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Crystallization and preliminary X-ray crystallographic studies of the N-terminal domain of human ribosomal protein L7a (RPL7a)

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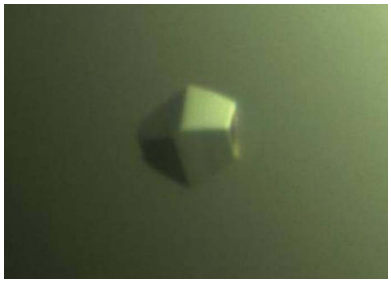

Ribosomal proteins are a major component of ribosomes, which catalyze protein synthesis. One ribosomal protein, L7a (RPL7a), which is a component of the 60S large ribosomal subunit, has additional functions involved in cell growth and differentiation that occur *via* interaction with human thyroid hormone receptor (THR) and retinoic acid receptor (RAR) and in turn inhibit the activities of the two nuclear hormone receptors. In this study, the N-terminal domain of human RPL7a was overexpressed in *Escherichia coli* using an engineered C-terminal His tag. The N-terminal domain of human RPL7a was then purified to homogeneity and crystallized at 293 K. X-ray diffraction data were collected to a resolution of 3.5 Å from a crystal belonging to the tetragonal space group $P4_122$ or $P4_322$ with unit-cell parameters a = 92.28, b = 92.28, c = 236.59 Å.

1. Introduction

Ribosomes are the particles that catalyze protein synthesis in all organisms (Maguire & Zimmermann, 2001; Steitz, 2010). The codons of the mRNA are exposed on ribosomes to allow aminoacyl-tRNA binding, which leads to the incorporation of amino acids into the growing polypeptide chain in accordance with the genetic information (Blanchard, 2009). Functional ribosomes are composed of two distinct subunits, large and small, and about 70% of the mass of the ribosome consists of RNA known as ribosomal RNA (rRNA), while a third of the protein is ribosomal protein (RP). In humans, 80 ribosomal proteins in males and 79 ribosomal proteins in females have been identified, without knowing their precise function (Warner & Nierras, 1998). The ribosomal proteins are named in accordance with the subunit of the ribosome to which they belong, with the proteins belonging to the large subunit being denoted L1–L44 and those belonging to the small subunit being denoted S1–S31. Many ribosomal proteins, particularly those of the large subunit, including ribosomal protein L7a (RPL7a), are composed of a globular surface-exposed RNA-binding domain that binds to the rRNA core to stabilize its structure. Although the critical activities of decoding and peptide transfer are rRNA-based, ribosomal proteins also play an important role in the process of protein synthesis (Wool, 1996). In addition to their function as ribosomal proteins, many ribosomal proteins have a particular function outside the ribosome involving various cellular processes such as replication, transcription, RNA processing, DNA repair and even inflammation (Wool, 1996; Yamamoto, 2000). Moreover, recent data have shown that the abnormal expression of some ribosomal proteins is linked to carcinogenesis (Ebert *et al.*, 2008; Vaarala *et al.*, 1998).

60S ribosomal protein L7a (RPL7a), a component of the 60S large ribosomal subunit, also plays a critical role in stabilizing ribosomes by binding to rRNA (De Falco *et al.*, 1993; Huxley & Fried, 1990). This protein appendage is highly mobile, interacts with elongation factors and is involved in eliciting their GTPase activity (Maguire & Zimmermann, 2001). RPL7a contains two distinct RNA-binding domains: one encompassing amino acids 52–100 and the other encompassing amino acids 101–161 (Russo *et al.*, 2005). In addition to its function in the ribosome, this protein may also be involved in cell growth and differentiation by interacting with human thyroid

hormone receptor (THR) and retinoic acid receptor (RAR) and in turn inhibiting the activities of the two nuclear hormone receptors (Burris *et al.*, 1995). It has recently been shown that the N-terminal domain of RPL7a binds directly to transglutaminase 2 (TG2) and regulates the activity of TG2 (unpublished data).

