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Coiled-coil dimerization of the LOV2 domain of the blue-light photoreceptor phototropin 1 from *Arabidopsis thaliana*

A key role in signal transduction and dimerization mediated by Per–Arnt–Sim (PAS) domains is played by α -helical linkers that flank the structurally similar α/β cores of these domains. However, crystal-packing forces and the different construct lengths and sequences of the PAS domains influence the final length and orientation of the linkers relative to the core and create uncertainty in the exact mechanism of the linker function. Thus, structural characterization and comparison of the linkers within isolated PAS-domain constructs and/or full-length PAS-containing proteins is important for clarification of the mechanism. The plant blue-light photoreceptors phototropins possess two N-terminal flavin mononucleotide-based light, oxygen or voltage (LOV) domains (LOV1 and LOV2) that comprise a subclass of the PAS family and one C-terminal serine/threonine kinase domain whose enzymatic activity is regulated by blue light. The dark-adapted state crystal structures of the *Arabidopsis thaliana* phototropin 1 and phototropin 2 LOV1-domain constructs flanked by an N-terminal A' α helix and the structure of the phototropin 2 core LOV2 domain are known. Here, the crystal structure of the *A. thaliana* phototropin 1 LOV2 domain has been determined in its dark-adapted state. The core is flanked by an N-terminal A' α helix and a C-terminal J α helix similar to those in the previously reported structure of *Avena sativa* phototropin 1 LOV2. In contrast to the monomeric *A. sativa* LOV2, *A. thaliana* LOV2 is a dimer in which two A' α helices adopt a scissor-like orientation at the dimer interface and form a short α -helical coiled coil. The J α helix predominantly interacts with the β -sheet and plays a role in coiled-coil formation and dimerization.

1. Introduction

Arabidopsis thaliana phototropin 1 (phot1) and phototropin 2 (phot2) are photosensory proteins that contain two N-terminal light, oxygen or voltage (LOV) domains (LOV1 and LOV2) and one C-terminal serine/threonine kinase domain (Sakai *et al.*, 2001; Kagawa *et al.*, 2001; Kinoshita *et al.*, 2001; Sakamoto & Briggs, 2002; Takemiya *et al.*, 2005; Folta & Spalding, 2001; Christie *et al.*, 1998). Both LOV domains belong to the Per–Arnt–Sim (PAS) domain superfamily (Taylor & Zhulin, 1999; Henry & Crosson, 2011; Möglich *et al.*, 2009b), noncovalently bind flavin mononucleotide (FMN) in their dark state (Taylor & Zhulin, 1999) and possess a conserved α/β fold of their core flanked by α -helical linkers at their N-termini and C-termini (Harper *et al.*, 2003; Halavaty & Moffat, 2007). Although LOV1 and LOV2 undergo identical reversible photochemical reactions (Kasahara *et al.*, 2002; Salomon *et al.*, 2000), they play different roles in signal transduction and oligomerization by phototropins (Christie *et al.*, 2002; Matsuoka & Tokutomi, 2005; Nakasako *et al.*, 2004, 2008; Salomon *et al.*, 2004; Okajima *et al.*, 2012; Nakasone *et al.*, 2008; Christie, 2007).

The photocycle in plant phototropins begins with the light-driven formation of a covalent flavin-cysteinyll adduct between the C4a atom of FMN and the S γ atom of a conserved cysteine residue (Cys512 in *A. thaliana* LOV2) in their LOV domains (Salomon *et al.*, 2000; Swartz *et al.*, 2001; Iwata *et al.*, 2002; Halavaty & Moffat, 2007). Formation of this covalent bond triggers small structural changes in the FMN-binding pocket and its vicinity (Crosson & Moffat, 2002;

Table 1

Data-collection and refinement statistics.

Values in parentheses are for the highest resolution shell.

