Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary crystallographic studies of a ribosomal protein L30e from the hyperthermophilic archaeon *Thermococcus celer*

Kam-Bo Wong,[a]* Wooi Koon Wang,[b] Mark R. Proctor,[b] Mark Bycroft[b] and Yu Wai Chen[b]

[a]Department of Biochemistry, The Chinese University of Hong Kong, Shatin, Hong Kong, and [b]Cambridge University Chemical Laboratory and Centre for Protein Engineering, MRC Centre, Hills Road, Cambridge CB2 2QH, United Kingdom

Correspondence e-mail: kbwong@cuhk.edu.hk

Received 24 January 2001
Accepted 14 March 2001

Ribosomal protein L30e from the hyperthermophilic archaeon *Thermococcus celer* is a good model for the study of the thermostability of proteins. It has been overexpressed, purified and crystallized using the hanging-drop vapour-diffusion method using PEG 8000 as precipitant at 290 K. The crystal belongs to the hexagonal space group $P6_1/P6_5$, with unit-cell parameters $a = b = 48.32$, $c = 86.42$ Å. The asymmetric unit contains a single molecule of L30e, with a corresponding crystal volume per protein mass (V_M) of 2.68 Å^3 Da^{-1} and a solvent content of 54%. A complete data set diffracting to 1.96 Å resolution was collected from a single crystal at 100 K.

1. Introduction

L30e belongs to one of the protein components of the large subunit of the ribosome. The L30e protein contains an RNA-binding motif that is conserved in protein families across different genomes. They include archaeal and eukaryotic ribosomal proteins (L30e, L7Ae/S6e and S12e), yeast omnipotent translation termination suppressors (SUP1), a yeast nuclear protein (Nhp2) and an *Escherichia coli* ribosomal protein-modification enzyme (RimK; Koonin *et al.*, 1994). In yeast, the ribosomal protein L30e is not just a constituent of ribosome. It also negatively regulates the splicing of pre-mRNA and translation of mRNA by binding to its own mRNA transcript (Eng & Warner, 1991; Vilardell & Warner, 1994, 1997).

It is interesting to understand the 'rules' of structural adaptation by which proteins from thermophilic organisms remain stable and active at high temperatures. This is not only of academic interest but also has potential applications in biotechnology (Adams & Kelly, 1998). For example, industrial enzymatic processes can be made more cost-effective if the enzymes can be engineered to remain active at elevated temperatures. The ribosomal protein L30e from *T. celer*, a hyperthermophilic archaeon that grows at an optimal temperature of 358 K, is an excellent model for this kind of study. The protein is highly thermostable – it resists unfolding at over 363 K and has a melting temperature of 383 K (Wong *et al.*, unpublished results). In contrast, the yeast L30e undergoes irreversible denaturation at 318 K (Mao & Williamson, 1999).

To the best of our knowledge, the only L30e structure reported is that from yeast (both free and RNA-bound), which was determined by NMR spectroscopy (Mao *et al.*, 1999; Mao & Williamson, 1999). The yeast (a mesophile) L30e adopts a three-layer $\alpha/\beta/\alpha$ sandwich fold and has the same topology as that of the ribosomal protein L7Ae from *Haloarcula marismortui*, being part of the 2.4 Å crystal structure of the large ribosomal subunit (Ban *et al.*, 2000). The amino-acid sequence of *T. celer* L30e shares 30 and 25% identity with yeast L30e and *H. marismortui* L7Ae, respectively. A comparison of homologous protein structures from thermophilic and mesophilic organisms will be the most intuitive way to reveal the structural determinants of thermostability of thermophilic proteins. As a first step toward structure determination, we report the overexpression, crystallization and preliminary crystallographic analysis of L30e from *T. celer*, for which diffraction data to 1.96 Å resolution were collected.

2. Expression and purification

The DNA sequence encoding the L30e protein was subcloned into a modified pRSET A vector (Invitrogen) without the polyhistidine tag. The expression plasmid was transformed to an *E. coli* C-41 strain (Miroux & Walker, 1996). The bacteria culture was grown in 2×TY medium at 310 K until the absorbance at 600 nm reached 0.4. Protein expression was induced by addition of isopropyl-β-D-thiogalactopyranose to 0.5 m*M*. Cells were harvested after 16 h and then lysed by sonication in buffer *A* (50 m*M* Tris pH 7.2, 0.5 m*M* phenylmethylsulfonyl fluoride, 5 m*M* dithiothreitol). The solution was centrifuged at 44 000*g* for 15 min. The cell extract was heated to 353 K for 10 min to denature and precipitate unwanted heat-labile proteins from the *E. coli* host. It was then centrifuged at 44 000*g* for

30 min. The supernatant was loaded onto a heparin affinity column pre-equilibrated with buffer *A*. The protein was eluted using a linear gradient of 0–1.0 *M* NaCl in buffer *A* over a volume of 100 ml. The purified protein was dialysed against the sample buffer (20 m*M* sodium acetate, 0.5 *M* Na_2SO_4 pH 5.6).

The concentrated protein sample (20 mg ml^{-1}), originally prepared for NMR studies, was found to crystallize readily in an NMR tube after storage at 277 K for several weeks. This observation prompted us to attempt crystallization screening for this protein.

