Crystal structure of human programmed cell death 10 complexed with inositol-(1,3,4,5)-tetrakisphosphate: A novel adaptor protein involved in human cerebral cavernous malformation

Jingjin Ding, Xiaoyan Wang, De-Feng Li, Yonglin Hu, Ying Zhang, Da-Cheng Wang

National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, People's Republic of China

ABSTRACT

Programmed cell death 10 (PDCD10) is a novel adaptor protein involved in human cerebral cavernous malformation, a common vascular lesion mostly occurring in the central nervous system. By interacting with different signal proteins, PDCD10 could regulate various physiological processes in the cell. The crystal structure of human PDCD10 complexed with inositol-(1,3,4,5)-tetrakisphosphate has been determined at 2.3 Å resolution. The structure reveals an integrated dimer via a unique assembly that has never been observed before. Each PDCD10 monomer contains two independent domains: an N-terminal domain with a new fold involved in the tight dimer assembly and a C-terminal four-helix bundle domain that closely resembles the focal adhesion targeting domain of focal adhesion kinase. An eight-residue flexible linker connects the two domains, potentially conferring mobility onto the C-terminal domain, resulting in the conformational variability of PDCD10. A variable basic cleft on the top of the dimer interface binds to phosphatidylinositide and regulates the intracellular localization of PDCD10. Two potential sites, respectively located on the two domains, are critical for recruiting different binding partners, such as germinal center kinase III proteins and the focal adhesion protein paxillin.

© 2010 Elsevier Inc. All rights reserved.

Introduction

Adaptor proteins are attracting more interest due to their essential roles in governing multiple protein cross-talk and signaling specificity of numerous signal transduction pathways that mediate various physiological processes in the cell [1,2]. They can recruit diffrent signal proteins to a specific location and regulate the interactions between these binding partners, thus driving the formation of larger signaling complexes. Adaptor proteins tend to lack any intrinsic catalytic activity but instead consist of different protein–protein and protein–lipid-interaction modules. For example, the Src-homology 2 (SH2) domains recognize specific sequences within their binding partners that contain phosphotyrosine residues, and Src-homology 3 (SH3) domains bind proline-rich sequences within specific peptide sequence contexts of their binding partners. Lipid-interaction modules, such as the pleckstrin-homology (PH) domains, bind phoshatidylinositois and determine the localization of the corresponding adaptor proteins [3].

Programmed cell death 10 (PDCD10) was initially characterized as a gene whose expression was upregulated upon the induction of apoptosis in human myeloid cell lines [4]. Later, PDCD10 was identified as the cerebral cavernous malformation 3 (CCM3) gene, in which loss-of-function mutations could result in cerebral cavernous malformation (CCM) [5]. CCM is a common vascular lesion found mostly in the central nervous system. Individuals with CCM may be under a risk of focal hemorrhage, resulting in headaches, seizures and stroke in midlife [6]. The protein encoded by the PDCD10 gene was predicted to be an adaptor protein because it lacks any known catalytic domains. As a novel adaptor protein, PDCD10 was identified as a component of the larger CCM signaling complex, which consists of KRIT1 (Krev1/Rap1A Interaction Trapped 1, also known as CCM1), OSM (Osmosensing Scaffold for MEKK3, also known as CCM2) and PDCD10 [7,8]. Both proliferative and apoptotic functions have been identified for this adaptor protein [9,10]. Recently, PDCD10 has been found to localize to the Golgi apparatus, forming a complex with proteins of the germinal center kinase III (GCKIII) family, which are involved in signal pathways of cell orientation and migration [11]. Although understanding of the physiological role of PDCD10 has increased, the structural basis for the function of PDCD10 remains unknown and is of great interest.
Here we present the crystal structure of PDCD10 complexed with inositol-(1,3,4,5)-tetrakisphosphate (4IP) and discuss the unique dimeric assembly, possible conformational variability and phosphatidylinositide binding of this novel adaptor protein. The potential sites of PDCD10 for binding partner recruitment are also investigated.

