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Cloning, recombinant production, crystallization and preliminary X-ray diffraction analysis of SDF2-like protein from *Arabidopsis thaliana*

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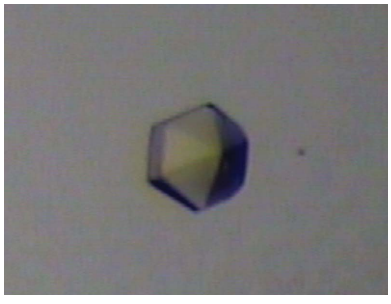
The stromal-cell-derived factor 2-like protein of *Arabidopsis thaliana* (AtSDL) has been shown to be highly up-regulated in response to unfolded protein response (UPR) inducing reagents, suggesting that it plays a crucial role in the plant UPR pathway. AtSDL has been cloned, overexpressed, purified and crystallized using the vapour-diffusion method. Two crystal forms have been obtained under very similar conditions. The needle-shaped crystals did not diffract X-rays, while the other form diffracted to 1.95 Å resolution using a synchrotron-radiation source and belonged to the hexagonal space group $P6_1$, with unit-cell parameters $a = b = 96.1$, $c = 69.3$ Å.

1. Introduction

The unfolded protein response (UPR) is a conserved mechanism by which all eukaryotic cells preserve homeostasis of the endoplasmic reticulum (ER) during ER stress conditions (Ron & Walter, 2007; Vitale & Boston, 2008). SDF2-type proteins [SDF2 and SDF2-like 1 (SDF2L1) of 23 and 23.6 kDa, respectively] have been identified in mouse and humans (Hamada *et al.*, 1996; Fukuda *et al.*, 2001). The expression of SDF2L1 has been reported to be significantly up-regulated in the response to ER stress-inducing agents (Fukuda *et al.*, 2001). Mouse SDF2L1 interacts with ERdj3, an ER Hsp40 co-chaperone, and associates with other molecular chaperones and folding enzymes to form a multiprotein complex in the ER even in the absence of high amounts of misfolded proteins (Bies *et al.*, 2004; Meunier *et al.*, 2002). In *Arabidopsis thaliana* a single SDF2-type protein (AtSDF2-like; AtSDL; gi:18400721) of 23.9 kDa (218 residues) is present. Expression of the AtSDF2-like gene has also been shown to be up-regulated in response to UPR-inducing reagents (Martinez & Chrispeels, 2003; Kamauchi *et al.*, 2005), suggesting a conserved function in the UPR of animals and plants.

SDF2-type proteins contain an N-terminal signal peptide and three motifs referred to as MIR motifs (Hamada *et al.*, 1996; Fukuda *et al.*, 2001). MIR motifs are named after three of the protein families in which they occur: protein *O*-**m**annosyltransferases (PMT), **i**nositol 1,4,5-triphosphate receptors (IP_3R) and **r**yanodine receptors (RyR) (Ponting, 2000). Although they are mainly arranged in several copies, these ∼50-residue sequences have been proposed to be independent structural units that provide a surface necessary for the function, interaction and regulation of the proteins listed above (Ponting, 2000). To date, structural information for MIR-motif-containing proteins is only available for IP_3R (Bosanac *et al.*, 2002, 2005) and for a protein of unknown function from *Caenorhabditis elegans* (PDB code 1t9f; J. Symersky, S. Li, R. Bunzel, N. Schormann, D. Luo, W. Y. Huang, S. Qiu, R. Gray, Y. Zhang, A. Arabashi, S. Lu, C. H. Luan, J. Tsao, L. DeLucas & M. Luo, unpublished work). Although the three-dimensional structures revealed that MIR motifs fold into a β-trefoil domain, their precise function has still to be unravelled.

In order to provide insight into the functional role of SDF2-type proteins and the MIR motifs, we set out to determine the three-dimensional structure of AtSDL by X-ray crystallography. Here, we report the cloning, expression, purification, crystallization and preliminary crystallographic analysis of native AtSDL.



