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# Crystallization and preliminary crystallographic studies of RhoGDI in complex with the radixin FERM domain

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The Rho guanine nucleotide-dissociation inhibitor (RhoGDI) is a general regulator that forms a complex with the GDP-bound form of Rho-family GTPases and suppresses their activation. The FERM domains of ERM (ezrin/radixin/moesin) proteins bind to RhoGDI and dissociate Rho from RhoGDI. The formation of a complex between RhoGDI and the FERM domain is an important step in the regulatory cycle of Rho activation. In this study, crystals of RhoGDI complexed with the FERM domain of radixin were obtained. The crystals of the binary complex belong to the space group $P2_12_12$, with unit-cell parameters $a$ = 130.9 (2), $b$ = 151.2 (2), $c$ = 71.2 (1) Å, and contain two protein complexes in the crystallographic asymmetric unit. A 2.9 Å resolution data set was collected using synchrotron radiation at SPring-8.

## 1. Introduction

The Rho-family small GTPases participate in the regulation of the actin cytoskeleton and various cell-adhesion events (Van Aelst & D'Souza-Schorey, 1997; Hall, 1998; Kaibuchi *et al.*, 1999). They act as molecular switches, cycling between an active GTP-bound and an inactive GDP-bound state. The GTP/GDP cycle is regulated by three different classes of proteins, guanine nucleotide-exchange factors (GEFs), GTPase-activating proteins (GAPs) and guanine nucleotide-dissociation inhibitors (GDIs). GEFs and GAPs stimulate the GTP/GDP exchange reaction and GTP hydrolytic reaction, respectively (Hall, 1990; Takai *et al.*, 1995; Cerione & Zheng, 1996). RhoGDI is a molecule that interacts specifically with the GDP-bound form of Rho GTPases and regulates their activities by preventing nucleotide dissociation (Takai *et al.*, 1995; Ueda *et al.*, 1990; Fukumoto *et al.*, 1990). Rho–GDP in complex with RhoGDI is not activated by RhoGEFs (Takai *et al.*, 1995; Hart *et al.*, 1991; Yaku *et al.*, 1994; Ozaki *et al.*, 1996), suggesting that another factor works in the activation of Rho GTPases. ERM (ezrin/radixin/moesin) proteins are general cross-linkers between cortical actin filaments and the plasma membrane and have the ability to stimulate the release of Rho GTPase from GDIs. *In vitro*, the N-terminal half (corresponding to the FERM domain) of radixin dissociates Rho–GDP from RhoGDI by interacting directly with RhoGDI in the Rho–GDP–RhoGDI complex (dissociation constant of $\sim$0.6 $\mu M$) and thereby enhances the ability of RhoGEFs to induce GDP/GTP exchange (Takahashi *et al.*, 1997).

In order to understand the recognition mechanism of RhoGDI by the FERM domain and the general molecular mechanisms by which ERM proteins release Rho GTPases from RhoGDI, we have been engaged in the structural determination of the complex between RhoGDI and the radixin FERM domain. Here, we report the purification, crystallization and preliminary crystallographic results of the protein complex between full-length RhoGDI and the radixin FERM domain.

## 2. Materials and methods

Bovine RhoGDI (residues 1–204; 23.4 kDa) was overexpressed in BL21(DE3)RIL cells harbouring plasmid pGEX-2T as a fusion protein with glutathione S-transferase (GST) (Takahashi *et al.*, 1997). The cells were disrupted by sonication at 277 K in a solution containing 200 m$M$ NaCl, 10 m$M$ Tris–HCl pH 7.8 and 1 m$M$ EDTA. The supernatant was applied onto a GST affinity column with glutathione Sepharose 4B (Pharmacia Biotech, Uppsala, Sweden). The GST tag was cleaved by digestion with 10 units ml$^{-1}$ human thrombin (Nippon Ham, Japan) for 5 h at 294 K on the affinity column. The cleaved proteins were collected and further purified by HiTrap Q (Pharmacia Biotech). The purity of the protein was monitored by 12.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) and the gels were

