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Crystallization and preliminary X-ray data analysis of a DJ-1 homologue from *Arabidopsis thaliana* (*At*DJ-1D)

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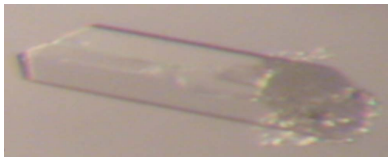
A DJ-1 homologue protein from *Arabidopsis thaliana* (*At*DJ-1D) belongs to the DJ-1/ThiJ/Pfpl superfamily and contains two tandem arrays of DJ-1-like sequences, but no structural information is available to date for this protein. *At*DJ-1D was expressed in *Escherichia coli*, purified and crystallized for structural analysis. A crystal of *At*DJ-1D was obtained by the hanging-drop vapour-diffusion method using 0.22 *M* NaCl, 0.1 *M* bis-tris pH 6.5, 21% polyethylene glycol 3350. *At*DJ-1D crystals belonged to the monoclinic space group $P2_1$, with unit-cell parameters $a = 56.78$, $b = 75.21$, $c = 141.68$ Å, $\beta = 96.87^\circ$, and contained a trimer in the asymmetric unit. Diffraction data were collected to 2.05 Å resolution. The structure of *At*DJ-1D has been determined using the multiple-wavelength anomalous dispersion (MAD) method.

1. Introduction

The DJ-1/ThiJ/Pfpl superfamily includes a broad spectrum of proteins from most organisms which are characterized by having a highly conserved DJ-1 domain. DJ-1 (also known as PARK7) was first identified as a novel oncogene in mouse (Nagakubo *et al.*, 1997) and was later identified as an autosomal recessive gene of Parkinson's disease (PD; Bonifati *et al.*, 2003; Wilson *et al.*, 2003; Mo *et al.*, 2008). Human DJ-1 is known to be involved in many different biochemical functions such as oncogenesis (Nagakubo *et al.*, 1997), neuroprotection (Park *et al.*, 2005; Kim *et al.*, 2005), male fertility (Okada *et al.*, 2002), control of protein–RNA interactions (van der Brug *et al.*, 2008) and modulation of androgen receptor transcription activity (Takahashi *et al.*, 2001; Tillman *et al.*, 2007). Furthermore, DJ-1 possesses protease and chaperone activities (Olzmann *et al.*, 2004; Shendelman *et al.*, 2004) and plays an established role in cellular protection against oxidative stress (Canet-Avilés *et al.*, 2004; Gu *et al.*, 2009; Kim *et al.*, 2005; Martinat *et al.*, 2004; Meulener *et al.*, 2006). Other members of the DJ-1/ThiJ/Pfpl superfamily from other organisms have been also characterized. However, their precise biochemical functions have not been as fully studied as those of the human homologue.

In the model plant *Arabidopsis thaliana*, six genes encoding homologues of human DJ-1 have been identified: *At*3g14990 (*At*DJ-1A), *At*1g53280 (*At*DJ-1B), *At*4g34020 (*At*DJ-1C), *At*3g02720 (*At*DJ-1D), *At*2g38860 (*At*DJ-1E) and *At*3g54600 (*At*DJ-1F). Of these plant DJ-1 homologues, *At*DJ-1A and *At*DJ-1C have recently been characterized. *At*DJ-1A carries the functionally conserved DJ-1 amino-acid residues associated with PD conditions and confers protection against oxidative stress by cytosolic superoxide dismutase activation (Xu *et al.*, 2010). In contrast, *At*DJ-1C, which does not have the highly conserved cysteine, is a chloroplast-targeted protein and is essential for chloroplast development (Lin *et al.*, 2011). No functional information on the other plant homologue proteins is available to date.

Interestingly, these plant DJ-1 homologues carry two full-length DJ-1 polypeptides arranged in tandem. Considering that members of the DJ-1/ThiJ/Pfpl superfamily in bacteria and mammals contain only one DJ-1 domain (Wei *et al.*, 2007) and form a dimer [or sometimes higher oligomers; Wilson *et al.*, 2005; Canet-Avilés *et al.*, 2004; Du *et*



al., 2000; YhbO from *Escherichia coli* (B. Claude, C. Abergel & J.-M. Claverie, unpublished work; PDB entry 1oi4)], these plant homologues have a unique structure of two DJ-1 domains in a tandem array, which distinguishes them from the other DJ-1 superfamily members. It seems that this oligomerization is important for function (Wei *et al.*, 2007; Wilson *et al.*, 2005). In order to understand their unique structural arrangement of two DJ-1 domains in tandem, one of these six plant DJ-1 homologues, *At*DJ-1D (*At*3g02720), which has 25% sequence identity to human DJ-1, was selected for structural study. Here, we report the overexpression from *Escherichia coli*, purification and crystallization of *At*DJ-1D. A crystal of *At*DJ-1D diffracted to 2.05 Å resolution and the structure has been solved by the multiple-wavelength anomalous dispersion (MAD) method.

