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Crystallization and preliminary X-ray characterization of full-length *Chlamydomonas reinhardtii* centrin

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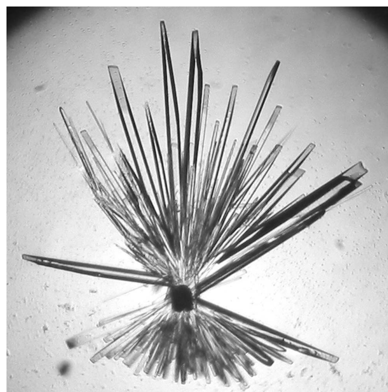

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Chlamydomonas reinhardtii centrin is a member of the EF-hand calcium-binding superfamily. It is found in the basal body complex and is important for flagellar motility. Like other members of the EF-hand family, centrin interacts with and modulates the function of other proteins in a calcium-dependent manner. To understand how *C. reinhardtii* centrin interacts with its protein targets, it has been crystallized in the presence of the model peptide melittin and X-ray diffraction data have been collected to 2.2 Å resolution. The crystals are orthorhombic, with unit-cell parameters a = 52.1, b = 114.4, c = 34.8 Å, and are likely to belong to space group $P2_12_12$.

1. Introduction

Centrin is a 19.4 kDa member of the EF-hand calcium-binding superfamily. It is found in the microtubule-organizing center (MTOC) of various eukaryotic organisms, including yeast, algae, mice and humans (Salisbury, 1995). The MTOC is an organelle that regulates the active cellular processes of the microtubule-based cytoskeleton, such as chromosomal segregation, cytokinesis, fertilization, cellular morphogenesis, cell motility and intracellular trafficking. In the green algae *Chlamydomonas reinhardtii*, centrin is a component of the nucleus basal body connector and the distal striated fiber. These structures consist of calcium-sensitive contractile fibers that serve to reorient and move the flagella.

Centrin is composed of two independent calcium-binding EF-hand domains and a tethered helix, with sequence homology to calmodulin and other members of the EF-hand superfamily (Ortiz *et al.*, 2005). Each domain is structurally and functionally independent, with different target protein-binding specificities. Studies have shown that in the absence of Ca^{2+} centrin occupies a mixture of closed conformations. Binding of a single ion in site IV is sufficient to drastically alter the equilibrium in conformation, promoting the occupancy of an open conformation (Hu *et al.*, 2004). Nevertheless, an exchange between closed and open conformations remains even at saturating levels of Ca^{2+}. It has been proposed that the C-terminal domain of centrin serves as an anchor, while the N-terminal domain interacts with distinct protein targets in a Ca^{2+}-dependent manner, consistent with a model in which it serves as a bridging/assembly factor in the MTOC (Salisbury, 2004). Crystal structures have been determined of full-length centrin proteins from yeast (Li *et al.*, 2006), mouse (Park *et al.*, 2006) and human (Thompson *et al.*, 2006) in complex with target peptides. Additionally, NMR structures are available of the isolated N-terminal (Sheehan *et al.*, 2006) and C-terminal (Hu & Chazin, 2003) domains of *C. reinhardtii* centrin. No structure of full-length *C. reinhardtii* centrin is currently available.

Our efforts are aimed towards understanding the structure–function relationship of this protein and its interaction with target peptides. Therefore, in order to understand the nature of the interactions between centrin and its target proteins within the MTOC, the model peptide melittin (MLT) was used to help crystallize centrin in order to study its structure by X-ray diffraction. Melittin is a 26-amino-acid peptide isolated from honeybee venom with sequence GIGAVLKVLTTGLPALISWIKRKRQQ. This peptide has been

Table 1
X-ray data-collection and processing statistics.

Values in parentheses are for the highest resolution shell (2.38–2.30 Å).

Space group	$P2_12_12$
Unit-cell parameters (Å)	a = 52.08, b = 114.36, c = 34.82
Wavelength (Å)	1.5418
Resolution range (Å)	20–2.30 (2.38–2.30)
Unique reflections	9588 (879)
Redundancy	6.4 (6.2)
Completeness (%)	97.3 (95.6)
$R_{\rm merge}$† (%)	6.5 (48.9)
Average $I/\sigma(I)$	10.7 (3.9)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the ith measured diffraction intensity and $\langle I(hkl)\rangle$ is the mean of the intensity for Miller index hkl.

used previously as a model of target proteins binding to other centrin proteins (Cox *et al.*, 2005; Durussel *et al.*, 2000).

2. Experimental procedures and results

2.1. Expression and purification

Details of the expression and purification of *C. reinhardtii* centrin have been described previously (Pastrana-Rios *et al.*, 2002). Briefly, centrin was overexpressed from plasmid pt7-5 in *Escherichia coli* strain BL21 (λDE3). Transformed *E. coli* cells were grown in 2×YT medium containing 50 µg ml^{-1} ampicillin at 310 K. At an $OD_{600\,nm}$ of 0.8, the culture was induced with 0.5 m*M* IPTG. Cells were harvested by centrifugation and frozen 4 h after induction.

Frozen cells were thawed in lysis buffer containing a cocktail of protease inhibitors and lysed by sonication. The lysate was centrifuged at 10 000 rev min^{-1} for 15 min at 277 K. The supernatant was recovered and adjusted to 2 m*M* $CaCl_2$ and 4 m*M* $MgCl_2$; this was followed by a second ultracentrifugation at 30 000 rev min^{-1} for 30 min at 277 K. The filtered supernatant was then applied onto a Phenyl-Sepharose CL-4B (Pharmacia Inc., USA) column which was washed and eluted with an EGTA-containing buffer. Fractions were analyzed by SDS–PAGE and fractions containing centrin were pooled, concentrated, re-equilibrated in buffer (40 m*M* Tris, 2 m*M* $CaCl_2$, 1 m*M* DTT, 0.08% NaN_3 pH 7.4) and subjected to anion-exchange chromatography using a High Q column (Bio-Rad, Hercules, California, USA). Elution using an NaCl gradient was monitored at 280 nm, fractions were collected and aliquots were analyzed by Agilent Microfluidics. Fractions containing pure centrin were pooled and concentrated. The purity of the protein at this stage was estimated to be >98% by SDS–PAGE.

