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Structure-based design and confirmation of peptide ligands for neuronal polo-like kinase to promote neuroregeneration

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ABSTRACT

Neuronal polo-like kinase (nPLK) is an essential regular of cell cycle and differentiation in nervous system, and targeting nPLK has been established as a promising therapeutic strategy to treat neurological disorders and to promote neuroregeneration. The protein contains an N-terminal kinase domain (KD) and a C-terminal Polo-box domain (PBD) that are mutually inhibited by each other. Here, the intramolecular KD–PBD complex in nPLK was investigated at structural level *via* bioinformatics analysis, molecular dynamics (MD) simulation and binding affinity scoring. From the complex interface two regions representing separately two continuous peptide fragments in PBD domain were identified as the hot spots of KD–PBD interaction. Structural and energetic analysis suggested that one (PBD peptide 1) of the two peptides can bind tightly to a pocket nearby the active site of KD domain, which is thus potential as self-inhibitory peptide to target and suppress nPLK kinase activity. The knowledge harvesting from computational studies were then used to guide the structural optimization and mutation of PBD peptide 1. Consequently, two of three peptide mutants separately exhibited moderately and considerably increased affinity as compared to the native peptide. The computationally modeled complex structures of KD domain with these self-inhibitory peptides were also examined in detail to unravel the structural basis and energetic property of nPLK-peptide recognition and interaction.

1. Introduction

The neural signaling network is orchestrated by various functional proteins involved in nervous system. An important among these neuroproteins is the neuronal polo-like kinase (nPLK), which is a Ser/Thr protein kinase that plays critical roles in nerve cycle and proliferation. Accumulated evidences suggested that nPLK is a central mediator of activity-dependent change in molecular composition and morphology of synapses, which can be dynamically regulated in neurons by synaptic activity at mRNA and/or protein levels; induction of nPLK may cause global dampening of synaptic strength following heightened neuronal activity (Seeburg et al., 2005). Currently, nPLK has become a well studied neurokinase because of its tight association with diverse neurological disorders; overexpression and activation of nPLK in central nervous system (CNS) are involved in the aberrations of cell cycle control in neurological pathogenesis. For example, increased nPLK implicates aberrations in cell cycle control in Alzheimer's disease (Harris et al., 2000) and inhibition of the kinase can considerably reduce β-amyloid-induced neuronal cell death in this disease (Song et al., 2011). In addition, nPLK has been found as a dominant kinase involved in the phosphorylation of α-synuclein in Lewy bodies, which are one of the hallmarks of Parkinson's disease neuropathology (Aubele et al., 2013). Thus, targeting nPLK has been established as a promising therapeutic strategy to treat neurological disorders (Garuti et al., 2012) and to promote neuroplasticity, neurogenesis and neuroregeneration (Seeburg et al., 2008; Seeburg and Sheng, 2008). Although a number of chemical inhibitors such as NMS-P937 (Beria et al., 2011) and PL-2 (Ahn et al., 2015) have been successfully developed to target and suppress the kinase, most of them exhibited low selectivity and high toxicity. In addition, frequently observed somatic mutations in nPLK kinase domain may also cause drug resistance to these small-molecule agents (Simizu and Osada, 2000). Instead, biologic therapeutics has recently been raised as a promising strategy to target protein kinases with good biocompatibility and low risk of side effect (Grant, 2009).

The nPLK contains an N-terminal kinase domain (KD) and a C-terminal Polo-box domain (PBD) that are mutually inhibited by each other. Crystallographic study revealed that the PBD binds and rigidifies the hinge region of the KD in a distinct conformation from

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that of the phosphopeptide-bound PBD, which sheds light on the autoinhibitory mechanism of the kinase (Xu et al., 2013). Based on the crystal structure of nPLK in autoinhibitory state we herein performed bioinformatics analysis to dissect the structural basis and energetic property of intramolecular interaction between the KD and PBD. Several protein fragments were derived from the interaction site of PBD that can be considered as self-inhibitory peptides to target KD, which were then computationally modified and optimized to improve their binding capability. We also employed fluorescence-based assays to determine the binding affinity of few promising candidates to KD.

2. Materials and methods

2.1. Bioinformatics tools used

KFC2 (Zhu and Mitchell, 2011): The KFC2 is a knowledge-based hot spot prediction method based on interface solvation, atomic density and plasticity features, which utilized supervised learning approach to provide a set of hierarchical rules for hot spot classification (Darnell et al., 2008). Here, we employed KFC2 to identify hot-spot residues at the KD–PBD interaction interface of nPLK crystal structure. These residues will be used to define key peptide fragments that are able to bind at the interface independently.