Data collection	
Wavelength (Å)	0.9002
Temperature (K)	105
Space group	<i>P</i> 4 ₁ 2 ₁ 2
Unit-cell parameters (Å, °)	<i>a</i> = <i>b</i> = 63.4, <i>c</i> = 171.3, $\alpha = \beta = \gamma = 90.0$
Resolution range (Å)	30.00–2.75 (2.80–2.75)
No. of unique reflections	8574 (399)
<i>R</i> _{merge} (%)	7.0 (58.0)
Completeness (%)	87.9 (86.6)
<i>I</i> / <i>σ</i> (<i>I</i>)	17.5 (2.2)
Multiplicity	3.3 (3.6)
Wilson <i>B</i> factor (Å ²)	66.3
Refinement	
Resolution range (Å)	29.72–2.75 (2.82–2.75)
No. of reflections	8128 (545)
<i>R</i> _{work} / <i>R</i> _{free} (%)	23.0/29.0 (29.0/47.0)
No. of protein molecules/non-H atoms	2/2466
No. of ligands/non-H atoms	2/62
No. of water O atoms	29
Mean temperature factor (Å ²)	48.4
Coordinate deviation	
R.m.s.d., bonds (Å)	0.01
R.m.s.d., angles (°)	1.31
Ramachandran plot†	
Most favored (%)	88.1
Allowed (%)	11.1
Generously allowed (%)	0.7
Disallowed (%)	0.0

† Statistics are based on PROCHECK (Laskowski *et al.*, 1993).

Halavaty & Moffat, 2007; Harper *et al.*, 2003) that ultimately activate autophosphorylation of phototropin by the kinase domain (Crosson *et al.*, 2003). The photocycle is completed in the dark by slow spontaneous rupture of the covalent bond. Discovery of a C-terminal *Jα* helix flanking the core of the *Avena sativa* LOV2 domain (Harper *et al.*, 2003) revealed that light-mediated displacement of the *Jα* helix from the core β -sheet and unfolding of *Jα* is an early step in autophosphorylation. Crystal-packing forces impeded these substantial structural changes in crystals of *A. sativa* LOV2(404–546) (denoted AsLOV2; Halavaty & Moffat, 2007). However, formation of the covalent adduct in the crystals resulted in the rearrangement of the hydrogen-bonding interactions in a linker region N-terminal to the core that contains a short α -helix denoted the A' α helix (Halavaty & Moffat, 2007). Upon light illumination, the A' α helix may unfold first and then displace the *Jα* helix (Zayner *et al.*, 2012). In contrast, Takeda *et al.* (2013) have shown that the A' α helix unfolds after disruption of the *Jα* helix. Numerous crystal structures of homologous PAS domains show that the orientation and length of the flanking α -helices might depend on the specific PAS sequence and be affected by the length of the construct and/or by crystal packing [PDB entries 3sw1 (Circolone *et al.*, 2012), 2gj3 (Key *et al.*, 2007), 2v0u (Halavaty & Moffat, 2007), 3e4o (Zhou *et al.*, 2008), 1d06 (Miyatake *et al.*, 2000), 3cwf (Chang *et al.*, 2010), 1xj3 (Key & Moffat, 2005), 4hj4 (Conrad *et al.*, 2013), 4gcz (Diensthuber *et al.*, 2013), 3c2w (Yang *et al.*, 2008) and 3ewk (Ukaegbu & Rosenzweig, 2009)]. The flanking linkers form α -helical coiled coils and/or bundles through which signal transduction and dimerization is facilitated (Little *et al.*, 2012; Slavny *et al.*, 2010; Lee *et al.*, 2008; Etzkorn *et al.*, 2008; Nan *et al.*, 2010; Key *et al.*, 2007; Park *et al.*, 2004; Miyatake *et al.*, 2000; Diensthuber *et al.*, 2013; Wang *et al.*, 2013). Given the structural diversity of the flanking regions, a comparative sequence–structure analysis is increasingly important for understanding the exact mechanism(s) of their function, particularly since LOV domains are increasingly being used for optogenetic applications (Strickland *et al.*, 2008, 2012; Wu *et al.*,

et al., 2009; Christie, Gawthorne *et al.*, 2012; Möglich *et al.*, 2009a; Möglich & Moffat, 2010; Drepper *et al.*, 2011).

We have determined the crystal structure at 2.75 Å resolution of the dark-adapted state of the *A. thaliana* phot1 LOV2 domain construct spanning residues 452–615, denoted AtLOV2, that includes the N-terminal A' α helix and the C-terminal *Jα* helix. The A' α helix largely contributes to dimerization through an α -helical coiled coil. Despite its spatial proximity to the A' α helix, the *Jα* helix is not part of the coiled coil, but does appear to be involved in the coiling and dimerization of AtLOV2.

