

Complex Structures of an NTPase OsYchF1 Reveal the Working Mechanisms of the Ancestral YchF Subfamily G Proteins

Xiao-Rong LI, Wei WU and Zhong-Zhou CHEN*

Beijing Advanced Innovation Center for Food Nutrition and Human Health, State Key Laboratory of Agrobiotechnology, China Agricultural University, Beijing 100193, China

chenzhongzhou@cau.edu.cn

*Corresponding author

Keywords: Small G proteins, YchF, Molecular basis, Complex structure.

Abstract. G proteins are involved in almost all aspects of the cellular regulatory pathways through their ability to bind and hydrolyze GTP. The YchF subfamily are the core universally conserved G proteins and are present in all kingdoms of life. YchF proteins, interestingly, possess the unique ability to bind both ATP and GTP. However, the biological significance and working mechanism of such a highly conserved G proteins have long eluded researchers. Here, we have solved the structures of the apo forms of a YchF homolog in rice (OsYchF1) at four conditions and its complexes with ATP and GTP by using X-ray crystallography. These structures perfectly elucidate the conformational changes of OsYchF1 caused by different pH, ATP or GTP binding. Compared with other YchF homologs, OsYchF1 is the first YchF subfamily structure which completely displays three functional regions: G2/switch I, G3/switch II and Loop A. Furthermore, by comparing the conformational changes of the apo-OsYchF1 between weakly acidic and weakly alkaline conditions and of the ligand-bound versions, a working mechanism for the YchF protein's ability to transduce signal is revealed. This discovery has a remarkable impact on our understanding of the structure-function relationships of the YchF subfamily of G proteins in all kingdoms of life. Human YchF homolog OLA1 is a multifunctional molecular switch-like ATPase and this regulatory ATPase is a potential novel target for antioxidative and antineoplastic therapy. Our results provide a structural basis to elucidate these molecular regulators, and may reveal novel insights into the understanding of cellular defense systems as well as expose new therapeutic targets for antioxidant and antineoplastic drugs.

Introduction

GTP-binding proteins (G proteins/GTPases) are widely distributed molecular switches and play important roles in diverse fundamental biological processes in living organisms, including signal transduction, cell division, development, intracellular transport, translation, and others [1]. Based on sequence and structure similarities, the GTPase superclass can be divided into more than fifty families. Within these families, a core group of eight universally conserved GTPases are found in all domains of life, including YihA, YchF, HflX, IF-2, EF-Tu, EF-G, Ffh, and FtsY [2]. However, the functions and working mechanisms of some ancestral G proteins, such as YchF, which are believed to play essential roles in cellular processes, are still unknown.

All G proteins contain a G domain (composed of the G1–G5 motifs) for GTP binding and hydrolysis [3]. The G4 motif (canonical sequence: NKxD) confers the specificity for binding

GTP. In YchF proteins, the noncanonical G4 sequence (NxxE) is correlated with the loss of nucleotide binding specificities [4]. In some cases, ATP is the preferred ligand [5]. YchF proteins derived from bacterial, yeast, and human are all defined as an ATPase. Rice YchF protein (OsYchF1) significantly differs from other YchF homologs in that it has the same ability to bind and hydrolyze GTP and ATP.

Results

We have resolved the structures of the apo forms of OsYchF1 at weakly acidic and alkaline conditions and its complexes with AMPPNP (adenylyl-imidodiphosphate) and GMPPNP (guanylyl-imidodiphosphate) [6] (Fig. 1). The OsYchF1 structure, as the first crystal structure of the plant YchF homolog, shares the conserved domains of YchF subfamily proteins, including the G domain, helical domain and TGS domain (Fig. 1A). However, OsYchF1 possesses significantly different functional regions: G2/switch I, G3/switch II and Loop A, and the apo-OsYchF1 is the first YchF subfamily structure which displays the above three functional areas completely (Figure 1B). The noncanonical G4 motif ($_{230}\text{NMSE}_{233}$) makes OsYchF1 have more room to accommodate both ATP and GTP. Our structures of OsYchF1 in complex with AMPPNP and GMPPNP show that the guanine base interacting with binding pocket is mainly through water-mediated hydrogen bonds, so the guanine base is bound ~ 2 Å shallower than the adenine base in the binding pocket of OsYchF1.

