Biochemical and Biophysical Research Communications 446 (2014) 399–403

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Structural insights into PDZ-mediated interaction of NHERF2 and LPA_2, a cellular event implicated in CFTR channel regulation

Joshua Holcomb [a], Yuanyuan Jiang [a], Guorong Lu [a], Laura Trescott [a], Joseph Brunzelle [b], Nualpun Sirinupong [c], Chunying Li [a], Anjaparavanda P. Naren [d,*], Zhe Yang [a,*]

[a] Department of Biochemistry and Molecular Biology, Wayne State University School of Medicine, Detroit, MI, USA
[b] Advanced Photon Source, Argonne National Lab, Argonne, IL, USA
[c] Nutraceuticals and Functional Food Research and Development Center, Prince of Songkla University, Hat-Yai, Songkhla, Thailand
[d] Department of Pediatrics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

ARTICLE INFO

Article history:
Received 25 February 2014
Available online 12 March 2014

Keywords:
NHERF2
LPA_2
X-ray crystallography
CFTR
Scaffold protein
Cystic fibrosis

ABSTRACT

The formation of CFTR–NHERF2–LPA_2 macromolecular complex in airway epithelia regulates CFTR channel function and plays an important role in compartmentalized cAMP signaling. We previously have shown that disruption of the PDZ-mediated NHERF2–LPA_2 interaction abolishes the LPA inhibitory effect and augments CFTR Cl^- channel activity in vitro and in vivo. Here we report the first crystal structure of the NHERF2 PDZ1 domain in complex with the C-terminal LPA_2 sequence. The structure reveals that the PDZ1–LPA_2 binding specificity is achieved by numerous hydrogen bonds and hydrophobic contacts with the last four LPA_2 residues contributing to specific interactions. Comparison of the PDZ1–LPA_2 structure to the structure of PDZ1 in complex with a different peptide provides insights into the diverse nature of PDZ1 substrate recognition and suggests that the conformational flexibility in the ligand binding pocket is involved in determining the broad substrate specificity of PDZ1. In addition, the structure reveals a small surface pocket adjacent to the ligand-binding site, which may have therapeutic implications. This study provides an understanding of the structural basis for the PDZ-mediated NHERF2–LPA_2 interaction that could prove valuable in selective drug design against CFTR-related human diseases.

© 2014 Elsevier Inc. All rights reserved.

1. Introduction

Cystic fibrosis transmembrane conductance regulator (CFTR) is a cAMP-regulated chloride (Cl^-) channel primarily localized at the apical surfaces of epithelial cells lining the airway, gut and exocrine glands [1,2]. CFTR is responsible for transepithelial salt and water transport and plays critical roles in maintaining fluid homoeostasis, airway fluid clearance, and airway submucosal glands secretion in both healthy and disease phenotypes [3,4]. Growing evidence suggests that CFTR interacts directly or indirectly with other ion channels, transporters, scaffolding proteins, protein kinases, effectors, and cytoskeletal elements to form macromolecular complexes at specialized subcellular domains [5,6]. These dynamic protein–protein interactions regulate CFTR channel function as well as its localization and processing within cells [7,8]. We have shown that CFTR, lysophosphatidic acid receptor 2 (LPA_2), and Na^+/H^+ exchanger regulatory factor-2 (NHERF2) form macromolecular complexes at the plasma membrane of gut epithelia, which functionally couple LPA_2 signaling to CFTR-mediated Cl^- transport [9]. LPA_2 is a G protein-coupled receptor that binds the lipid signaling molecule LPA and mediates diverse cellular responses such as cell proliferation and platelet aggregation [10]. NHERF2 is a PDZ domain-containing protein that typically functions as a scaffold to cluster transporters, receptors, and signaling molecules into supramolecular complexes [11]. We have demonstrated that LPA inhibits both CFTR-mediated Cl^- transport through the LPA_2-mediated Gi pathway in a compartmentalized manner in cells and CFTR-dependent cholera toxin-induced mouse intestinal-fluid secretion in vivo [9]. We also demonstrated that disruption of the PDZ-mediated NHERF2–LPA_2 interaction abolishes the LPA inhibitory effect and augments CFTR Cl^- channel activity in Calu-3 cells and also in fluid secretion from pig tracheal submucosal glands [12]. These findings imply that targeting the PDZ-mediated NHERF2–LPA_2 interaction could provide new strategies for therapeutic interventions of CFTR-associated diseases [8,12].

* Corresponding authors. Address: 540 E. Canfield Street, Detroit, Michigan 48201, USA. Fax: +1 313 577 2765 (Z. Yang). Address: 3333 Burnet Avenue, Cincinnati, OH 45229, USA (A.P. Naren).

E-mail addresses: zyang@med.wayne.edu (Z. Yang), anaren@cchmc.org (A.P. Naren).

http://dx.doi.org/10.1016/j.bbrc.2014.02.128
0006-291X/© 2014 Elsevier Inc. All rights reserved.