2. Materials and methods

2.1. Protein expression and purification

First-strand *At*DJ-1D cDNA was prepared *via* reverse transcriptase polymerase chain reaction using SuperScript II RT (Invitrogen Inc., Burlington, Ontario, Canada). Primers were designed using the forward primer 5′-GCGGATCCATGGCGAACTCGAGAACTG-TC-3′ and the reverse primer 5′-GCGTCGACTTAGAATGAAAC-CTGAATGCC-3′. The PCR product was digested with *Bam*HI and *Sal*I restriction enzymes and ligated into the *Bam*HI and *Sal*I restriction sites of a pET-28a expression plasmid (Novagen, Madison, Wisconsin, USA) to produce recombinant *At*DJ-1D with a His tag and a T7 tag at the N-terminus (MGSSHHHHHHSSGLVPRGS-HMNSMTGGQQMGRGS). The DNA sequence of the inserted *At*DJ-1D was confirmed by DNA sequencing. The calculated molecular weight of the tagged monomer was 45.2 kDa and the theoretical isoelectric point was 5.8.

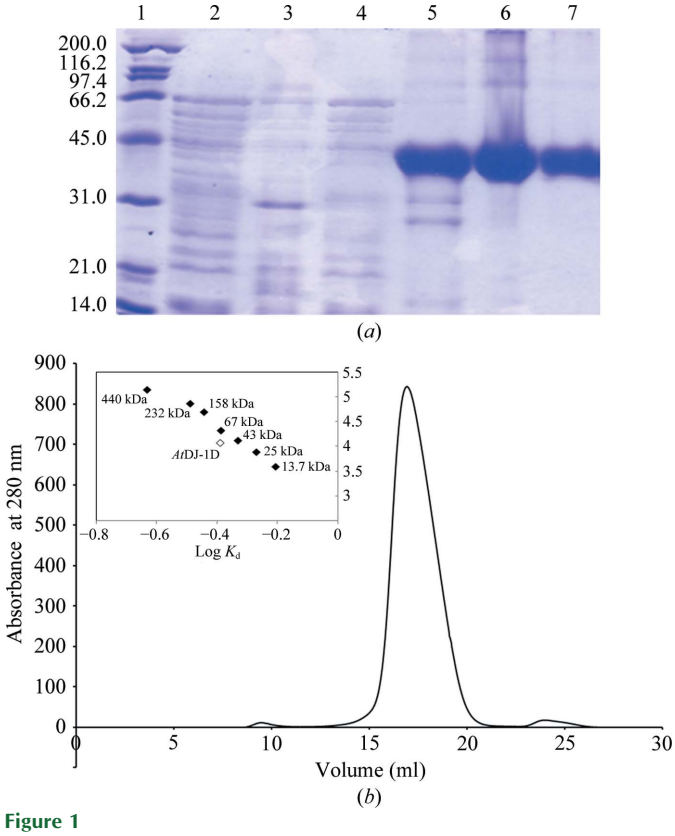


Figure 1
(*a*) SDS–PAGE analysis of *At*DJ-1D at different steps of the purification process. Lane 1, marker (labelled in kDa); lane 2, total cell lysate; lane 3, supernatant after cell lysis; lane 4, pellet after cell lysis; lane 5, proteins from an Ni–NTA affinity column; lane 6, proteins separated after anion-exchange chromatography; lane 7, proteins separated after Superdex 200 chromatography (8.5 µg protein used). (*b*) Superdex 200 size-exclusion chromatography of *At*DJ-1D. A single peak corresponding to a monomer was observed. The scale at the bottom indicates the elution volume. Inset, semi log plot of the molecular mass of the standard proteins used *versus* their log K_d values.

