Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallographic characterization of the radixin FERM domain bound to the C-terminal region of the human Na^+/H^+-exchanger regulatory factor (NHERF)

Shin-ichi Terawaki,[a] Ryoko Maesaki,[a] Kengo Okada[a] and Toshio Hakoshima[a,b]*

[a]Structural Biology Laboratory, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 630-0192, Japan, and [b]CREST, Japan Science and Technology Corporation, 8916-5 Takayama, Ikoma, Nara 630-0192, Japan

Correspondence e-mail: hakosima@bs.aist-nara.ac.jp

Received 12 June 2002
Accepted 28 October 2002

Radixin is a member of the ERM proteins, which cross-link plasma membranes and actin filaments. The N-terminal FERM domains of ERM proteins interact with Na^+/H^+-exchanger regulatory factors (NHERFs), which are PDZ-containing adaptor proteins, to modulate the ion-channel activity. Here, crystals of complexes between the radixin FERM domain and the C-terminal regions of NHERF and NHERF2 have been obtained. The crystals of the FERM–NHERF complex were found to belong to space group $P2_12_12_1$, with unit-cell parameters a = 69.38 (2), b = 146.27 (4), c = 177.76 (7) Å. The crystal contains four complexes in the asymmetric unit. An intensity data set was collected to a resolution of 2.50 Å.

1. Introduction

Ezrin/radixin/moesin (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). At these cell-surface structures, ERM proteins play a role as cross-linkers between plasma membranes and actin filaments (Arpin *et al.*, 1994; Bretscher, 1999; Mangeat *et al.*, 1999; Tsukita & Yonemura, 1999). 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 protein 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 FERM domain directly binds the cytoplasmic domains of adhesion molecules such as the intercellular adhesion molecules ICAM-1, ICAM-2 and ICAM-3 of the immunoglobulin superfamily, the cell-surface hyaluronate receptor CD44 and the cell-surface glycoprotein CD43 (Helander *et al.*, 1996; Yonemura *et al.*, 1993, 1998; Tsukita *et al.*, 1994). Interestingly, the FERM domain also binds the Na^+/H^+-exchanger regulatory factor (NHERF) and NHERF2, which are adaptor proteins for ion channels. NHERF, which is also called ERM-binding phosphoprotein 50 (EBP50; Reczek *et al.*, 1997), is necessary for the protein kinase A regulation of Na^+/H^+-exchanger 3 (NHE3; Weinman *et al.*, 1995). NHERF2 is the second member of this family and is also called the Na^+/H^+-exchanger 3 kinase A regulatory factor (E3KARP; Yun *et al.*, 1997, 1998). NHERF and NHERF2 display 55% sequence identity.

Human NHERF is a 358-residue protein containing two PSD-95/Dlg/ZO-1 homology (PDZ) domains (residues 11–97 and 150–237) followed by ~120 C-terminal residues. The first PDZ domain of NHERF interacts with four carboxy-terminal residues of the cytoplasmic domains of NHE3 and other ion channels such as the cystic fibrosis transmembrane conductance regulator, which functions as a cAMP-regulated chloride channel, and the β_2 adrenergic receptor (Hall *et al.*, 1998; Short *et al.*, 1998; Wang *et al.*, 1998). These NHERF-interactive membrane proteins are linked to the actin cytoskeleton by interaction between the C-terminal region of NHERF and the FERM domain of ERM proteins, which directly bind actin filaments (Reczek & Bretscher, 1998). Here, we report the first crystallization and preliminary crystallographic studies of the radixin FERM domain complexed with the C-terminal region of NHERF (the FERM–NHERF complex) and with the C-terminal region of NHERF2 (the FERM–NHERF2 complex).

2. Method and results

2.1. Protein preparation

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 *et al.*, 2000). The purified samples were verified with matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy (MALDI–TOF MS; PerSeptive Inc.) and N-terminal analysis (M492; Applied Biosystems). The peptides corresponding to

the C-terminal regions of human NHERF and NHERF2 were purchased from Sawady Technology (Tokyo, Japan). A binding assay of these peptides to the FERM domain was performed by surface plasmon resonance measurements using a BIAcore Biosensor instrument (BIAcore 3000; Pharmacia Biosensor). Each biotinylated peptide was immobilized on the surface of an SA sensor chip. The purified FERM domain was injected onto the peptide surfaces under a constant flow (30 µl min^{-1}). The kinetic parameters were evaluated using the *BIA* evaluation software (Pharmacia). We tested peptides from the C-terminal region encompassing about 50 residues and found that the NHERF peptide, which consists of 28 C-terminal residues (^{331}KERAHQKRSSKRAPQMDWSKKNELFSNL^{358}), binds the radixin FERM domain with high affinity (K_d = 7.5 n*M*; data not shown). The NHERF2 peptide (^{310}KEKARAMRVNKRAPQMDWNRKREIFSNF^{337}) also binds the radixin FERM domain, with K_d = 32.7 n*M*. These results were consistent with the previous reports indicating that the minimum binding regions of NHERF and NHERF2 to ERM proteins contain 30 C-terminal residues (Yun *et al.*, 1998; Reczek & Bretscher, 1998). For crystallization, the unbiotinylated peptides were dissolved to 5.3 m*M* concentration in a buffer containing 70 m*M* NaCl and 10 m*M* Na MES pH 6.8, 1 m*M* DTT.

