Phosphorylation-induced conformational change responsible for the function of a myosin phosphatase inhibitor, CPI-17

Shin-ya Ohki a,*, Masumi Eto b, Rei Takada c, Masato Shimizu c, David L. Brautigan b, Masatsune Kainoshoc,d,*

a Center for Nano Materials and Technology, Japan Advanced Institute of Science and Technology (JAIST), 1-1 Asahidai, Tatsunokuchi, Ishikawa 923-1292, Japan
b Center for Cell Signaling, University of Virginia School of Medicine, Box 800577 Charlottesville, VA 22908, USA
c Tokyo Metropolitan University, 1-1 Minami-ohsawa, Hachioji, Tokyo 192-0397, Japan
d CREST (JST), 1-1 Minami-ohsawa, Hachioji, Tokyo 192-0397, Japan

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Abstract

The structures of CPI-17 (Protein kinase-C dependent protein phosphatase-1 (PP1) inhibitor of 17 kDa) in an inactive and an active form have been determined by multidimensional NMR spectroscopy. Comparison of the two structures revealed how the molecular switch turns on at atomic resolution. Using the NMR structure of CPI-17 in the active form, the binding with catalytic domain of PP1 (PP1c) was simulated and the binding model is proposed in this report. When the phospho-Thr38 docks to the catalytic site of PP1, possible interactions for the tight binding are found: one is electrostatic interaction between a negatively charged cluster on phospho-CPI-17 and an acidic groove of PP1c, and the other is hydrophobic interaction between a hydrophobic surface area of phospho-CPI-17 and a hydrophobic groove of PP1c.

Keywords: CPI-17; PP1c; Modeling; PKC; Smooth muscle; Purkinje cell

1. Introduction

Reversible phosphorylation on proteins is known to play a significant role in cellular signal transduction. Various biological functions including cell-cycle progression, transcriptional regulation, and muscle contraction are regulated by phosphorylation and dephosphorylation [1]. One of the most famous examples is smooth muscle contraction. It is controlled by reversible phosphorylation on myosin. Its dephosphorylation is achieved by myosin phosphatase (MP), and the activity of MP is modulated by a small inhibitory protein named CPI-17 (Protein kinase-C dependent protein phosphatase-1 inhibitor of 17 kDa). Phosphorylation of Thr38 of CPI-17 increases over 103-fold in inhibitory potency for MP [2]. CPI-17 is exclusively expressed in smooth muscle and brain [2], and suggested to function controlling vascular tone [3] and maintaining cerebellar memory [4]. A series of biochemical experiments suggested that this inhibition is achieved by the direct binding of phospho-Thr38 on CPI-17 to the catalytic site of MP [5–7]. But a lack of three-dimensional structure of CPI-17 prevents further understanding of the reaction mechanism at atomic resolution.

MP is classified into type-1 of protein phosphatase-1 (PP1). Over 30 kinds of inhibitory proteins of PP1 have been reported [8], whereas no three-dimensional structure of the inhibitors has been solved. Moreover that CPI-17 has a unique amino acid sequence also makes it difficult to build a model structure.

As a first step in understanding the molecular basis, we have determined the solution NMR structures of CPI-17 and its mutant. The functional domain, residue 22–120 [6,7], was employed for our NMR study. Because of the difficulty in NMR sample preparation of phospho-CPI-17, we have employed a mutant as a model of phospho-CPI-17. We compared the 1H–15N HSQC of wild type CPI-17, partially phosphorylated CPI-17, and CPI-17 with a single mutation to introduce a negative charge at the residue 38.
There was a switch in the protein structure due to either Asp substitution or phosphorylation, thus we solved the solution NMR structure of the CPI-17 T38D mutant as a model for the active (phospho-) conformation.

2. Materials and methods

$^{13}$C and $^{15}$N doubly isotope-labeled proteins (wild and T38D) were expressed in E. Coli and purified by the procedure published previously [2,6]. $^{15}$N labeled proteins were also obtained. Isotope-aided triple resonance multidimensional NMR experiments were performed on Bruker DRX600 and Avance800 NMR spectrometers. Temperature of the samples during all NMR experiments was kept at 25°C. A set of NMR data for structure determination was recorded for each protein [9]. All NMR data were processed with NMRPipe [10] and analyzed with PIPP [11]. Distance and dihedral constraints were employed to calculate the solution structures and residual dipolar coupling constants were used to refine the structures [12]. Pf1 phage was employed for partially alignment of protein molecules. Structure calculation was carried out using X-PLOR ver.3.851 [13].

