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Purification, nanocrystallization and preliminary X-ray analysis of a C-terminal part of tropomodulin protein 1, isoform *A*, from *Caenorhabditis elegans*

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The C-terminal part of tropomodulin protein 1, isoform *A*, from *Caenorhabditis elegans* was expressed in *Escherichia coli* and purified to homogeneity. Optimized from the initial nanoscreen, crystals grew to dimensions of 0.25 × 0.15 × 0.15 mm at 277 K using 28.0%(*v*/*v*) PEG 400 as the precipitant by the hanging-drop vapor-diffusion technique. A data set of 94.9% completeness was collected to a resolution of 1.98 Å at 100 K using a synchrotron X-ray source (SER-CAT). The crystals belong to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 31.7, b = 50.6, c = 107.1 Å, and contained one molecule per asymmetric unit.

1. Introduction

Proteins that interact with actin play an important role in regulating the functions of the actin filament in maintaining cell integrity, intracellular organization, cell division and cell endocytosis as well as striated muscle contraction (Schoenenberger *et al.*, 2002; Almenar-Queralt *et al.*, 1999). First isolated from red blood cells, tropomodulin, a pointed end capping protein, blocks both elongation and depolymerization at the slow-growing end of tropomyosin-actin filaments (Gregorio *et al.*, 1995). The N-terminal half of erythrocyte-tropomodulin (e-tropomodulin) from chicken did not show a definite folded structure in solution, while the C-terminal half could be identified as a tight domain (Kostyukova *et al.*, 2001). The N-terminal half of tropomodulin from chicken or human is responsible for binding the N-terminal region of tropomyosin and the interaction may cause conformational changes in each protein (Kostyukova *et al.*, 2001; Vera *et al.*, 2000).

Four isoforms of tropomodulin have been identified in humans as well as their orthologs in mice (Cox & Zoghbi, 2000; Cox *et al.*, 2001). Different types of orthologs are involved in *Drosophila* (Mardahl-Dumesnil & Fowler, 2001; Dye *et al.*, 1998). The tropomodulin protein 1, isoform *A*, of *Caenorhabditis elegans* was identified in the genome-sequence project (The CES Consortium, 1998). A comparison of the protein sequence with chicken e-tropomodulin shows only 34% identity and 58% similarity.

Structural studies of tropomodulin from *C. elegans* will help to determine the mechanism of the dynamic assembly of proteins at the pointed end of actin filaments and to unveil the evolutionary pathway of tropomodulin among different life forms. Here, we report the first crystallization and preliminary X-ray crystallographic analysis of *C. elegans* tropomodulin.

2. Results and discussion

2.1. Protein production

The C-terminal fragment (residues 199–392) of tropomodulin protein 1, isoform *A*, of *C. elegans* was subcloned into expression vector pET28b (Novagen) and expressed in *Escherichia coli* strain BL21(DE3)plySs (Novagen). The expressed recombinant protein will contain a His_6-tag of 20 residues at the N-terminus. Cells containing the expression plasmids were cultured for about 3 h in Luria–Bertani broth (LB) with 50 mg l^{-1} kanamycin at 310 K. When the OD_{595} of the culture liquid attained 0.6, isopropyl-1-thio-β-D-galactopyranoside (IPTG) was added to a final concentration of 0.4 m*M* to induce production of the recombinant protein. The cell culture was continued for another 15 h at 291 K. After centrifugation at 7000*g* for 12 min at 277 K, cells were harvested and resuspended in a buffer consisting of 20 m*M* Tris–HCl, 5 m*M* imidazole, 500 m*M* NaCl pH 7.9. Using a Fisher Scientific 550 sonic dismembrator, cells were sonicated on ice. The crude lysates were centrifuged for 30 min at 15 000*g* and 277 K and supernatants were removed and stored at 277 K. A Chelating Sepharose Fast Flow (Amersham Pharmacia) nickel column was saturated with 50 m*M* $NiSO_4$ solution and then equilibrated with a buffer consisting of 20 m*M* Tris–HCl, 5 m*M* imidazole, 500 m*M* NaCl at pH 7.9. The supernatants were applied to the column and the unbound proteins were washed out of the column using a buffer consisting of 10 m*M* Tris–HCl, 500 m*M* NaCl, 60 m*M* imidazole pH 7.9. The bound proteins were

