Biochemical and Biophysical Research Communications 418 (2012) 122–127

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Crystal structures of the *Arabidopsis thaliana* abscisic acid receptor PYL10 and its complex with abscisic acid

Demeng Sun [a,1], Haipeng Wang [a,1], Minhao Wu [a], Jianye Zang [a], Fangming Wu [b,*], Changlin Tian [a,b,*]

[a] Hefei National Laboratory for Physical Sciences at Microscale, School of Life Sciences, University of Science and Technology of China, Hefei, Anhui 230026, China
[b] High Magnetic Field Laboratory, Chinese Academy of Sciences, Hefei, Anhui 230031, China

ARTICLE INFO

Article history:
Received 8 December 2011
Available online 8 January 2012

Keywords:
ABA receptor
PYR/PYL/RCAR family
Phytohormone
Helix-grip fold
START domain

ABSTRACT

Abscisic acid (ABA) is one of the most essential phytohormones, and plays an important role in growth and development regulation, as well as in stress responses. The PYR/PYL/RCAR family (PYL for short)—comprised of 14 proteins in *Arabidopsis*—was recently identified as soluble ABA receptors that function in the perception and transduction of ABA signaling. In this work, the crystal structures of PYL10 were determined in the apo- and ABA-bound states, with respective resolutions of 3.0 and 2.7 Å. Surprisingly, a closed CL2 conformation was observed in the apo–PYL10 structure, which was different from a previously reported open CL2 conformation. A putative two-conformation dynamical equilibrium model was proposed to explain PYL10's constitutive binding to PP2Cs in the apo-state and its increased PP2C binding ability in the ABA-bound state.

1. Introduction

Abscisic acid is an important phytohormone that regulates numerous developmental processes and adaptive stress responses in plants [1,2]. The soluble ABA receptor family (PYR/PYL/RCAR proteins) was discovered independently by two groups [3,4]; the family comprises 14 members (PYR1 and PYL1-13) in *Arabidopsis thaliana* that share a characteristic START (star-related lipid transfer) domain [5]. Soon after this discovery, a large biochemical and structural research effort—focusing mainly on PYR1, PYL1, and PYL2—revealed the molecular mechanism behind the interactions between PYLs and downstream PP2Cs (type 2C protein phosphatases), and their inhibition of the phosphatase activity of PP2Cs such as ABI1, ABI2, and HAB1 [6–10]. Upon binding of ABA, two conserved loops in PYLs (termed as gate and latch [10] or CL2 and CL3 [6]) undergo significant conformational change, and thus create a protein surface suitable for PP2C association [6–10]. The substrate binding site for PP2C is blocked in the ABA–PYL–PP2C complex, which impairs the PP2C-mediated inhibition of downstream SnRK2 kinases, and leads to the accumulation of active SnRK2, possibly by autophosphorylation [6,9,10]. The activated SnRK2 can then activate the transcription and expression of relevant genes [11,12].

On the other hand, some members of this ABA receptor family may be able to constitutively interact with and inhibit PP2C [3,13]. Very recently, this was confirmed by Hao and colleagues through a systematic biochemical characterization of PYL proteins [14]. A subclass of PYL proteins, represented by PYL10 (UniProt accession: Q8H1R0), were able to constitutively bind and inhibit the phosphatase activity of PP2Cs, even in the absence of ABA. Their PP2Cs binding ability would be further stimulated by binding to ABA [14]. Their crystal structure of apo–PYL10 showed an open conformation in the CL2 loop, despite that a closed CL2 conformation was anticipated [14].

Here, we report the crystal structure of ligand-free PYL10 with a resolution of 3.0 Å. Interestingly, our apo–PYL10 structure showed a closed CL2 conformation, as expected by Hao and colleagues [14]. Further examination showed that the conformation of the CL2 loop was influenced by the CL3 loop of the other PYL10 molecule in the same asymmetric unit. To interpret the previously reported biochemical results, we proposed a putative two-conformation dynamical equilibrium model of PYL10. We also determined the crystal structure of the ABA–PYL10 complex with a resolution of 2.7 Å, which has not been reported previously.

