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Crystal Structure of a Multi-domain Immunophilin from *Arabidopsis thaliana*: A Paradigm for Regulation of Plant ABC Transporters

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FKBP42 is a membrane-anchored immunophilin playing a critical role in morphogenesis and development of higher plants. We present the X-ray structure of the cytoplasmic portion of FKBP42 comprising both the FKBP-like domain and the TPR domain at 2.85 Å resolution. The data shed light on the probable binding modes of key interaction partners, including HSP90 and two classes of ABC transporters. The resulting models provide a structural background for further investigation of the unique biological properties of this protein.

Keywords: FKBP42; *Arabidopsis thaliana*; X-ray crystallography; HSP90; ABC transporter

Introduction

The FK506-binding proteins (FKBPs) represent an ancient and ubiquitous protein family named after the role of several members as primary targets of FK506-type immunosuppressants in animal and human cells.[1] The FKBP–drug complex has been shown to block calcineurin (PP2B)-mediated signal transduction leading to an inhibition of the T cell-dependent immune response.[2] Based on this activity, the FKBPs, together with the family of cyclophilins, have also been termed "immunophilins".

Another feature shared by many FKBPs is the ability to act as peptidylprolyl *cis-trans* isomerases (PPIases), which implicates these proteins in peptide folding and chaperoning processes.[3] Mammalian FKBP12, which comprises a single FKBP domain, has been shown to interact with different types of calcium release channels[4,5] and to modulate their gating behavior. Moreover, it associates with the type I receptors for transforming growth factor β (TGFβ)-family cytokines,[6] where it prevents ligand-independent activation. Multi-domain FKBPs are structurally characterized by additional protein modules, typically a tripartite tetratricopeptide repeat (TPR) domain and a calmodulin binding motif, in addition to one or more FKBP domains. This group is exemplified by mammalian FKBP52, which is the major immunophilin of the multiprotein glucocorticoid receptor complex.[7] The crystal structure of human FKBP52 has been published recently.[8]

Members of the FKBP family have also been identified in plants. The crystal structure of *At*FKBP13, a single-domain FKBP localized in the thylakoid lumen of *Arabidopsis thaliana* chloroplasts, has been determined.[9] However, structural information on multi-domain FKBPs from plants is still unavailable.

*At*FKBP42, also termed TWISTED DWARF1 (TWD1) due to the reduced height and disoriented growth of null mutants, is a type II membrane protein comprising 365 amino acid residues. In addition to a single FKBP-type domain, it contains a tripartite TPR motif, a putative calmodulin binding site and a hydrophobic C-terminal membrane anchor.[10–12] In contrast to many other immunophilins, *At*FKBP42 is devoid of PPIase activity and does not display measurable affinity for FK506. The TPR region of the protein binds to *At*HSP90, comparable to the complexes formed of multi-domain immunophilins and HSP90 in mammalian cells.[10] Recent experimental evidence has revealed a crucial role of HSP90 in plant development and

Abbreviations used: ABC, ATP-binding cassette; FKBP, FK506-binding protein; HSP90, 90 kDa heat shock protein; HOP, HSP90 organizing protein; aa, amino acid residues; PDB, Protein Data Bank; ICD, intracellular domain; MDR, multidrug resistance protein; MRP, MDR-related protein; NBD, nucleotide-binding domain; PP2B, protein phosphatase 2B; PPIase, peptidylprolyl *cis-trans* isomerase; TGFβ, transforming growth factor β; TWD1, TWISTED DWARF1.

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phenotypic plasticity, including the response to environmental stresses and pathogen infection.[13]

The FKBP domain of *At*FKBP42 has been demonstrated to physically interact with plasma membrane-localized ABC transporters *At*PGP1 and *At*PGP19,[11] whereas the TPR domain appears to be responsible for functional association with vacuolar transporters *At*MRP1 and *At*MRP2.[12]

Phytohormones of the auxin family represent essential regulators of plant growth and development. The predominant auxin, indole 3-acetic acid, is synthesized at the shoot apex and undergoes a basipetal transport that is crucial for the establishment of plant polarity.[14] Recently, *At*PGP1 and *At*PGP19 have been shown to directly mediate cellular auxin efflux.[15,16] Since double mutants *atpgp1/atpgp19* display a subset of phenotypic features of *twd1*, including a reduction of polar auxin transport,[11] it seems reasonable to assume that impaired cell elongation and disoriented growth of *twd1* plants result from non-sufficient auxin transport. Indeed, *At*FKBP42 has recently been demonstrated to regulate auxin transport by *At*PGP1.[17]