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199                GSHGT TFNGIMQSYV PRIVPDEPDN DTDVESCINR LREDDTDLKE
241 VNINNMKRVS KERIRSLIEA ACNSKHIEKF SLANTAISDS EARGLIELIE TSPSLRVLNV
301 ESNFLTPELL ARLLRSTLVT QSIVEFKADN QRQSVLGNQV EMDMMMAIEE NESLLRVGIS
361 FASMEARHRV SEALERNYER VRLRRLGKDP NV
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Figure 1
The amino-acid sequence corresponding to the recombinant C-terminal protein of *C. elegans* tropomodulin, isoform *A*, used in crystallization (starting at the arrow). The additional three amino acids (lighter print) in front of the N-terminal end remained from the expression vector pET28b (Novagen) after thrombin cleavage.

eluted with a buffer consisting of 20 m*M* Tris–HCl, 500 m*M* NaCl, 100 m*M* EDTA pH 7.9. The concentration of the eluted protein was determined by a Bio-Rad protein assay. The cleavage of the N-terminal histidine tag was conducted at 295 K overnight using 1 U thrombin (Sigma-Aldrich) per 16.7 mg of recombinant protein in the elution buffer. The completeness of cleavage was checked by SDS–PAGE. Three residues remained at the N-terminus of the purified protein after thrombin cleavage (Fig. 1). The sample was then passed through a Superdex-75 Hiload 16/60 size-exclusion column (Amersham Biosciences), which was pre-equilibrated with a buffer consisting of 20 m*M* HEPES, 150 m*M* NaCl pH 8.0. The eluent from the largest peak was collected. Using an Amicon ultracentrifugal filter with a 10 kDa cutoff, the sample buffer in the protein solution was exchanged with a buffer consisting of 10 m*M* HEPES, 0.01% sodium azide pH 7.5. The protein solution was finally concentrated to 13.7 mg ml^{-1}.

2.2. Nanoscreen and crystallization optimization

Initial crystallization screening was carried out by the sitting-drop vapor-diffusion method (Adachi *et al.*, 2003) using a Nano-Screen robot (designed at the University of Alabama at Birmingham, USA) at 277 K. 0.041 µl protein solution mixed with 0.079 µl reservoir solution was equilibrated against 70 µl reservoir screening solution in a Corning 384-well crystallization plate. Precipitant reservoir solutions from several commercial kits were used for screening. The mother reservoir liquid from the kits was diluted four times with double-distilled water prior to mixing with the protein solution to ensure accurate dispensing in nanolitre volumes. The orthorhombic shaped crystals appeared under the condition of Natrix kit (Scott *et al.*, 1995) No. 47 in 4 d. During a period of two weeks, optimization was conducted using hanging drops made up of 1 µl protein solution mixed with an equal volume of reservoir solution equilibrated against 1000 µl reservoir solutions in 24-well VDX plates (Hampton Research) by manual operation. Under the final refined reservoir condition of 28.0%(*v*/*v*) polyethylene glycol (PEG) 400, 100 m*M* KCl, 10 m*M* $MgCl_2$, 100 m*M* Tris–HCl pH 8.0, crystals grew to dimensions of 0.25 × 0.15 × 0.15 mm (Fig. 2) within 4 d at 277 K. Prior to data collection, crystals were transferred to an artificial mother liquor containing 20% glycerol and flash-cooled in liquid nitrogen.

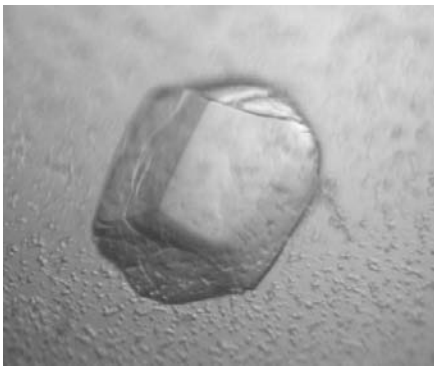

Figure 2
A crystal of the C-terminal part of tropomodulin protein 1, isoform *A*, from *C. elegans*. Approximate dimensions are 0.25 × 0.15 × 0.15 mm.

