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Structure of the thioredoxin-fold domain of human phosducin-like protein 2

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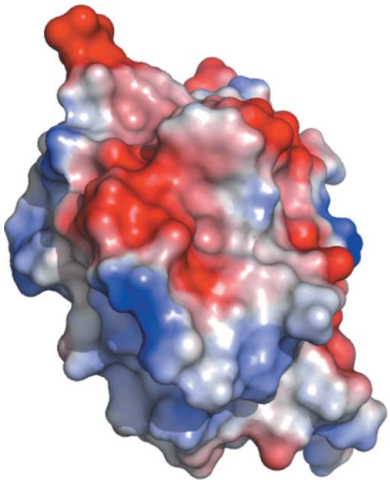


Human phosducin-like protein 2 (hPDCL2) has been identified as belonging to subgroup II of the phosducin (Pdc) family. The members of this family share an N-terminal helix domain and a C-terminal thioredoxin-fold (Trx-fold) domain. The X-ray crystal structure of the Trx-fold domain of hPDCL2 was solved at 2.70 Å resolution and resembled the Trx-fold domain of rat phosducin. Comparative structural analysis revealed the structural basis of their putative functional divergence.

1. Introduction

The thioredoxin-fold (Trx-fold) protein-structure classification (SCOP; http://scop.mrc-lmb.cam.ac.uk; Murzin *et al.*, 1995) was characterized based on the structure of *Escherichia coli* Trx1 (Holmgren *et al.*, 1975). The classic Trx fold consists of a single domain with a central five-stranded mixed β-sheet flanked by two α-helices on each side. The secondary-structure elements are arranged in the order $\beta\alpha\beta\alpha$-$\beta\alpha\beta\beta\alpha$, with $\beta4$ antiparallel to the other strands. A set of protein families containing this fold have been identified in SCOP, including Trx, glutaredoxin (Grx; Sun *et al.*, 1998), protein disulfide isomerase (PDI; Tian *et al.*, 2006), glutaredoxin *S*-transferase (Reinemer *et al.*, 1991), calsequestrin (Wang *et al.*, 1998) and phosducin (Pdc; Gaudet *et al.*, 1996).

Phosducin is a 28 kDa cytosolic protein which was first found in retinal extracts (Lolley *et al.*, 1977). It was originally identified as a modulator of heterotrimeric G-protein signalling in the retina (Schroder & Lohse, 1996). Subsequently, a series of Pdc-like proteins (PhLPs) were identified in vertebrates and lower eukaryotes that constitute the Pdc family (Blaauw *et al.*, 2003). The members of this family share an N-terminal helix domain and a C-terminal Trx-fold domain (Gaudet *et al.*, 1996; Stirling *et al.*, 2007) and can be divided into three subgroups (Blaauw *et al.*, 2003). Subgroup I includes Pdc and PhLP1, subgroup II contains PhLP2 and subgroup III contains PhLP3 (Blaauw *et al.*, 2003; Willardson & Howlett, 2007). The proteins of subgroup I are the best characterized. Crystal structures of the Pdc–$G_t\beta\gamma$ complex (Gaudet *et al.*, 1996, 1999; Loew *et al.*, 1998) showed that Pdc could block G-protein signalling by disrupting the interaction between $G_t\alpha$ and $G_t\beta\gamma$. PhLP1 contains an 11-residue sequence corresponding to the conserved $G_t\beta\gamma$-binding motif of Pdc in the N-terminal domain (Miles *et al.*, 1993; Blaauw *et al.*, 2003), which has been reported to play a similar role in regulating other G-protein $\beta\gamma$ subunits (Loew *et al.*, 1998) and to bind and regulate chaperonin-containing Tcp1 (CCT; Martin-Benito *et al.*, 2004). Proteins in subgroup III have been identified as modulators of CCT and to play roles in actin and tubulin biogenesis in yeast (McLaughlin *et al.*, 2002) and microtubule function in *Caenorhabditis elegans* (Stirling *et al.*, 2007). Proteins in subgroup II were reported to be essential in *Dictyostelium discoideum* and yeast (Blaauw *et al.*, 2003; Stirling *et al.*, 2007). Moreover, recent findings have indicated that most members of the Pdc family act as cochaperones with CCT (Willardson & Howlett, 2007).

