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Crystallization and preliminary X-ray characterization of a novel calcium-binding protein AtCBL2 from *Arabidopsis thaliana*

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A new family of calcineurin B-like calcium-binding proteins has recently been identified in *Arabidopsis thaliana*. AtCBL2, a member of this family, has been crystallized in the presence of calcium ions using polyethylene glycol as a precipitant at 293 K. The crystals belong to space group $C222_1$, with unit-cell parameters a = 83.9, b = 118.1, c = 49.1 Å. The asymmetric unit contains one molecule, with a V_M of 2.36 Å^3 Da^{-1} and a solvent content of 48%. Native diffraction data to 2.1 Å resolution have been collected using synchrotron radiation at SPring-8.

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1. Introduction

Calcium ions play a crucial role as a second messenger in intracellular signalling and control a wide range of cellular processes. In plants, many extracellular stimuli such as light, drought and high salt concentrations elicit changes in the cytosolic calcium concentration (Bush, 1995; Trewavas & Malho, 1998).

Arabidopsis thaliana calcineurin B-like protein (AtCBL) is a novel calcium-binding sensor protein in plants and shows sequence similarity to both the regulatory subunit of the protein phosphatase calcineurin (calcineurin B) and the neuronal Ca^{2+} sensor (NCS) in animals (Liu & Zhu, 1998; Kudla *et al.*, 1999). The protein family has multiple EF-hand motifs for Ca^{2+} binding and interacts with a group of serine/threonine protein kinases called AtCIPKs (AtCBL-interacting protein kinases; Shi *et al.*, 1999; Halfter *et al.*, 2000). The C-terminal non-kinase domain of AtCIPK has a region that is conserved among different AtCIPK members and interacts with AtCBL (Albrecht *et al.*, 2001).

AtCBLs are encoded by a multigene family of at least ten members in *Arabidopsis* that have similar structural domains with small variations in the length of the coding regions and sequence identities ranging from 20 to 90% (Luan *et al.*, 2002). Different members of the AtCBL gene family play various roles in plant signal transduction processes in response to different stress conditions. AtCBL1 is highly inducible by drought, cold and wounding (Kudla *et al.*, 1999), and AtCBL4 or salt overly sensitive 3 (SOS3) is responsive to salt resistance (Liu & Zhu, 1998). The AtCBL4–AtCIPK24 (SOS2) complex activates the plasma membrane Na^+-transporter SOS1 through phosphorylation (Qiu *et al.*, 2002; for a review, see Zhu, 2002). In addition, SCaBP5 (AtCBL1)–PKS3 (AtCIPK15) is shown to be a negative regulator of abscisic acid signalling (Guo *et al.*, 2002).

The expression profile of AtCBL2 in response to light suggests that AtCBL2 is involved in light transduction in *Arabidopsis*. AtCBL2 strongly interacts with AtSR1 (AtCIPK14; Nozawa *et al.*, 2001) induced by light stimuli (Chikano *et al.*, 2001). Despite the great importance of intracellular signalling pathways in plants, no structural information has been obtained for the AtCBL/AtCIPK system. Here, we report the crystallization and preliminary crystallographic study of AtCBL2 as the first step toward elucidating the AtCBL/AtCIPK signalling mechanism in atomic detail.

2. Methods and results

2.1. Expression and purification

Recombinant AtCBL2 from *A. thaliana* was expressed in *Escherichia coli* BL21(DE3) pLysS cells harbouring plasmid pDEST15 (Lifetech) as a fusion protein with glutathione *S*-transferase (GST). The cells were disrupted by sonication at 277 K. The supernatant was applied onto a GST affinity column of glutathione Sepharose 4B (Pharmacia Biotech) and then washed with 50 m*M* Tris–HCl buffer pH 7.5 containing 100 m*M* NaCl, 1 m*M* $CaCl_2$ and 1 m*M* dithiothreitol (DTT). The fusion protein absorbed to the resin was cleaved by human α-thrombin (Funakoshi) at 2 units per millilitre for 14 h at 277 K. The cleaved proteins were collected for further purification by two column-chromatographic steps using HiTrap Q and Superdex 75 (Pharmacia Biotech). The purified protein was finally obtained as a single