2. Materials and methods

2.1. Cloning, expression, purification and crystallization of AtLOV2

AtLOV2 was amplified from the genomic DNA and cloned into the pET-28c vector (EMD Serono, Rockland, Massachusetts, USA) *via* *Nde*I/*Hind*III restriction sites, expressed in *Escherichia coli* BL21 (DE3) cells (NEB, Ipswich, Massachusetts, USA) and purified as described previously (Halavaty & Moffat, 2007). In brief, the BL21 (DE3) cells were grown to an OD₆₀₀ of 0.7 at 310 K at 200 rev min^{−1} followed by overnight induction at 289 K with 1 mM isopropyl β -D-1-thiogalactopyranoside (IPTG) in the dark. After induction, the cells were spun down and ruptured by sonication in 20 mM Tris–HCl buffer pH 7.4, 20 mM NaCl (buffer A) containing an EDTA-free protease-inhibitor cocktail tablet (Roche Diagnostic, Mannheim, Germany). The soluble fraction was loaded onto a Ni–NTA column and washed with 20 mM imidazole in buffer A. AtLOV2 was eluted with 250 mM imidazole in buffer A followed by overnight dialysis against buffer A. The N-terminal purification tag was cleaved by incubating the protein in the dark with thrombin (EMD Millipore, Merck, Darmstadt, Germany) according to the standard manufacturer's protocol. The extent of cleavage was assayed by passing the reaction mixture over a Ni–NTA column and analysing the pooled fractions by SDS–PAGE. Cleaved AtLOV2 was further purified on a Superdex 200 gel-filtration column (GE Healthcare, Piscataway, New Jersey, USA) in buffer A plus 15% glycerol (buffer B).

AtLOV2 (21 mg ml^{−1}) was crystallized in buffer B by mixing it with a candidate crystallization condition in a 1:1 volume ratio in the dark. Crystals suitable for data collection and structure determination were obtained by the sitting-drop vapor-diffusion technique using a condition from The JCSG+ Suite (Qiagen, Valencia, California, USA) consisting of 40 mM potassium phosphate, 16%(w/v) PEG 8000, 20%(v/v) glycerol at 287 K. Crystals of different sizes appeared in between one and two weeks. Prior to data collection, crystals were flash-cooled in liquid nitrogen in the dark.

2.2. Data collection and structure determination

Monochromatic oscillation X-ray data were collected at station 14-BM-C of BioCARS at the Advanced Photon Source (APS), Argonne National Laboratory (ANL) and were processed with *HKL*-3000 (Minor *et al.*, 2006). The structure was determined by molecular replacement using *Phaser* (McCoy *et al.*, 2007) from the *CCP4* package (Winn *et al.*, 2011) using AsLOV2 as a search model (PDB entry 2v0u; Halavaty & Moffat, 2007). The initial model was rebuilt with *ARP/wARP* (Morris *et al.*, 2003), corrected in *Coot* (Emsley & Cowtan, 2004; Emsley *et al.*, 2010), refined with *REFMAC* v.5.5 (Murshudov *et al.*, 2011) and validated with the *ADIT* validation (<http://deposit.pdb.org/validate/>) and *MolProbity* (Chen *et al.*, 2010; Davis *et al.*, 2007) servers. Data-processing and structure-determination statistics are given in Table 1. The atomic coordinates were deposited in the PDB as entry 4hhd. Structure figures were