2.3. X-ray analysis

The X-ray diffraction data were collected at SER-CAT, beamline 22-ID, Advanced Photon Source, Argonne National Laboratory using a MAR165 CCD area detector. A total of 360 frames with a 0.5° oscillation angle were collected at 100 K using a wavelength of 0.9840 Å and a crystal-to-detector distance of 150 mm (Fig. 3). Data were processed with *HKL*2000 (Otwinowski & Minor, 1997). Statistics of data collection and processing are summarized in Table 1.

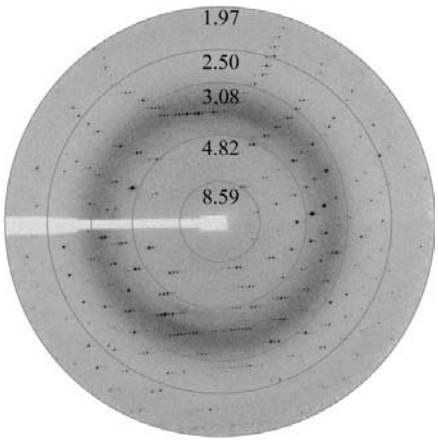

Figure 3
A 0.5° oscillation X-ray diffraction pattern from a crystal of tropomodulin protein 1, isoform *A*, from *C. elegans*. The resolution is 1.98 Å at the edge of the image.

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

Values in parentheses refer to the last resolution shell.

Space group	$P2_12_12_1$
Unit-cell parameters (Å)	a = 31.7, b = 50.6, c = 107.1
Resolution range (Å)	31–1.98
Total reflections	83380
Unique reflections	12221 (764)
Completeness (%)	94.9 (60.9)
Redundancy	6.8 (2.9)
Average $I/\sigma(I)$	11.7 (3.9)
R_{sym}† (%)	0.049 (0.212)

† $R_{sym} = \sum_{hkl} |I_i(hkl) - \langle I_i(hkl)\rangle| / \sum_{hkl} I_i(hkl)$, where $\langle I_i(hkl)\rangle$ is the average of the i intensity measurements.

The 194 amino-acid fragment from tropomodulin protein 1, isoform *A*, of *C. elegans* could be aligned with its counterpart from chicken tropomodulin-e with only 34% identity. Crystallization of the chicken tropomodulin-e fragment (residues 160–344) was crystallized with 15%(*v*/*v*) PEG 400, 0.1 *M* MES–NaOH buffer pH 6.5 and 6 m*M* $ZnSO_4$ (Krieger *et al.*, 2001). The presence of $ZnSO_4$ was critical in order to grow good-quality crystals. The space group of the chicken tropomodulin crystals is *R*3, $a = b = 69.6$, $c = 101.3$ Å, with one molecule per asymmetric unit. The morphology and the altered crystallization conditions of the *C. elegans* tropomodulin crystals suggested a different crystal form, probably involving different protein packing. The farthest reflection observed was at 1.8 Å resolution. The data set was fully processed to 1.98 Å resolution with a completeness of 94.9%, an R_{sym} of 0.049, an average $I/\sigma(I)$ of 11.7 and an overall mosaicity of 0.5°. The crystals belonged to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 31.7, b = 50.6, c = 107.1 Å. The Matthews coefficient V_M was calculated to be 2.15 Å^3 Da^{-1}, corresponding to a solvent content of 42% (Matthews, 1968) if one protein molecule is present in the asymmetric unit.

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South East Regional Collaborative Access Team (SER-CAT) at the Advanced Photon Source, Argonne National Laboratory. Supporting institutions may be found at http://www.ser.anl.gov/new/index.html. Use of the Advanced Photon Source was supported by the US Department of Energy, Basic Energy Sciences, Office of Science under Contract No. W-310-109-Eng-38.

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