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Crystal structure of the polo-box domain of polo-like kinase 2

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ABSTRACT

Polo-like kinase 2 (PLK2) is a crucial regulator in cell cycle progression, DNA damage response, and neuronal activity. PLK2 is characterized by the conserved N-terminal kinase domain and the unique C-terminal polo-box domain (PBD). The PBD mediates diverse functions of PLK2 by binding phosphorylated Ser-pSer/pThr motifs of its substrates. Here, we report the first crystal structure of the PBD of PLK2. The overall structure of the PLK2 PBD is similar to that of the PLK1 PBD, which is composed by two polo boxes each contain β6α structures that form a 12-stranded β sandwich domain. The edge of the interface between the two polo boxes forms the phosphorylated Ser-pSer/pThr motifs binding cleft. On the hand, the peripheral regions around the core binding cleft of the PLK2 PBD is distinct from that of the PLK1 PBD, which might confer the substrate specificity of the PBDs of the polo-like kinase family.

1. Introduction

Polo-like kinases (PLKs) are key players in multiple biological processes including cell cycle progression, DNA replication and DNA damage response, cilia disassembly, centriole biogenesis and duplication, and neuronal activity [1–6]. Currently five mammalian PLK family members, PLK1–5, have been identified [7]. Despite their distinct biological functions, all the five PLK family members are characterized by an N-terminal kinase domain and a C-terminal polo-box domain (PBD). The kinase domain is highly similar to the kinase domains of other serine–threonine protein kinases, while the PBD is unique to the PLK family [8], which regulates the kinase activity, subcellular localization, and substrate interactions through binding the phosphorylated Ser-pSer/pThr motifs of its target proteins [9,10]. The PBD of PLK1 (PBD1) has been extensively studied and characterized based on the determined structures of the PBD in complex with the phosphopeptides [10–12] or in complex with the kinase domain of PLK1 [13], but much less is known about the PBDs of other members than about that of PLK1 [14].

Among the five members, PLK2 is the more closely related to PLK1, which has been shown to play crucial roles in the nervous system such as synaptic regulation [15] and neuronal protection [16]. PLK2 regulates synaptic plasticity by mediating degradation of SPAR (spine-associated RapGAP) via ubiquitin–proteasome pathway, and might protect neuron by mediating degradation of α-synuclein via lysosome-autophagy pathway. Both neuronal functions of PLK2 depend on its C-terminal PBD to bind the phosphorylated substrates and lead to their degradation [15,16]. The PBD of PLK2 (PBD2) bind phosphorylated Ser-pSer/pThr motifs as the closely related PLK1 but with much lower affinity [17]. However, the understanding of the molecular basis for the substrate specificity of the PBDs has been hindered by the lack of the crystal structure of the PBD of PLK2. To address this problem, we determined the crystal structure of the PBD of PLK2 at 2.6 Å resolution, which provide structural insight into understand the substrate specificity of the PBDs of PLKs.

2. Materials and methods

2.1. Protein expression and purification

The DNA fragment of human PLK2 (residues 451–685) was amplified by PCR and cloned into the *EcoRI* and *XhoI* sites of expression vector pGEX-6p-1 or pGEX-6p-2. Proteins were expressed in BL21 (DE3) pLysS strains. Point mutations were introduced using Easy mutagenesis system (TransGen Biotech). Cells were grown to an optical density of 0.6 at 37 °C with vigorous shaking and then cooled to 25 °C, induced by a final concentration 0.1 mM IPTG. The cells were harvested and frozen at −80 °C. The cells were thawed in buffer A (20 mM Tris, 500 mM NaCl, 1 mM DTT pH = 8.0) and lysed by addition of 0.1 mg/ml DNase I (sigma), $MgCl_2$, 1 mg/ml Lysozyme (sigma) and 0.1% Triton X-100 [18]. The lysate was centrifuged at 13000*g* for 30 min and the supernatant was filtered

