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Crystallization and preliminary X-ray diffraction studies of the calcium-binding protein CalD from *Streptomyces coelicolor*

Calcium ions play an important regulatory role in eukaryotes. However, the regulatory roles of Ca^{2+} in prokaryotes are poorly understood. CalD, an 18 kDa calcium-binding protein from the model actinomycete *Streptomyces coelicolor* A3(2), was purified and crystallized for structure determination by X-ray crystallography. Crystals of CalD that were suitable for X-ray diffraction were obtained using the hanging-drop vapour-diffusion method and diffraction data were collected in-house to 1.56 Å resolution. The crystals belonged to space group $P2_12_12_1$, with unit-cell parameters $a = 32.9$, $b = 51.0$, $c = 87.0$ Å, $\alpha = \beta = \gamma = 90.0^\circ$. There is one protein molecule per asymmetric unit.

1. Introduction

The important and diverse regulatory roles of Ca^{2+} in eukaryotic cells are mediated by a large family of EF-hand calcium-binding proteins (CaBPs; Clapham, 1995; Strynadka & James, 1989). These helix-loop-helix EF-hand-containing proteins (Babu *et al.*, 1988) can be divided into two groups according to the function induced by Ca^{2+} : calcium sensors convey the Ca^{2+} signal through an ion-induced conformational change for the recognition of target molecules (Ikura *et al.*, 1992), while calcium buffers interact with the metal ions for transportation roles in eukaryotes that are less sensitive to Ca^{2+} loading (Ikura, 1996).

In contrast to eukaryotes, calcium-mediated regulatory roles are less evident in prokaryotes, despite accumulated evidence that Ca^{2+} is involved in a myriad of physiologically important bacterial activities, including cell division (Yu & Margolin, 1997), chemotaxis (Tisa & Adler, 1995; Watkins *et al.*, 1995) and cell differentiation (Norris *et al.*, 1991; Michiels *et al.*, 2002; Yonekawa *et al.*, 2001). Calerythrin, a well studied CaBP from *Saccharopolyspora erythraea*, was the first prokaryotic calcium-binding protein identified to contain four canonical calcium-binding EF-hand motifs (Leadlay *et al.*, 1984; Swan *et al.*, 1987) and was proposed to function as a Ca^{2+} buffer rather than as a Ca^{2+} sensor (Cox & Bairoch, 1988).

It has recently been reported that the CaBP from *Streptomyces coelicolor*, a model actinomycete responsible for producing most natural antibiotics used in medicine, is involved in the regulation of spore germination and aerial hypha formation (Wang *et al.*, 2008). Cell-differentiation processes have been established to be calcium-dependent and calcium-mediated in actinomycetes (Natsume *et al.*, 1989; Natsume & Marumo, 1992). The preliminary X-ray diffraction analysis of an 18 kDa calcium-binding protein from *S. coelicolor* A3(2) named CalD (SCO4411) offers a fundamental structural basis for understanding the possible regulatory roles of Ca^{2+} in prokaryotes. The low sequence similarity (less than 28%) between CalD and other prokaryotic EF-hand proteins (Bylsma *et al.*, 1992; Michiels *et al.*, 2002; Swan *et al.*, 1987; Tossavainen *et al.*, 2003; Yonekawa *et al.*, 2001) suggests that it has a potentially distinct and significant function in physiological processes. Here, we report the expression, purification and crystallization of CalD. Preliminary crystallographic analysis shows that CalD is a stable monomer and is suitable for X-ray crystallographic studies.

2. Materials and methods

2.1. Protein expression and purification

We obtained the original pET-44b-CalD plasmid used to express the CalD gene (SCO4411) from Shenglan Wang (Institute of Microbiology, Chinese Academy of Sciences) and subcloned the full-length CalD gene (residues 1–170) into the bacterial expression vector pGEX-6p-1 (GE Healthcare) with *Bam*HI and *Eco*RI restriction sites. The new recombinant plasmid pGEX-6p-1-CalD, which introduced a N-terminal GST tag for protein purification by glutathione *S*-transferase affinity chromatography, was transformed into *Escherichia coli* strain BL21 (DE3).