Although several structures of prokaryotic ribosomes and eukaryotic ribosomes have been identified to date (Ben-Shem *et al.*, 2010; Ramakrishnan, 2010; Steitz, 2010), no high-resolution structures of RPL7a are currently available and the low-resolution RPL7a structures do not contain the N-terminus. In the present study, we overexpressed, purified and crystallized the N-terminal domain of RPL7a as a first step towards elucidating the molecular structure and the function of the N-terminal domain of RPL7a. Details regarding the atomic structure of RPL7a should help us to understand the function of RPL7a, especially the regulation mechanism of the binding of the N-terminal domain of RPL7a to TG2.

2. Materials and methods

2.1. Expression and purification

To express C-terminally His-tagged enzyme, the coding region for human ribosomal protein L7a (RPL7a) was amplified by PCR using P1 (5′-GGG**CATATG**ATGCCGAAAGGAAAGAAG-3′) and P2 (5′-GGG**CTCGAG**GAGGTCTCTTTTGGGCTGG-3′) primers. The PCR product was then digested with restriction enzymes (*Nde*I/*Xho*I) and inserted into vector pET26b that had been cut with the same restriction enzymes. The plasmid was transformed into BL21 (DE3) *Escherichia coli* competent cells and its expression was induced by treating the bacteria with 0.5 m*M* isopropyl *β*-D-1-thio-galactopyranoside (IPTG) overnight at 293 K. Cells expressing the N-terminal domain of RPL7a were pelleted by centrifugation, resuspended and lysed by sonication in 50 ml lysis buffer (20 m*M* Tris pH 7.9, 500 m*M* NaCl and 20 m*M* imidazole). The lysate was then centrifuged at 16 000 rev min^{-1} for 1 h at 277 K, after which the supernatant fractions were applied onto a gravity-flow column (Bio-Rad) packed with Ni–NTA affinity resin (Qiagen). Next, the unbound bacterial proteins were removed from the column using lysis buffer (20 m*M* Tris pH 7.9, 500 m*M* NaCl and 20 m*M* imidazole). The C-terminally His-tagged N-terminal domain of RPL7a was eluted from the column using elution buffer (20 m*M* Tris buffer pH 7.9, 500 m*M* NaCl and 250 m*M* imidazole). The elution fractions were then collected on a 0.5 ml scale to 2 ml. The collected N-terminal domain of RPL7a was then applied onto a Superdex 75 gel-filtration column (GE Healthcare) that had been pre-equilibrated with a solution consisting of 20 m*M* Tris pH 8.0, 50 m*M* NaCl and 1 m*M* DTT. The N-terminal domain of RPL7a (molecular weight 6702.9 Da) eluted at around 19 ml and was collected and concentrated to 3–4 mg ml^{-1}. The protein concentration was measured using a protein-assay kit (Bio-Rad) and was determined using the Bradford method (Bradford, 1976). The peak was then confirmed to contain the N-terminal domain of RPL7a by SDS–PAGE.

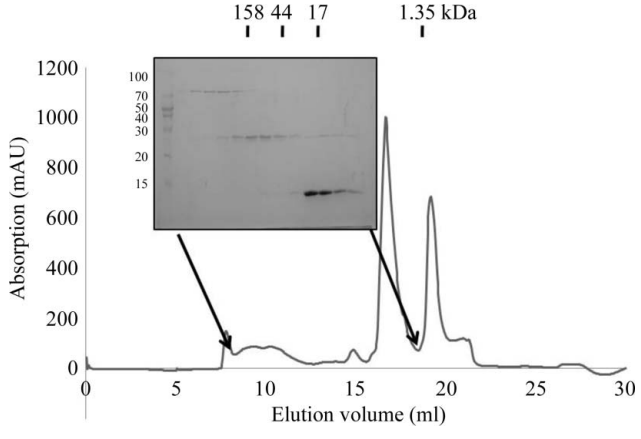

Figure 1
Gel-filtration chromatography and SDS–PAGE (inset) of the N-terminal domain of RPL7a.