2. Material and methods

2.1. Cloning and protein expression

The AtSDL coding region (base pairs +70 to +657) was amplified by polymerase chain reaction (PCR) from the full-length cDNA clone U14608 (Arabidopsis Biological Resource Center; Yamada *et al.*, 2003) using primers 790, 5′-GACAGATCTGCCTCCGCCGCTGCTTC-3′, and 791, 5′-CAGCTCGAGCTATTACTTGCTGCTCTCATTAAGGG-3′. The PCR product was digested with *Bgl*II and *Xho*I, cloned into the *Bam*HI and *Xho*I sites of vector pGEX-6P-1 (GE Healthcare) and analysed by sequence analysis. The resulting construct encodes an in-frame fusion of a GST tag followed by a PreScission protease-cleavage site at the N-terminus of the AtSDL protein (residues 24–218) devoid of its N-terminal signal sequence. The recombinant protein was expressed in *Escherichia coli* strain BL21 (DE3). Cells were grown for 3 h at 310 K in LB (lysogeny broth) medium containing ampicillin (100 mg l^{-1}). Protein expression was induced using 0.4 m*M* isopropyl *β*-D-1-thiogalactopyranoside (IPTG) and took place overnight at 291 K. Cells were harvested by centrifugation for 30 min at 3500*g* and 277 K and were frozen at 193 K.

2.2. Purification

Cell pellets were resuspended in lysis buffer containing 300 m*M* NaCl, 50 m*M* Tris pH 8.0, 0.1 m*M* EDTA, 1 m*M* phenylmethylsulfonyl fluoride (PMSF) and 1 m*M* benzamidine and passed through an M-110L Microfluidizer (Microfluidics). The cell lysate was centrifuged for 30 min at 23 000*g* and 277 K. Subsequently, the supernatant was passed through a 0.45 µm filter and applied onto a 1 ml GSTrap HP column (GE Healthcare). The GSTrap HP column was washed with 15 column volumes of lysis buffer and the protein was eluted in lysis buffer containing 20 m*M* glutathione and 1 m*M* dithiothreitol (DTT). The protein solution was dialysed against PreScission protease-cleavage buffer (150 m*M* NaCl, 50 m*M* Tris pH 7.5, 1 m*M* EDTA, 1 m*M* DTT). The GST moiety was then cleaved by GST-tagged PreScission protease (GE Healthcare) digestion for 10 h at 277 K and AtSDL was released with four additional residues (GPLG) at the N-terminus. To remove the affinity tag and the PreScission protease, the protein solution was loaded onto two coupled 1 ml GSTrap HP columns and the flowthrough was collected. The fractions containing AtSDL were pooled and further purified on a Superdex 200 16/60 size-exclusion column (GE Healthcare) equilibrated in 150 m*M* NaCl, 10 m*M* Tris pH 7.5. Protein-containing fractions were pooled and analysed by 12% Coomassie-stained polyacrylamide gel electrophoresis (SDS–PAGE). The protein was immediately used for crystallization experiments.

2.3. Crystallization

Prior to crystallization trials, the protein was concentrated to a final concentration of 12.8 mg ml^{-1} (in 150 m*M* NaCl, 10 m*M* Tris pH 7.5) in an Amicon Ultrafree-MC, VV 0.1 µm (Millipore).

Initial crystallization trials as well as optimization of the crystallization conditions were performed using custom screens and the hanging-drop vapour-diffusion method in 24-well plates (Greiner) with 500 µl reservoir solution and 2 µl drops set up using a 1:1 ratio of protein to reservoir solution. Prior to X-ray analysis, crystals were briefly soaked in a cryoprotectant solution consisting of the reservoir solution supplemented with 20%(*v*/*v*) ethylene glycol. Crystals were flash-cooled in liquid nitrogen prior to diffraction experiments.