Figure 1

The AtLOV2 structure. (a) Superposition of the two AtLOV2 chains: in chain A the PAS core is shown in yellow, the A'α helix in light blue and the Jα helix in light green, while in chain B the PAS core is shown in brown, the A'α helix in dark blue and the Jα helix in dark green. (b) Coordination of FMN (chain A). (c) Top, superposition of AtLOV2 [chain A; colored as in (a)] and AsLOV2 (the PAS core is shown in gray, the A'α helix in magenta and the Jα helix in dark cyan). Bottom, superposition of AtLOV2 (chain A) and AtLOV1 (the PAS core is shown in gray in chain A and white in chain B; the A'α helix is in magenta for both chains). (d) Sequence alignment of plant phototropins showing the N- and C-terminal portions of the LOV2 domain only. Secondary-structure elements of AtLOV2 are displayed with the A'α helix from chain A and the Jα helix from chain B. The UniProtKB identifiers are as follows: *Arabidopsis thaliana*, O48963; *Avena sativa*, O49003; *Oryza sativa*, O49003; *Zea mays*, O48547; *Pisum sativum*, P93489; *Phaseolus vulgaris*, Q5DW43; *Vicia faba*, Q8H935. The hydrophobic residues at positions a and d of the heptad repeat in the A'α helix and its preceding N-terminal regions are shown in blue and the Jα helix and its following C-terminal regions are shown in green.

Figure 2

Dimerization of AtLOV2. (a) AtLOV2 dimer in which the flanking linker helices form an α -helical coiled coil. (b) Dimerization interface of AtLOV2. Residues with one star belong to the A' α helix and those with two stars belong to the J α helix; unlabeled residues are from the PAS core domain. (c) Varied dimerization modes of AtLOV2, *Rhodospirillum rubrum* LOV (RsLOV; PDB entry 4hj4; Conrad *et al.*, 2013) and the *Bacillus subtilis* LOV domain of FixL (PDB entry 4gc2; the histidine kinase domain of the chimera was omitted for clarity; Diensthuber *et al.*, 2013). The A' α helix is colored light blue (chains A) and dark blue (chains B) and the J α helix is depicted in light green (chains A) and dark green (chains B). The LOV core domains of the displayed structures are shown in gray. The K α helix C-terminal to the J α helix is shown in red for both chains in the RsLOV structure.

prepared with *PyMOL* (DeLano, 2010) and *LigPlot*⁺ (Laskowski & Swindells, 2011). Multiple sequence alignments were performed with *ClustalW* (Thompson *et al.*, 1994) and figures were generated with *ESPrpt2.2* (Gouet *et al.*, 1999).

3. Results and discussion

The asymmetric unit of the *AtLOV2* crystal contains two monomers denoted chain *A* and chain *B* (r.m.s.d. of 0.9 Å over 144 C α atoms; Fig. 1*a*). The flanking A' α and J α helices emerge on the same side of the β -sheet of each *AtLOV2* monomer and vary in length. The FMN is embedded within the core of *AtLOV2* and interacts with conserved residues (Fig. 1*b*). The side chain of Cys512 poised to form the light-mediated covalent bond to the C4a atom of FMN is in the dark-state conformation (Halavaty & Moffat, 2007; Supplementary Fig. S1¹). The core of *AtLOV2* resembles those of the phot1 *AtLOV1* (r.m.s.d. of 1.2 Å over 105 C α atoms; PDB entry 2z6c; Nakasako *et al.*, 2008), the phot2 *AtLOV1* (r.m.s.d. of 0.8 Å over 105 C α atoms; PDB entry 2z6d; Nakasako *et al.*, 2008), the phot2 *LOV2* (r.m.s.d. of 0.9 Å over 108 C α atoms; PDB entry 4eep; Christie, Hitomi *et al.*, 2012) and the phot1 *AsLOV2* (r.m.s.d. of 0.5 Å over 102 C α atoms; PDB entry 2v0u; Halavaty & Moffat, 2007), thus confirming the high degree of structure conservation among LOV cores. However, the relative orientations and lengths of the flanking regions vary markedly among the crystal structures of LOV domains and evidently depend on the construct sequence/length and crystal packing (Figs. 1*a* and 1*c*).