Synthetic melittin was generously supplied by Dr Frank Prendergast of the Mayo Clinic and Foundation at Rochester, Minnesota, USA. The lyophilized peptide was dissolved in H_2O and dialyzed against 0.1 *N* HCl to remove trifluoracetic acid (TFA; Graff *et al.*, 1997; Pastrana-Rios, 2001). This peptide sample was then dialyzed a second time against 50 m*M* HEPES, 150 m*M* NaCl, 2 m*M* $MgCl_2$ and 2 m*M* $CaCl_2$ at pH 7.00. The molar extinction coefficient at 280 nm for this peptide was calculated to be 5500 M^{-1} cm^{-1} based on the single Trp residue within its sequence. The concentration of the resulting peptide sample was determined by UV absorption at 280 nm. For the centrin sample, purified protein was dialyzed against the same buffer (50 m*M* HEPES, 150 m*M* NaCl, 2 m*M* $MgCl_2$ and 2 m*M* $CaCl_2$ at pH 7.00) and its concentration was estimated by absorption at 274 nm using the measured extinction coefficient of 1310 M^{-1} cm^{-1} (Pastrana-Rios *et al.*, 2002). To prepare the crystallization sample, the purified centrin and MLT peptide solutions described above were mixed to render a centrin:MLT mol ratio of 1:1 and a total protein concentration of 25 mg ml^{-1}.

2.2. Crystallization

Sparse-matrix crystallization screening was carried out using the hanging-drop vapor-diffusion method at 298 K by mixing 1.5 µl protein solution with 1.5 µl precipitant solution in a VDX plate containing 750 µl precipitant solution in the reservior. Small clusters of thin needle crystals were observed after 2–5 d from a precipitant solution containing 50 m*M* HEPES buffer pH 7.5, 200 m*M* KCl and 35%(*v*/*v*) pentaerythritol propoxylate (5/4 PO/OH; Fig. 1). Crystals were only obtained under these conditions in the presence of the melittin peptide. In order to improve the size and quality of the crystals for data collection, an optimization grid screen was constructed by varying the concentration of the precipitant (5/4 PO/OH) *versus* the pH. Larger crystals grew in conditions with 40%(*v*/*v*) 5/4 PO/OH and a pH varying from 7.00 to 7.75 (Fig. 1).

2.3. X-ray data collection and analysis

A single needle crystal measuring ~400 × 40 × 20 µm was separated from a cluster and directly mounted at 93 K in a fiber loop for data collection. X-ray diffraction data were collected on a rotating copper-anode source with a Saturn92 CCD detector (Rigaku/MSC) and integrated and scaled using the *HKL* software suite (Otwinowski

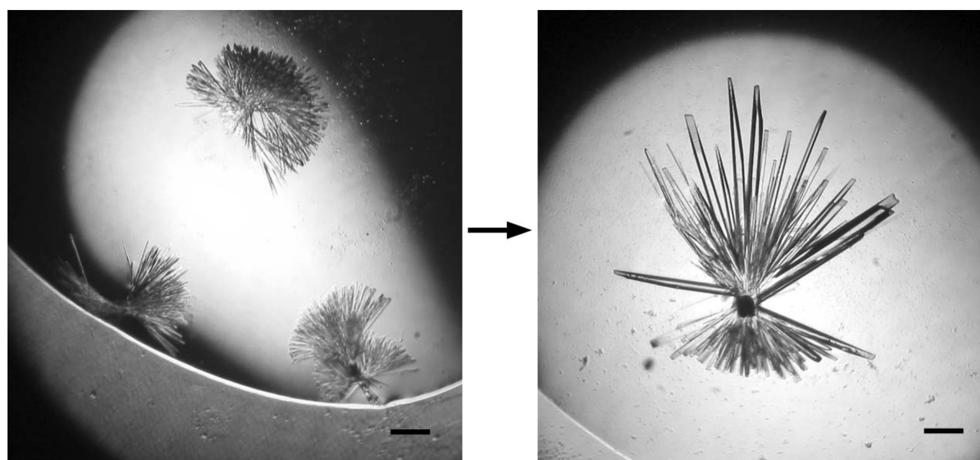

Figure 1
Optimization of needle crystals of *C. reinhardtii* centrin–melittin for X-ray data collection. The scale bar is 100 µm in length.

& Minor, 1997). A data set with 97.3% completeness was collected to 2.3 Å resolution with an overall $R_{\rm merge}$ of 6.5% when scaled as primitive orthorhombic. Data-collection statistics are summarized in Table 1. Systematic absences suggest the presence of two screw axes; therefore, the space group was tentatively assigned as $P2_12_12$. One centrin molecule (MW 19 459 Da) and one melittin peptide (MW 2847 Da) per asymmetric unit gave a calculated Matthews coefficient of 2.32 $Å^3\,Da^{-1}$, which corresponds to a solvent content of 47.1%. This value is within the range commonly observed for protein crystals (Matthews, 1968).

Attempts are currently under way to solve the structure by molecular replacement with available structures of the EF-hand domain-containing proteins centrin and calmodulin. A detailed analysis of the structure will follow structure determination and refinement of the model and will be reported elsewhere.

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