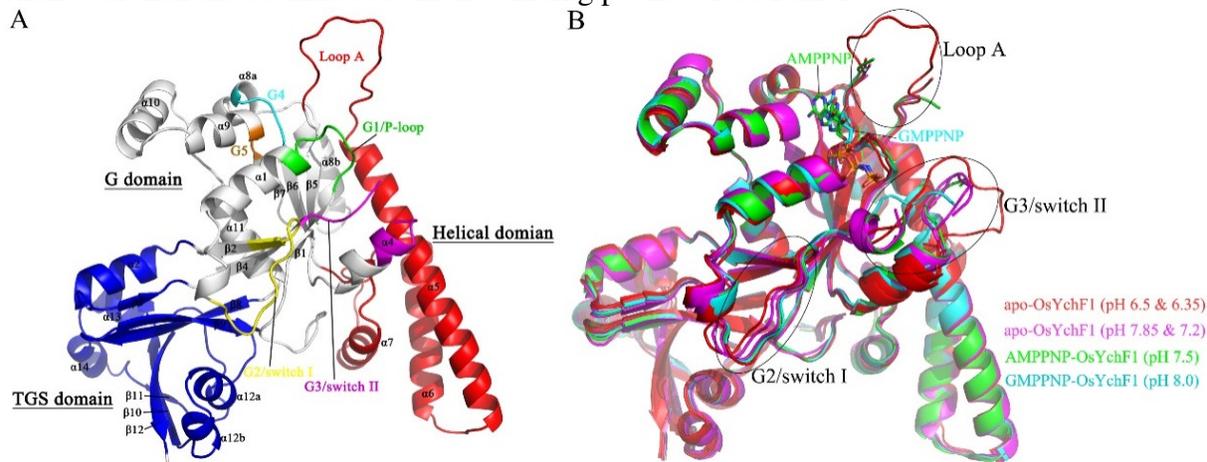


Figure 1. The overall structures of OsYchF1.

(A) The general structure of OsYchF1. A cartoon representation of apo-OsYchF1 structure showing its three domains: a G domain (white), a helical domain (red), and a C-terminal TGS domain (blue). The G1–G5 motifs of the G domain are color-coded in green, yellow, magenta, cyan, and orange, respectively. (B) Structural comparisons of apo-OsYchF1 at weakly acidic (pH 6.5/6.35) and weakly alkaline (pH 7.85/7.2) conditions and AMPPNP-OsYchF1, GMPPNP-OsYchF1 complexes. Structures are indicated and colored as follows: red, apo-OsYchF1 (pH 6.5/6.35); magenta, apo-OsYchF1 (pH 7.85/7.2); green, AMPPNP-OsYchF1; cyan, GMPPNP-OsYchF1. AMPPNP/GMPPNP bound to the nucleotide binding site of the OsYchF1 are shown in a stick representation. Three functional regions are shown in black ovals.

Significant conformational changes occur in the G3/switch II region, when comparing all

solved structures of OsYchF1 (Fig. 2). At weakly alkaline conditions (corresponding to the physiological condition of normal cells), there is a helix- $\alpha 3$ in the G3/switch II region (Box 1 of Fig. 2). In a lower pH value (pH 6.5/6.35), the helix- $\alpha 3$ is unfolded and $\alpha 4$ swings away from the nucleotide-binding site (Box 2 of Fig. 2). The reason is the imidazole ring of His-115 in helix- $\alpha 4$ is protonated, thereby disrupting the hydrophobic interactions that stabilize $\alpha 3$. OsYchF1 may not bind any nucleotide at weakly acidic conditions, for the side chain of Lys-37 in the P-loop extends to the opposite direction of the nucleotide binding site (Box 5 of Fig. 2). The structure of AMPPNP-OsYchF1 at pH 7.5 is virtually identical to that of apo-OsYchF1 at pH 7.85/7.2. However, it is likely that Loop A should move toward the nucleotide binding site region when AMPPNP binds to OsYchF1 (Box 3 of Fig. 2). Binding of GMPPNP induces the unfolding of $\alpha 3$ and the swinging of helix- $\alpha 4$ toward the nucleotide-binding site so that the amide of Gly-97 is repositioned to form a hydrogen bond with the γ -phosphate of GMPPNP (Box 4 of Fig. 2). This may mean that the intracellular OsYchF1 binding of GTP requires the involvement of other regulatory factors.

