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Crystallization and preliminary crystallographic studies of L30e, a ribosomal protein from *Methanocaldococcus jannaschii* (*MJ*1044)

Sarani Rangarajan,[a] Jeyaraman Jeyakanthan,[b,c] Palappetty Mridula,[a] Keiko Sakamoto,[b] Yoshiaki Kitamura,[b] Yoshihiro Agari,[b] Akeo Shinkai,[b] Akio Ebihara,[b] Seiki Kuramitsu,[b,d] Shigeyuki Yokoyama[b,e,f] and Kanagaraj Sekar[a,g]*

[a]Bioinformatics Centre, Indian Institute of Science, Bangalore 560 012, India, [b]RIKEN SPring-8 Center, Harima Institute, 1-1-1 Kouto, Sayo, Hyogo 679-5148, Japan, [c]National Synchrotron Radiation Research Center, 101 Hsin-Ann Road, Hsinchu Science Park, Hsinchu 30076, Taiwan, [d]Graduate School of Science, Osaka University, Toyonaka, Osaka 560-0043, Japan, [e]Genomic Sciences Center, Yokohama Institute, RIKEN, 1-7-22 Suehiro-cho, Tsurumi, Yokohama 230-0045, Japan, [f]Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan, and [g]Supercomputer Education and Research Centre, Indian Institute of Science, Bangalore 560 012, India

Correspondence e-mail: sekar@serc.iisc.ernet.in, sekar@physics.iisc.ernet.in

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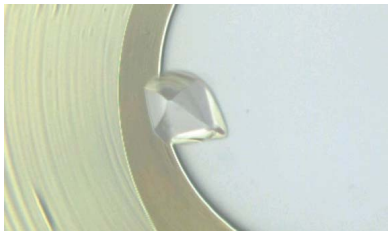

In view of the biological significance of understanding the ribosomal machinery of both prokaryotes and eukaryotes, the L30e ribosomal protein from *Methanocaldococcus jannaschii* was cloned, overexpressed, purified and crystallized using the microbatch-under-oil method with the crystallization conditions 40% PEG 400, 0.1 *M* MES pH 6.0 and 5% PEG 3000 at 291 K. A diffraction-quality crystal (0.20 × 0.20 × 0.35 mm) was obtained that belonged to the primitive tetragonal space group $P4_3$, with unit-cell parameters a = 46.1, b = 46.1, c = 98.5 Å, and diffracted to a resolution of 1.9 Å. Preliminary calculations reveal that the asymmetric unit contains two monomers with a Matthews coefficient (V_M) of 2.16 Å^3 Da^{-1}.

1. Introduction

An integral part of the cellular machinery, the ribosome is a complicated dynamic machine responsible for protein synthesis. Although enormous efforts have been made to understand the structure of a complete ribosome and the roles of its numerous components, the RNA–protein complex is not fully understood. The archaeal 70s ribosome consists of two protein subunits, one large (50s) and another small (30s), defined by their Svedberg coefficients, which reflect the rate of sedimentation of the molecule in the solvent. Each subunit of the ribosome contains several ribosomal RNAs (rRNAs). For example, 16s rRNAs are part of the 30s subunit and 25s rRNAs and 5s rRNAs are part of the 50s subunit. Initially, it was believed that the proteins, rather than the RNA, were the enzymatic components of the ribosome. However, the present understanding is that one of the main functions of the ribosomal proteins is to act as scaffolding in order to ensure the correct placement of the RNA, thereby enabling the RNA to catalyse the reactions. In fact, the three-dimensional structures of both the subunits have been solved (Wimberly *et al.*, 2000; Ban *et al.*, 2000). However, since much remains to be known about the interactions and the functions of various parts of the complex, a study of individual proteins would be very useful.

The L30e protein from the 50s subunit has been found to be conserved in many archaeal and eukaryotic genomes (Lecompte *et al.*, 2002). Furthermore, a stringent double autoregulation function in yeast (Chao *et al.*, 2003; Mao & Williamson, 1999) implies that the function of the L30e protein is important, if not crucial, to the ribosome. It is known that the L30e protein is found in the 50s subunit near the binding site of the elongation factor Tu, at the interface between the two subunits, and is involved in binding to the 5S rRNA (Ban *et al.*, 2000). In this regard, a study of the multiple functions of the conserved RNA-binding domain revealed the L30e protein to be responsible for the formation of bridges between the large and small ribosomal subunits (Vilardell *et al.*, 2000). A recent study of the dynamic behaviour of the ribosomal protein L30e in the eukaryotic ribosome suggested the same (Halic *et al.*, 2005). The L30e protein is involved in the selenocystine-recoding mechanism in eukaryotes (Chavatte *et al.*, 2005) and it is believed that it may also have a similar function in archaea. Two previous three-dimensional structures of L30e from archaea have been obtained from the thermophilic organism *Bacillus stearothermophilus* (Wilson *et al.*, 1986) and more

recently from the extreme thermophile *Thermococcus celer* (Chen *et al.*, 2003; Wong *et al.*, 2003). In fact, a recent study focused on the thermostability of the L30e protein from *T. celer* indicated that this property to partly be a consequence of electrostatic interactions (Lee *et al.*, 2005). However, there is no clear indication of the effect of other extreme conditions on the protein.