In general, PDZ domains mediate protein interactions by recognizing the C-terminal sequence of target proteins and binding to the targets through a canonically and structurally conserved PDZ peptide-binding pocket [13]. Based on the residues at positions 0 and −2 of the peptides (position 0 referring to the C-terminal residue), early studies have grouped PDZ domains into two major specificity classes: class I, (S/T)X(V/I/L) (X denoting any amino acid); class II, (F/Y)X(F/V/A) [14–16]. However, more recent mounting evidence indicates that PDZ specificity is unexpectedly complex and diverse, with the PDZ domain family recognizing up to seven C-terminal ligand residues and forming at least 16 unique specificity classes [17]. The complexity of PDZ-peptide interactions is further exemplified by the facts that many PDZ domains can bind to multiple ligands of different peptide classes and that single peptides are capable of binding to distinct PDZ domains [17]. This complex picture of PDZ-peptide interactions raises a challenging problem regarding how PDZ domains, structurally simple protein-interaction modules, achieve binding promiscuity and specificity concomitantly, the nature of which remains obscure. In this context, we present the crystal structure of NHERF2 PDZ1 in complex with the LPA_2 C-terminal peptide MDSTL. The structure reveals that the LPA_2 peptide binds to PDZ1 in an extended conformation with the last four residues making specific side chain contacts. Comparison of the PDZ1–LPA_2 structure to the structure of PDZ1 in complex with a different peptide suggests that the binding diversity of PDZ1 is facilitated by the conformational flexibility in the peptide-binding pocket. This study provides the structural basis of the PDZ-mediated NHERF2–LPA_2 interaction and could be valuable in the development of novel therapeutic strategies against CFTR-related human diseases.

2. Materials and methods

2.1. Protein expression and purification

A DNA fragment encoding the human NHERF2 PDZ1 (residues 9–90) was amplified by PCR using the full-length human NHERF2 cDNA as a template. The C-terminal extension MDSTL that corresponds to residues 347–351 of human LPA_2 was created by inclusion of 15 extra bases in the reverse primer. The PCR products were cloned in the pSUMO vector containing an N-terminal His6-SUMO tag. The resulting clone was transformed into *Escherichia coli* BL21 Condon Plus (DE3) cells for protein expression. The transformants were grown to an OD600 (optical density at 600 nm) of 0.4 at 37 °C in LB medium, and then induced with 0.1 mM isopropylthio-β-D-galactoside at 15 °C overnight. The cells were harvested by centrifugation and lysed by French Press. The soluble fraction was then subjected to Ni^{2+} affinity chromatography purification, followed by the cleavage of the His6-SUMO tag with yeast SUMO Protease 1. PDZ1 proteins were separated from the cleaved tag by a second Ni^{2+} affinity chromatography and further purified by size-exclusion chromatography. Finally, the proteins were concentrated to 20–30 mg/ml in a buffer containing 20 mM Tris–HCl (pH 8.0), 150 mM NaCl, 1 mM β-mercaptoethanol (BME), and 5% glycerol.

2.2. Crystallization, data collection and structure determination

Crystals were grown by the hanging-drop vapor-diffusion method by mixing the protein (~20 mg/ml) with an equal volume of a reservoir solution containing 100 mM HEPES, pH 7.0, 0.2 M potassium thiocyanate (KSCN), 25% PEG3350 at 20 °C. Crystals typically appeared overnight and continued to grow to their full size in 2–3 days. Prior to X-ray diffraction data collection, crystals were cryoprotected in a solution containing the mother liquor and 25% glycerol and flash cooled in liquid nitrogen. The data were collected at 100 K at beamline 21-ID-F at the Advanced Photon Source (Argonne, IL) and processed and scaled using the program XDS [18]. Crystals belong to the space group $P2_1$ with unit cell dimensions $a = 26.4$ Å, $b = 40.3$ Å, $c = 37.1$ Å, $\beta = 107.4°$, and one molecule in the asymmetric unit (Table 1). The structure was solved by the molecular replacement method with the program PHASER [19] using the PDZ1–EDTSV structure (PDB code: 2OCS) as a search model. Structure modeling was carried out in COOT [20], and refinement was performed with PHENIX [21]. To reduce the effects of model bias, iterative-build OMIT maps were used during model building and structure refinement. The final models were analyzed and validated with Molprobity [22]. All figures of 3D representations of the PDZ1–LPA_2 structure were made with PyMOL (www.pymol.org).

Table 1
Crystallographic data and refinement statistics.

Data	
Space group	$P2_1$
Cell parameters (Å)	
a	26.4
b	40.3
c	37.1
Wavelength (Å)	1.2719
Resolution (Å)	24.2–1.34 (1.37–1.34)
R_{merge} [a]	0.039 (0.250) [b]
Redundancy	4.1 (4.0)
Unique reflections	17,966
Completeness (%)	99.8 (99.6)
$\langle I/\sigma \rangle$	15.3 (3.0)
Refinement	
Resolution (Å)	24.2–1.34 (1.37–1.34)
Molecules/AU	1
R_{work} [c]	0.145 (0.268)
R_{free} [d]	0.177 (0.275)
Ramachandran plot	
Residues in favored	97.9%
Residues in allowed	2.1%
RMSD	
Bond lengths (Å)	0.007
Bond angles (°)	1.2
No. of atoms	
Protein	1347
Peptide	73
Water	143
Chloride	2
B-factor ($Å^2$)	
Protein	17.2
Peptide	17.7
Water	28.5
Chloride	20.2
SCN	12.4

[a] $R_{merge} = \Sigma|I - \langle I \rangle|/\Sigma I$, where I is the observed intensity and $\langle I \rangle$ is the averaged intensity of multiple observations of symmetry-related reflections.
[b] Numbers in parentheses refer to the highest resolution shell.
[c] $R_{work} = \Sigma|F_o - F_c|/\Sigma|F_o|$, where F_o is the observed structure factor, F_c is the calculated structure factor.
[d] R_{free} was calculated using a subset (5%) of the reflection not used in the refinement.

2.3. Protein data bank accession number

Coordinates and structure factors have been deposited in the Protein Data Bank with accession number 4P0C.

3. Results and discussion

3.1. Specificity determinants of NHERF2–LPA_2 interaction

The overall structure of NHERF2 PDZ1 is similar to other PDZ domains [16,23], consisting of six β strands (β1–β6) and two

Download English Version:

https://daneshyari.com/en/article/10755447

Download Persian Version:

https://daneshyari.com/article/10755447

Daneshyari.com