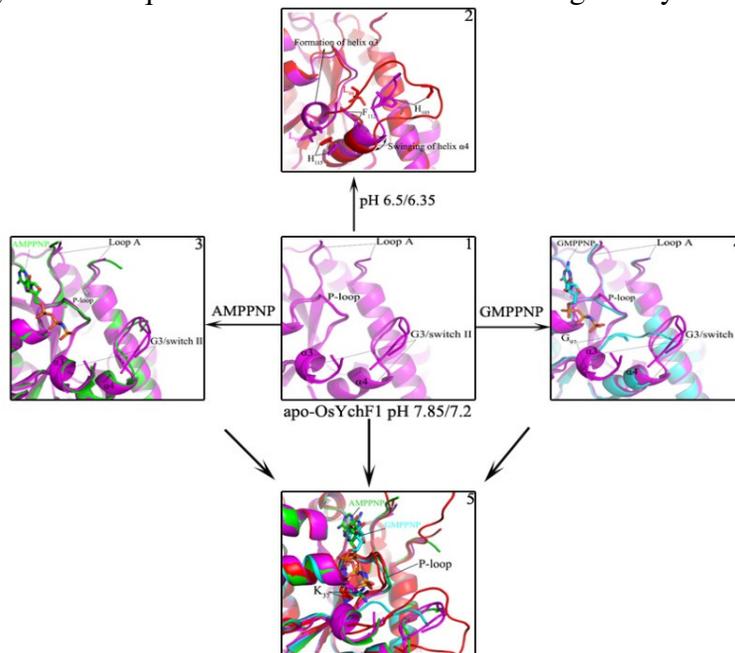


Figure 2. Structural changes of the G3/switch II region.

Box 1: the G3/switch II and Loop A regions of apo-OsYchF1 at pH 7.85/7.2 (purple). There is a helix- $\alpha 3$ in the G3/switch II region, which is stabilized by the hydrophobic interactions between those residues. Box 2: Structure comparison of the G3/switch II region of apo-OsYchF1 at pH 7.85/7.2 (purple) and pH 6.5/6.35 (red). Acidic condition makes $\alpha 3$ unfolded and an extended loop is formed. Box 3: Structure comparison of apo-OsYchF1 at pH 7.85/7.2 (purple) and AMPPNP-OsYchF1 (green). Binding of AMPPNP may lead Loop A moving to the nucleotide binding site region. Box 4: Structure comparison of the G3/switch II region of apo-OsYchF1 at pH 7.85/7.2 (purple) and GMPPNP-OsYchF1 (cyan). Binding of GMPPNP induces the unfolding of $\alpha 3$ so that the amide of Gly-97 form a hydrogen bond with the γ -phosphate of GMPPNP. Box 5: Combination of boxes 1, 2, 3, 4. The side chain of Lys-37 in the P-loop extends to the opposite direction of the nucleotide binding site at weakly acidic conditions.