Model building of the complex between CPI-17 and PP1c was also carried out by using XPLOR ver.3.851. Both of proteins were treated as rigid-body. First, the side-chain of residue 38 of CPI-17 was ligated to the metal ions at the catalytic site of PP1c. Then, this binding is fixed and CPI-17 molecule was manually rotated against PP1c to find the best-fitting angle. Finally, energy minimization was applied onto the complex.

Fig. 1 shows the solution NMR structures of CPI-17 and its mutant, T38D. Both of proteins have 4-helix bundle topology in which residues 48–61 (A), 77–80 (B), 87–97 (C), and 104–115 (D) form the α-helices. The A- and D-helices are paired, and the other two, B- and C-helices, are also paired. These two components are packed as a ‘V’ shape by hydrophobic interaction including Trp55, Phe107, and Leu111. DALI [18], which is a program to find proteins in homologous folding, showed that this V-letter folding is

3. Results and discussion

3.1. Description of the structures

We have determined the solution NMR structures of CPI-17 (wild type) and its mutant (T38D). Structure calculation was carried out by using NOE, hydrogen bonding, dihedral angle, and $^1$H$^{15}$N dipole as structural restraints. Total restraints of structure determination was 1349 and 1379 for wild type and T38D, respectively. These numbers are enough to obtain well-defined structure for a protein with this molecular size (~100 residues). None of the final structures has distance violations greater than 0.5 Å, dihedral violations greater than 5°, or $^1$H$^{15}$N residual dipole violations greater than 2.0 Hz. Quality of the structures was checked with AQUA-Procheck [17]. The Ramachandran plot analysis of the final structures showed that only 1.7 ± 1.1 and 2.8 ± 1.2% of the non-Gly and non-Pro residues were in the disallowed regions for wild type and T38D, respectively.

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relatively a novel type, but the folding can be categorized into the left-hand 4-helix bundle with much lower threshold. Not so many proteins in the left-hand 4-helix bundle folding are recorded in Protein Data Bank (http://www.rcsb.org/pdb), thus CPI-17 is a new member of this minority group.

The phosphorylation site, Thr38, is located in a loop at the N-terminal end termed P-loop. In the wild type protein the side-chain of Thr38 has poor solvent accessibility. However, introduction of a negative charge at position 38 produces a conformational change of the protein, resulting in residue 38 being exposed to the solvent. The exposure of residue 38 is supposedly a key for the molecular switch.

Together with the swinging of the P-loop, two of the four helices (A- and B-helices) in the 4-helix bundle are rotated. The rotation seems to bury the cavity generated by movement of the P-loop. The other two helices, C- and D-helices, are not moved, thus found to form a scaffold in CPI-17.

The difference of the two structures described here is the conformational change induced by introduction of the negative charge at residue 38. This is the first insight how CPI-17 becomes an active form.

3.2. Binding model

Using the crystal structure of PP1c, which is a homolog of the catalytic subunit of MP, we also considered target binding of CPI-17. Various biochemical experiments suggested that phospho-Thr38 of CPI-17 binds to the catalytic site of PP1c. Thus we put the side-chain of phospho-Thr38 onto the catalytic site of PP1c, and rotated the molecules to find the possible binding surface. We treated the mainchain of molecules as rigid. Fig. 2 shows a possible binding model of phospho-CPI-17 and PP1c. In this model, two pairs of protein surface regions are found to be responsible for formation of the complex.

One is electrostatic interaction between a negatively charged cluster on the surface of phospho-CPI-17 and an acidic groove of PP1c. The other is hydrophobic interaction between a hydrophobic surface area of phospho-CPI-17 and a hydrophobic groove of PP1c.

4. Conclusion

In conclusion, we determined the structures of CPI-17 in the inactive and active form using heteronuclear multidimensional NMR spectroscopy. Comparison of these structures revealed how the molecular switch turns on. To our best knowledge, this is the first three-dimensional structure of PP1 inhibitory proteins. These preliminary results allow us to the next stage in understanding the biological function of CPI-17.

Using the structure of T38D, we also built a structure of the complex between phospho-CPI-17 and PP1c. The model will give us a valuable information how to design small molecules which can control PP1c activity. More generally, the atomic resolution-level knowledge obtained here is important for the rational design of new nano-materials using biological materials.

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Fig. 2. A possible binding model between CPI-17 and PP1c. The structure of PP1c was obtained from PDB (1FJM). The molecular surfaces were colored red (negatively charged), blue (positively charged), and white (hydrophobic). Three pairs of binding regions are indicated by circles and horizontal lines.
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