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Crystallization and preliminary crystallographic analysis of the central domain of *Drosophila* Dribble, a protein that is essential for ribosome biogenesis

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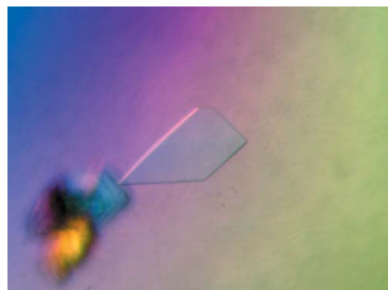

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Dribble (DBE) is a *Drosophila* protein that is essential for ribosome biogenesis. Bioinformatics analysis revealed a folded central domain of DBE which is flanked by structural disorder in the N- and C-terminal regions. The protein fragment spanning amino-acid residues 16–197 ($DBE_{16–197}$) was produced for structural determination. In this report, the crystallization and preliminary X-ray diffraction data analysis of the $DBE_{16–197}$ protein domain are described. Crystals of $DBE_{16–197}$ were grown by the sitting-drop vapour-diffusion method at 289 K using ammonium phosphate as a precipitant. The crystals belonged to space group $P2_12_12_1$. Data were collected that extended to beyond 2 Å resolution.

1. Introduction

Dribble (DBE; Gene ID 33269) is a *Drosophila* protein that represents a class of evolutionarily conserved proteins in higher eukaryotes (Chan *et al.*, 2001; Xin *et al.*, 2005; Gromadka *et al.*, 2004; Sasaki *et al.*, 2000). This class of proteins is essential for early ribosomal RNA (rRNA) processing (Chan *et al.*, 2001) and ribosome assembly (Sasaki *et al.*, 2000). It has previously been shown that the yeast orthologue of DBE, Krr1p, is a member of the small-subunit (SSU) processome (Dragon *et al.*, 2002; Bernstein *et al.*, 2004). The SSU processome is not only an early ribosome intermediate but is also actively involved in pre-rRNA processing. Having no endonuclease activity, this class of proteins is believed to play a scaffolding role in linking both the specific regions of rRNA and protein factors together for pre-rRNA cleavage. The proposed scaffolding role of this protein family is supported by the fact that DBE possesses RNA-binding affinity (Yiu *et al.*, 2006) and Krr1p interacts with a range of ribosomal proteins as well as other factors that are essential for ribosome biogenesis (Grandi *et al.*, 2002). Biophysical studies on DBE demonstrated that a protein domain exists in the central part of the protein that is flanked by structurally disordered N- and C-terminal regions (Yiu *et al.*, 2006). *DisProt* VL3H disorder-prediction analysis (Peng *et al.*, 2005) suggested that approximately amino-acid residues 20–200 are likely to be ordered. Furthermore, both Pfam (Finn *et al.*, 2008) and SUPERFAMILY (Wilson *et al.*, 2009) database searches demonstrated that DBE contains a KH-like domain that approximately spans amino-acid residues 120–200 (Yiu *et al.*, 2006). However, the structural identity of the $DBE_{20–119}$ fragment is currently unknown. A DBE protein fragment spanning amino-acid residues 16–197 was generated with the aim of studying the protein architecture of this family of proteins and, more importantly, shedding light on their molecular functions (Chan *et al.*, 2001; Xin *et al.*, 2005; Gromadka *et al.*, 2004; Sasaki *et al.*, 2000).

2. Experimental procedures

2.1. Expression and purification

A bacterial expression construct, pET-HS-$DBE_{16–197}$, encoding amino-acid residues 16–197 (20.9 kDa) of DBE was generated. The primers used were 5′-AAC TCG ACC GGT GGA GTG GAC AAT

GCG TGG TCC-3′ and 5′-AAC TCG CTC GAG TTA TCA GGG GTG CAC ATT GTT CAT-3′. pET-HS is a homemade bacterial expression vector derived from pET3d (Novagen). The DBE_{16-197} fragment carries an N-terminal His/SUMO (HS) tag that can be removed by a specific protease SENP1C derived from the SUMO-specific protease SENP1 (Xu *et al.*, 2006). The sequence of the HS tag was MRGSHHHHHHMSDQEAKPSTEDLGDKKEGEYIL-KKVIGQDSSEIHFKVKMTTHLKKLKESYCQRQGVPMNSLR-FLFEGQRIADNHTPKELGMEEEDVIEVYQEQTGG. No extra residues were left on DBE_{16-197} after proteolytic cleavage of the HS tag. *Escherichia coli* BL21 (DE3) pLysS cells transformed with the pET-HS-DBE_{16-197} construct were grown in LB medium at 310 K. Recombinant protein expression was induced by 0.1 m*M* isopropyl β-D-1-thiogalactopyranose at 295 K with shaking for 16–20 h. The cells were then harvested by centrifugation and lysed by sonication in 50 m*M* sodium phosphate pH 7.4, 300 m*M* sodium chloride, 50 m*M* imidazole and 1 m*M* phenylmethylsulfonyl fluoride. The total lysate was centrifuged at 20 000*g* for 30 min at 277 K. The supernatant was collected and loaded onto an Ni^{2+}-charged HiTrap IMAC column (GE Biosciences) equilibrated with 50 m*M* sodium phosphate pH 7.4, 300 m*M* sodium chloride, 50 m*M* imidazole and 1 m*M* phenylmethylsulfonyl fluoride. Using an ascending linear gradient of imidazole from 50 to 300 m*M* over 100 ml, the HS-DBE_{16-197} protein eluted from ∼220 m*M* imidazole onwards. The specific protease SENP1C was used at an enzyme:substrate molar ratio of 1:500 to cleave the HS tag from the DBE_{16-197} protein fragment. The reaction was carried out at room temperature for 30 min. The DBE_{16-197} fragment was released after SENP1C cleavage; this fragment was then separated from the cleaved HS tag by loading onto an Ni^{2+}-charged HiTrap IMAC column equilibrated with 50 m*M* sodium phosphate pH 7.4, 300 m*M* sodium chloride, 50 m*M* imidazole and 1 m*M* phenylmethylsulfonyl fluoride and the DBE_{16-197} fragment was collected in the flowthrough. The protein was then concentrated and loaded onto a HiLoad 26/60 Superdex 75 column (GE Biosciences) equilibrated with 50 m*M* sodium phosphate pH 7.4, 400 m*M* sodium chloride. The purified DBE_{16-197} fragment was subsequently concentrated to 10 mg ml^{-1} under the same buffering conditions as described for the Superdex 75 column purification step and subjected to protein crystallization.