Discussion

Our structures of apo-OsYchF1 at weakly acidic and alkaline conditions indicate that the G3/switch II region has a pH-dependent conformational changes. Meanwhile, this region is a very important functional area of G proteins, which can interact with other effectors. *E. coli* YchF homolog also has a pH-dependent ATPase activity and ATPase specific activity is maximal at a pH equal to or above 7.5 and significantly declines at a pH below 6.7 [7]. The G3/switch II motif is highly conserved in the YchF subfamily, so we can reasonably speculate that the YchF subfamily may all have a conformational change process of pH dependence. This pH-dependent structural changes of YchF subfamily illustrate that cytoplasm pH value may affect the function of YchF proteins *in vivo*, including human YchF homolog OLA1 (Obg-like ATPase 1). The published structure of OLA1 has no interpretable electron density for three functional regions involved in the binding and hydrolysis of NTP of YchF subfamily [4]. Our structures clearly show the three conserved sequence motifs. Therefore, our structures provide a structural basis for functional study of human OLA1. The overexpression of OLA1 has been observed in many types of human malignancy [8]. OLA1 is a regulatory protein that interacts with downstream effector protein(s) and exerts its downstream functions by the conformational switch between the ADP- and ATP-bound forms. The YchF proteins have a slow endogenous NTPase activity. GTPase activating proteins (GAPs) accelerate the slow endogenous GTP hydrolytic activity of G proteins and promote the formation of the GDP-bound, inactive conformation [9]. OsGAP1 can interact with OsYchF1, and stimulate its NTPase activities [10]. The highly conserved histidine residue in the Loop A is critical for the NTPase activity of the YchF proteins [7]. On the basis of these reported and our structural results, here, we propose a simple model for the mechanism of NTP binding by the YchF proteins (Fig. 3). As a molecular switches, the YchF proteins generally cycle between a NTP-bound “on” state and a NDP-bound “off” state. YchF proteins may no longer bind to NTP at cytoplasm pH value below 6.7. When faced with some sorts of regulatory signaling, GAPs bind to the Loop A of YchF proteins, activating YchF proteins NTPase activity. The hydrolysis of NTP is accompanied with the long helix domain, which has a lever-like structure, outward loosening and then YchF proteins release of effectors, transfer to another location. Altogether, YchF proteins use its NTP ligand to transduce signal within cells. Our data therefore gave the first report to our knowledge demonstrating the importance of ATP binding in determining the different functions of YchF homologs. In general, ATPases are attractive drug targets, and a number of ATPase inhibitors are already on the market [11]. OLA1, as a P-loop ATPase, would appear to be drugable. The role of OLA1 as a novel negative regulator of the antioxidant response and cell proliferation has potential therapeutic implications. Consequently, the crystal structures of YchF subfamily facilitate the structure-based design of small molecular inhibitors.

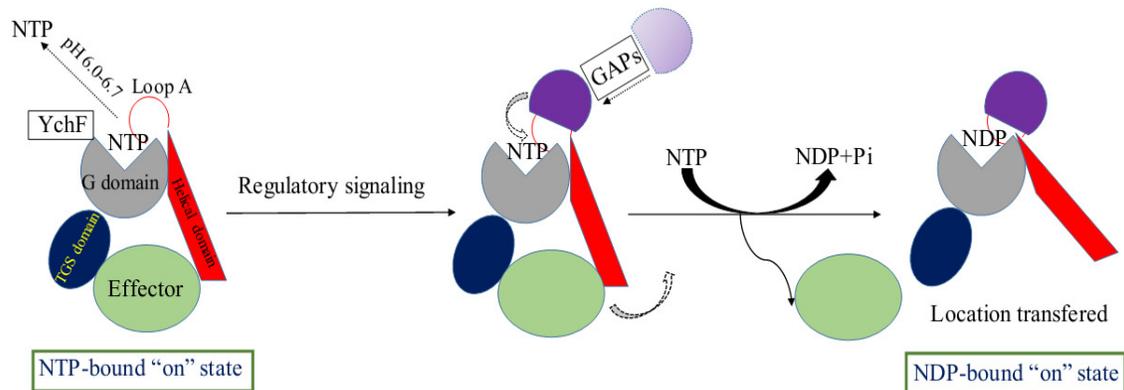


Figure 3. Working model of YchF proteins.

YchF proteins contain G domain (gray), TGS domain (blue), helical domain (red), where G domain binds to the nucleotide substrate. NTP-bound YchF proteins are in its “on” state. When cytoplasm pH value is below 6.7, YchF proteins may no longer bind NTP. Some regulatory signaling will induce GAPs to bind to the Loop A, activating YchF proteins NTPase activity, then YchF proteins release its effector and shift to a new location. In short, YchF proteins use its ligand to transduct signal within cells.

Acknowledgement

This work was supported financially by National Key Research and Development Program of China (2016YFC1200400) and National Natural Science Foundation of China (31370720, 31570725 and 91519332).

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