Figure 1
Crystals of ribosomal protein L30e from *T. celer* with maximum dimensions 0.1 × 0.1 × 1.5 mm.

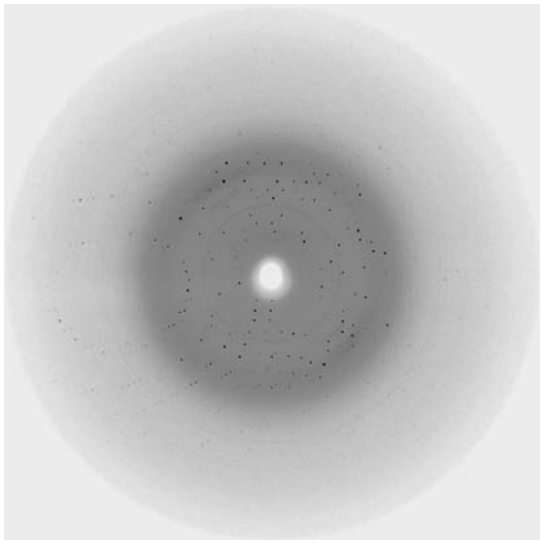

Figure 2
Diffraction image (Φ = 60.0–61.0°) of the ribosomal protein L30e from *T. celer*.

3. Crystallization and X-ray analysis

Initial screening for crystallization conditions was performed with Hampton Research Crystal Screens I and II at 290 K using freshly prepared protein samples. Crystals for diffraction data collection were grown using the hanging-drop vapour-diffusion method at 290 K by mixing 1 µl of 24 mg ml^{-1} protein sample with 1 µl of reservoir solution containing 50 m*M* KH_2PO_4, 20%(*w*/*v*) polyethylene glycol (PEG) 8000 on siliconized cover slips over 500 µl reservoir solution. Hexagonal needle crystals grew to maximum dimensions of 0.1 × 0.1 × 1.5 mm (Fig. 1). Cryoprotection was achieved by diffusing 1 µl of 50%(*w*/*v*) PEG 8000 into the droplets containing the crystals and equilibrating for 2 min at room temperature. The crystals were then cooled in a nitrogen stream at 100 K (Oxford Cryosystems Cryostream). A complete set of diffraction data (Fig. 2), consisting of 126 frames, was collected from a single crystal in 1° oscillation steps at 100 K using a Cu *Kα* X-ray source on a MAR 345 scanner at the Medical Research Council Laboratory of Molecular Biology, Cambridge, England.

The diffraction data were processed with *IPMOSFLM* (Leslie, 1992) and were merged, scaled and reduced with programs (*SCALA* and *TRUNCATE*) from the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994). Pseudo-precession images generated with *HKLVIEW* (*CCP*4 suite) revealed 6/*m* Laue symmetry and reflection conditions $l = 6n$ were observed, indicating the presence of a sixfold screw axis parallel to the *l* axis. The space group belongs to one of the enantiomorphic pair $P6_1$ or $P6_5$, with unit-cell parameters $a = b = 48.32$, $c = 86.42$ Å. The asymmetric unit contains one molecule of protein, giving a crystal volume per protein mass (V_M; Matthews, 1968) of 2.68 Å^3 Da^{-1} and a solvent content of 54%. The statistics for data processing are summarized in Table 1. Structure solution by molecular replacement is under way.

Table 1
Data-processing statistics for L30e from *T. celer*.

Values in parentheses are for the highest resolution shell (2.07–1.96 Å).

Resolution (Å)	24.2–1.96
No. of measurements (no cutoff)	62022
No. of unique reflections	8226
Multiplicity	7.5 (7.3)
Completeness (%)	99.9 (99.9)
R_{merge}† (%)	8.2 (34.3)
Mean $I/\sigma(I)$	7.6 (2.1)

† $R_{merge} = \sum_{hkl} |I - \langle I \rangle| / \sum_{hkl} I$.

WKW is supported by a studentship from the Universiti Putra Malaysia and the Ministry of Science and Technology (Malaysia). YWC is supported by a grant from the Wellcome Trust.

References

Adams, M. W. W. & Kelly, R. M. (1998). *Trends Biotechnol.* **16**, 329–332.
Ban, N., Nissen, P., Hansen, J., Moore, P. B. & Steitz, T. A. (2000). *Science*, **289**, 905–920.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Eng, F. J. & Warner, J. R. (1991). *Cell*, **65**, 797–804.
Koonin, E. V., Bork, P. & Sander, C. (1994). *Nucleic Acids Res.* **22**, 2166–2167.
Leslie, A. G. W. (1992). *Jnt CCP4/ESF–EACMB Newsl. Protein Crystallogr.* **26**.
Mao, H., White, S. A. & Williamson, J. R. (1999). *Nature Struct. Biol.* **6**, 1139–1147.
Mao, H. & Williamson, J. R. (1999). *J. Mol. Biol.* **292**, 345–359.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Miroux, B. & Walker, J. E. (1996). *J. Mol. Biol.* **260**, 289–298.
Vilardell, J. & Warner, J. R. (1994). *Genes Dev.* **8**, 211–220.
Vilardell, J. & Warner, J. R. (1997). *Mol. Cell Biol.* **17**, 1959–1965.