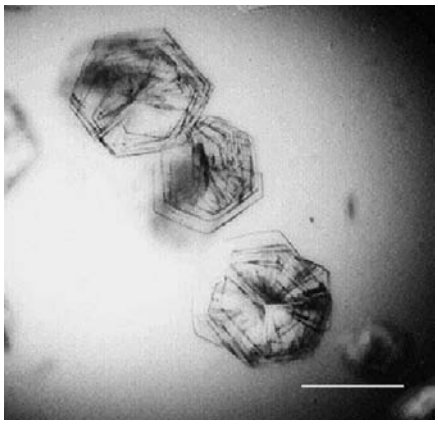

**Figure 1**
Crystals of the complex between RhoGDI and the radixin FERM domain. The scale bar indicates 0.5 mm.

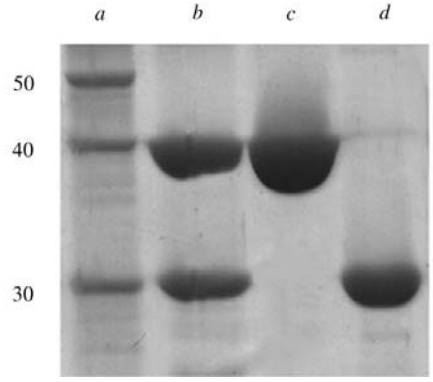


**Figure 2**
SDS–PAGE of molecular-weight markers (lane *a*), the complex crystals (lane *b*), the radixin FERM domain (lane *c*) and RhoGDI (lane *d*).

stained with Coomassie brilliant blue. The purified protein was concentrated to 26 mg ml$^{-1}$ using a Centricon 10 ultrafiltration membrane (Amicon, Beverly, MA, USA). Using this purification scheme, 10 mg of purified protein was obtained from 5 g of wet cells. N-terminal analysis (M492, Applied Biosystems) and matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy (MALDI-TOF MS; PerSeptive) were performed to validate the purity of the resulting protein. N-terminal analysis revealed that the protein has four additional residues (Gly-Ser-Gly-Thr) at the N-terminus.

The FERM domain (residues 1–310, 36.7 kDa) of mouse radixin was also expressed as a fusion protein with glutathione S-transferase in BL21(DE3)RIL cells (Matsui *et al.*, 1998). Details of the purification and verification of the protein have been described previously (Hamada *et al.*, 2000). The purified bovine RhoGDI (26 mg ml$^{-1}$) and the FERM domain of mouse radixin (24 mg ml$^{-1}$) were mixed in a 1:1 molar ratio in a solution of 230 m*M* NaCl, 6 m*M* MES pH 6.8 and 1 m*M* DTT. Crystallization conditions were screened using the hanging-drop vapour-diffusion method at 277 K. It was confirmed by SDS–PAGE and MALDI-TOF MS that the crystals contained both RhoGDI and the FERM domain of radixin as a 1:1 complex.

X-ray diffraction data were collected with a MAR CCD detector installed on the BL41XU beamline (Kamiya *et al.*, 1995) at SPring-8 using crystals flash-frozen in cryobuffer. The data collection was performed with an angular range of 180°, a step size of 1.0° and an exposure time of 10 s. The wavelength was set to 1.00 Å. The camera was fixed at a distance of 160 mm. All data were processed and scaled with the programs *MOSFLM* (Leslie *et al.*, 1986) and *SCALA* (Collaborative Computational Project, Number 4, 1994), respectively.

## 3. Results and discussion

Crystals of the binary complex were obtained in several days at 277 K by mixing the complex with an equal volume of reservoir solution containing 8% polyethylene glycol 8000 (PEG 8K), 40 m*M* sodium cacodylate pH 6.5, 80 m*M* magnesium acetate and 10% glycerol (Fig. 1). Crystals were thoroughly washed with the reservoir solution and dissolved in an aliquot of water for analysis by SDS–PAGE. The gels showed that the crystals contained the 1:1 complex of RhoGDI and the radixin FERM domain (Fig. 2). The morphology of the crystals consisted of clusters of plates, which we disassembled into several single crystals having typical dimensions of approximately 0.5 × 0.2 × 0.03 mm. All diffraction data were collected from crystals cooled to 100 K after soaking for several minutes in a cryobuffer containing 20% glycerol, 16% PEG 8K, 80 m*M* sodium cacodylate pH 6.5 and 160 m*M* magnesium acetate. The crystals diffracted to 2.9 Å resolution and formed in space group $P2_12_12$, with unit-cell parameters $a$ = 130.9 (2), $b$ = 151.2 (2), $c$ = 71.2 (1) Å. The total number of measured reflections was 516 798, which gave 32 141 unique reflections. The resulting data gave an $R_{\rm merge}$ of 8.1% (43.7% for the outer shell, 2.90–3.06 Å) with a completeness of 98.8% (99.7% for the outer shell). The multiplicity of reflections was 4.9 (5.2 for the outer shell) and the crystal mosaicity was 0.77°. The crystal was estimated to contain two protein complexes in the asymmetric unit with a $V_{\rm M}$ value (Matthews, 1968) of 2.87 Å$^3$ Da$^{-1}$, corresponding to a solvent content of 57%. Structural analysis of the crystals using molecular-replacement and/or multiple isomorphous replacement methods is in progress.

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