Dynamic and Thermodynamic Response of the Ras Protein Cdc42Hs upon Association with the Effector Domain of PAK3

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Abstract

Human cell division cycle protein 42 (Cdc42Hs) is a small, Rho-type guanosine triphosphatase involved in multiple cellular processes through its interactions with downstream effectors. The binding domain of one such effector, the actin cytoskeleton-regulating p21-activated kinase 3, is known as PBD46. Nitrogen-15 backbone and carbon-13 methyl NMR relaxation was measured to investigate the dynamical changes in activated GMPPCP·Cdc42Hs upon PBD46 binding. Changes in internal motion of the Cdc42Hs, as revealed by methyl axis order parameters, were observed not only near the Cdc42Hs–PBD46 interface but also in remote sites on the Cdc42Hs molecule. The binding-induced changes in side-chain dynamics propagate along the long axis of Cdc42Hs away from the site of PBD46 binding with sharp distance dependence. Overall, the binding of the PBD46 effector domain on the dynamics of methyl-bearing side chains of Cdc42Hs results in a modest rigidification, which is estimated to correspond to an unfavorable change in conformational entropy of approximately −10 kcal mol^{-1} at 298 K. A cluster of methyl probes closest to the nucleotide-binding pocket of Cdc42Hs becomes more rigid upon binding of PBD46 and is proposed to slow the catalytic hydrolysis of the γ phosphate moiety. An additional cluster of methyl probes surrounding the guanine ring becomes more flexible on binding of PBD46, presumably facilitating nucleotide exchange mediated by a guanosine exchange factor. In addition, the Rho insert helix, which is located at a site remote from the PBD46 binding interface, shows a significant dynamic response to PBD46 binding.
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Introduction

Human cell division cycle homolog protein 42 (Cdc42Hs)* is a member of the Ras family of small guanosine triphosphatases (GTPases). Interactions with various proteins allow the Rho GTPases to be highly coordinated “molecular switches”. Activated Cdc42Hs regulates a variety of cellular responses, principally involving actin polymerization, intracellular trafficking and cell growth regulation, through interactions with multiple downstream effector proteins [1]. Through their regulatory interactions with guanine nucleotide exchange factors (GEFs), GTPase-activating proteins (GAPs) and guanine nucleotide dissociation inhibitors (GDIs), GTPases cycle between an activated, guanosine triphosphate (GTP)-bound state and an inactive, guanosine diphosphate (GDP)-bound state [2,3].

All members of the Rho subfamily of GTPases share a common fold consisting of five α-helices, a central β-sheet and a conserved nucleotide-binding pocket [4]. Activation of members of this family results in a structural response, mainly in two regions of the protein, termed Switches I and II, that are central to GTP hydrolysis and binding of downstream effector proteins [5]. Many of the effector proteins bind the active, GTP-bound form of Cdc42Hs via a Cdc42/Rac interactive binding (CRIB) motif that binds between the two switch regions of Cdc42Hs and forms an additional strand to the central β-sheet. One such

CRIB-containing effector protein family is the actin cytoskeleton-regulating p21-activated serine/threonine kinase 3 (PAK3) family. Members of this family are implicated in numerous human diseases [6–8] and are some of the most thoroughly studied effectors of Cdc42Hs [9].

The GTP-activated state of Cdc42Hs initiates cellular signaling cascades via PAK3 kinase CRIB-binding domain, which inhibits the GTPase activity of Cdc42Hs [10]. A significant structural feature of the Rho family members is the presence of an α-helix containing the so-called Rho insert region [11]. This helix has been implicated in the intracellular signaling of the membrane associated activated GTPase. The signaling is proposed to initiate in response to a reorientation of the Rho insert helix with the subsequent activation of the GDI, an effector protein of the Rho GTPases [12,13]. Important structural features of Cdc42Hs are highlighted in Fig. 1. The structural response of the activated GTPase Cdc42Hs to binding the downstream PAK3 effector PBD46 is localized to the binding interface [14,15]. The ensuing cascade of molecular events requires a more distal response than the structural changes might suggest. The dynamical response of activated Cdc42Hs to effector binding Cdc42Hs is the focus of this study.