Table 1
Diffraction data statistics for RPL7a crystals.

Values in parentheses are for the highest resolution shell.

X-ray source	BL-6C at PAL
Wavelength (Å)	1.2398
Space group	$P4_122$ or $P4_322$
Unit-cell parameters (Å)	a = 92.28, b = 92.28, c = 236.59
Resolution limits (Å)	50–3.5
No. of observations	172998
No. of unique reflections	48524
Mean $I/\sigma(I)$	11.6 (3.4)
Completeness (%)	98.9 (96.6)
$R_{\rm merge}$ (%)†	11 (26.2)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the ith observation of reflection hkl and $\langle I(hkl)\rangle$ is the weighted average intensity for all observations i of reflection hkl.

2.2. Crystallization

The crystallization conditions were initially screened at 293 K by the hanging-drop vapour-diffusion method using screening kits from Hampton Research (Crystal Screen, Crystal Screen 2, Natrix, MembFac, SaltRX and Index HT) and deCODE Biostructures (Wizard I, II, III and IV). Initial crystals were grown on plates by equilibrating a mixture containing 1 µl protein solution (3–4 mg ml^{-1} protein in 20 m*M* Tris pH 8.0, 50 m*M* NaCl and 1 m*M* DTT) and 1 µl reservoir solution No. 4 from the Wizard II kit (deCODE Biostructures; 2.0 *M* ammonium sulfate, 0.2 *M* sodium chloride and 0.1 *M* cacodylate pH 6.5) against 0.4 ml reservoir solution. Crystallization was further optimized using a range of protein concentrations, ammonium sulfate concentrations and pH and using additive screening. Crystals appeared within 3 d and grew to maximum dimensions of 0.1 × 0.1 × 0.05 mm (Fig. 1) in the presence of 2.0 *M*

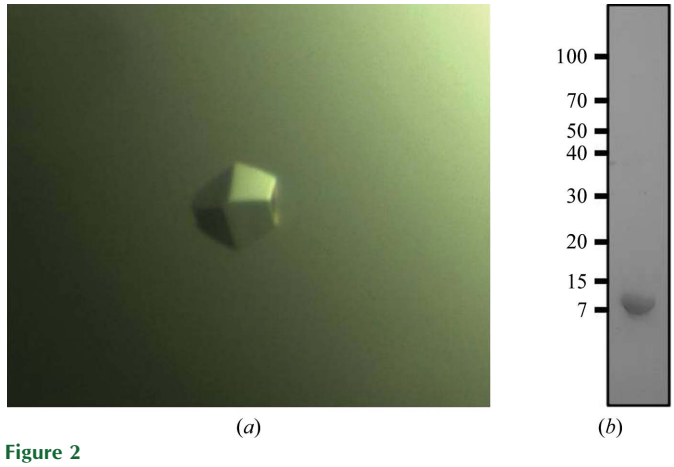

Figure 2
(*a*) A crystal of the N-terminal domain of RPL7a. Crystals were grown in 3 d in the presence of 2.0 *M* ammonium sulfate, 0.2 *M* sodium chloride, 0.1 *M* potassium chloride and 0.1 *M* cacodylate pH 6.3. The approximate dimensions of the crystals were 0.1 × 0.1 × 0.05 mm. (*b*) The content of the crystals was confirmed by dissolving crystals after washing and loading the solution onto the SDS–PAGE.

