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Crystallographic characterization of the membrane-binding domain of radixin

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Radixin is a protein which cross-links plasma membranes and actin filaments and thus forms membrane-associated cytoskeleton. The radixin N-terminal domain, which is responsible for membrane association, has been purified and crystallized by vapour diffusion with polyethylene glycol 6000. The crystals belong to space group $P4_12_12$ or $P4_32_12$, with unit-cell parameters $a = b = 96.36$, $c = 133.16$ Å, and diffract to a resolution of 3.0 Å.

1. Introduction

Radixin, which was originally isolated from rat liver tissue as a component of cell-to-cell adherens junctions (Tsukita *et al.*, 1989), is a protein cross-linking plasma membranes and actin filaments. The protein is a member of the ezrin/radixin/moesin (ERM) protein family, which has been found in eukaryotic cells from *Caenorhabditis elegans* to human cells (Tsukita & Yonemura, 1999). Previous immunofluorescence studies of cultured cells have revealed that these ERM proteins are localized at cell-surface structures such as microvilli, microspikes, ruffling membranes and cell-adhesion sites (Bretscher, 1983; Sato *et al.*, 1991, 1992; Amieva & Furthmayr, 1995; Franck *et al.*, 1993; Serrador *et al.*, 1997). It has been shown that at these sites actin filaments are associated with plasma membranes. The N-terminal halves of ERM proteins display high sequence homology (~85% identity) and bind the cytosolic parts of integral membrane proteins, thus playing a role in cell adhesion (Funayama *et al.*, 1991; Tsukita *et al.*, 1994; Hirao *et al.*, 1996). To date, three membrane proteins, a cell-surface hyaluronate receptor CD44, a cell-surface glycoprotein CD43 and an intercellular adhesion molecule ICAM, have been characterized as possible partners of the ERM proteins (Yonemura *et al.*, 1998). The C-terminal halves of ERM proteins bind F-actin through their conserved actin-binding sites consisting of 34 residues at the C-terminal ends. Recent studies have shown that several other proteins exhibit sequence similarity to the N-terminal domains of the ERM proteins. These proteins consist of members of the band 4.1 protein superfamily (Takeuchi *et al.*, 1994) and a subset of protein tyrosine phosphatases such as PTP-D1 (Moller *et al.*, 1994). Therefore, these conserved N-terminal domains are nowadays referred to as the FERM (4.1 and ERM) domain, which is thought to form a new class of modules mediating protein–protein interactions. Here, we report the purification and crystallization of the radixin FERM domain.

2. Materials and methods

The FERM domain of mouse radixin, containing the N-terminal 310 residues, was expressed in BL21(DE3)RIL cells harbouring plasmid pGEX4T-3 as a fusion protein with glutathione S-transferase (GST; Matsui *et al.*, 1998). The cells were disrupted by sonication at 277 K. The supernatant was applied onto a GST affinity column with glutathione Sepharose 4B (Pharmacia Biotech) and was washed with 20 m*M* MES–Na buffer pH 6.8 containing 200 m*M* NaCl, 1 m*M* EDTA and 1 m*M* dithiothreitol (DTT). The fusion protein adsorbed to the resin was cleaved by human thrombin (Nippon Ham) at 10 units ml^{-1} for 14 h at 277 K. The cleaved proteins were collected for further purification by two column-chromatographic steps using Hitrap Q and Hitrap S (Pharmacia Biotech). Purity was monitored by 15% polyacrylamide gel electrophoresis and the gels were stained with Coomassie brilliant blue.

The purified samples were verified with matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy, MALDI–TOF MS (JMS ELITE, PerSeptive Inc.) and N-terminal analysis (M492, Applied Biosystems). The N-terminal analysis revealed that the protein has two additional residues, glycine and serine, at the N-terminus. MALDI–TOF MS of the sample resulted in a single peak of 37 924.8 Da, which corresponds to the calculated value of 37 919 Da. Gel filtration was performed using Superose 12 (Pharmacia Biotech) with 20 m*M* MES–Na buffer (pH 6.8) containing 200 m*M* NaCl and 1 m*M* DTT. The molecular sizes were calculated from a calibration curve obtained from the elution profiles of a set of standard proteins.

The purified protein was concentrated to 20 mg ml^{-1} for crystallization. Crystallization

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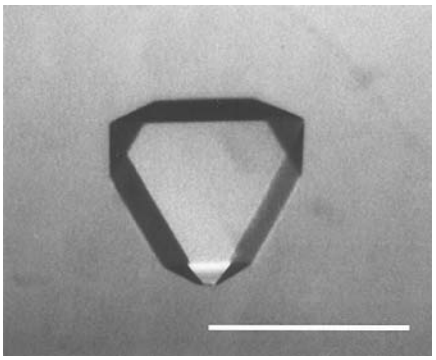

Figure 1
A crystal of the radixin FERM domain. The scale bar indicates 0.5 mm.

conditions were screened using the hanging-drop vapour-diffusion method. X-ray diffraction data of the crystals were collected at 288 K with a Rigaku R-AXIS IV imaging-plate area detector using Cu $K\alpha$ radiation generated by a Rigaku RU-300H rotating-anode X-ray generator and also using a Weissenberg camera for macromolecules installed on the BL6A beamline at the Photon Factory (PF) at Tsukuba, Japan. The data collection at the PF was performed with an angular range of 70°, a step size of 2.5° and an exposure time of 160 s. The wavelength was set to 1.00 Å. The camera was set to a crystal-to-film distance of 430 mm. Intensity data were processed with the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). One crystal was used for each data collection at the laboratory and at the PF. The self-rotation functions (Rossmann & Blow, 1962) were calculated using the program *POLARRFN* from the *CCP*4 package (Collaborative Computational Project, Number 4, 1994).

3. Results and discussion

The best crystals of the radixin FERM domain were obtained from a solution containing 10 mg ml^{-1} protein, 50 m*M* MES–Na pH 6.0, 2% polyethylene glycol 6000 (PEG 6K), 150 m*M* NaCl and 0.5 m*M* DTT equilibrated against 4% PEG 6K and 100 m*M* MES–Na pH 6.0 at 277 K (Fig. 1). Crystals belonged to space group $P4_12_12$ or $P4_32_12$, with unit-cell parameters $a = b = 96.36$ (12), $c = 133.16$ (13) Å. Initially, X-ray intensity data were collected in the laboratory using the R-AXIS IV image-plate detector. This data collection gave a set of intensity data to a resolution of 3.0 Å. X-ray intensity data were subsequently collected on the BL6A beamline at the PF. This data collection improved the completeness of the intensity data, although no significant improvement in the resolution was obtained for the present crystals. The total number of measured reflections was 52 320, which gave 12 482 unique reflections. The resulting data gave an $R_{\rm merge}$ of 6.5% with a completeness of 95.3% (91.7% for the outer shell, 3.1–3.0 Å). The redundancy of reflections was 4.05 and the crystal mosaicity was 0.1°.

The present crystals were estimated to contain one or two molecules in the asymmetric unit, with a V_m value of 4.22 or 2.11 Å^3 Da^{-1}, respectively. Gel-filtration experiments indicated that the radixin FERM domain exists as a monomer in solution (data not shown). We calculated self-rotation functions (Rossmann & Blow, 1962) to search for non-crystallographic symmetry with several Patterson sphere radii. However, we failed to find significant peaks on the maps. Non-crystallographic symmetry axes, if present, may possibly run along one of the crystallographic axes. Alternatively, it is possible that there is no non-crystallographic symmetry.

Structural analysis of the crystals using the multiple isomorphous replacement method is in progress.

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