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Crystallization and preliminary X-ray analysis of PH1566, a putative ribosomal RNA-processing factor from the hyperthermophilic archaeon *Pyrococcus horikoshii* OT3

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A putative ribosomal RNA-processing factor consisting of two KH domains from *Pyrococcus horikoshii* OT3 (PH1566; 25 kDa) was crystallized by the sitting-drop vapour-diffusion method using PEG 3000 as the precipitant. The crystals diffracted X-rays to beyond 2.0 Å resolution using a synchrotron-radiation source. The space group of the crystals was determined as primitive orthorhombic $P2_12_12_1$, with unit-cell parameters a = 45.9, b = 47.4, c = 95.7 Å. The crystals contain one molecule in the asymmetric unit (V_M = 2.5 Å^3 Da^{-1}) and have a solvent content of 50%.

1. Introduction

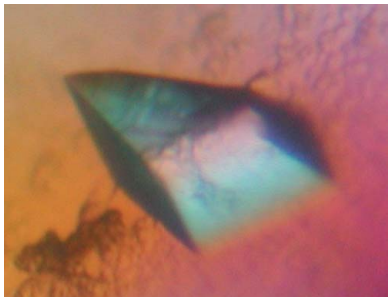
The biogenesis of ribosomal subunits in eukaryotes includes the synthesis, maturation and assembly of four ribosomal RNAs (rRNAs) and ~75 ribosomal proteins (Eichler & Craig, 1994; Kressler *et al.*, 1999; Venema & Tollervey, 1999; Lalev *et al.*, 2000). rRNAs are matured by successive endo- and exoribonucleolytic cleavages of pre-rRNAs (Kressler *et al.*, 1999; Venema & Tollervey, 1999) by rRNA-processing factors that contain one or more KH domains, which are a type of RNA-binding domain (Burd & Dreyfuss, 1994).

The rRNA-processing factor Dim2p is a core constituent of small ribosomal units in yeast nuclear 40S pre-ribosome as well as seven other major non-ribosomal proteins: Rrp12p, Tsr1p, Enp1p, Hrr25p, Nob1p, Dim1p and Rio2p (Schafer *et al.*, 2003). Dim2p is required for pre-rRNA processing at cleavage sites A_1 and A_2 and for 18S rRNA dimethylation (Vanrobays *et al.*, 2004) and its homologs have been found from archaea to metazoans. However, only one tertiary structure has been determined thus far for Dim2p-homologous proteins: the crystal structure of APE0754 from the aerobic hyperthermophilic archaeon *Aeropyrum pernix* (R. Zhang, T. Skarina, A. Savchenko, A. Edwards & A. Joachimiak, unpublished results). Here, we report the crystallization of another Dim2p homologue, PH1566 from the anaerobic hyperthermophilic archaeon *Pyrococcus horikoshii* OT3, which has 26 and 34% amino-acid sequence identity with Dim2p and APE0754, respectively.

2. Methods and results

2.1. Expression and purification

The gene of the Dim2p homologue (PH1566) was identified in the *P. horikoshii* OT3 genome, amplified by PCR and transformed into the expression vector pET-28a (Novagen). Recombinant PH1566 with an N-terminal His tag was overexpressed in *Escherichia coli* strain Rosetta (DE3) (Novagen). Cells were harvested by centrifugation, resuspended in lysis buffer (50 m*M* Tris–HCl pH 8.5, 0.3 *M* NaCl) and disrupted by sonication. The *E. coli* lysate was then incubated at 343 K for 30 min. After centrifugation, PH1566 was purified from the supernatant using Ni Sepharose 6 Fast Flow (Amersham Biosciences). After the removal of the His tag by thrombin digestion, PH1566 was further purified by two column chromatography steps: Resource Q 6-ml and Superdex 200 HR 10/30 (Amersham Biosciences).



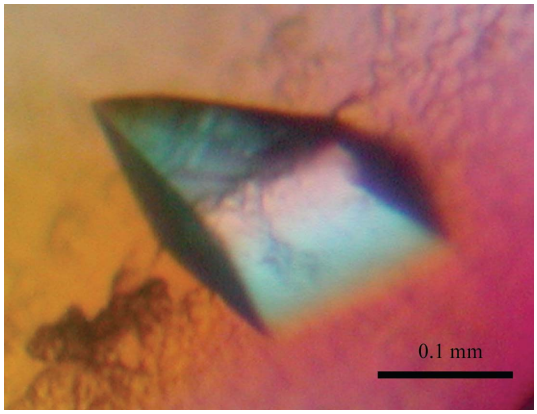


Figure 1
A crystal of PH1566 grown at 293 K using PEG 3000 as precipitant.