In humans, five family members, hPdc, hPhLP1, hPDCL2, hPDCL3 and hPhLP3, have been recognized to date. We determined the crystal structure of the Trx-fold domain of hPDCL2, a member of subgroup II, at 2.70 Å resolution. To date, its detailed function has not been characterized.

2. Materials and methods

2.1. Cloning and protein expression

The gene encoding the Trx-fold domain (residues 88–209) of hPDCL2 (*hPDCL2*; GenBank Accession No. BC034431) was amplified by PCR from the human cDNA library (Proteintech Group Inc.). The amplified DNA was cloned into a pET29a-derived expression vector between *Nco*I and *Not*I restriction sites, which were incorporated into the sequences of the sense and antisense primers 5′-GCC**CCATGGC**AAATTTGGAGAATTAAG-3′ and 5′-GCC-**GCGGCCGC**TTAGTTTTCTTCCAAATC-3′, respectively (shown in bold). The resulting construct was verified by sequencing and contained an MHHHHHHG tag at the N-terminus of the protein as a result of the cloning strategy. *Escherichia coli* BL21 (DE3) strain cells were transformed with this construct and cultured in 600 ml LB medium containing 10 µg ml^{-1} kanamycin at 310 K until the OD_{600} reached 0.6. Protein expression was induced by adding isopropyl β-D-1-thiogalactopyranoside (IPTG) to a final concentration of 0.2 m*M* for 20 h at 291 K. Cells were harvested by centrifugation at 7330*g* for 10 min and resuspended in cold lysis buffer (20 m*M* Tris–HCl pH 8.0, 50 m*M* NaCl). The suspension was sonicated and clarified by centrifugation at 29 000*g* for 25 min at 277 K.

2.2. Protein purification

The supernatant was loaded onto 2 ml Ni–NTA affinity resin (Qiagen) pre-equilibrated with lysis buffer at 289 K. After washing with 20 ml lysis buffer plus 10 m*M* imidazole, the target protein was eluted with lysis buffer containing 250 m*M* imidazole and then further purified on a HiLoad 16/60 Superdex 75 column (Amersham Biosciences) equilibrated with lysis buffer at 289 K. The fractions containing the target protein were verified using SDS–PAGE.

Table 1
Data-collection and refinement statistics.

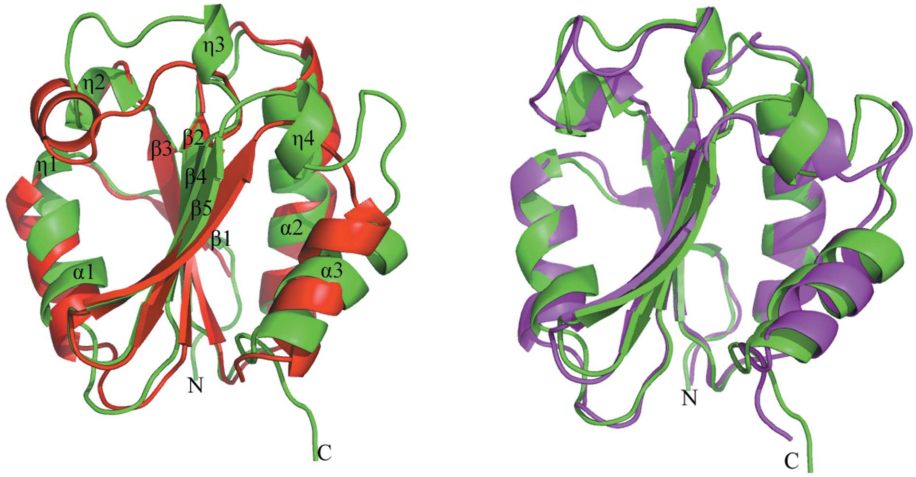
Values in parentheses are for the highest resolution shell.