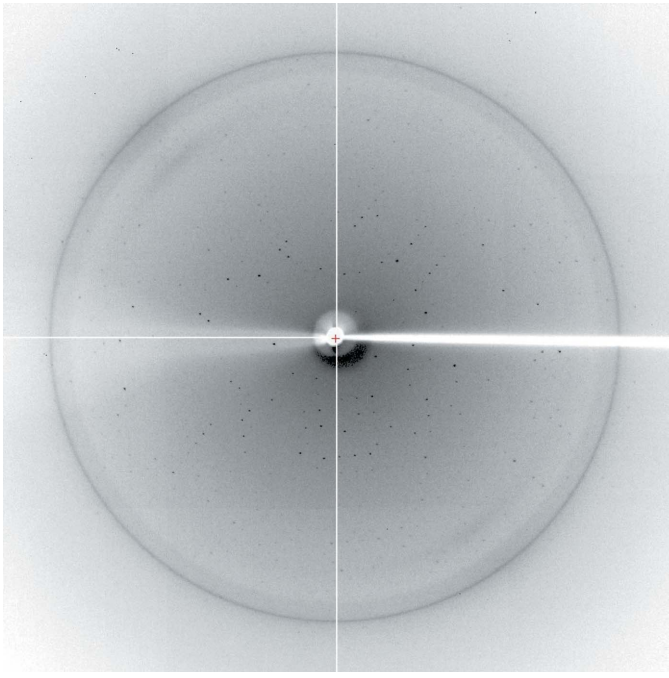

Figure 3
A diffraction image (1° oscillation) of the N-terminal domain of RPL7a with 3.5 Å resolution limit.

ammonium sulfate, 0.2 *M* sodium chloride, 0.1 *M* potassium chloride and 0.1 *M* cacodylate pH 6.3. The crystals diffracted to a resolution of 3.5 Å.

2.3. Crystallographic data collection

For data collection, the crystals were soaked for 5 s in a solution corresponding to the reservoir solution supplemented with 8%(*v*/*v*) glycerol and were then cooled in liquid nitrogen. A 3.5 Å resolution native diffraction data set was collected on beamline BL-6C at the Pohang Accelerator Laboratory (PAL), Republic of Korea. The data sets were indexed and processed using *HKL*-2000 (Otwinowski & Minor, 1997). The diffraction data are shown in Table 1.

3. Results and discussion

A two-step purification method consisting of affinity chromatography followed by gel-filtration chromatography produced homogenous N-terminal domain of human RPL7a, which was analyzed by SDS–PAGE (Fig. 1). No contaminating bands were observed upon SDS–PAGE analysis. Assuming that the RPL7a band contains ∼20 µg protein and the detection limit of SDS–PAGE is 0.1 µg, the purity of the complex was >99%. The calculated monomeric molecular weight of the N-terminal domain of human RPL7a including the C-terminal tag was 6702.9 Da and it eluted at approximately 19 ml, suggesting that it exists as a monomer in solution.

An initial needle-shaped crystal was obtained from condition No. 4 of the Wizard II kit (deCODE Biostructures Group) and diffracted poorly. Optimization of the crystallization conditions using additive screening kits (Hampton) and a range of concentrations of protein and precipitate and pH led to tetragonal diffracting crystals using 0.1 *M* potassium chloride (Fig. 2). The optimized crystals grew to dimensions of 0.1 × 0.1 × 0.05 mm in 3 d and diffracted to 3.5 Å resolution (Fig. 3). The crystals belonged to space group $P4_122$ or $P4_322$, with unit-cell parameters $a = 92.28$, $b = 92.28$, $c = 236.59$ Å.

The possible number of molecules in the crystallographic asymmetric unit is 12–22. The Matthews coefficient (V_M) was calculated to be between 1.86 Å^3 Da^{-1} (for 12 molecules in the asymmetric unit) and 3.41 Å^3 Da^{-1} (for 22 molecules in the asymmetric unit), corresponding to a solvent content of 33.89–63.94% (Matthews, 1968). Diffraction data statistics are given in Table 1. The data set was indexed and processed using *HKL*-2000 (Otwinoski & Minor, 1997). The molecular-replacement phasing method was performed using the *CNS* program (Brünger *et al.*, 1998) using the structure of lipase B (PDB code 1lbt; Uppenberg *et al.*, 1995), which is the most closely related structure to the N-terminal domain of RPL7a (19% amino-acid sequence identity), as a search model.

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