Preliminary X-Ray Crystallographic Analysis of Centrin from Ciliate *Euplotes octocarinatus*

Xiao-jing He[1], Liu Li[2], Hai Hou[2], Yaqin Zhao[1], Aihua Liang[1,*] and Zihe Rao[2,*]

[1]*Institute of Biotechnology, Key Laboratory of Chemical Biology and Molecular Engineering of Ministry of Education, Shanxi University, Taiyuan 030006,China;* [2]*Laboratory of Structural Biology, Tsinghua University, Beijing, 100084, China & National Laboratory of Macromolecules, Institute of Biophysics, Chinese Academy of Science, Beijing, 100101, China.*

Abstract: Centrins are four-EF-hand Ca^{2+}-binding proteins, which belong to the CaM super family. The centrin from ciliate *Euplotes Octocarinatus* has been expressed in Escherichia coli, purified and crystallized using the hanging-drop method. Rod-like crystals were grown and diffracted to 2.0 Å. The crystals belong to space group $P2_12_12_1$ and the unit-cell parameters are a=34.442 Å, b=48.954 Å, c=72.583 Å.

Keywords: Centrin, crystallization, preliminary X-ray studies.

1. INTRODUCTION

The number, direction and polarity of microtubules in eukaryotic cells are usually organized by a unique microtubule-organizing centre (MTOC) [1]. Alteration of the MTOC duplication could severely perturb the bipolar spindle formation, and this blocks the cell in the G2/M phase. In contrast, hyper amplification of the centrosome is associated with cellular transformation and cancer [2, 3]. About 150-200 proteins are permanently or temporarily part of this matrix and among them are several regulatory proteins such as centrin [4]. Centrin was first identified in unicellular green algae *Chlamydomonas reinhardtii,* where it is located in the basal bodies and in calcium-sensitive contractile fibers associated with the basal bodies [5]. It is a ubiquitous highly conserved protein in diverse evolutionary lineages, including algal, higher plants, invertebrate and mammalian cells [4, 6]. It is an acidic protein of 19.5 kDa, which belongs to the highly conserved EF-hand CaM super family of Ca^{2+}-binding proteins.

The high degree of conservation and the ubiquity of the protein in all species investigated so far suggest that centrin is essential for proper cell function. It has been shown to be required for the normal duplication and separation of the microtubule-organizing center (MTOC) [7-9]. Mutations and complete deletions of the centrin gene have shown that the protein is required for proper cell division [10-13]. They also play fundamental roles in contraction of centrin-based fiber systems in eukaryotic cells [8, 9, 14].

MTOC of the unicellular protozoa ciliate is not structurally defined. The microtubules of spindle pole body (SPB) in *Euplotes* were organized by the microtubule-organizing centre in karyolymph [15]. The centrin of *Euplotes (*EoCen) assembles into calcium-sensitive contractile cytoskeletal systems and plays an important role in the cell. In this report, we describe the purification**,** crystallization and preliminary X-ray diffraction studies of the recombinant centrin of *Euplotes octocarinatus.*

2. MATERIALS AND METHODS

2.1 Protein Expression and Purification

The centrin gene obtained through amplification of macronuclear DNA of the ciliate *Euplotes octocarinatus* was inserted into the GST-fusion expression vector pGEX-6p [16]. High levels of fusion protein were produced in *Escherichia coli* BL21 and then harvested. Recombinant bacteria pellets were suspended in PBS and lysed by sonication. The soluble cell lysate, obtained by centrifugation at 15000rev $min^{-1}$for 30min, was a Glutathione Sepharose^{TM} 4B column pre-equilibrated with PBS. The GST-EoCen fusion protein was digested by PreScission protease (Amersham Biosciences) overnight at 4°C on the column. The fraction with target protein was eluted by PBS and concentrated, then passed through Mono Q (20 mM Mes, 50 mM NaCl, pH 6.5) for further purification.

2.2 Crystallization and Preliminary X-Ray Studies

Crystallization screens of EoCen were initially performed with commercially available reagents (Crystal Screen I and II, PEG/ION, Hampton Research) using the hanging-drop vapour-diffusion method at 291K. The purified protein was concentrated using a 10 K ultrafiltration membrane (Filtron) to 20 mg ml^{-1} in a solution containing 10 mM $CaCl_2$. Protein concentration was determined by UV absorbance at 280nm with an extinction coefficient of 0.260 $M^{-1}cm^{-1}$(based on the amino-acid sequence). 1 μl of protein solution were mixed with 1 μl of reservoir solution and equilibrated against 0.2 ml of the reservoir solution. The rod-like crystals were obtained after one month.