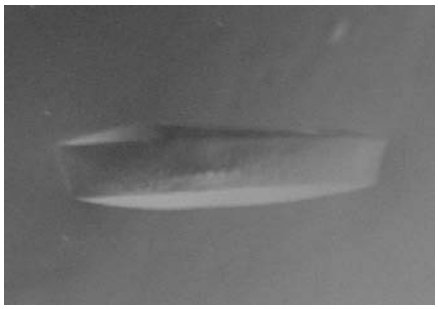

Figure 1
A crystal of AtCBL2. Its approximate dimensions are 0.1 × 0.1 × 0.5 mm.

band stained with Coomassie brilliant blue in SDS–PAGE.

2.2. Crystallization

Purified protein in 5 m*M* Tris–HCl buffer pH 7.5 containing 100 m*M* NaCl, 1 m*M* DTT and 1 m*M* $CaCl_2$ was concentrated to about 6 mg ml^{-1} in a Centricon-10 concentrator (Amicon) at 277 K. All crystallization experiments were carried out with the hanging-drop vapour-diffusion method using 24-well tissue-culture plates (Sumitomo Bakelite Co.). Preliminary crystallization conditions were established using Hampton Research Crystal Screen kits 1 and 2 and PEG/Ion screen at 277 and 293 K. Initial crystals were obtained using PEG/Ion screen solution No. 4, containing 0.2 *M* lithium chloride and 20%(*w*/*v*) polyethylene glycol (PEG) 3350, at 293 K. Thin plate-like crystals grew within a few days. Optimization of the condition to 0.1 *M* Na MES buffer pH 6.0, 0.2 *M* lithium chloride and 10% PEG 8000 greatly improved the quality and the size of the crystals. Finally, crystals suitable for high-resolution X-ray crystallographic analysis were obtained at 293 K in a few days (Fig. 1) when drops containing equal volumes (1.5 µl) of protein (6 mg ml^{-1}) and reservoir solution were equilibrated against 0.4 ml reservoir solution. Crystals were soaked in the crystallization buffer with 20% glycerol as a cryoprotectant.

Table 1
Data-collection statistics.

Values in parentheses are for the highest resolution shell.

X-ray source	SPring-8 BL45XU
Wavelength (Å)	1.0200
Space group	$C222_1$
Unit-cell parameters (Å)	a = 83.9, b = 118.1, c = 49.1
Resolution range (Å)	39.9–2.1 (2.17–2.10)
$R_{\rm merge}$† (%)	6.0 (28.6)
Average $I/\sigma(I)$	7.9 (2.4)
No. of observations	80055
No. of unique reflections	14502
Data completeness (%)	98.8 (98.8)
Redundancy	5.5 (5.3)
Crystal mosaicity (°)	0.5

† $R_{\rm merge} = \sum_h \sum_i |I(h)_i - \langle I(h)\rangle| / \sum_h \sum_i I(h)_i$, where $I(h)$ is the intensity of reflection h, $\sum_h$ is the sum over all the measured reflections and $\sum_i$ is the sum over i measurements of the reflection.

2.3. Data collection and analysis

The crystals belonged to space group $C222_1$, with unit-cell parameters a = 83.9, b = 118.1, c = 49.1 Å. The presence of one AtCBL2 molecule with a molecular weight of 25.8 kDa in the asymmetric unit gives a crystal volume per protein mass $V_{\rm M}$ of 2.36 Å^3 Da^{-1} and a solvent content of 48%, which lie within the ranges usually found for protein crystals (Matthews, 1968). X-ray diffraction data of the native crystal (~0.1 × 0.1 × 0.5 mm) were collected to 2.1 Å resolution at 100 K on the Rigaku Jupiter 210 CCD system at beamline BL45XU of SPring-8, Japan. The data were processed using *CrystalClear* (Pflugrath, 1999). Table 1 summarizes the statistics of data collection.

AtCBL2 shows 23% sequence identity with human calcineurin B (PDB code 1aui; Kissinger *et al.*, 1995). Molecular replacement using human calcineurin B as a search model has been attempted with *AMoRe* (Navaza, 1994), but so far without success. Structural analysis by isomorphous replacement combined with anomalous diffraction is in progress.

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