The association of proteins with ligands is a central aspect of biochemical work and has a complex thermodynamic origin [16,17]. A fundamental consideration of the thermodynamics of ligand binding is the potential contribution from changes in protein conformational entropy and has generated much discussion and speculation over the past few decades [18–22]. It is only recently that experimental methods and analytical strategies have emerged to allow determination of changes in protein conformational entropy upon a change in functional state and use of comprehensive measures of changes in internal protein motion as a "dynamical proxy" for the conformational entropy that they reflect [23–26].

The first experimental demonstration of large-scale changes in internal protein motion due to the binding of a ligand to a protein examined the interaction of calcium-activated calmodulin with peptides corresponding to calmodulin-binding domains of regulated proteins [27]. With the use of a simple model-dependent "oscillator inventory" interpretation [28], the changes in the fast motion (nanosecond-to-picosecond timescale) of the methyl-bearing side chains of calmodulin suggested that changes in the conformational entropy of calmodulin were on the order of the free energy of binding [27]. Subsequently, a linear correlation between the total entropy of binding and the apparent conformational entropy was observed across a family of calmodulin complexes, suggesting that evolution has employed the variation in conformational entropy to adjust the free energy of binding [24]. More recently, it has become clear that changes in NMR-derived order parameters can indeed be used as a quantitative proxy for conformational entropy and may be generally useful as an "entropy meter" [25,26]. Molecular dynamics simulations also suggest that the linear relationship between the order parameter and the calculated rotamer entropy of the methyl-bearing side chains has simple physical origins [26].

Whether large contributions to the free energy of formation of protein–protein complexes by changes in conformational entropy are pervasive remains an

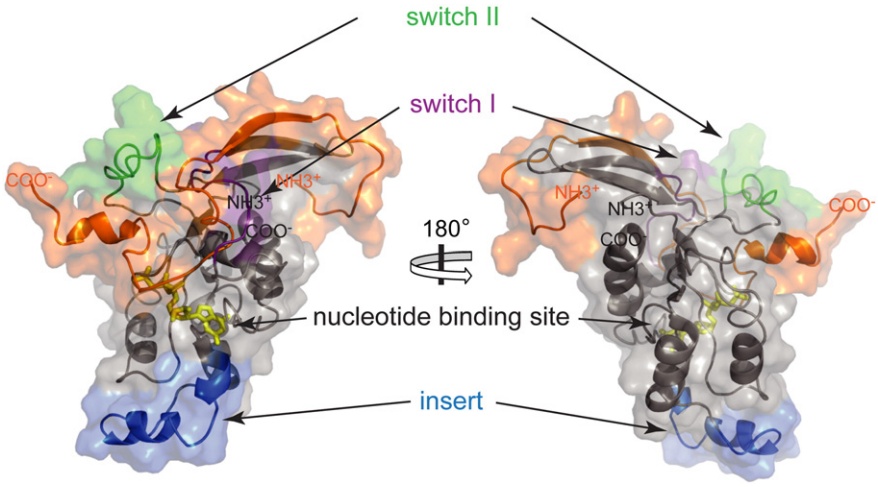

Fig. 1. Views of the structure of Cdc42Hs·GMPPCP bound to PBD46 [14] (PDB ID: 1EES). Cdc42Hs is depicted with gray ribbons and PBD46 is depicted in orange ribbons; the surface of the complex is colored as the protein or peptide. Three regions of interest in the Cdc42Hs protein are designated by colors. Switch I [55] is in purple, Switch II [55] is in green and the Rho insert region [5] is in blue. The GTP analog (GMPPCP) shown in yellow is absent from the deposited model owing to insufficient structural restraints [14] and was therefore modeled here using a homologous binary complex [54] (PDB ID: 2QRZ). Structural renderings in this and subsequent figures were generated using the program PyMOL [89].

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