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Crystallization and preliminary crystallographic analysis of the ezrin FERM domain

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Ezrin is a member of the ERM (ezrin/radixin/moesin) family of proteins that cross-link the actin cytoskeleton to the plasma membrane and that also function, both upstream and downstream of the small G-protein Rho, in signaling cascades that regulate the assembly of actin stress fibers. In this study, crystals were obtained of the amino-terminal 297 residues (referred to as FERM) of ezrin. The crystals of the FERM domain of ezrin belong to the monoclinic space group $P2_1$, with unit-cell parameters a = 48.5, b = 112.8, c = 66.3 Å, β = 102.3°, and contain two molecules in the asymmetric unit. A 2.3 Å data set was collected using synchrotron radiation at CHESS A1.

1. Introduction

Ezrin belongs to the ERM [ezrin (Bretscher, 1983; Gould *et al.*, 1986)/radixin (Tsukita *et al.*, 1989; Funayama *et al.*, 1991)/moesin (Lankis & Furthmayr, 1991)] family of proteins that serve to mediate the cross-linking of the actin cytoskeleton and the plasma membrane (reviewed in Bretscher, 1999). Ezrin, radixin and moesin are found in vertebrates and while they share ~75% sequence identity, they show differences in their primary tissue distributions. Various lines of evidence indicate that the ERMs function as versatile scaffolds, interacting with a variety of signalling molecules, including the 85 kDa regulatory subunit of PI3-kinase (designated p85; Gautreau *et al.*, 1999), the multifunctional regulator RhoGDI (Hirao *et al.*, 1996), EBP50 (Reczek & Bretscher, 1998) and the tumour suppressor hamartin (Lamb *et al.*, 2000). However, despite seemingly functional redundancy, only ezrin is phosphorylated in response to epidermal growth factor, platelet-derived growth factor and hepatocyte growth factor (Kreig & Hunter, 1992; Jiang *et al.*, 1995; Crepaldi *et al.*, 1997) at Tyr353 (ezrin numbering). Furthermore, although the ERM family members share a common Tyr at position 145, only ezrin is phosphorylated in response to growth-factor stimulation.

Recently, structures of other members of the FERM family have been reported. These include the FERM domains of moesin (Edwards & Keep, 2001), radixin (Hamada *et al.*, 2000), band 4.1 (Han *et al.*, 2000) and the dormant FERM domain of moesin bound to the C-terminal ERM activation domain or CERMAD (Pearson *et al.*, 2000). These studies revealed that the FERM domain is a clover-shaped molecule consisting of three structural domains (lobes F1, F2 and F3). Residues 2–82 (lobe F1) possess a ubiquitin-like fold, residues 83–195 (lobe F2) fold into a topology like that of acylCoA-binding protein (Kragelund *et al.*, 1993) and residues 196–297 (lobe F3) adopt the pleckstrin homology (PH)/phosphotyrosine-binding (PTB) fold. The active FERM domains of radixin and moesin each reveal a localized conformational change in lobe F3 (residues 162–171) and movement in lobe F2 (residues 139–145) compared with dormant moesin.

Combining the structure for the FERM domain of ezrin with those of the other FERMs will allow us to identify those structural changes that are a consequence of activation as opposed to sequence differences or crystal packing interactions. Also, the higher resolution of our structural analysis should provide more details regarding the structural changes involved in activation. Finally, comparisons between active radixin and moesin will highlight the unique structural features of ezrin.

2. Expression and purification of the recombinant FERM domain of ezrin

2.1. Expression

The vector preparation and expression of the FERM domain of human ezrin (residues 1–297) has been described previously (Reczek *et al.*, 1997). However, owing to poor protein yield, the construct of the human ezrin FERM was transformed into *Escherichia coli* BL21-DE3 (Novagen) and cultured using 4 l fermentors. Protein expression was induced using 100 µ*M* IPTG when the cell suspension had reached an OD_{600} of 0.6. The fermentors were switched to 298 K and induced for 12 h. Cells were harvested by centrifugation at 6000 rev min^{-1} for 10 min. Cell pellets were stored at 200 K.

2.2. Purification

Purification of the FERM domain of ezrin was modified from previous methods (Reczek *et al.*, 1997). The modified purification consisted of resuspension in 180 m*M* KH_2PO_4 buffer containing 0.20 *M* phenylmethanesulfonyl fluoride, 10 mg ml^{-1} aprotonin and leupeptin, and 0.25 *M* benzamidine. 1 mg ml^{-1} of lysozyme (Sigma–Aldrich) was added to the lysis suspension. When lysis was complete, DNase I (Roche) was added to 0.05 mg ml^{-1} followed by the addition of 0.4 mg ml^{-1} of deoxycholate (Sigma–Aldrich). The lysate was cleared by centrifugation and applied to a hydroxyapatite column; ezrin was eluted with a 180–800 m*M* KH_2PO_4 gradient. The ezrin was then dialyzed against 20 m*M* MES, 180 m*M* NaCl and applied to an SP-Sepharose column, with elution occurring in an 180–800 m*M* NaCl gradient (Fig. 1). Optimal protein expression yielded 250 mg in 8 l of culture. Purified ezrin FERM was concentrated to ∼20 mg ml^{-1}, flash-frozen in liquid N_2 and stored at 200 K.