With this in mind, the L30e protein from the oxygen-intolerant methane-producing archaeon *Methanocaldococcus jannaschii* was cloned from the *MJ*1044 gene, expressed, purified and crystallized. The nitrogen-fixing *M. jannaschii* is of particular importance and interest since the archaeon thrives under extreme conditions. Not only is it hyperthermophilic with an optimal growth temperature of 358 K, but it also survives pressures of over 20 MPa. Thus, the proposed study would be of great biological interest as it would reveal the effect of both temperature and pressure on the structure of the L30e protein from *M. jannaschii*.

2. Materials and methods

2.1. Cloning, expression and purification

The *MJ*1044 gene from *M. jannaschii* strain DSM 2661 was amplified by genomic polymerase chain reaction (PCR) using 5′-GGAATTcatatgAGAAGGAGGGAGAATATGG-3′ (*Nde*I site in lower case) and 5′-GGAATTggatccTTATTATTCACCACCTTCCT-TTTTCTCT-3′ (*Bam*HI site in lower case) as primers and the amplified fragment was cloned under the control of the T7 promoter (*Nde*I–*Bam*HI site) of *Escherichia coli* expression vector pET-21a(+) (Novagen, Madison, Wisconsin, USA) with no purification tag. The plasmid was used to transform *E. coli* Rosetta (DE3) cells. The transformants were cultured at 310 K overnight in a medium containing 1.0%(*w*/*v*) polypeptone, 0.5%(*w*/*v*) yeast extract, 0.5%(*w*/*v*) NaCl and 100 µg ml^{-1} ampicillin pH 7.0. The cells were lysed by sonication in 20 m*M* Tris–HCl pH 8.0 containing 500 m*M* NaCl, 5 m*M* β-mercaptoethanol and 1 m*M* phenylmethylsulfonyl fluoride. The cell lysate was heat-treated at 343 K for 13 min and then centrifuged (15 000*g*) for 30 min at 277 K to precipitate nonthermostable contaminant proteins from the host cells. After the supernatant had been applied onto a HiPrep 26/10 Desalting column (GE Healthcare Biosciences) pre-equilibrated with 20 m*M* Tris–HCl pH 8.0 for buffer exchange, the protein sample was loaded onto a Toyopearl SuperQ-650M column (Tosoh) pre-equilibrated with 20 m*M* Tris–HCl pH 8.0. The protein bound to the column was eluted with a linear NaCl gradient from 0 to 300 m*M*. The L30e protein-containing fraction was subjected to buffer exchange with 10 m*M* potassium phosphate buffer pH 7.0 and loaded onto a Bio-Scale CHT2-I column (Bio-Rad) pre-equilibrated with the same buffer. The protein bound to the column was eluted with a linear potassium phosphate gradient from 10 to 500 m*M* at neutral pH. The L30e was subjected to gel filtration on a HiLoad 16/60 Superdex 200 pg column (GE Healthcare Biosciences) pre-equilibrated with 20 m*M* Tris–HCl and 200 m*M* NaCl pH 8.0. The protein-containing fraction was collected and the purified protein was concentrated using a Vivaspin 5K (Sartorius). Finally, dithiothreitol (DTT) was added to a final concentration of 1 m*M* DTT pH 8.0. The final concentration of the protein, determined by measuring the absorbance at 280 nm (Kuramitsu *et al.*, 1990), was 20.5 mg ml^{-1}.

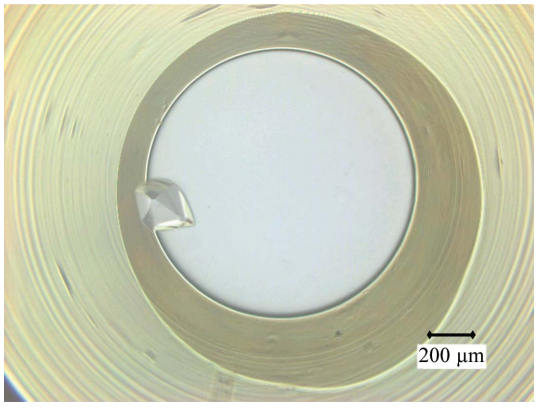

Figure 1
Crystal of L30e from *M. jannaschii*.