2. Materials and methods

2.1. Protein expression, purification and crystallization

The PYL10 gene from *Arabidopsis thaliana* was cloned into the pET-21b(+) expression vector (Novagen) and expressed in

* Corresponding authors. Fax: +86 551 5591149 (F. Wu), +86 551 3600441 (C. Tian).
E-mail addresses: fmwu@hmfl.ac.cn (F. Wu), cltian@ustc.edu.cn (C. Tian).
[1] These authors contributed equally to this work.

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doi:10.1016/j.bbrc.2011.12.145

Table 1
Data collection and refinement statistics.

	Apo–PYL10	ABA–PYL10
Data collection		
Space group	P3121	P3121
Unit-cell parameters (Å, °):	$a = b = 80.47$, $c = 124.93$ $\alpha = \beta = 90$, $\gamma = 120$	$a = b = 68.33$, $c = 63.40$ $\alpha = \beta = 90$, $\gamma = 120$
Resolution (Å)	50.0–3.00 (3.05–3.00)	43.3–2.70 (2.76–2.70)
Number of unique reflections	9834	4929
Completeness (%)	98.2 (99.4)	99.2 (100)
Redundancy	4.2 (4.2)	5.3 (5.8)
Average $I/\sigma I$	12.7 (3.2)	40.6 (3.7)
R_{merge}[a] (%)	13.4 (52.1)	5.5 (29.6)
Refinement		
Resolution (Å)	46.5–3.00	59.17–2.70
R_{work} (No. of reflections)	22.0 (8679)	23.7 (4435)
R_{free} (No. of reflections)	28.8 (970)	27.4 (480)
R.M.S.D. bond lengths (Å)	0.010	0.010
R.M.S.D. bond angles (°)	1.403	1.402
Number of non-hydrogen atoms		
Protein	2451	1208
ABA	None	19
Water	34	11
Sulfate ion	5	None
Average B-factors (Å²)		
Protein	34.93	67.64
ABA	None	63.68
Water	27.88	65.93
Sulfate ion	46.91	None
Ramachandran plot[b]		
Favored (98%) regions	98.7%	97.4%
Allowed (>99.8%) regions	100%	100%

[a] $R_{merg} = |I_i - \langle I \rangle| / |I_i|$ where I_i is the intensity of the ith measurement, and $\langle I \rangle$ is the mean intensity for that reflection.
[b] Statistics for the Ramachandran plot from an analysis using MolProbity [21].

Escherichia coli strain BL21(DE3)RP (Stratagene). The transformed strain was incubated at 37 °C until the OD_{600} value reached 0.6–0.8; it was then induced using IPTG (isopropyl β-D-thiogalactopyranoside, Sigma) at 25 °C for 12 h. The harvested cells were lysed by sonication (300 cycles, 2 s per cycle, 1:2 pulse/pause at 30% amplitude of 750 W) in lysis buffer (70 mM Tris–HCl, pH 8.0, 300 mM NaCl) which was supplemented with 5.6 mM β-ME. The over-expressed proteins were purified using Ni-NTA affinity column (QIAGEN) and eluted by binding buffer (20 mM Tris–HCl, pH 8.0, 200 mM NaCl) supplemented with 300 mM imidazole and 5.6 mM β-ME. The protein was further purified by gel filtration chromatography (which was performed using a Superdex 75 10/300 GL column (GE healthcare), run on an ÄKTA system) in the buffer of 20 mM Tris–HCl, pH 8.0, 200 mM NaCl and 2 mM DTT. The fractionally collected proteins were concentrated using an Amicon Ultra filter (10,000 MWCO, Millipore).