Cells were grown in Luria–Bertani (LB) medium with 100 µg ml^{−1} ampicillin at 310 K and were induced with 0.2 mM IPTG at an OD₆₀₀ of ~0.6 for functional expression. After growth for a further 4 h, the cells were harvested by centrifugation. After resuspension in 40 ml 1×PBS (phosphate-buffered saline; 10 mM sodium phosphate pH 7.4, 150 mM NaCl), cells were lysed by sonication on ice (medium power output). The debris was removed by centrifugation (30 min at 27 000g, 277 K) and the supernatant was loaded onto a glutathione column filled with Glutathione Sepharose 4B (GE Healthcare) equilibrated with 1×PBS. The GST-tagged protein was eluted with 300 mM NaCl in 1×PBS buffer and cleaved with 200 µg GST-rhinovirus 3C protease at 277 K overnight, which left five residues (GPLGS) at the N-terminus before the CalD protein. Fractions containing CalD lacking the GST tag were pooled and desalted by ultrafiltration for further purification using Resource Q anion-exchange chromatography columns (Amersham-Pharmacia Biotech) pre-equilibrated with 20 mM HEPES pH 7.5. The protein was washed with five column volumes of 20 mM HEPES pH 7.5 and eluted with a linear 0–1.0 M gradient of NaCl in 20 mM HEPES pH 7.5. The flowthrough containing CalD fractions with $A_{280}/A_{254} > 1.25$ was collected and concentrated. The target proteins were then further applied onto a Superdex 75 gel-filtration column (Pharmacia) pre-equilibrated and eluted with 20 mM HEPES pH 7.5, 150 mM NaCl. Fractions containing CalD (tested by SDS–PAGE) were collected and concentrated to 20 mg ml^{−1} by ultrafiltration (10 kDa molecular-weight cutoff). The purified CalD with high purity and homogeneity was analyzed by 15% SDS–PAGE (Fig. 1) and stored in 20 mM HEPES pH 7.5.

Figure 1
15% SDS–PAGE analysis of CalD purified using a Superdex 75 column. Lanes 1 and 2 contain protein at different concentrations. Lane M contains molecular-weight markers (kDa).

Table 1

Data-collection statistics.

Values in parentheses are for the outer resolution shell.

Wavelength (Å)	1.5418
Space group	$P2_12_12_1$
Unit-cell parameters (Å, °)	$a = 32.9, b = 51.0, c = 87.0,$ $\alpha = 90.0, \beta = 90.0, \gamma = 90.0$
Resolution range (Å)	50–1.56 (1.61–1.56)
Total reflections	91632
Unique reflections	21409 (1683)
Mean redundancy	4.3 (3.5)
Completeness (%)	98.9 (94.6)
$R_{\text{merge}}^{\dagger}$ (%)	5.4 (33.7)
Average $I/\sigma(I)$	11.9 (2.8)

$\dagger R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of the i th observation of reflection hkl and $\langle I(hkl) \rangle$ is the mean intensity of the reflections.

2.2. Crystallization

Crystallization trials were carried out in custom-made 16-well plates at 291 K using the hanging-drop vapour-diffusion method. Initial crystallization screening was carried out with Crystal Screens I and II (Hampton Research). A 1 µl droplet consisting of 20 mg ml^{−1} protein in 20 mM HEPES pH 7.5 was mixed with an equal volume of reservoir solution and equilibrated against 200 µl reservoir solution in a sealed chamber. Initial needle-like crystals of CalD appeared in Crystal Screen I condition No. 45 (18% PEG 8000, 0.1 M sodium cacodylate pH 6.5, 0.2 M zinc acetate) after 1 d and this condition was used for further optimization by variation of the precipitant concentration, buffer pH and protein concentration. Following optimization, thin tablet-like CalD crystals which diffracted well were obtained using the following conditions: 14% (w/v) PEG 8000, 0.1 M sodium cacodylate pH 6.5, 0.2 M zinc acetate (Fig. 2).

2.3. Data collection and processing

A single CalD crystal was harvested using a nylon loop (Hampton Research), flash-cooled to 100 K in a nitrogen-gas stream and used for data collection. A cryo-solvent was not used in the flash-cooling of the CalD crystals, since the precipitant in the crystallization solution was able to serve as a cryo-solvent. Complete diffraction data were collected at 100 K with 1° oscillation per image on an R-Axis IV⁺⁺ image-plate detector using an in-house Rigaku MicroMax-007 rotating-anode X-ray generator (Fig. 3). The data were indexed and scaled using the programs *DENZO* and *SCALEPACK* from the *HKL-2000* package (Table 1).

Figure 2
Crystal of CalD from *S. coelicolor* grown by the hanging-drop vapour-diffusion method in 14% (w/v) PEG 8000, 0.1 M sodium cacodylate pH 6.5, 0.2 M zinc acetate.

Figure 3

A typical X-ray diffraction pattern from a CalD crystal collected on an R-AXIS IV⁺⁺ image-plate detector.

3. Results and discussion

The initial needle-like crystals exhibited high mosaicity and were not good enough for data collection. Following optimization of the initial conditions, plate-like CalD crystals which diffracted well and were suitable for data collection were obtained by reducing the concentration of the precipitant and lowering the protein concentration to 10 mg ml⁻¹. Diffraction data were collected in-house from the plate-like crystals to 1.56 Å resolution (Fig. 3, Table 1). The crystals belonged to space group $P2_12_12_1$, with unit-cell parameters $a = 32.9$, $b = 51.0$, $c = 87.0$ Å, $\alpha = \beta = \gamma = 90.0^\circ$ (Table 1). Initial analysis of the crystal solvent content using the Matthews coefficient (Matthews, 1968) suggested that the asymmetric unit contains one molecule with 39.4% solvent content (Matthews coefficient 2.03 Å³ Da⁻¹). Attempts at MAD phasing based on selenomethionine substitution are in progress.

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