GalaxyPepDock (Lee et al., 2015): The peptide docking method GalaxyPepDock was proposed recently by Lee et al. to model the binding mode of flexible peptide ligands to their protein receptors based solved protein–peptide complex structures. The method performs similarity-based docking by finding templates from experimentally determined structures and building models using energy-based optimization in consideration of flexibility. Here, the method was employed to redock those key fragments defined by KFC2 to the interface regions of KD.

FlexPepDock (London et al., 2011): The FlexPepDock is a high-resolution protein–peptide complex refinement protocol implemented within the Rosetta environment (Raveh et al., 2011), which has been shown to be able to accurately refine the peptide structure starting from the initial position nearby its native conformation, allowing full flexibility to the peptide and side-chain flexibility to protein receptor (Raveh et al., 2010). Here, the method was used to refine the coarse-grained structure models of redocked KD–peptide complexes.

QSAR-improved PPRCP (Han et al., 2013): The QSAR-improved PPRCP was designed for general-purpose scoring of protein–peptide binding affinities based on their complex structures, which combines unsupervised knowledge-based statistical potential derived from hundreds of interfacially diverse, non-redundant protein–peptide complex structures and supervised quantitative structure-activity relationship (QSAR) modeling trained by known protein–peptide affinity data. Here, we employed the scoring function to explore KD–peptide binding potency in a high-throughput manner.

2.2. Molecular dynamics simulations

Molecular dynamics (MD) simulations of KD–peptide complexes were performed using GROMACS package (Hess et al., 2008) with Amber03 force field (Duan et al., 2003). Complex was dissolved in a TIP3 P solvent environment (Jorgensen et al., 1983) with periodic boundary conditions. Default protonation states were assigned for all ionizable residues in the complex system. Counter-ions of Na^+ were added to keep neutralization of the system. For each simulation, the complex structure was first relaxed by steepest-descent and conjugate-gradient energy minimizations. Then, the system was equilibrated with 1-ns MD simulations under a constant volume ensemble (NVT) with a harmonic position restraint applied on heavy atoms of the solute and 1-ns simulations under a constant pressure ensemble (NPT) without any restraint. Subsequently, 50-ns production simulations were carried out under NPT ensemble (Yu et al., 2014). The linear constraint solver (LINCS) algorithm (Hess et al., 1997) was employed to constrain all covalent bonds containing hydrogen atoms. Berendsen's coupling algorithm (Berendsen et al., 1984) was used to maintain temperature and pressure. The long-range electrostatic interactions were treated by particle mesh Ewald (PME) strategy (Darden et al., 1993), while the van der Waals interactions were calculated at a cutoff of 15 Å.

The molecular mechanics-generalised Born/surface area (MM-GB/SA) method (Guimarães and Cardozo, 2009) was employed to

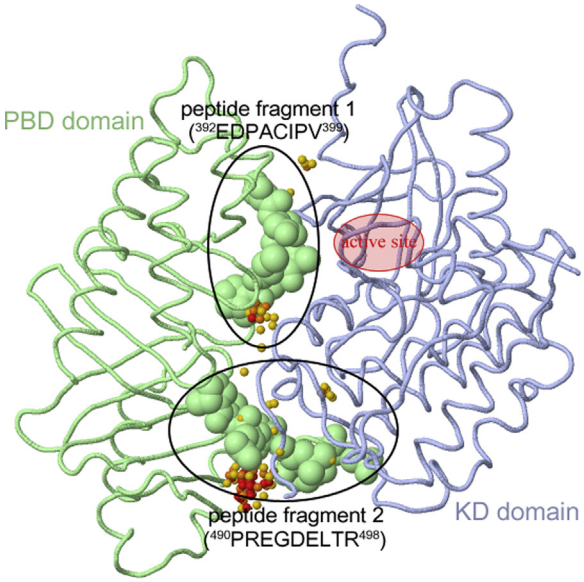

Fig. 1. The hot spot detection at the complex interface of KD–PBD intramolecular interaction in nPLK crystal structure (PDB: 4J7B) using KFC2 server (Zhu and Mitchell, 2011). Only the hot-spot residues of PBD domain at the interface are shown in Ball style, which can be clustered into two regions separately corresponding to two peptide fragments of the domain.