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Structural studies on molecular recognition for the radixin FERM domain

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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. These proteins have been found in eukaryotic cells. In cultured cells, ERM proteins are coexpressed in cell-adhesion sites, ruffling membranes and cleavage furrows where actin filaments associate with plasma membranes.

Radixin, which was originally isolated from rat liver as a component of cell-cell adherens junctions, consistent of 583 amino-acid residues with three domains, the FERM (4.1 and ERM) domain (residue 1-310), a central helical domain (residues 311-470) and a C-terminal tail domain (477-583) which binds F-actin. The amino-acid sequence of the FERM domain are 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.

FERM domains interact with a variety of proteins; PDZ-containing adaptor proteins such as Na^+/H^+ exchanger 3 kinase A regulatory protein (NHERF) and ERM-binding phospho-protein 50 (NHERF2), the cytoplasmic tail of L-selectin that regulates the recruitment of native lymphocytes from the bloodstream to secondary lymphoid organs, and other different adhesion molecules (*e.g.* ICAM-1, -2, -3 of the immunoglobulin superfamily, the cell-surface hyaluronate receptor CD44, and the cell-surface glycoprotein CD43).

Here, crystals of complexes between the radixin FERM domain and the C-terminal regions of NHERF and NHERF2 have been obtained.

Human NHERF is 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.

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 the ERM proteins, which directly bind actin filaments.

Method and results

The radixin FERM domain and the NHERF peptide were mixed in a 1:1 molar ratio in a solution of 185 mM NaCl, 10 mM Na MES pH 6.8 and 1 mM DTT. Crystallization condition were searched using the hanging-drop vapor-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 mM Na HEPES pH 7.5. The crystals grew to maximal dimensions of 0.5 x 0.2 x 0.1 mm. 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. A peak of 3400.9 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.

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. 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. It was also confirmed by MALDI-TOF MS that the crystals contain both the radixin FERM domain and the NHERF2 peptide.

X-ray diffraction data of the FERM-NHERF complex were collected from flash-frozen crystals using synchrotron. The data collection was performed with a total oscillation range of 180 degree with a step size of 0.5 degree. Crystals were found to diffract to a resolution of 2.5 angstrom and belong to space group $P2_12_12_1$, with unit-cell parameters a=69, b=146, c=177 angstrom.

X-ray diffraction data of the FERM-NHERF2 complex were collected from flash-frozen crystals using synchrotron. The data collection was performed with a total oscillation range of 90 degree with a step size of 0.5 degree. Crystals were found to diffract to a resolution of 3.0 angstrom and belong to space group $P2_12_12_1$, with unit-cell parameters a=68, b=143, c=177 angstrom, which were nearly isomorphous to the crystals of the FERM-NHERF complex.

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