The plasmid was transformed into *Escherichia coli* strain BL21 (DE3) for protein expression. A 10 ml aliquot of an overnight culture was seeded into 1000 ml fresh M9 minimal medium (6 g Na_2HPO_4, 3 g KH_2PO_4, 1 g NH_4Cl, 0.5 g NaCl, 2 g glucose, 2 m*M* $MgSO_4$ and 0.1 m*M* $CaCl_2$ per litre of solution) containing 30 mg ml^{-1} kanamycin and grown to an OD_{600} of 0.6 at 303 K. The protein was expressed at 288 K for 3 d with 1 m*M* isopropyl *β*-D-1-thiogalactopyranoside (IPTG). Cells were harvested by centrifugation at 6520*g* for 6 min at 277 K, suspended in binding buffer (50 m*M* NaH_2PO_4, 0.5 *M* NaCl, 5 m*M* imidazole and 5 m*M* *β*-mercaptoethanol per litre of solution, pH 8.0) and disrupted by sonication. The crude lysate was centrifuged at 15 930*g* for 1 h at 277 K. The clear supernatant was then filtered (Qualitative filter paper, Advantec) and applied onto a column of Nickel Sepharose 6 Fast Flow (GE Healthcare, Sweden) pre-equilibrated with the binding buffer. The column was washed first with 20 column volumes of binding buffer and then with two column volumes of washing buffer (50 m*M* Tris–HCl pH 8.0, 0.5 *M* NaCl, 20 m*M* imidazole, 5 m*M* *β*-mercaptoethanol). The recombinant *At*DJ-1D was eluted with 50 m*M* Tris–HCl pH 8.0, 0.1 *M* NaCl, 0.3 *M* imidazole and 5 m*M* *β*-mercaptoethanol. Fractions containing *At*DJ-1D were pooled, concentrated and exchanged into 50 m*M* Tris–HCl pH 8.0, 10% glycerol and 1 m*M* DTT by ultrafiltration (Centriprep YM-30, Millipore Corporation, Bedford, Massachusetts, USA). The *At*DJ-1D was further purified by anion-exchange chromatography on a Resource 15Q column (GE Healthcare, Piscataway, New Jersey, USA) and eluted with a linear gradient of 0–500 m*M* NaCl in 50 m*M* Tris–HCl pH 8.0, 10% glycerol and 1 m*M* DTT. The fractions of the major peak were concentrated by ultrafiltration (Centriprep YM-30, Millipore Corporation, Bedford, Massachusetts, USA). The *At*DJ-1D was finally purified by Superdex 200 size-exclusion chromatography (GE Healthcare, Piscataway, New Jersey, USA) equilibrated with 20 m*M* Tris–HCl pH 8.0, 150 m*M* NaCl, 10% glycerol and 1 m*M* DTT. *At*DJ-1D eluted as a monomer and was sufficiently pure for crystallization as judged by SDS–PAGE (Fig. 1). The fractions were pooled and concentrated to a final concentration of 8.5 mg ml^{-1} in 20 m*M* Tris–HCl pH 8.0, 10% glycerol and 1 m*M* DTT by ultrafiltration (Microcon YM-30, Millipore Corporation, Bedford, Massachusetts, USA). All purification steps were carried out at 293 K with ice-cooled buffers. The protein concentration was determined by the Bradford assay using bovine serum albumin as a standard. The N-terminal His tag and T7 tag were not removed for crystallization.

2.2. Crystallization

For crystallization trials of *At*DJ-1D, Crystal Screen, Crystal Screen 2 and Index (Hampton Research, California, USA) and Wizard Screens I and II and Cryo I and II (Emerald BioStructures, Bainbridge Island, Washington, USA) and laboratory solutions were used. Crystallization was set up in 72-well plates at 291 K using the microbatch method under Al's oil (D'Arcy *et al.*, 2003). For each screen, equal volumes (1 µl) of protein solution (8.5 mg ml^{-1}) and reservoir solution were mixed and equilibrated under the oil. Crystals were initially produced in the condition Index solution No. 71 (0.2 *M* NaCl, 0.1 *M* bis-tris pH 6.5, 25% polyethylene glycol 3350).

Table 1
Data statistics.

Values in parentheses are for the highest resolution shell.