*Address correspondence to these authors at the Laboratory of Structural Biology, Tsinghua University, Beijing, 100084, China & National Laboratory of Macromolecules, Institute of Biophysics, Chinese Academy of Science, Beijing, 100101, China; Fax: 86-351-7011499; E-mail, aliang@sxu.edu.cn, Fax: 86-10-6277-3145; E-mail, raozh@xtal.tsinghua.edu.cn

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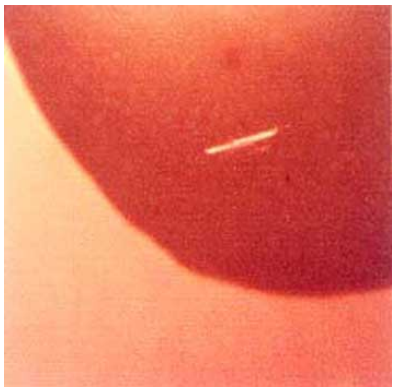

Figure 1. Typical crystal of EoCen grown using the hanging-drop method in 20% PEG 3350, 0.2 M NaCl.

Data collection of EoCen (Ca^{2+}) was performed in-house on a Rigaku Micro-007 rotating copper-anode X-ray generator with a RAIXS IV++ image-plate detector. The crystal was mounted on a nylon loop and flash-frozen in a cold nitrogen-gas stream at 100K. Data were indexed and scaled using DENZO and SCALEPACK [17].

3. RESULTS AND DISCUSSION

Good quality, well diffracting crystals could be obtained from a condition containing 0.2M NaCl, 20%PEG 3350 (Figure **1**). Eocen crystals belong to space group $P2_12_12_1$ and diffract to 2.01 Å. The unit-cell parameters are a=34.4 Å, b=48.9Å, c=72.6 Å (Figure **2**). Assuming the presence of one molecule in the asymmetric unit, the solvent content is estimated to be about 19.4% and the Matthews coefficient (V_M) is about 1.5 $Å^3$ Da^{-1} [18]. The relevant data-collection statistics are summarized in Table **1**. Structure solution is in progress and we are preparing for expression of a Se–Met derivative protein suitable for phase determination by the multi-wavelength anomalous diffraction (MAD) method.

Table 1. Data-collection and processing statistics of eocen

Space group	**P2 (1) 2(1) 2(1)**
Unit-cell parameters (Å)	a=34.4 b=48.9 c=72.5
Wavelength (Å)	1.5418
Resolution (Å)	2.01
Reflections observed	28223
Unique reflections	8576
Redundancy	3.4(3.0)
Completeness (%)	98.7 (89.0)
Rmerge(%)	7.9 (47.6)
I/sigma	12.3 (3.1)

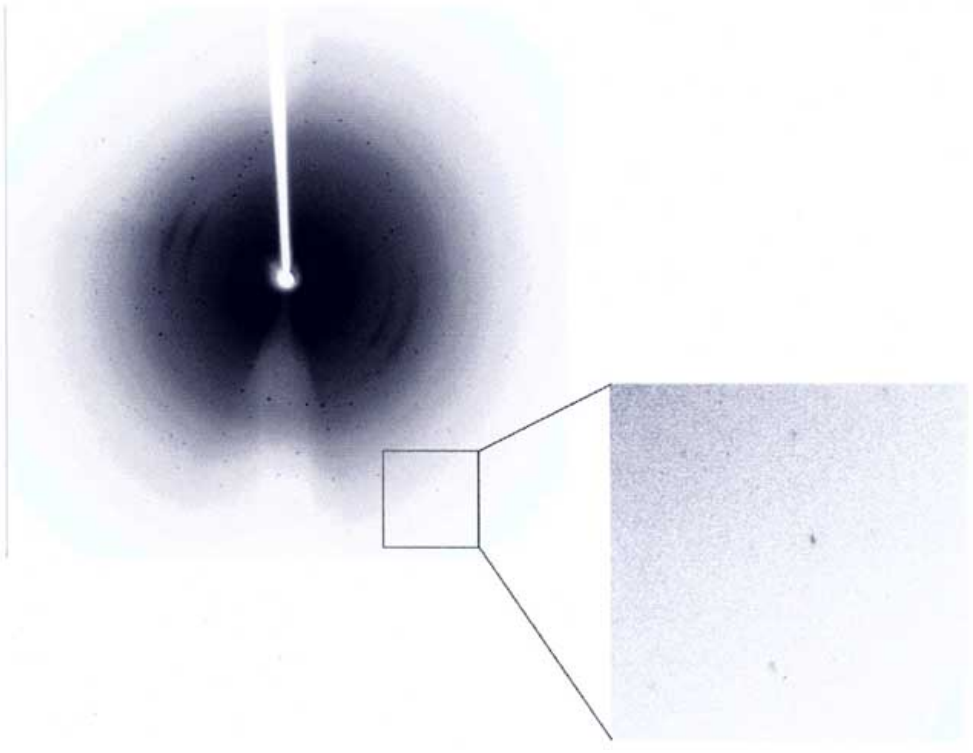

Figure 2. A typical X-ray diffraction pattern from a crystal of centrin. The diffraction image was collected on a MAR Research image-plate detector. An enlarged image of the area is beside it.

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