2.2. Crystallization

The radixin FERM domain and the NHERF peptide were mixed in a 1:1 molar ratio (each at 0.33 m*M*) in a solution of 185 m*M* NaCl, 10 m*M* Na MES pH 6.8 and 1 m*M* DTT. Crystallization conditions were searched using the hanging-drop vapour-diffusion method at 277 K. Crystals of the complex were obtained in 3 d by combining 1 µl of protein solution with 1 µl of reservoir solution containing 10% polyethylene glycol 4000 (PEG 4K), 5% 2-propanol, 100 m*M* Na HEPES pH 7.5. The crystals grew to maximal dimensions of 0.2 × 0.5 × 0.1 mm (Fig. 1). The obtained crystals were re-dissolved in an aliquot of water for MALDI–TOF MS in order to verify that the crystals contain both the radixin FERM domain and the NHERF peptide. We observed a peak of 3400.4 Da corresponding to the calculated value of 3400.9 Da for the NHERF peptide, as well as a peak corresponding to the radixin FERM domain. The crystals were transferred stepwise into a cryoprotective solution containing 20% PEG 200, 20% PEG 4K, 10% 2-propanol and 100 m*M* Na HEPES pH 7.5 for flash-cooling.

Crystals of the complex between the radixin FERM domain and the NHERF2 peptide were obtained under a condition similar to that for the FERM–NHERF complex. The radixin FERM domain and the NHERF2 peptide were mixed in a 1:1 molar ratio (each of 0.45 m*M*) in the same solution as for the FERM–NHERF complex. Crystals of the complex were obtained in two weeks by combining 1.3 µl of protein solution with 0.7 µl of the same reservoir solution as for the FERM–NHERF complex. The crystals grew to maximal dimensions of 0.2 × 0.4 × 0.01 mm. It was also confirmed by MALDI–TOF MS that the crystals contain both the radixin FERM domain and the NHERF2 peptide. We observed a peak of 3507.82 Da corresponding to the calculated value of 3508.09 Da for the NHERF2 peptide, as well as a peak corresponding to the radixin FERM domain. The crystals were transferred stepwise into the same cryoprotective solution as used for the FERM–NHERF complex crystals.

2.3. Data collection and processing

X-ray diffraction data of the FERM–NHERF complex were collected from flash-frozen crystals using an ADSC Quantum 4R detector installed on the BL40B2 beamline at SPring-8. The data collection was performed with a total oscillation range of 180°, with a step size of 0.5° for an exposure time of 60 s. The camera distance was 180 mm. Crystals were found to diffract to a resolution of 2.5 Å and to belong to space group $P2_12_12_1$, with unit-cell parameters a = 69.38 (2), b = 146.27 (4), c = 177.76 (7) Å. All data were processed with the programs *MOSFLM* (Leslie, 1992) and *SCALA* (Collaborative Computational Project, Number 4, 1994). The total number of observed reflections was 1 059 886, which gave 63 273 unique reflections. The resulting data gave an R_{merge} of 6.5% (34.9% for the outer shell, 2.5–2.64 Å) with a completeness of 99.2% (99.2% for the outer shell). The estimated mosaicity of the crystal was 0.30°. Assuming the presence of four complexes in the asymmetric unit, the calculated value of the crystal volume per protein mass (V_M; Matthews, 1968) is 2.81 $Å^3$ Da^{-1}. This value corresponds to a solvent content of approximately 56%.

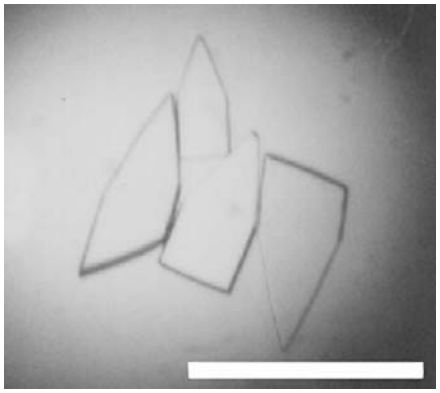

Figure 1
Crystals of the complex between the radixin FERM domain and the C-terminal peptide of NHERF. The scale bar indicates 0.5 mm.