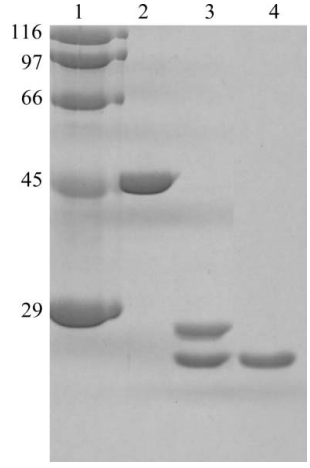
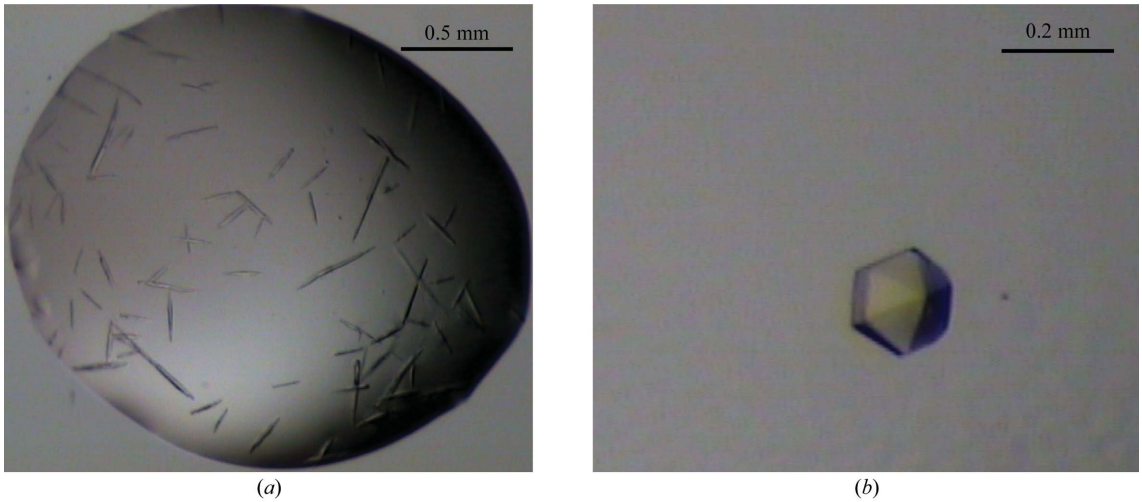


Figure 1
Purification of AtSDL. The samples were analyzed on Coomassie-stained SDS–PAGE. Lane 1, high-molecular-weight standards (labelled in kDa); lane 2, GST-AtSDL fusion protein (48 kDa); lane 3, GST-tag (26 kDa) and AtSDL (22 kDa) after protease cleavage; lane 4, AtSDL after size-exclusion chromatography.



(*a*) (*b*)

Figure 2
Typical crystals of AtSDL grown in (*a*) 0.1 *M* HEPES pH 7.7, 0.2 *M* ammonium sulfate and 20%(*w*/*v*) PEG 3350 and (*b*) 0.1 *M* HEPES pH 7.7, 0.2 *M* ammonium sulfate and 22.5%(*w*/*v*) PEG 3350.

