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Crystallographic characterization of the radixin FERM domain bound to the cytoplasmic tail of the adhesion protein ICAM-2

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Radixin is a member of the ERM proteins, which cross-link plasma membranes and actin filaments. The FERM domains located at the N-terminal regions of ERM proteins are responsible for membrane association through direct interactions with the cytoplasmic domains of integral membrane proteins. Here, crystals of the complex between the radixin FERM domain and the full-length cytoplasmic tail (28-residue peptide) of intercellular adhesion molecule 2, ICAM-2, have been obtained. The crystals were found to belong to space group $P3_121$ or $P3_221$, with unit-cell parameters $a = b = 100.44\ (9)$, $c = 99.49\ (6)$ Å, and contain one complex in the crystallographic asymmetric unit. An intensity data set was collected to a resolution of 2.60 Å.

1. Introduction

Radixin is a member of the ezrin/radixin/moesin (ERM) proteins, which generally act as cross-linkers between the plasma membranes and actin filaments (Arpin *et al.*, 1994; Bretscher, 1999; Mangeat *et al.*, 1999; Tsukita & Yonemura, 1999). These proteins have been found in eukaryotic cells from *Caenorhabditis elegans* to human. In cultured cells, ERM proteins are coexpressed in cell-surface structures such as microvilli, cell-adhesion sites, ruffling membranes and cleavage furrows where actin filaments associate with plasma membranes (Sato *et al.*, 1991, 1992; Amieva & Furthmayr, 1995; Franck *et al.*, 1993; Serrador *et al.*, 1997). Radixin, which was originally isolated from rat liver as a component of cell–cell adherens junctions (Tsukita *et al.*, 1989), consists of 583 amino-acid residues with three domains, the FERM (4.1 and ERM) domain (residues 1–310), a central helical domain (residues 311–470) and a C-terminal tail domain (residues 477–583) which binds F-actin. The amino-acid sequence of the FERM domain is highly conserved (~85%) among ERM proteins and is responsible for membrane association by direct binding to the cytoplasmic domain or tail of integral membrane proteins. At present, intercellular adhesion molecules ICAMs (ICAM-1, ICAM-2 and ICAM-3) of the immunoglobulin superfamily, a cell-surface glycoprotein CD43 and a cell-surface hyaluronate receptor CD44 have been characterized as binding partners of the ERM proteins (Helander *et al.*, 1996; Yonemura *et al.*, 1993, 1998; Tsukita *et al.*, 1994). Here, we report the first crystallization and preliminary crystallographic studies of the complex between the radixin FERM domain and the cytoplasmic tail of ICAM-2.

2. Methods and results

2.1. Protein purification

The FERM domain (residues 1–310, 36.7 kDa) of mouse radixin was expressed in BL21(DE3)RIL cells containing plasmid pGEX4T-3 as a fusion protein with glutathione S-transferase (GST) (Matsui *et al.*, 1998). Details of the purification scheme of this domain have been described previously (Hamada, Matsui *et al.*, 2000). In brief, the fusion protein was purified using a GST affinity column with glutathione-Sepharose 4B (Pharmacia Biotech). The protein, cleaved by thrombin, was purified further by two ion-exchange columns, Hitrap Q and Hitrap S (Pharmacia Biotech), and concentrated to 24 mg ml^{-1} in a solution containing 300 m*M* NaCl, 10 m*M* Na MES (pH 6.8) and 1 m*M* dithiothreitol (DTT). The purified samples were verified by matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy (MALDI-TOF MS; PerSeptive Inc.) and N-terminal analysis (M492, Applied Biosystems).

The peptide corresponding to the full-length cytoplasmic tail of the mouse ICAM-2 was purchased from Sawady Technology (Tokyo, Japan) in a highly purified grade. This peptide consists of 28 residues (^{1}HWHRRRTGTY GVLAAWRRL PRAFRARPV^{28}) with a molecular weight of 3440 Da and strongly binds the radixin FERM domain with a

dissociation constant of nanomolar order (K_d = 6.6 n*M*, data not shown). For crystallization, the peptide was dissolved to 3 m*M* concentration in a buffer containing 70 m*M* NaCl and 10 m*M* Na MES pH 6.8.

2.2. Crystallization

The prepared radixin FERM domain and the ICAM-2 peptide were mixed in a 1:1 molar ratio (each 0.45 m*M*) in a solution of 270 m*M* NaCl, 10 m*M* Na MES pH 6.8 and 0.9 m*M* DTT. Crystallization conditions were scanned using the hanging-drop vapour-diffusion method at 277 K. Crystals of the complex were obtained in 4 d by combining 2 µl of protein solution with 2 µl of reservoir solution containing 4% polyethylene glycol 6000 (PEG 6K) and 100 m*M* Na MES pH 6.0. The crystals reached maximal dimensions of 0.4 × 0.3 × 0.2 mm (Fig. 1). It was confirmed by MALDI-TOF MS that the crystals contained both the radixin FERM domain and the ICAM-2 peptide. The crystals were transferred stepwise into a cryoprotective solution containing 35% glycerol, 7% PEG 6K and 50 m*M* Na MES pH 6.0 for flash-cooling.

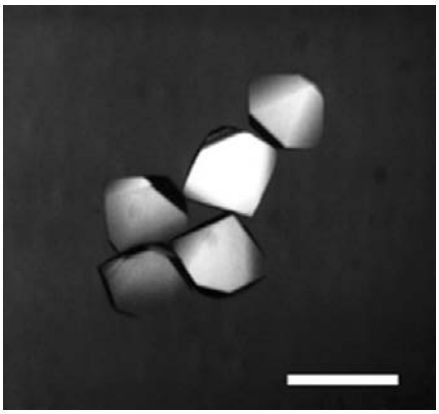

Figure 1
Crystals of the complex formed by the radixin FERM domain with the cytoplasmic tail peptides of ICAM-2. The scale bar indicates 0.5 mm.

2.3. Data collection and processing

All diffraction data were collected from crystals cooled to 100 K in a cold nitrogen stream using an R-AXIS IV image-plate detector on an FR-C X-ray generator (Rigaku). The data collection was performed with a total oscillation range of 106°, with a step size of 2.0° for an exposure time of 30 min. The camera distance was 150 mm. Crystals were found to diffract to a resolution of 2.6 Å and to belong to space group $P3_121$ or $P3_221$, with unit-cell parameters $a = b = 100.44$ (9), $c = 99.49$ (6) Å. All data were processed using the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). The total number of observed reflections was 158 273, which gave 17 729 unique reflections. The resulting data gave an $R_{\rm merge}$ of 9.8 (30.0% for the outer shell, 2.60–2.69 Å) with a completeness of 97.3% (91.5% for the outer shell). The redundancy of reflections was 4.9 (3.4 for the outer shell) and the crystal mosaicity was estimated to be 0.45°. The present crystals were estimated to contain one complex in the asymmetric unit ($Z = 6$), with a $V_{\rm M}$ value (Matthews, 1968) of 3.50 Å^3 Da^{-1}. The solvent content of these crystals was approximately 65%. The free form of the radixin FERM domain crystallized as tetragonal crystals (Hamada, Matsui *et al.*, 2000; Hamada, Shimizu *et al.*, 2000) which also had a relatively high solvent content (71%).

Structural analysis of the crystal by molecular replacement using the free radixin FERM domain (Hamada, Shimizu *et al.*, 2000) is in progress.

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