The availability of the three-dimensional structure of *At*FKBP42 is expected to provide a starting point for detailed investigations of the complexes with physiologically relevant target proteins, in particular plant ABC transporters. As a first step, we have crystallized the N-terminal part of *At*FKBP42 (aa 1–180) containing the FKBP-like domain and determined its three-dimensional structure.[18,19] Here we report the X-ray structure of the water-soluble portion of the molecule (aa 1–339), which in addition to the FKBP domain contains the putative TPR modules. Novel features of this structure as compared to known multi-domain immunophilins and TPR proteins from mammals and plants are discussed with respect to their functional correlates. In particular, we address the properties of the conserved binding site for HSP90-type chaperones and present structural models illustrating the interaction of *At*FKBP42 with the ABC transporters *At*PGP1 and *At*MRP1.

Results and Discussion

Structure of AtFKBP42

The cytoplasmic portion of *At*FKBP42 (aa 1–339) was expressed and purified as outlined in Materials and Methods. Crystals belonging to space group $P2_12_12$ were obtained using ammonium sulfate and PEG400 as precipitating agents. The X-ray structure could be solved by isomorphous replacement incorporating anomalous scattering information and complemented by molecular replacement (see Materials and Methods). Figure 1 shows a ribbon representation of the *At*FKBP42(1–339) structure, with colors reflecting the mean atomic temperature factor for each residue. The N-terminal part of the molecule (left) displays an FKBP-type fold consisting of a five-stranded antiparallel β-sheet wrapped around a short α-helix, and is essentially identical to our previously published structure of the isolated domain[19] (r.m.s. deviation 0.73 Å for C^{α} positions of aa 35–164). The C-terminal segment, on the other hand, represents a helical bundle (right) as expected from the early sequence-based classification as a TPR domain.[10] The N-terminal portion of the molecule (aa 1–34) could not be traced in the electron density maps and is thus likely to be disordered. The same is true for the C-terminal stretch (aa 293–339), which contains the putative calmodulin-binding site. The two domains are linked by an extended loop, which is very flexible as judged by relatively high temperature factors and weak electron density in this region. Moreover, high mobility is evident for the termini of the TPR helices and the connecting loops, with an overall increase towards the C terminus of the molecule. The temperature factors that we determined for the TPR domain of *At*FKBP42(1–339) (mean $B = 70$ Å^2 for aa 172–292) are significantly higher than those found in crystal structures of other large immunophilins.[8,20,21] Although we cannot exclude that this discrepancy may partially be related to issues in data scaling, the individual crystal packing environments are likely to restrain conformational mobility to a different extent. Recent NMR studies on the TPR module of protein phosphatase 5 (Ppp5) have provided insight into the conformational dynamics of this type of domain.[22,23] The authors found that

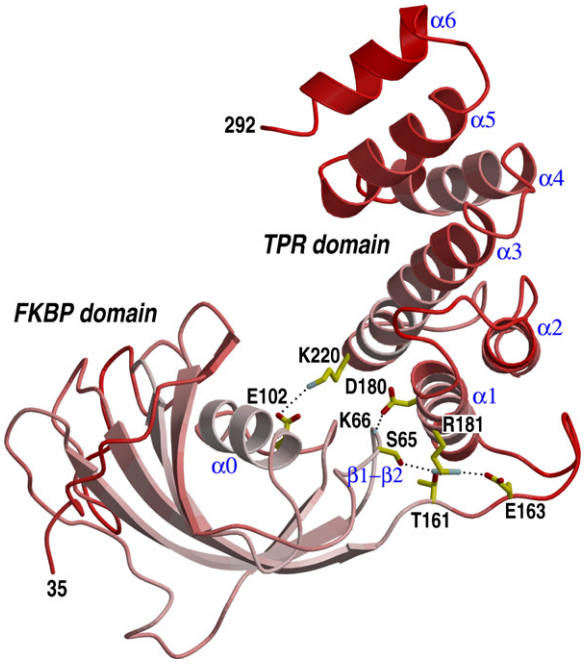

Figure 1. Ribbon representation of the *At*FKBP42 (1–339) crystal structure determined here. The mean *B* factors per residue are indicated by a color gradient from white (mean $B < 35$ Å^2) to red (mean $B > 90$ Å^2). Side-chains involved in hydrogen bonds at the domain interface are drawn in stick mode. Hydrophobic contacts (see the text) are omitted for clarity.