X-ray diffraction data of the FERM–NHERF2 complex were collected from flash-frozen crystals using a MAR CCD detector installed on the BL41XU beamline at SPring-8 with a total oscillation range of 82.5°, with a step size of 0.5° for an exposure time of 5 s. Crystals were found to belong to space group $P2_12_12_1$, with unit-cell parameters a = 68.23 (2), b = 143.69 (4), c = 177.65 (7) Å, which were nearly isomorphous to the crystals of the FERM–NHERF complex. Intensity data at 3.0 Å resolution were processed in a similar manner to those of the FERM–NHERF complex. The total number of observed reflections was 642 361, which gave 45 938 unique reflections with an R_{merge} of 10.0% (26.2% for the outer shell, 3.0–3.16 Å) and a completeness of 97.1% (97.1% for the outer shell). Structural analyses of both crystals by molecular replacement using the free radixin FERM domain are in progress.

We would like to thank Dr K. Hamada for his advice in protein preparation and J. Tsukamoto for technical support in performing MALDI–TOF MS analysis. This work was partly supported by Grants in Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan to TH (12490024). RM was supported by a research fellowship for Young Scientists from the Japan Society for the Promotion of Science.

References

Amieva, M. R. & Furthmayr, H. (1995). *Exp. Cell Res.* **219**, 180–196.
Arpin, M., Algrain, M. & Louvard, D. (1994). *Curr. Opin. Cell Biol.* **6**, 136–141.
Bretscher, A. (1999). *Curr. Opin. Cell Biol.* **11**, 109–116.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Franck, Z., Gary, R. & Bretscher, A. (1993). *J. Cell. Sci.* **105**, 219–231.
Hall, R. A., Ostedgaard, L. S., Premont, R. T., Blitzer, J. T., Rahman, N., Welsh, M. J.,

Lefkowitz, R. J. (1998). *Proc. Natl Acad. Sci. USA*, **95**, 8496–8501.
Hamada, K., Shimizu, T., Matsui, T., Tsukita, S., Tsukita, S. & Hakoshima, T. (2000). *EMBO J.* **19**, 4449–4462.
Helander, T. S., Carpen, O., Turunen, O., Kovanen, P. E., Vaheri, A. & Timonen, T. (1996). *Nature (London)*, **382**, 265–268.
Leslie, A. G. W. (1992). *Jnt CCP4/ESF–EACBM Newsl. Protein Crystallogr.* **26**.
Mangeat, P., Roy, C. & Mertin, M. (1999). *Trends Cell Biol.* **9**, 187–192.
Matsui, T., Maeda, M., Doi, Y., Yonemura, S., Amano, M., Kaibuchi, K., Tsukita, S. & Tsukita, S. (1998). *J. Cell. Biol.* **140**, 647–657.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Reczek, D., Berryman, M. & Bretscher, A. (1997). *J. Cell. Biol.* **139**, 169–179.
Reczek, D. & Bretscher, A. (1998). *J. Biol. Chem.* **273**, 18452–18458.
Sato, N., Funayama, N., Nagafuchi, A., Yonemura, S., Tsukita, S. & Tsukita, S. (1992). *J. Cell. Sci.* **103**, 131–143.
Sato, N., Yonemura, S., Obinata, T., Tsukita, S. & Tsukita, S. (1991). *J. Cell. Biol.* **113**, 321–330.
Serrador, J. M., Alonso-Lebrero, J. L., del Pozo, M. A., Furthmayr, H., Schwartz-Albiez, R., Calvo, J., Lozano, F. & Sanchez-Madrid, F. (1997). *J. Cell. Biol.* **138**, 1409–1423.
Short, D. B., Trotter, K. W., Reczek, D., Kreda, S. M., Brestscher, A., Boucher, R. C., Stutts, M. J. & Milgram, S. L. (1998). *J. Biol. Chem.* **273**, 19797–19801.
Tsukita, S., Hieda, Y. & Tsukita, S. (1989). *J. Cell. Biol.* **108**, 2369–2382.
Tsukita, S., Oishi, K., Sato, N., Sagara, J., Kawai, A. & Tsukita, S. (1994). *J. Cell. Biol.* **126**, 391–401.
Tsukita, S. & Yonemura, S. (1999). *J. Biol. Chem.* **274**, 34507–34510.
Wang, S., Raab, R. W., Schatz, P. J., Guggino, W. B. & Li, M. (1998). *FEBS Lett.* **427**, 103–108.
Weinman, E. J., Steplock, D., Wang, Y. & Shenolikar, S. (1995). *J. Clin. Invest.* **95**, 2143–2149.
Yonemura, S., Hirao, M., Doi, Y., Takahashi, N., Kondo, T., Tsukita, S. & Tsukita, S. (1998). *J. Cell. Biol.* **140**, 885–895.
Yonemura, S., Nagafuchi, A., Sato, N. & Tsukita, S. (1993). *J. Cell. Biol.* **120**, 437–449.
Yun, C. H., Lamprecht, G., Forster, D. V. & Sidor, A. (1998). *J. Biol. Chem.* **273**, 25856–25863.
Yun, C. H., Oh, S., Zizak, M., Steplock, D., Tsao, S., Tse, C. M.,Weinman, E. J. & Donowitz, M. (1997). *Proc. Natl Acad. Sci. USA*, **94**, 3010–3015.