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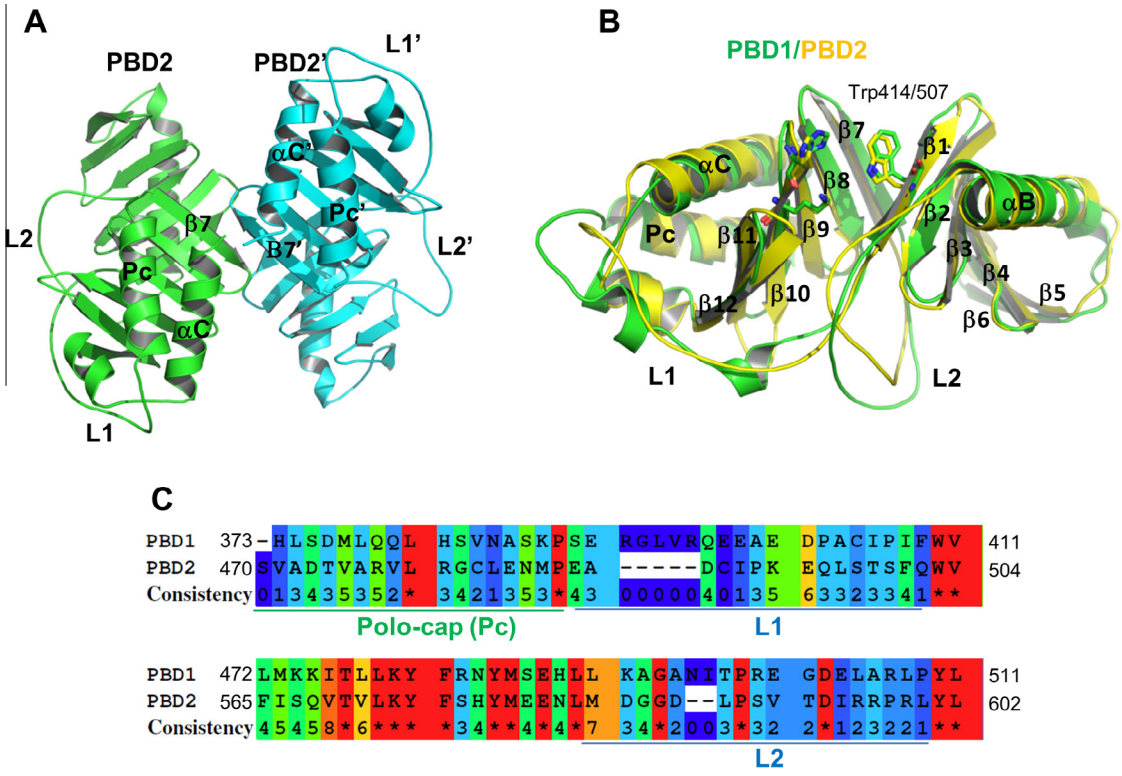

Fig. 1. Overall structure of PBD2. (A) The dimeric structure of PBD2 in one asymmetric unit. (B) Superposition of the structures of PBD1 (green) (PDB code 1UMW) [10] and PBD2 (yellow). (C) Sequence alignment of the Polo-cap, L1, and L2 regions of PBD1 and PBD2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

through 0.45 μm filter. The supernatant was loaded into glutathione–Sepharose column (GE Healthcare), washed with buffer A and eluted with 20 mM glutathione in buffer A. The GST tag was digested with 3C precision protease overnight. The digestion was reloaded onto glutathione–Sepharose column. The flowthrough was collected and concentrated and then loaded onto a gel-filtration column (Superdex200, GE Healthcare). The protein was concentrated into 9 mg/ml and stored at −80 °C.

2.2. Crystallization and structure determination

Initially crystal screening was performed using sitting-drop method. Small crystals were obtained overnight using Index and Crystal Screens (Hampton Research, CA, USA). Crystals were grown at 20 °C in hanging drops using Index 28 containing 35% v/v Tacsimate TM pH 7.0. The initial diffraction data was collected at beamline BL17U of the Shanghai Synchrotron Radiation facility (λ = 0.9793 Å) at 100 K. The crystal structure of PBD2 451–685 was determined by molecular replacement using Phaser-PHENIX [19,20] suite based on the crystal structure of PBD1 (PDB code 1UMW) [10] encompassing residues 373–595. The refinement of the initial solutions with manually built in Coot [21] and refined by Refmac [22] in CCP4 [23] yielded experimental electron-density map for model building. The final atomic model of the PBD2 was refined to an R factor of 0.186 and an R_{free} value of 0.247 at 2.6 Å resolution. The protein in the figures was rendered with PyMol [24].

2.3. Fluorescence polarization assay

Binding assays were carried out essentially as described previously [25]. In brief, FITC-labeled phospho-peptide FITC-GPMQTSpTPKNG (10 nM) (GL Biochem Shanghai Ltd) was titrated by the PBD of PLK2 in a buffer containing 20 mM Tris, and 200 mM NaCl, pH 8.0. All the experiments were performed in 96-well black plates using Envision multilabel reader (Perkin Elmer). Plates were read one hour after mixing of all assay components.

3. Results and discussion

3.1. Overall structure of the polo-box domain$_{451–685}$

According to the sequence alignment between PBD1 and PBD2, PBD2 containing residues 451–685 was constructed, purified and crystallized. The crystal structure was determined by molecular replacement using the crystal structure of the PBD1 (PDB code 1UMW) [10] as the template, which was solved at 2.6 Å in the I23 space group with a dimer in the asymmetric unit (Fig. 1A and Table 1). The structure of PBD2 contains residues 470–682, residues at the two extreme terminuses were not observed in the solved crystal structure.

The overall structure of PBD2 resembles the structure of PBD1 (Fig. 1B), composed of two tandem polo boxes (PB1 and PB2) that have identical β6α fold. The N-terminal extension (residues 470–486) forms the helical Polo-cap (Pc) that wraps around PB2 and connects to PB1 via a linker region (residues 487–502, or L1), while PB1 and PB2 are connected by a secondary linker region (residues 584–600, or L2). Structural superposition reveals an RMSD of 0.99 Å for 152 equivalent α-carbons between PBD2 and PBD1 despite their low sequence identity (about 32%). The major structural variations locate at the two linker regions L1 and L2, which is consistent with the low sequence homology in these regions between PBD2 and PBD1 (Fig. 1C).

3.2. The conservation of the phosphopeptide binding cleft

Both PBD2 and PBD1 bind phosphopeptides containing a Ser-pSer/pThr motif, but PBD2 binds such peptides with lower binding