Data collection	
Resolution range (Å)	26.14–2.70 (2.80–2.70)
Space group	$P3_1$
Temperature (K)	100
Wavelength (Å)	1.5418
Unit-cell parameters	
a (Å)	44.59
b (Å)	44.59
c (Å)	141.97
α (°)	90.00
β (°)	90.00
γ (°)	120.00
Unique reflections	9164 (895)
Completeness (%)	93.9 (88.6)
Redundancy	2.7 (2.0)
Mean $I/\sigma(I)$	10.22 (1.53)
R_{merge}†	0.175 (0.614)
Refinement	
Resolution range (Å)	18.88–2.70
R_{cryst}‡	0.236
R_{free}‡	0.276
Matthews coefficient (Å^3 Da^{-1})	2.79
Solvent content (%)	55.88
Mean temperature factor (Å^2)	42.30
R.m.s.d. bond lengths (Å)	0.012
R.m.s.d. bond angles (°)	1.221
Ramachandran statistics	
Residues in most favoured regions (%)	90.1
Residues in additional allowed regions (%)	9.0
Residues in generously allowed regions (%)	0.9
Residues in disallowed regions (%)	0

† $R_{merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $\langle I(hkl)\rangle$ is the mean intensity of i reflections with intensities $I_i(hkl)$ and common indices hkl. ‡ R_{cryst} and $R_{free} = \sum_{hkl} ||F_o| - |F_c|| / \sum_{hkl} |F_o|$; R_{free} was calculated for a 4.7% set of reflections excluded from refinement.

2.3. Crystallization and X-ray data collection

Purified protein solution (20 m*M* Tris–HCl pH 8.0, 50 m*M* NaCl) was concentrated to 8 mg ml^{-1} by ultrafiltration (Millipore Amicon) and treated with 4 m*M* dithiothreitol (DTT) for 1 h. Crystallization conditions were screened by the sitting-drop vapour-diffusion method at 291 K using Crystal Screens I and II (Hampton Research). The drops consisted of 1 µl protein solution and 1 µl reservoir solu-



(*a*) (*b*)

Figure 1
Overall structure superposition of the hPDCL2 Trx-fold domain (green) with (*a*) hTrx1 (PDB code 1aiu; red) and (*b*) the Trx-fold domain of rPdc (PDB code 2trc; magenta). The secondary-structure elements are labelled sequentially in (*a*). The figures were prepared using *PyMOL* (DeLano, 2002).

tion and were equilibrated against 100 µl reservoir solution using a 48-well sitting-drop plate (XtalQuest, Beijing). After optimization, crystals suitable for X-ray diffraction grew within 3 d in a condition consisting of 28%(*w*/*v*) PEG 8000, 0.2 *M* ammonium sulfate. Crystals were flash-frozen in liquid nitrogen using a cryoprotectant consisting of the reservoir solution plus 25% glycerol. Diffraction data were collected to 2.70 Å resolution at 100 K using an in-house Rigaku MM007 X-ray generator (λ = 1.5418 Å) with a MAR Research 345 detector at the School of Life Sciences, University of Science and Technology of China (USTC; Hefei, People's Republic of China). The program *HKL*-2000 (Otwinowski & Minor, 1997) was used for data processing and scaling and data-collection statistics are shown in Table 1. The $R_{\rm merge}$ value is relatively high owing to the diffraction anisotropy of the crystal. The crystal belongs to space group $P3_1$, with a Matthews coefficient of 2.79 Å^3 Da^{-1} and a solvent content of 55.88% (Matthews, 1968), suggesting the presence of two molecules in an asymmetric unit.

2.4. Structure determination and refinement

Structure solution was achieved by molecular replacement using *Phaser* from the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994) with the NMR structure of the Trx-like domain of mouse Pdc-like protein 2 (mPDCL2; PDB code 2dbc) as a search model. The programs *Coot* (Emsley & Cowtan, 2004) and *REFMAC*5 (Murshudov *et al.*, 1997) were used to build and refine the final model, which consists of residues 88–205.

