the binding of thiostrepton to rRNA (Xing & Draper, 1996). Chemical probing, NMR studies and modelling have suggested a possible thiostrepton binding site on 23S rRNA. Thiostrepton binds in the cleft located between the N-terminal domain of L11 and the hairpin-loop region formed by rRNA helices H43–H44 (Egebjerg *et al.*, 1989; Wimberly *et al.*, 1999; Jonker *et al.*, 2007). However, the structural details of the L11–rRNA–thiostrepton complex are unknown.

The structures of the full-length bacterial protein L11 and its C-terminal domain have been solved by NMR (Markus *et al.*, 1997; Ilin *et al.*, 2005). The structures of the full-length bacterial protein and its C-terminal domain bound to the 58-nucleotide fragment of 23S rRNA have been determined by X-ray crystallography (Conn *et al.*, 1999; Wimberly *et al.*, 1999). The structure of the C-terminal domain of archaeal L11 was determined for the first time in the structure of the 50S ribosomal subunit from *Haloarcula marismortui* (Klein *et al.*, 2004). In 2013, during refinement of this structure, L11NTD was also visualized within the 50S subunit but with two missing

segments (Gabdulkhakov *et al.*, 2013). The electron-density map corresponding to the L11NTD region has low quality because of the mobility of this domain. Our attempts to determine the structure of full-length L11 in complex with the specific fragment of 23S rRNA and a two-domain fragment of the ribosomal protein P0 were not successful (Mitroshin *et al.*, 2013). Here, we report the crystallization and preliminary crystallographic analysis of the isolated archaeal ribosomal protein L11. The goal of the investigation is to obtain a complete model of the archaeal protein L11 and to analyze the mobility of its N-terminal domain.

2. Materials and methods

2.1. Protein purification

The gene encoding the ribosomal protein L11 from *Methanococcus jannaschii* (MjaL11) was cloned in pET-11c (Shcherbakov *et al.*, 2006) and then overexpressed in *E. coli* BL21(DE3)/pUBS520 cells at 310 K using the method of Studier *et al.* (1990). The cell pellet was disrupted by sonication on ice. Cell debris and ribosomes were removed by two consecutive steps of centrifugation (12 000g for 30 min and 300 000g for 50 min). Thermolabile *E. coli* proteins were removed by heat treatment at 348 K for 20 min and subsequent centrifugation. Purification of MjaL11 was performed using a series of chromatographic steps (described in further detail in Mitroshin *et al.*, 2013). The purified MjaL11 protein sample was pooled, exchanged into a buffer consisting of 10 mM Tris–HCl pH 7.5, 50 mM NaCl, 1 mM dithiothreitol and concentrated.

To obtain selenomethionine-derivatized (SeMet) MjaL11, the methionine-auxotrophic *E. coli* strain B834(DE3)/pUBS520 was transformed by the expression vector containing the gene for MjaL11. The transformed cells were cultivated in M9 minimal medium in the presence of selenomethionine at 310 K. The induction of gene expression as well as the isolation and purification of SeMet MjaL11 were performed using the same protocol as for the unmodified protein.

The homogeneity and RNA-binding activity of MjaL11 were verified by nondenaturing gel electrophoresis as described in Shcherbakov *et al.* (2006).

2.2. Protein crystallization

Screening for initial crystallization conditions was performed at 293 K using the sitting-drop vapour-diffusion method in 96-well plates. Drops consisting of 0.5 μ l protein solution (30 mg ml^{−1}) and 0.5 μ l reservoir solution were equilibrated against 50 μ l reservoir solution. Commercial crystallization solution sets from Molecular Dimensions (United Kingdom), Hampton Research (USA) and Qiagen (Germany) were used in the screening experiment. After a few days, initial crystals were obtained in condition No. 49 of the MemMeso (Molecular Dimensions, United Kingdom) crystallization solution set consisting of 0.1 M sodium citrate pH 5.0, 0.1 M MgCl₂, 30% polyethylene glycol (PEG) 500 DME.

Optimization of crystallization conditions was performed at 293 K using the vapour-diffusion method with hanging drops in 24-well plates. Hanging drops on the cover glass were prepared by mixing 2 μl protein solution (30 mg ml⁻¹) and 1 μl reservoir solution. The hanging drop was vapour-equilibrated against 0.5 ml reservoir solution. Crystals appeared after 5 d (Fig. 1*a*). The approximate dimensions of the crystals were 150 $\times$ 100 $\times$ 70 μm . Crystals of SeMet MjaL11 were obtained in the same conditions and grew to dimensions of 100 $\times$ 70 $\times$ 50 μm (Fig. 1*b*).

2.3. Data collection and processing

Crystals were fished out with a nylon loop and soaked for a short time in cryosolution consisting of 0.1 M sodium citrate pH 5.0, 0.1 M MgCl₂, 50% PEG 600. The crystals were then flash-cooled at 100 K in a nitrogen-gas stream. Native and SeMet data sets were collected on the ID23-1 beamline at the European Synchrotron Radiation Facility (ESRF), Grenoble, France (Nurizzo *et al.*, 2006) equipped with a Pilatus 6M detector. Data collection was controlled by the *MxCuBE* system (Gabadinho *et al.*, 2010) and the strategy was calculated by *BEST* (Bourenkov & Popov, 2010). Diffraction data

Table 1

Data collection and processing.

