FEBS Open Bio 4 (2014) 496–509

FEBS openbio

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

Molecular mechanisms in the selective basal activation of pyrabactin receptor 1: Comparative analysis of mutants

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ARTICLE INFO

Article history:
Received 21 March 2014
Revised 10 May 2014
Accepted 13 May 2014

Keywords:
Pyrabactin resistance
Abscisic acid signaling
Constitutively active mutations
Molecular dynamics simulations
Essential collective dynamics analysis

ABSTRACT

Pyrabactin receptors (PYR) play a central role in abscisic acid (ABA) signal transduction; they are ABA receptors that inhibit type 2C protein phosphatases (PP2C). Molecular aspects contributing to increased basal activity of PYR against PP2C are studied by molecular dynamics (MD) simulations. An extensive series of MD simulations of the apo-form of mutagenized PYR1 as a homodimer and in complex with homology to ABA-insensitive 1 (HAB1) phosphatase are reported. In order to investigate the detailed molecular mechanisms mediating PYR1 activity, the MD data was analyzed by essential collective dynamics (ECD), a novel approach that allows the identification, with atomic resolution, of persistent dynamic correlations based on relatively short MD trajectories. Employing the ECD method, the effects of select mutations on the structure and dynamics of the PYR1 complexes were investigated and considered in the context of experimentally determined constitutive activities against HAB1. Approaches to rationally design constitutively active PYR1 constructs to increase PP2C inhibition are discussed.

1. Introduction

In 2009, two research groups independently reported the discovery of a family of abscisic acid (ABA) -binding proteins in *Arabidopsis thaliana*, containing as many as 14 members known as PYR1 (pyrabactin resistance 1) and PYL (PYR1-like) [1] or RCAR (regulatory component of ABA response) receptors [2]. In one instance this was enabled by the identification of pyrabactin as an inhibitor of ABA-mediated developmental and stress response effects in phenotypic screens [1]. Pyrabactin was shown to mediate its inhibition through the pyrabactin receptor 1 protein (PYR1). Further analyses showed that PYR1 and its associated homologs play a central role in ABA signal transduction, as the ABA receptors, acting via inhibition of protein phosphatases of the type 2C (PP2C) variety [1–3].

Structural studies have demonstrated that when ABA binds to the receptor, interactions between the agonist and two flexible loops (Lβ3β4 and Lβ5β6 named gate and latch, respectively) form a unique receptor surface (Fig. 1A) that can interact with the phosphatases [4,5]. Three independent studies also demonstrated that a subset of the ABA receptors exhibit constitutive activity against PP2Cs *in vitro* [1,6,7]. Although PYR1 and PYLs 1–3 show very weak basal activity, PYL4 was found to be active against HAB1 [7], whereas other family members such as PYLs 5–6 and 8–10 are constitutively active. Based on analogy to the PYL10's gate sequence, recent work demonstrated that replacing residue V87 in PYL2's gate-latch region by the bulkier L87 equivalent from PYL10 partially increased PYL2's constitutive inhibition of PP2C [7]. Further in this vein, a less rational but more successfully applied broad based mutagenic screening approach to the development of constitutive active ABA receptors was recently reported [8]. In this instance probing of 39 residues around ligand pocket in the ABA receptor led to the identification of 10 sites in the gate-latch and C-terminal helix regions involved in mediating basal receptor activity. Interestingly, although single-site mutations were sufficient to

Abbreviations: MD, molecular dynamics; ECD, essential collective dynamics; PCA, principal component analysis; WT, wild type; ABA, A8S abscisic acid; PDB, Protein Data Bank; PP2C, phosphatase type 2C; PYR1, pyrabactin resistance 1; PYL, PYR1-like; RCAR, regulatory component of ABA response; CA, constitutively active; HAB1, homology to ABA insensitive 1; PYV, pyrabactin or $C_{16}H_{13}BrN_2O_2S$; P2M, *N*-(pyridin-2-ylmethyl) naphthalene-1-sulfonamide or $C_{16}H_{14}N_2O_2S$

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http://dx.doi.org/10.1016/j.fob.2014.05.001
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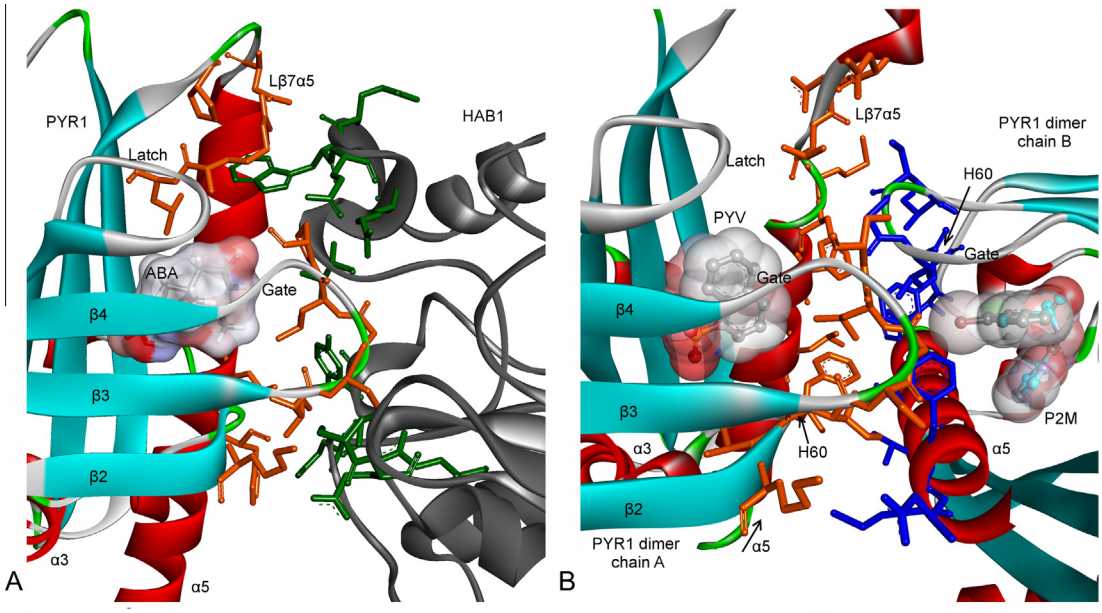