3. Result and discussion

3.1. Overall structure

The Trx-fold domain of hPDCL2 adopts a canonical Trx fold comprising a five-stranded central β-sheet flanked by seven helices (Fig. 1). The secondary-structure elements are arranged in the order $\beta\eta\alpha\beta\alpha\beta\eta\eta\beta\beta\eta\alpha$, with β4 antiparallel to the other strands. The r.m.s.d.

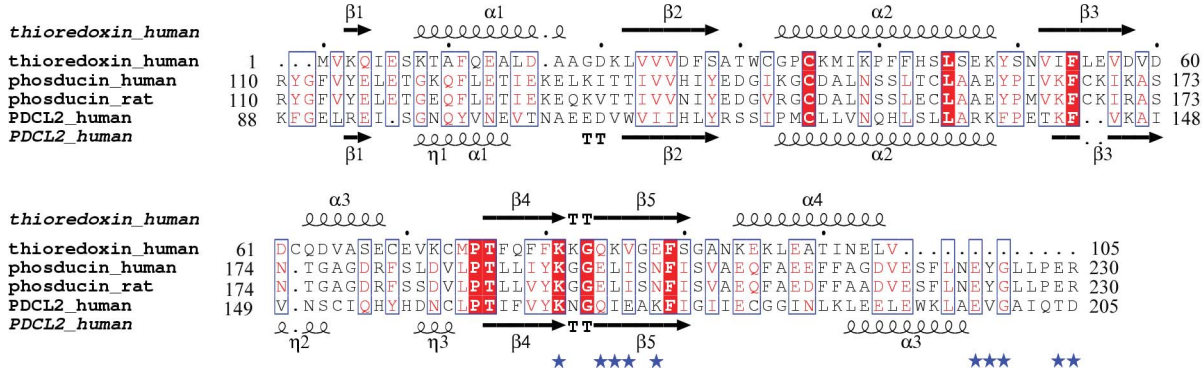


Figure 2
Structure-based sequence alignment. The sequences of hTrx1, hPdc, rPdc and hPDCL2 were obtained from the Swiss-Prot database (accession Nos. P10599, P20941, P20942 and Q8N4E4, respectively). The alignment region of each sequence is labelled at the N- and C-termini. The secondary structure of hTrx1 is shown at the top and that of the hPDCL2 Trx-fold domain is shown at the bottom. Alignments were performed using the programs *MULTALIN* (Corpet, 1988) and *ESPript* (Gouet *et al.*, 2003). Conserved residues are marked in red. Conserved residues in the Trx-fold domain of rPdc that contact G protein are labelled with blue stars.