The first six N-terminal residues in chain *B* were not resolved and its A' α helix is thus shorter than in chain *A* (Figs. 1*a* and 1*c*). The A' α helix in chain *A* curves slightly. Its sequence has an irregular heptad repeat of apolar residues characteristic of α -helical coiled coils (Fig. 1*d*). However, the length of the hydrogen bonds in the proximity of these residues is not significantly shorter than elsewhere in the helix (Parry *et al.*, 2008; Conway & Parry, 1990), yet the hydrogen-bond distances are set by the current 2.75 Å resolution, *i.e.* they are affected by the quality of the final model. The van der Waals interactions between the N-terminal residues of the A' α helix (chain *A*) and the residues of the H β –I β loop (chain *B*) may contribute to the bending of the helix. The interactions of the J α helix with the PAS core are nearly identical in *AtLOV2* and *AsLOV2* (Halavaty & Moffat, 2007; Harper *et al.*, 2003; Figs. 1*a* and 1*c*). A multiple full-length sequence alignment of the plant phototropins and structural comparison of *AtLOV2* and *AsLOV2* suggest that plant LOV2 sequences with a shorter I β –J α junction (six residues, as in *AsLOV2*) may have a shorter J α helix, whereas those with a longer junction (ten residues, as in *AtLOV2*) may adopt a longer helix (Fig. 1*d*). In the *AtLOV2* structure the disordered residues 581–592 of the junction in chain *A* and the partially ordered residues 580–586 in chain *B* presumably adopt a loop conformation, whereas residues 587–592 in chain *B* form part of the J α helix that is slightly bent (Supplementary Fig. S1).

The dimer of *AtLOV2* has a total buried surface area of 4500 Å² contributed in each monomer by 23 residues from the A' α helix, 20 residues from the PAS core and three residues from the J α helix (Figs. 2*a* and 2*b*). Similar scissor-like interactions between even shorter A' α helices have been reported in PAS/LOV structures both in the presence and the absence of the J α helix [PDB entries 2gj3 (Key *et al.*, 2007) and 3sw1 (Circolone *et al.*, 2012)], in which dimerization is additionally stabilized by direct β -sheet-to- β -sheet contacts. Direct contacts are also found in short constructs which lack both the

flanking A' α and J α helices [PDB entries 1byw (Morais Cabral *et al.*, 1998) and 1g28 (Crosson & Moffat, 2001); Supplementary Fig. S1], but direct contacts are absent in the *AtLOV2* structure owing to the folding pattern of the J α helix. The role of the A' α helix in dimerization of the current *AtLOV2* construct is apparent, although the solution behavior of our *AtLOV2*(452–615) construct is unknown. Nakasone *et al.* (2006) showed that the *AtLOV*(449–586) construct that possesses the A' α helix and lacks the J α helix dimerizes in its dark state in a concentration-dependent manner. In contrast, the *AtLOV2*(449–661) construct in which both flanking helices are present is a monomer even at high concentrations (Nakasone *et al.*, 2008). Although the A' α helices in *AtLOV2* and *AsLOV2* are superimposable, the A' α helix in *AsLOV2* spans only one turn, whereas the length of the structurally ordered N-terminus of *AsLOV2* is half that of *AtLOV2*. In *AsLOV2*, Phe403 (remaining from the purification tag and replacing a conserved Asp403) lies in a hydrophobic pocket at the crystal contact (not shown). It thus may prevent extension of the A' α helix and bring about the monomeric form of *AsLOV2*. Some role of the J α helix in the dimerization of *AtLOV2* is possible, but details cannot be explicitly obtained from the current *AtLOV2* structure. The sequences N-terminal to the A' α helix and C-terminal to the J α helix show heptad periodicity and helical propensity based on the *PSIPRED* prediction (Jones, 1999; Buchan *et al.*, 2010). They potentially form the extended or additional α -helical coiled coils and/or α -helical bundles found at the dimerization interface of certain PAS/LOV domains [Fig. 2*c* and Supplementary Fig. S1; PDB entries 4gcz (Diensthuber *et al.*, 2013), 3c2w (Yang *et al.*, 2008), 4hj4 (Conrad *et al.*, 2013) and 3e4o (Zhou *et al.*, 2008)].

Overall, our structure supports numerous findings on the role of the flanking linker regions in the dimerization of PAS/LOV domains. Similar to the homologous PAS/LOV domains, these linkers exhibit amphipathic character and pack together in coiled-coil/ α -helical bundle structures to facilitate the dimerization of *AtLOV2*. However, it still remains unclear exactly how *AtLOV2* dimerizes, since we and others have explicitly shown that variations in sequence, construct design and crystal contacts undoubtedly affect the relative orientation and length of the flanking regions and ultimately the dimerization mode of these sensor domains.

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¹ Supplementary material has been deposited in the IUCr electronic archive (Reference: SW5066).

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