Material and methods

Cloning, expression, and purification

The truncated DNA comprising residues 8–212 of the human PDCD10 gene (GenBank: NP_009148) was amplified from the reverse transcriptase-polymerase chain reaction products of human hematopoietic stem cell by PCR and subcloned into a pET22b(+) vector (Novagen) using Nde I and Xho I restriction sites. The recombinant protein was overexpressed as a fusion protein with a C-terminal six-histidine tag in Escherichia coli BL21(DE3). The cells were grown in LB medium supplemented with 100 μg/mL ampicillin at 310 K until the culture reached an OD600 of 0.6, and expression was induced with 0.5 mM IPTG at 289 K overnight. The harvested cells were resuspended in lysis buffer (50 mM NaH2PO4, pH 8.0; 300 mM NaCl; 10 mM imidazole) with 0.1 mM PMSF and lysed by sonication. The lysate was clarified by centrifugation and purified on a nickel-affinity column followed by size-exclusion chromatography using a Superdex 200 column (Amersham Pharmacia). The purified protein was concentrated to 40 mg/ml and stored in 20 mM Tris–HCl pH 8.0, 150 mM NaCl, 0.2 mM EDTA, 5 mM DTT at 193 K. The selenomethionine-substituted (SeMet) derivative was produced by expression in E. coli methionine auxotrophic strain B834(DE3). The purification procedure of the SeMet derivative was the same as that of the native protein.

Crystallization

The crystallization experiments were conducted using the hanging-drop vapor-diffusion method at 293 K with 2 μl drops containing 1 μl protein solution and 1 μl reservoir solution equilibrated over 0.5 ml reservoir solution. Initial crystallization screening was performed using Index kit (Hampton Research). After two days, two conditions produced microcrystals: (i) 3.0 M NaCl and 100 mM Bis–Tris pH 5.5 and (ii) 200 mM ammonium sulfate, 25% (w/v) PEG 3350 and 100 mM Bis–Tris pH 5.5. Well-shaped crystals were obtained in the optimized conditions by varying the concentration of precipitants, the pH, and the type and concentration of additives. However, the crystals diffracted poorly to 8 Å on a Rigaku FRE diffraction system using a Rigaku R-Axis IV++ image plate detector. In an attempt to produce better crystals, we added inositol-(1,3,4,5)-tetrakisphosphate (4IP, Avanti Polar Lipids) to the protein to a final concentration of 5 mM and reset the crystallization trials. Well-diffracting crystals of both the native protein and the SeMet derivative were obtained under the following condition: 30% (v/v) ethylene glycol, 100 mM sodium cacodylate pH 6.0. Diamond-shaped crystals (300 /C2/C2/C200 m) typically appeared in 24 h.

Data collecting and processing

All diffraction data were collected at the beamline 17A (BL17A) at the Photon Factory (Tsukuba, Japan) using an ADSC Quantum-270 charge-coupled device (CCD) detector. Prior to data collection, PDCD10 crystals were transferred to a cryoprotectant solution containing 20% (v/v) dimethyl sulfoxide, 25% (v/v) ethylene glycol, 100 mM sodium cacodylate pH 6.0 and soaked for about 2 min. The crystals were then flash-frozen in a nitrogen-gas stream at
95 K on nylon cryoloops (Hampton Research). The native data were collected at a wavelength of 1.0000 Å with 2.3 Å resolution. The 3-wavelength MAD data from a SeMet derivative crystal diffracted up to 2.7 Å resolution. MOSFLM and SCALA from the CCP4 program suite [12] were used to process, reduce and scale the diffraction data. Data statistics are summarized in Table 1.