3. Crystallization

Purified ezrin FERM was thawed and dialyzed against 20 m*M* Tris–HCl pH 8.0 and 300 m*M* NaCl. The protein was then concentrated to ∼30 mg ml^{-1}. Initial crystallization screening experiments were carried out at 291 K using the screens of Jancarik & Kim (1991) and Cudney *et al.* (1994). Crystalline growth only took place in experiments containing PEG 4K as precipitant buffered with Tris at 291 K. Various polyethelene glycols (PEG 2K, 3K, 4K, 8K) and polyethylene glycol monomethyl ethers (PEG MME 2K and 5K) were then investigated as precipitants in the concentration range 10–25%. Glycerol and 2-propanol were found to improve the size of the crystals. Final crystals were grown at 291 K using the hanging-drop method by mixing 2 µl of ezrin FERM domain with 2 µl of reservoir solution containing 10–15%(*w*/*v*) monomethyl ether PEG 2000 (Fluka), 15%(*w*/*v*) glycerol, 10%(*w*/*v*) 2-propanol, 0.1 *M* Na HEPES pH 8.1. The crystals grew to approximately 100 × 200 × 400 µm in size in 2 d (Fig. 2).

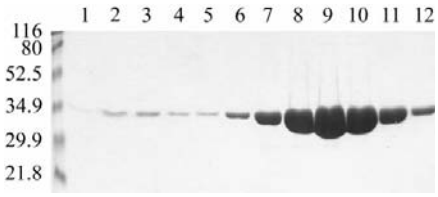

Figure 1
SDS–PAGE pattern showing the migration of purified recombinant ezrin FERM after final SP-Sepharose purification. Lane 1 corresponds to molecular-weight markers (values are given in kDa).

Table 1
Data-collection statistics.

Values in parentheses refer to the last resolution shell (2.4–2.3 Å).

Space group	$P2_1$
Unit-cell parameters (Å, °)	a = 48.5, b = 112.8, c = 66.3, β = 102.3.
Resolution range (Å)	26.0–2.3
Unique reflections	31885
$I/\sigma(I)$	7.1 (2.8)
$R_{\rm meas}$ (%)	6.6 (35)
Completeness (%)	91
Multiplicity	2.5
Solvent content (%)	51

4. Data collection and analysis

For cryoprotection experiments, crystals were soaked in solutions containing reservoir solution supplemented with 15% PEG 2K and 15% glycerol. Initial attempts to gradually increase the cryoprotection solution concentration led to a decrease in the diffraction resolution and the crystals were prone to cracking. Cryocooling was performed by mounting single crystals in CrystalCap Copper Magnetic cryoloops (Hampton Research, CA, USA) followed by immediate immersion in liquid N_2 and storage at 100 K until use. X-ray diffraction data collection using cryocooled crystals was carried out on station A1 at the Cornell High Energy Synchrotron Source (CHESS) using a Quantum 2K CCD detector at 100 K. The crystal-to-detector distance was 200 mm with a wavelength of 0.928 Å. 180 images were taken with 1° oscillations. Data processing and integration were performed with the *MOSFLM* package and scaling was performed with the *SCALA* program (Collaborative Computational Project, Number 4, 1994).

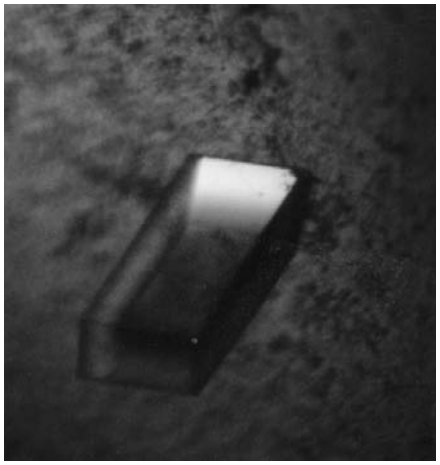

Figure 2
Monoclinic crystal of native ezrin FERM domain grown using the hanging-drop vapour-diffusion method

5. Results and discussion

The crystals have the symmetry of space group $P2_1$ and unit-cell parameters a = 48.5, b = 112.8, c = 66.3 Å, β = 102.3°. With the assumption of two molecules in the asymmetric unit, a value for the Matthews parameter of 2.53 Å^3 Da^{-1} is obtained, with a corresponding solvent content of approximately 51%. A set of native diffraction data extending to a resolution limit of 2.3 Å [31 885 unique reflections with $I/\sigma(I)$ = 7.1 for all data and $I/\sigma(I)$ = 2.8 for the last resolution shell (2.4–2.3 Å), $R_{\rm meas}$ = 6.6% and 91% completeness] was used for the determination of the crystal structure (Table 1). The high degree of amino-acid sequence identity between moesin and radixin FERM domains (∼85%) will enable us to use the molecular-replacement method for determination of the ezrin FERM domain. The crystal structure of the ezrin FERM domain will offer insight as to why, despite their sequence similarity, only ezrin and not its closely related family members are phosphorylated in response to growth-factor stimulation. Furthermore, owing to our higher resolution analysis, we will be able to draw more specific conclusions as to the method of activation of the ERM proteins.

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