The purified apo–PYL10 was concentrated to 7 mg/mL in 20 mM Tris–HCl, pH 8.0, 200 mM NaCl and 2 mM DTT. Crystals of apo- and ABA-bound PYL10 were grown at 295 K in 48-well plates (Xtal-Quest Co.), using the sitting-drop vapor diffusion method. One microliter of protein solution was mixed with an equal volume of reservoir solution, and the resulting droplet was equilibrated against 100 μL of reservoir solution. Apo–PYL10 crystallized in the well buffer containing 0.1 M Bis–Tris–HCl, pH 5.5, 0.2 M $(NH_4)_2SO_4$, and 25% PEG 3350. To obtain crystals of ABA-bound PYL10, apo–PYL10 was mixed with (+)-ABA (Sigma–Aldrich) at a molar ratio of 1:3, and concentrated to 9 mg/mL for co-crystallization. Crystals were obtained in the well buffer containing 0.1 M Bis–Tris–HCl, pH 6.5, and 20% PEG monoethyl ether 5000. The size and the shape of the crystals are presented in Supplementary Fig. S1.

2.2. Data collection, processing, structure determination and refinement

X-ray diffraction data were collected for both apo- and ABA-bound PYL10 at beamline BL17U at the SSRF (Shanghai Synchrotron Radiation Facility). Crystals were harvested, then soaked in a cryoprotectant solution containing crystallization buffer supplemented with 20% glycerol. The crystals were then flash-cooled in liquid nitrogen, and X-ray diffraction data was collected at 100 K. The observed reflections were reduced, merged and scaled with the program HKL-2000 [15].

Atomic coordinates of the apo-PYL2 (PDB ID: 3KDH) was used for molecular replacement with the program PHASER [16] into the data of apo–PYL10. The model completeness was done in COOT [17] and refinement was performed by REFMAC5 [18] with non-crystallographic symmetry (NCS) restraints in the early stage and CNS program (version 1.2) [19]. The structure of ABA-bound PYL10 was determined by molecular replacement with the program MOLREP [20] using the apo–PYL10 structure as the search model. The initial model was further refined using the program REFMAC5 [18], and rebuilt by using the σ_A-weighted electron-density maps with coefficients 2mFo−DFc and mFo−DFc in the program COOT [17]. The stereochemistry of the structure was checked by MolProbity [21]. The data collection and structure refinement statistics are summarized in Table 1.

3. Results

3.1. Overall structures of apo- and ABA-bound PYL10

Apo– and ABA–PYL10 were both crystallized in the P3121 space group. The structure of apo–PYL10 was refined to a resolution of 3.0 Å, with R_{work} and R_{free} values of 22.5% and 28.1%, respectively (Table 1). There were two molecules in each asymmetric unit (Supplementary Fig. S2). Similar to other members of the PYL family, the structure of PYL10 adopts a helix-grip fold (like the START domains [5]), which is mainly composed of seven anti-parallel β-strands and two α helices with the following topology: β1–α2–η1–β2–β3–β4–β5–β6–β7–α3 (η stands for a 3_{10}-helix) (Fig. 1A). The topology diagram was generated using PDBsum [22], and is shown in Supplementary Fig. S3. It should be mentioned that the N-terminal 24 residues—which correspond to the α1 helix in PYR1, PYL1 and PYL2—were missing in the density map. The structure of the ABA–PYL10 complex was determined by molecular replacement, using apo–PYL10 as the search model. The structure was refined to a resolution of 2.7 Å, with R_{work} and R_{free} values of 23.7% and 28.4%, respectively (see Table 1). It's noteworthy that there is large discrepancy of data quality between apo–PYL10 and ABA–PYL10. The Wilson B-factor of apo–PYL10 is only 37.6, while the Wilson B-factor of ABA–PYL10 is 78.0. So it is reasonable that average B-factor of apo–PYL10 is 29.8 and that of ABA–PYL10 is 66.4. One (+)-ABA molecule was coordinated in the putative PYL ligand-binding pocket (Fig. 1B) through hydrogen bond and *van der Waals* interactions, similar to the ligand binding mode of ABA to other known PYL family members. The carboxylate of ABA accepted one hydrogen bond from the side chain amine group of K56 (Fig. 1C). No water-mediated hydrogen bond was found, probably due to the relatively low resolution of the structure. The complex was also maintained via *van der Waals* contacts between ABA and the side-chains of F58, I59, L79, L83, I104, I106, L113, Y116, F155, V156, L159 and I160 (Fig. 1D). All of these residues were highly conserved in all 14 PYL proteins (Supplementary Fig. S4).