Structure determination and refinement

The structure of PDCD10 was determined by the MAD method using SOLVE [13]. Eleven Se atoms were found per asymmetric unit. Phasing extension to 2.3 Å resolution were performed using DM [12]. Automated model building was completed using RE-SOLVE [14,15], which provided a 50%-complete initial model. The remaining residues of the final model were manually built with Coot [16] and O [17]. The structure was refined to 2.3 Å resolution using PHENIX [18]. Translation libration screw (TLS) refinement was used in the final cycles of the refinement. The two domains of PDCD10 were processed separately as two TLS groups. The 4IP molecule was identified at the last stage of refinement according to the omit-annealing electron density maps. The original coordinates of 4IP were obtained from the HIC-Up database (http://xray.bmc.uu.se/hicup/). The quality of the final model was checked by MolProbity [19]. The statistics of the refinement and the

Fig. 1. Structural characteristics and unique dimeric assembly of PDCD10. (A) Overall structure of PDCD10 complexed with 4IP. The two monomers of PDCD10 are colored in green and yellow, respectively. The 4IP molecule is shown as a ball-and-stick model. The disordered loop in monomer B is shown as a broken line. (B) The structural architecture of the PDCD10 monomer. The N-terminal domain, the C-terminal domain, and the flexible linker are labeled and colored in orange, cyan and gray, respectively. The hinge (Lys69-Lys70) in α3 is also labeled. (C) Two N-terminal domains firmly inter-clasp with each other to form a compact six-helix bundle, mediating a unique dimeric assembly.

Fig. 2. Conformational variability based on C-terminal domain mobility. (A) C-terminal domain mobility. The orientation of the C-terminal domain varies approximately 30° from monomer A to monomer B. (B) Surface representation of the hetero-state (AB) observed in the present structure. (C) Surface representations of the closed-state (BB) and the open-state (AA) by modeling monomer A and B into homo-states. The size of the cleft on the top of the dimer interface varies with the conformational variety of PDCD10.
stereochemistry of the final model are summarized in Table 1. Figures were prepared using PYMOL (http://www.pymol.org/).

Results and discussion

Overall structure

The crystal structure of PDCD10 complexed with 4IP was determined at 2.3 Å resolution. The asymmetric unit contains two PDCD10 monomers (residues 11–211 for monomer A, residues 12–86 and 95–209 for monomer B), one 4IP molecule and 99 water molecules. The two monomers assemble into an integrated dimer via a unique assembly (Fig. 1A). Each monomer consists of seven α-helices (α1–α7), which fold into two independent domains, termed the N- and C-terminal domain (Fig. 1B). The N-terminal domain is comprised of three helices (α1, α2 and α3) and is shaped like a hook. Directed by the loop (Asn55–Gly57) and the hinge (Lys69–Lys70), the α3 helix is oriented at a 70° angle from the α2 helix, and forms a long arm that points to the C-terminal domain (Fig. 1B). The C-terminal domain, comprised of α4, α5, α6 and α7 helices, folds into a canonical four-helix bundle (Fig. 1B). All four helices are closely antiparallel. They are amphiphilic with the polar residues exposing their side-chains to the solvent and the non-polar residues extending their side-chains into the core of the bundle. An eight-residue loop long (residues 87–94), which is disorder in monomer B, creates a flexible linker connecting the two domains (Fig. 1B). This loop is like a cable and confers mobility onto the C-terminal domain.

The integrated dimeric structure of PDCD10 is shaped like a two-armed crane, in which the dimeric N- and C-terminal domains act as the base and carrier of the crane, respectively. The flexible linker connecting the two domains makes this system adjustable.