Fig. 1. PYR1 residues comprising the binding interfaces [10]: (A) – in PYR1–ABA–HAB1 complex, PDB ID 3QN1, with orange sticks showing PYR1 residues and green sticks showing HAB1 residues; and (B) – in PYV/P2M-bound PYR1 dimer, PDB ID 3NJO, with orange and blue sticks showing the residues of chain A and B, respectively. The ligands are depicted by translucent surfaces, colored according to the charge of ligand's atoms: red – positive charge, blue – negative charge, white – no charge. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

stabilize receptor-PP2C interactions (as detected by yeast-2-hybrid analysis), stacking of 3 and even 4 mutations was necessary to elicit basal receptor activation at a level comparable to ABA-stimulated receptor activity *in vitro*.

Recently it was demonstrated that ABA-receptors can adopt a homodimeric form in solution (in the absence of ABA, see Fig. 1B) and that this dimerization correlates with inhibition of basal receptor activity. In particular, ABA-free PYR1, PYL1 and PYL2 were found to be homodimeric, whereas PYLs 5–6 and 8–10 adopt a preferentially monomeric form [9,7]. It has also been shown [7] that mutation I88K in PYL2 both prevents homodimer formation in solution and increases its constitutive activity. Residue 60 in PYR1 has also been found to play a key role in determining the ABA-bound receptors' oligomeric state [9].

Molecular dynamics (MD) simulations and associated essential collective dynamics (ECD) analyses of wild type (WT) PYR1 in homodimeric and PP2C complexes reported in our earlier study [10], further support a 'competing complexes' model in which PYR1–PYR1 dimerization might be in competition with formation of PYR1–HAB1 complexes, in particular in the absence of ABA. Addition of ABA yielded an opposite effect on the dynamics of PYR1–HAB1 and PYR1–PYR1 complexes, constraining inter-molecular interactions in the former and destabilizing the latter. An in depth description of the ECD framework is available elsewhere [10–14].

Now, MD simulations and associated ECD analyses of mutations demonstrated, or predicted, to promote dissociation of the PYR1–PYR1 dimer or promote the constitutively active association of PYR1 with HAB1 are reported. Extensive MD simulations and experiments for multiple mutants of PYR1 considered in the context of complex formation with HAB1 versus homodimer were undertaken. Employing the ECD framework we investigate the effects of the mutations on structure and dynamics of the PYR1 complexes. We discuss promising PYR1 mutations and approaches to design constitutively active PYR1 constructs.

2. Results and discussion

2.1. Analysis of constitutively active PYR1 mutant constructs

A comparison of the interacting surfaces for PYR1 dimers versus the PYR1–ABA–HAB1 complex [3,15,16], based on our MD simulations analyses discussed elsewhere [10], shows that the interfaces are largely the same in these two complexes. Examples of PYR1 binding areas in crystallographic structure of in ABA-bound complex with HAB1 (PDB ID 3QN1) and PYV/P2M-bound dimer (PDB ID 3NJO) shown in Fig. 1A and B, respectively, indicate that the binding areas overlap significantly. PYR1 residues involved in these interactions, as identified by Accelrys VS, are listed in Table 1. The table also presents the inter-molecular interaction data for apo-PYR1 dimer for original X-ray crystallographic structure PDB ID 3K3K, as well as PYR1 residues contacting ABA for structure PDB ID 3K3K. It can be seen that similar interactions are found in loops Lα3β2, Lβ3β4, the gate, and helix α5 in PYR1–ABA–HAB1 complex and in both dimers. In apo-dimer, also the latch and the area of helix Lβ7α5 are involved in the interactions, similarly to the PYR1–ABA–HAB1 complex. A detailed comparative analysis of these interactions is presented elsewhere [10].

The overlap of the binding areas in PYR1 complexes with HAB1 and in the dimers complicates the task of identifying CA mutations of PYR1 that might drive it to preferentially bind to PP2Cs over forming homodimers in the absence of ABA. Thus activity and structural information from published reports [8,18], as well as available crystallographic structural data about ligand binding sites in PYL10 [7], residues involved in intermolecular interactions of PYL2 dimers [18] and interactions with phosphatase [18] were also considered. Fig. S1 (Suppl. Mat.) presents a sequence alignment for selected relevant PYR/PYL constructs, highlighting 42 identified sites involved in ligand (L), HAB1 phosphatase (P), or intermolecular dimer (D) PYR1 contacts. A detailed discussion of the considerations of these sites leading to the