Table 1
X-ray data-collection statistics for L30e ribosomal protein from *M. jannaschii*.

Values in parentheses are for the high-resolution shell.

Wavelength (Å)	1.0
Temperature (K)	100
Crystal system	Primitive tetragonal
Space group	$P4_3$
Unit-cell parameters (Å)	a = 46.1, b = 46.1, c = 98.5
Matthews coefficient (Å^3 Da^{-1})	2.16
Solvent content (%)	43
No. of molecules in ASU	2
Resolution range (Å)	50.0–1.9 (1.97–1.90)
No. of observed reflections	179337
No. of unique reflections	16282 (1595)
Completeness (%)	100 (100)
$R_{\rm merge}$† (%)	8.8 (54)
Multiplicity	11 (10)
Average $I/\sigma(I)$	26 (3)
Wilson B factor (Å^2)	23.0

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \overline{I(hkl)}|/\sum_{hkl}\sum_i I_i(hkl)$, where $I(hkl)$ is the intensity of reflection hkl, $\sum_{hkl}$ is the sum over all reflections and $\sum_i$ is the sum over i measurements of reflection hkl.

2.2. Crystallization experiments

The L30e ribosomal protein from *M. jannaschii* was crystallized at 291 K using the microbatch-under-oil (diffusion through paraffin oil) method with Nunc HLA plates (Nalge Nunc International). The purified protein was screened for preliminary crystallization conditions using commercially available sparse-matrix screens from Emerald Biosystems. The final crystallization condition was an equilibrated mixture containing 1 µl protein (20.5 mg ml^{-1} protein, 20 m*M* Tris–HCl pH 8.0, 200 m*M* NaCl and 1 m*M* DTT) and 1 µl reservoir solution containing 40% PEG 400, 0.1 *M* MES pH 6.0 and 5% PEG 3000 (Wizard Cryo I, condition No. 46) at 291 K. Crystals suitable for X-ray data collection appeared (Fig. 1) after a month and reached final dimensions of 0.20 × 0.20 × 0.35 mm.

2.3. Data collection

The crystal of native L30e protein grown under the conditions given above was soaked for a brief time in the precipitant solution and cooled in a nitrogen-gas stream at 100 K using the SPring-8 Precise Automatic Cryo-sample Exchanger (SPACE), which was controlled using the *BSS* beamline-scheduling software (Ueno *et al.*, 2004, 2005). Diffraction data were collected from the crystal using the RIKEN Structural Genomics Beamline I (BL26B1) at SPring-8 (Hyogo, Japan) with a Jupiter210 CCD detector (Rigaku MSC Co., Tokyo Japan). The crystal-to-detector distance was maintained at 180 mm. The crystal diffracted to 1.9 Å resolution. The data set was indexed and integrated using *DENZO* (Otwinowski & Minor, 1997) and scaled using *SCALEPACK* (Otwinowski & Minor, 1997).

3. Results and discussion

The L30e ribosomal protein from *M. jannaschii* consists of 110 amino acids and has an approximate molecular weight of 12 kDa. A native data set was collected in the resolution range 50.0–1.9 Å at 100 K using synchrotron radiation. A total of 179 337 measured reflections were merged into 16 282 unique reflections with an overall $R_{\rm merge}$ of 8.8% and a completeness of 100%. The crystal belongs to space group $P4_3$, with unit-cell parameters a = 46.1, b = 46.1, c = 98.5 Å. The relevant X-ray crystal parameters and data-processing statistics are summarized in Table 1. The Matthews coefficient $V_{\rm M}$ (Matthews, 1968) was calculated to be 2.16 Å^3 Da^{-1}, suggesting the presence of two molecules in the asymmetric unit and corresponding to a solvent content of 43%. The preliminary structure of the L30e protein was obtained by molecular-replacement calculations using the program *CNS* v.1.1 (Brünger *et al.*, 1998) with the atomic coordinates (PDB code 1w41) of the homologous protein from *T. celer* as the search model (Lee *et al.*, 2005). The search model had 44% sequence identity to the present protein. The molecular-replacement calculations were attempted in the two enantiomorphic space groups $P4_1$ and $P4_3$. The calculations in space group $P4_3$ revealed the presence of two monomers in the asymmetric unit. The molecules were further subjected to rigid-body refinement using *CNS* v.1.1 and the partially refined structures yielded crystallographic $R_{\rm work}$ and $R_{\rm free}$ values of 39% and 45%, respectively. A total of 5% of the reflections were exclusively retained for the calculation of $R_{\rm free}$ (Brünger, 1992). Further work is in progress. The detailed study of the three-dimensional structure should help in elucidating the factors responsible for the stability of the protein under high-temperature and high-pressure conditions.

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