Unique dimeric assembly by N-terminal domain

One of the notable structural characteristics of the PDCD10 dimer is located at the N-terminus. Similar to a pair of hooks, two N-terminal domains firmly inter-clasp with each other and form a compact six-helix bundle (Fig. 1C). A number of non-polar residues of α1, α2 and α3 helices point their hydrophobic side-chains into the center of the bundle and play an essential role in dimerization. Based on this unique dimeric assembly, an integrated PDCD10 dimer is constructed. A total of 6319 Å² of surface area is buried in the dimer interface, which stabilizes the dimer. Size-exclusion chromatography analysis reveals that PDCD10 is a homodimer in solution. However, a truncated constructs (residues 83–212), which include the flexible linker and the intact C-terminal domain, acts as a monomer in solution by size-exclusion chromatography analysis (data not shown). These data imply that the unique dimeric assembly of PDCD10 is completely mediated by the N-terminal domain, and the dimer is the basic unit of PDCD10 for both the structure and function. A search in the Dali database [20] with the single N-terminal domain or the dimeric N-terminal domains of PDCD10 did not reveal any proteins with a similar architecture. Thus, it could be concluded that the N-terminal domain of PDCD10 represents a new fold, and PDCD10 is a novel adaptor protein with a unique dimeric assembly by the N-terminal domain.

Conformational variability based on C-terminal domain mobility

The two domains of one PDCD10 monomer are very similar to their counterparts in the dimer-related monomer with r.m.s. deviations of 0.806 Å and 0.666 Å for N- and C-terminal domains, respectively. Structural superposition of the two monomers shows that the orientation of the C-terminal domain with respect to the N-terminal domain varies by approximately 30° from monomer A to monomer B (Fig. 2A). The flexible linker connecting the two domains should be the structural basis which induces the potential C-terminal domain mobility. Based on the C-terminal domain mobility, the PDCD10 dimer could adopt variable conformations. The PDCD10 structure observed in our experiment is a conformational hetero-state (AB) (Fig. 2B). Respectively, modeling monomer A and B into their conformational homo-states with the same dimeric assembly of AB, we could obtain two conformational homo-states. One is an open-state (AA), and the other is a closed-state (BB) (Fig. 2C). Referring to the hetero-state AB, the two C-terminal domains are shifted down and up about 30° for homo-states AA and BB (Fig. 2C). It is reasonable to propose that the variable dimer conformations similar to those mentioned above may be adopted by PDCD10 in some specific physiological conditions for achieving its functional roles.

A variable basic cleft for phosphatidylinositol binding

In the final structural model, a 4IP molecule, which is the head group of a biological phosphatidylinositol, was found binding in a basic cleft. The cleft is located on the top of the dimer interface.
between the two C-terminal domains (Fig. 3A). The binding pocket is mainly formed by four lysine residues (Lys70 and Lys186 of both monomers). The 4IP molecule interacts with the PDCD10 dimer through an extended hydrogen-bond network (Fig. 3B). The 1-phosphate group of inositol is exposed to the solvent, thus leaving room for the lipid chain when PDCD10 interacts with biological ligands, such as phosphatidylinositides. Interestingly, the size of this basic cleft is a significant site for binding to critical biological ligands. The binding pocket is mainly formed by four lysine residues (Lys70 and Lys186 of both monomers). The 4IP molecule interacts with the PDCD10 dimer through an extended hydrogen-bond network (Fig. 3B). It is reasonable to propose that the phosphatidylinositide binding affinity could vary along with the conformational variability of PDCD10. In fact, the rather diffuse electron density of the 4IP molecule in the final model indicates that the 4IP molecule is partially occupied and that PDCD10 does not bind 4IP with high affinity under the conformational hetero-state. Previous biochemical research has revealed that PDCD10 can bind phosphatidylinositides, which helps localize and stabilize its interaction with membrane-associated proteins [7]. Here we present the possible structural basis of phosphatidylinositide binding in PDCD10. The potential conformational variability of PDCD10 based on mobility of the C-terminal domain also provides the dynamic structural basis for regulation of the phosphatidylinositide binding, which in turn, mediates the intracellular localization and functional role of PDCD10.