Values in parentheses are for the outer shell.

	Native	SeMet
Diffraction source	ID23-1, ESRF	ID23-1, ESRF
Wavelength ($\text{\AA}$)	0.97625	0.97917
Temperature (K)	100	100
Detector	Pilatus 6M-F	Pilatus 6M-F
Crystal-to-detector distance (mm)	382.9	663.9
Rotation range per image ($^\circ$)	0.15	0.2
Total rotation range ($^\circ$)	119.1	360
Exposure time per image (s)	0.1	0.05
Space group	<i>I</i> 222	<i>I</i> 222
Unit-cell parameters ($\text{\AA}$, $^\circ$)	$a = 69.15$, $b = 88.05$, $c = 142.90$, $\alpha = \beta = \gamma = 90$	$a = 68.82$, $b = 88.57$, $c = 143.48$, $\alpha = \beta = \gamma = 90$
Mosaicity ($^\circ$)	0.235	0.262
Resolution range ($\text{\AA}$)	50.00–2.20 (2.25–2.20)	50.00–2.92 (3.00–2.92)
Total No. of reflections	94646 (14966)	119253 (5585)
No. of unique reflections	22254 (3481)	18162 (1203)
Completeness (%)	97.8 (96.0)	98.5 (87.4)
Multiplicity	4.25 (4.29)	6.56 (4.64)
$\langle I/\sigma(I) \rangle$	13.57 (1.85)	11.79 (2.00)
$R_{\text{r.i.m.}}^\dagger$	0.062 (0.850)	0.118 (0.743)
Overall <i>B</i> factor from Wilson plot ($\text{\AA}^2$)	61.1	68.2

$^\dagger R_{\text{r.i.m.}} = \sum_{hkl} \{N(hkl)/[N(hkl) - 1]\}^{1/2} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of the *i*th measurement of reflection *hkl* and $\langle I(hkl) \rangle$ is the average intensity of reflection *hkl*.

(*a*)

(*b*)

Figure 1

(*a*) Photograph of a crystal of the ribosomal protein MjaL11. (*b*) Photograph of crystals of the selenomethionine derivative of the ribosomal protein MjaL11.

were integrated and scaled in *XDS* (Kabsch, 2010). Crystallographic statistics are given in Table 1.

3. Results and discussion

The purified MjaL11 protein sample was concentrated for crystallization experiments. The purification protocol used allowed a homogeneous MjaL11 protein sample suitable for crystallization to be obtained.

The ribosomal protein L11 is an RNA-binding protein that binds to 23S rRNA (Egebjerg *et al.*, 1990). The functional RNA-binding activity of the protein was tested by the gel-shift method based on a change of the protein mobility in the gel. MjaL11 forms a tight homogeneous complex with a specific 74-nucleotide fragment of 23S rRNA containing helices H42–H44 as shown in Mitroshin *et al.* (2013).

Initial screening of crystallization conditions for the ribosomal protein MjaL11 was performed using commercial crystallization kits. Initial microcrystals were obtained in conditions consisting of 0.1 M sodium citrate pH 5.0, 0.1 M MgCl₂, 30% PEG 500 DME. To obtain larger crystals of MjaL11, optimization of the crystallization conditions was performed by using different PEGs and varying their concentrations. We managed to obtain single crystals using PEG 600 as a precipitant. The best crystals grew over a precipitant concentration range of 26–28%. The crystals diffracted to high resolution using synchrotron radiation (Fig. 2). Crystals of SeMet MjaL11 were obtained in the same conditions (0.1 M MgCl₂, 0.1 M sodium citrate pH 5.0, 27% PEG 600).

Determination of the positions of Se atoms and experimental phasing followed by density modification was carried out with *phenix.autosol* (Terwilliger *et al.*, 2009). The initial electron-density map after density modification was of sufficient quality to position and partially build two MjaL11

molecules in the asymmetric unit. The models were initially subjected to crystallographic refinement using *REFMAC5* (Murshudov *et al.*, 2011). Manual rebuilding of the models was carried out in *Coot* (Emsley *et al.*, 2010). An example of an electron-density map is shown in Fig. 3. Refinement of the structure is currently in progress and the structure will be published in the future.

This work may contribute to structural understanding of the archaeal ribosome and further refinement of the archaeal 50S subunit structure, in which the structure of L11 was not perfect (Ban *et al.*, 2000; Kavran & Steitz, 2007; Gabdulkhakov *et al.*, 2013).

Acknowledgements

This work was supported by the Program of RAS on Molecular and Cellular Biology. The diffraction experiments were performed on beamline ID23-1 at the European Synchrotron Radiation Facility (ESRF), Grenoble, France. We are grateful to the ESRF Structural Biology Group for providing assistance in using these beamlines. We are indebted to Dr A. Popov for help with the diffraction measurements.

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Figure 2
X-ray diffraction image of the MjaL11 protein crystal: concentric rings indicate resolutions (labelled in Å).

Figure 3
Fragment of an electron-density map at 2.9 Å resolution with a main-chain trace of the MjaL11 molecules. The $2|F_{\text{obs}}| - |F_{\text{calc}}|$ map is shown contoured at 2σ . NTD, N-terminal domain; CTD, C-terminal domain.

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