2.2. Crystallization

Crystallization-condition screening was carried out using the Crystal Screen 1 and 2 and Index kits from Hampton Research.

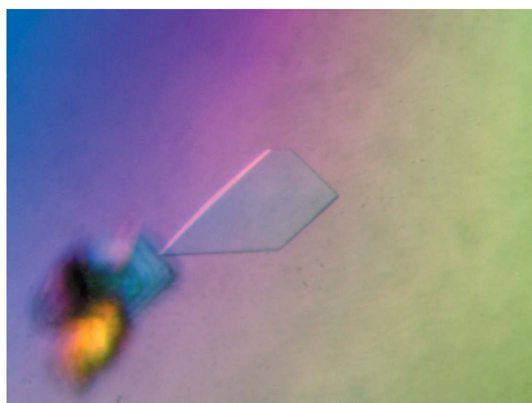

Figure 1
Protein crystals of DBE_{16-197}. Single thin crystals grew to dimensions of ∼0.3 × 0.4 × 0.02 mm after 4 d incubation.

Table 1
Data-collection and processing statistics.

Values in parentheses are for the highest resolution shell.

No. of crystals	1
Beamline	I04
Wavelength (Å)	0.9704
Detector	ADSC Q315
Crystal-to-detector distance (mm)	274.6
Rotation range per image (°)	0.5
Total rotation range (°)	280
Exposure time per image (s)	1.5
Resolution range (Å)	52.13–1.98 (2.09–1.98)
Space group	$P2_12_12_1$
Unit-cell parameters (Å, °)	a = 68.99, b = 78.76, c = 79.57
Mosaicity (°)	0.73
Total No. of measured intensities	322992
Unique reflections	30870
Multiplicity	10.5 (10.8)
Mean $I/\sigma(I)$	11.0 (3.9)
Completeness (%)	99.9 (100)
R_{merge}† (%)	9.6 (39.2)
R_{meas} or $R_{r.i.m.}$ (%)	10.1 (41.1)
Overall B factor from Wilson plot ($Å^2$)	25.5

† $R_{merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ and $\langle I(hkl)\rangle$ are the observed intensity and the mean intensity of symmetry-related reflections, respectively.

Crystallization of the DBE_{16-197} protein fragment was performed at 289 K using sitting drops consisting of 1 µl protein mixed with 1 µl reservoir solution in Greiner CrystalQuick plates. The initial crystallization condition was 0.4 *M* ammonium phosphate monobasic from Crystal Screen 1, which was then optimized by extensive grid screening. The best condition for crystal growth was found to consist of using a sitting drop comprising 2 µl protein solution and 4 µl precipitant solution equilibrated against 500 µl well solution consisting of 0.6 *M* ammonium phosphate pH 4.8 at 289 K. Crystals often grew as stacked plates; occasional single plates of dimensions ∼0.3 × 0.4 × 0.02 mm were obtained after 4 d incubation (Fig. 1).

2.3. Preliminary X-ray diffraction data analysis

Crystals were cryoprotected by soaking them in crystallization solution containing 30%(*v*/*v*) ethylene glycol for 1 min. X-ray diffraction data were collected on beamline I04 at the Diamond Light Source (UK) at 100 K using an ADSC Q315 detector. 720 images were collected using 0.5° oscillations at a wavelength of 0.9704 Å. Data processing was performed using *MOSFLM* (Leslie, 2006) and *SCALA* (Evans, 2006) *via CCP4i* (Potterton *et al.*, 2003). Pseudo-precession images revealed *mmm* Laue symmetry and reflection conditions $h = 2n$, $k = 2n$, $l = 2n$, suggesting that the space group was $P2_12_12_1$. The unit-cell parameters were $a = 68.99$, $b = 78.76$, $c = 79.57$ Å. Matthews coefficient (V_M; Matthews, 1968) analysis suggested that each asymmetric unit contained two protein molecules, giving a V_M value of 2.58 $Å^3$ Da^{-1} and a solvent content of 52.4%. Diffraction data were integrated and scaled to a maximum resolution of 1.98 Å. Statistics of data processing are summarized in Table 1. Attempts at phasing by multiple anomalous dispersion (MAD) using selenomethionine-substituted protein are currently under way.

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