Potential sites for binding partner recruitment

As a typical adaptor protein, PDCD10 contains a few potential sites for recruiting different binding partners. Checking the molecular surface of the dimeric N-terminal domains, we found two quasi 2-fold symmetric hydrophobic patches (termed Site I) (Fig. 4A and B). Site I is formed by three methionine residues (Met17 and Met20 of one monomer and Met83 of the other) and two tyrosine residues (Tyr23 and Tyr27). It stacks with its counterpart in the crystallographic symmetric molecule through broad hydrophobic contacts, which induce compact packing in the 4-fold direction (Fig. 4B). This hydrophobic patch has a large surface area suitable for potential intermolecular interactions. It has been reported that PDCD10 could interact with GCKIII kinases and stabilize them to promote Golgi assembly and cell orientation [11]. The binding site of PDCD10 for GCKIII kinases has been localized on the N-terminal domain [21]. Here the Site I of PDCD10 presents a potential site for GCKIII kinase binding and will be investigated in our future research.

The four-helix bundle fold represented by the C-terminal domain of PDCD10 is also found in some cell adhesion proteins. A Dali database search [20] reveals that the closest structural homologs of the C-terminal domain of PDCD10 are the focal adhesion targeting (FAT) domains of focal adhesion kinases such as FAK and Pyk2 (1K40 for FAK, Z-score = 14.8, r.m.s. deviation = 2.1 Å; 3GM2 for Pyk2, Z-score = 15.6, r.m.s. deviation = 1.8 Å). The FAT domains of FAK and Pyk2 mediate focal adhesion targeting of these two proteins by interacting with the LD motifs of the focal adhesion protein paxillin. Each FAT domain contains two hydrophobic patches that bind to different LD motifs on the two opposite faces of the four-helix bundle. Focal adhesion kinases are recruited to focal adhesion due to their interaction with paxillin, leading to activation of their kinase activities and transduction of adhesion signals [22–24]. Structural superposition of the C-terminal domain of PDCD10 with the FAT domains of focal adhesion kinases suggests that the hydrophobic patch on the α4β7 face for paxillin binding is conserved (termed Site II) and is composed of Ile131, Ala135, Ile138, Leu142, Val168, Ser171, Ser175 and Leu178 (Fig. 4A and C). It is surrounded by four conserved basic residues (Lys132, Lys138, Lys165 and Lys179) (Fig. 4C). However, the hydrophobic patch on the α4β7 face is occupied by the C-terminal part of α3 and a flexible linker that connects the two domains. This observation implies that Site II could potentially mediate paxillin binding in PDCD10 and could implicate PDCD10 in the cell adhesion.

Addendum

During the preparation of this manuscript, another group pre-published the structure of human CCM3 (an alias of PDCD10)
online in two different crystal forms [25]. The architecture of the PDCD10 monomer in their structures is generally similar to that in our complex structure. Their analysis focused on the dimerization of PDCD10 and the interactions of PDCD10 with CCM2 and paxillin via the C-terminal domain. In the present paper, we provide more comprehensive and detailed structural information of PDCD10, emphasizing the conformational variability of PDCD10 based on mobility of the C-terminal domain. Some potential sites for the localization and binding partner recruitment of PDCD10 are found, including a variable basic cleft for phosphatidylinositide binding and two hydrophobic patches on the N-terminal domain for GCKIII kinase binding. We anticipate that the insights gained from our analysis of PDCD10 will facilitate further understanding of the structural basis for the function of PDCD10.

Accession Numbers

The atomic coordinates and structure factors for the structure of human PDCD10 complexed with inositol-(1,3,4,5)-tetrakisphosphate have been deposited in the RCSB Protein Data Bank, with the accession code 3AJM.

Acknowledgements

The authors thank the Photon Factory of KEK (06G191), Tsukuba, Japan, and Shanghai Synchrotron Radiation Facility, Shanghai, for X-ray data collections. This work was supported by the Ministry of Science and Technology of China (Grant No. 2006CB1090102101CB910304) and the National Natural Science Foundation of China (Grant No. 30770433).

References