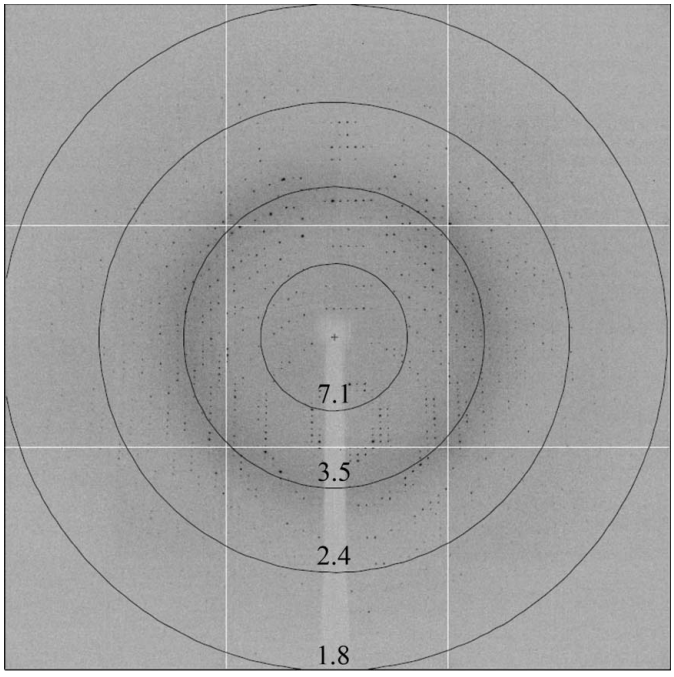


Figure 3
Diffraction pattern of AtSDL protein crystals. A 0.9° image is shown (crystal-to-detector distance of 245 mm). The resolution ranges specified by the rings are given in angstroms.

2.4. Data collection and phasing

Diffraction data were collected under cryogenic conditions (100 K; Oxford Cryosystems Cryostream) using an ADSC Q315R CCD detector on beamline ID23-eh1 at the European Synchrotron Radiation Facility (ESRF) in Grenoble, France. Data were processed with the program *MOSFLM* and scaled with *SCALA* from the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994).

3. Results and discussion

The region encompassing the three MIR motifs of the AtSDF2-like protein (residues 24–218) was cloned as a GST-fusion protein and overexpressed in BL21 (DE3) cells. After glutathione-affinity purification, digestion with PreScission protease and subsequent size-exclusion chromatography, the protein was almost pure as judged by Coomassie-stained SDS–PAGE, with AtSDL appearing as a single band at 22 kDa (Fig. 1). Size-exclusion chromatography revealed a homogeneous monomeric protein solution (data not shown).

The purified AtSDL was subjected to crystallization trials. Crystals appeared after one week at 293 K from reservoir solutions containing 0.1 *M* HEPES pH 7.7, 0.2 *M* ammonium sulfate and 20% or 22.5%(*w*/*v*) PEG 3350 (Fig. 2). Crystals grown in 20%(*w*/*v*) PEG 3350 had a needle-like shape and did not reveal any X-ray diffraction. Interestingly, crystals grown in the same buffer but in the presence of 22.5%(*w*/*v*) PEG 3350 had a hexagonal shape with typical dimensions of 100 × 100 × 30 µm and diffracted X-rays to 1.95 Å resolution. They belonged to the hexagonal space group $P6_1$, with unit-cell parameters $a = b = 96.1$, $c = 69.3$ Å. A complete data set was collected on ID23-eh1 at ESRF with an oscillation increment of 0.9° (over a total of 90°; Fig. 3). Data-collection statistics are summarized in Table 1.

Table 1
Data-collection statistics.

Values in parentheses are for the highest resolution shell.

Synchrotron-radiation source	ID23-eh1, ESRF
Wavelength (Å)	1.00
Space group	$P6_1$
Unit-cell parameters (Å)	$a = b = 96.1$, $c = 69.3$
Total No. of reflections	145886 (21463)
No. of unique reflections	26715 (3892)
Resolution (Å)	53.30–1.95 (2.06–1.95)
Completeness (%)	100 (100)
$\langle I/\sigma(I)\rangle$	14.0 (3.7)
$R_{\rm merge}$† (%)	11.6 (38.0)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$.

The Matthews coefficient and solvent content, which were estimated as 2.1 Å^3 Da^{-1} and 41.5%, respectively, are consistent with the presence of two monomers in the asymmetric unit (Matthews, 1968).

Molecular replacement was carried out with *MOLREP* (Collaborative Computational Project, Number 4, 1994) using PDB entry 1t9f, which displays 37% sequence identity with AtSDL, as a search model. A unique solution which located two AtSDL protein molecules in the asymmetric unit was obtained with a correlation coefficient of 39.8% and an *R* factor of 52.4% using diffraction data in the resolution range 53.30–2.5 Å. Refinement of the structure is currently in progress.

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