	SeMet derivative		
	Peak	Edge	Remote
Space group	$P2_1$		
Unit-cell parameters (Å, °)	a = 56.78, b = 75.21, c = 141.68, β = 96.87		
No. of chains in the asymmetric unit	3		
V_M (Å^3 Da^{-1})	2.38		
Solvent content (%)	48.43		
Wavelength (Å)	0.97954	0.97955	0.97186
Resolution (Å)	2.05 (2.17–2.05)	2.05 (2.12–2.05)	2.07 (2.14–2.07)
Unique reflections	73962 (6948)	73914 (6926)	73914 (6926)
Completeness (%)	99.2 (94.1)	99.1 (93.8)	99.5 (93.8)
R_{merge}† (%)	0.140 (0.497)	0.166 (0.733)	0.131 (0.510)
Multiplicity	3.6 (3.4)	3.6 (3.4)	3.6 (3.4)
$\langle I/\sigma(I)\rangle$	6.1 (4.6)	5.9 (4.1)	5.9 (4.1)
Figure of merit	0.45‡/0.80§		

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

Further screening to find optimal crystallization conditions was performed by hanging-drop vapour-diffusion trials, varying the salt and precipitant concentrations and the volume of the drop. The best crystals were grown at 291 K using *At*DJ-1D protein (8.5 mg ml^{-1}) in 20 m*M* Tris–HCl pH 8.0, 10% glycerol and 1 m*M* DTT and reservoir solution consisting of 0.22 *M* NaCl, 0.1 *M* bis-tris pH 6.5, 21% polyethylene glycol 3350. The drop consisted of 3 µl protein solution and 4 µl of a mixture of 4 µl reservoir solution and 1 µl Index Screen solution No. 44 (0.1 *M* urea) as an additive. Rod-shaped *At*DJ-1D crystals appeared after 2–3 d and grew to maximum dimensions of 0.4 × 0.3 × 0.2 mm (Fig. 2).

2.3. X-ray data collection

Before data collection, crystals were transferred for about 30 min into a cryosolution consisting of mother liquor supplemented with 15% ethylene glycol. The crystals were flash-cooled in liquid nitrogen. X-ray diffraction data were collected on beamline 6C1 of Pohang Accelerator Laboratory, Republic of Korea using an ADSC Quantum 210 CCD detector. For multiple-wavelength anomalous dispersion (MAD), three data sets were collected at wavelengths of 0.97954 Å (peak), 0.97955 Å (edge) and 0.98736 Å (remote) at 100 K. 180° of data were collected at each wavelength. All diffraction images were indexed, integrated and scaled using the *HKL*-2000 suite (Otwinowski & Minor, 1997). Data statistics are shown in Table 1. The positions of Se atoms were determined using *SOLVE* (Terwilliger & Berendzen, 1999). Density modification was performed with *RESOLVE* (Terwilliger, 2001).

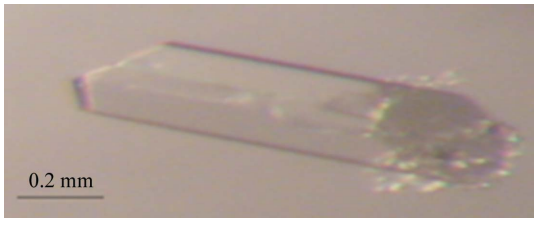


Figure 2
Crystal of *At*DJ-1D obtained by the hanging-drop method.

3. Results

We have established the expression, purification and crystallization of *At*DJ-1D. We collected a 2.05 Å resolution MAD data set at three wavelengths (peak, 0.97954 Å; edge, 0.97955 Å; remote, 0.98736 Å). Indexing indicated that the crystals of *At*DJ-1D belonged to the monoclinic space group $P2_1$, with unit-cell parameters a = 56.78, b = 75.21, c = 141.68 Å, β = 96.87° (Table 1). A solvent content of 48.4% and a Matthews coefficient V_M of 2.38 Å^3 Da^{-1} (Matthews, 1968) would indicate that the asymmetric unit contains three molecules. Phases were determined from the positions of 17 Se atoms located in *At*DJ-1D that were found from a total of 30 Se atoms (18 in *At*DJ-1D and 12 in the fusion tag) using *SOLVE* and *RESOLVE*. The structure of *At*DJ-1D was determined by the multiple-wavelength anomalous dispersion (MAD) method. The molecular weight of the protein was estimated to be about 45 kDa from Superdex 200 size-exclusion chromatography and that of the monomer of *At*DJ-1D was found to be approximately 45 kDa from SDS–PAGE, indicating that the protein exists as a monomer in solution (Fig. 1). However, *At*DJ-1D forms a trimer in the asymmetric unit in which the three monomers are related by noncrystallographic symmetry. It is not clear that the trimerization of *At*DJ-1D is related to its biological function. A detailed discussion of the refined structure will be published elsewhere.

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