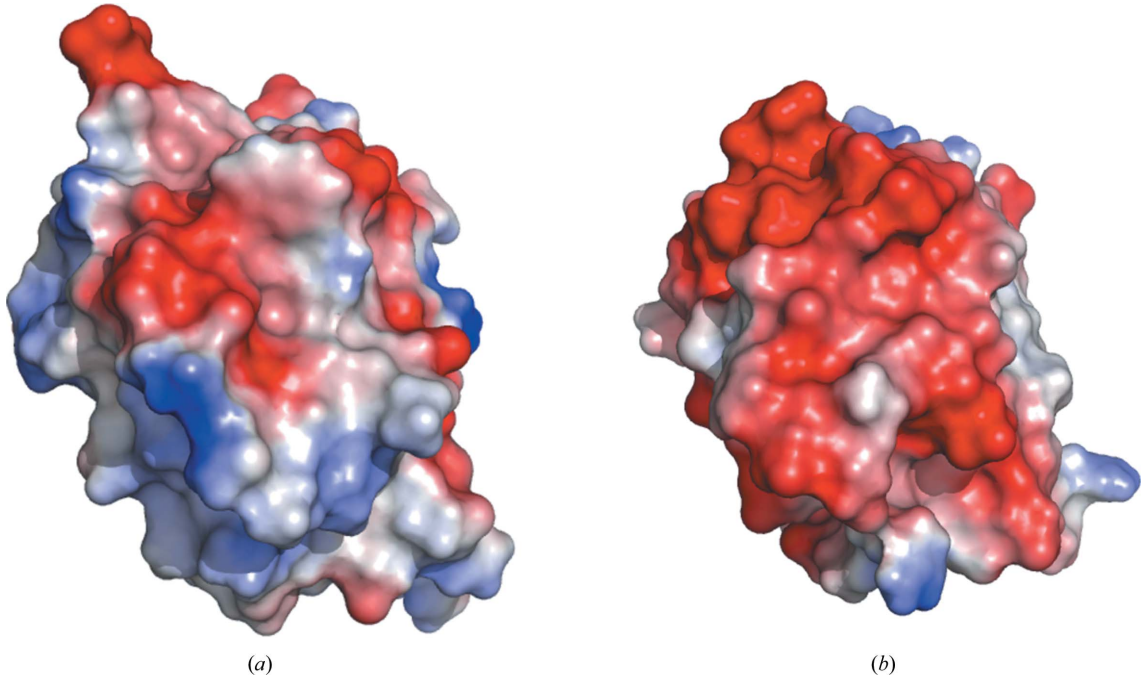


Figure 3
Electrostatic potential representations of the Trx-fold domains of (*a*) hPDCL2 and (*b*) rPdc. Figures were prepared using *PyMOL* (DeLano, 2002).

between the C^{α} atoms in this structure and the search model is 0.720 Å for 109 residues.

3.2. Comparison of the hPDCL2 Trx-fold domain and human Trx1

Thioredoxin is a small redox-active protein with a conserved catalytic active site (-Trp-Cys-Gly-Pro-Cys-) that undergoes reversible oxidation–reduction of two thiol groups aided by Trx reductase. The Trx-fold domain of hPDCL2 has a very low sequence homology to the classic Trx fold (19% identity and 35% similarity to human Trx1) and only contains one of the two conserved Trx active-site cysteines (Fig. 2). It can be superimposed on hTrx1 with an r.m.s. deviation (r.m.s.d.) of 1.726 Å between the C^{α} atoms for 87 residues and is extended compared with the classic Trx fold (Fig. 1*a*). Compared with the secondary-structure elements of hTrx1 (Fig. 2), helix α1 in the hPDCL2 Trx-fold domain is shorter and has an additional 3_{10}-helix η1 adjacent to its N-terminus; two 3_{10}-helices η2 and η3 with a loop between them substitute for helix α3 in hTrx1. Additionally, an extra 3_{10}-helix η4 was located between strand β5 and helix α3.

3.3. Comparison of Trx-fold domains between hPDCL2 and rPdc

The Trx-fold domain of hPDCL2 highly resembles that of rat Pdc based on a structure-homology search using the program *DALI* (Holm *et al.*, 2008). The sequence identity between the Trx-fold domains of hPDCL2 and rPdc is 24% and the corresponding r.m.s.d. between the C^{α} atoms is 1.194 Å for 97 residues (Figs. 1*b* and 2).

Although the Trx-fold domain of hPDCL2 is very similar to that of rPdc in overall structure, it contains some variations. Ten conserved residues in the rPdc Trx-fold domain were identified to bind G protein (Gaudet *et al.*, 1996). However, the Trx-fold domain of hPDCL2 only contains three of these ten conserved residues (Fig. 2), which indicates that this domain might not possess the ability to interact with G protein. Furthermore, the electrostatic potential of the hPDCL2 Trx-fold domain is greatly different from that of rPdc (Fig. 3). The Trx-fold domain of rPdc is highly negatively charged and the upper surface interacts with the β-subunit of G protein, whereas the opposite surface contributes to G$\beta\gamma$ translocation from the membrane (Gaudet *et al.*, 1996). The Trx-fold domain of hPDCL2 is less negatively charged than that of rPdc, especially at the surface corresponding to the membrane-interacting surface of rPdc.

In conclusion, the Trx-fold domain of hPDCL2 adopts a monothiol Trx-fold like that of rPdc in three-dimensional structure, but it lacks most of the conserved G-protein-interacting residues and exhibits a clear difference in electrostatic potential when compared with rPdc. Therefore, it could be hypothesized that hPDCL2 does not act as a modulator of G-protein signalling like rPdc.

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