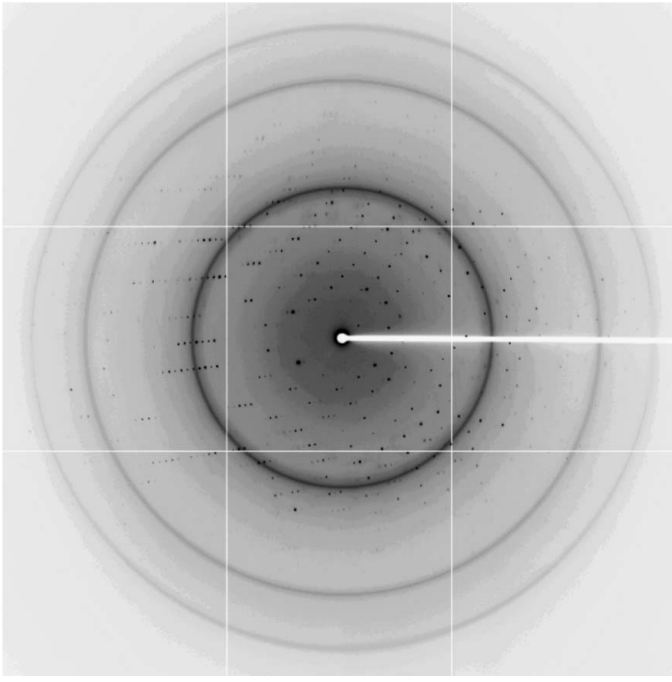


Figure 2
A diffraction image (1° oscillation) of the PH1566 crystal. The edge of the diffraction image corresponds to a resolution of 1.8 Å. The data were collected on BL41XU at SPring-8.

2.2. Crystallization

Crystallization trials were performed by the sitting-drop vapour-diffusion method using crystallization screening kits: Crystal Screens 1 and 2 (Hampton Research) and Wizard I and II (Emerald Biostructures). Crystals appeared in the presence of polyethylene glycol (PEG) 3000 as the precipitant. After refinement of the crystallization conditions, crystals suitable for X-ray analysis were obtained in one week by mixing 1.0 µl of the protein solution (20 mg ml^{-1} in 50 m*M* Tris–HCl pH 8.5, 0.05 *M* NaCl) and 1.0 µl of a reservoir solution consisting of 9%(*w*/*v*) PEG 3000, 100 m*M* cacodylate buffer pH 6.2 and 0.2 *M* $MgCl_2$. A drop was equilibrated against 500 µl reservoir solution at 293 K. Fig. 1 shows a typical crystal (0.2 × 0.1 × 0.1 mm).

Table 1
Crystal parameters of PH1566.

Values in parentheses are for the highest resolution shell.

X-ray source	SPring-8 BL41XU
Wavelength (Å)	1.000
Space group	$P2_12_12_1$
Unit-cell parameters (Å)	a = 45.9, b = 47.4, c = 95.7
Resolution range (Å)	50.0–2.00 (2.07–2.00)
Observed reflections	99494
Unique reflections	14815
Data completeness (%)	97.9 (99.2)
Redundancy	3.8 (3.5)
$R_{\rm merge}$†	0.056 (0.245)
$\langle I\rangle/\langle\sigma(I)\rangle$	39.9 (4.4)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle|/\sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the ith intensity measurement of reflection hkl, including symmetry-related reflections, and $\langle I(hkl)\rangle$ is its average.

2.3. X-ray data collection and processing

The crystal of PH1566 was picked up in a nylon loop (Hampton Research), transferred to a cryoprotective solution containing 20%(*v*/*v*) MPD, 7%(*w*/*v*) PEG 3000, 80 m*M* cacodylate buffer pH 6.2 and 0.16 *M* $MgCl_2$ and then mounted for flash-cooling at 100 K using a Rigaku cryostat. Diffraction data were collected at beamline BL41XU at SPring-8 (Harima, Japan) using an ADSC Quantum 315 detector. The wavelength was set to 1.000 Å and the crystal-to-detector distance was 250 mm. The crystals diffracted to beyond 2.0 Å resolution (Fig. 2). The diffraction data were indexed and scaled with *HKL*2000 (Otwinowski & Minor, 1997). The space group of the crystals was determined to be $P2_12_12_1$, with unit-cell parameters a = 45.9, b = 47.4, c = 95.7 Å. The crystals contain one protein molecule per asymmetric unit according to the Matthews coefficient ($V_{\rm M}$ = 2.5 Å^3 Da^{-1}; Matthews, 1968). The data statistics are given in Table 1.

Structural determination by molecular replacement using the coordinates of APE0754 (PDB code 1tua; R. Zhang, T. Skarina, A. Savchenko, A. Edwards & A. Joachimiak, unpublished results) as a search model is currently under way.

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