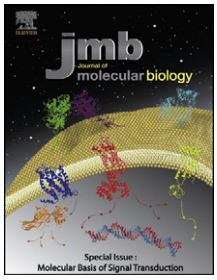

Signaling States of a Short Blue-Light Photoreceptor Protein PpSB1-LOV Revealed from Crystal Structures and Solution NMR Spectroscopy

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Abstract

Light–Oxygen–Voltage (LOV) domains represent the photo-responsive domains of various blue-light photoreceptor proteins and are widely distributed in plants, algae, fungi, and bacteria. Here, we report the dark-state crystal structure of PpSB1-LOV, a slow-reverting short LOV protein from *Pseudomonas putida* that is remarkably different from our previously published "fully light-adapted" structure [1]. A direct comparison of the two structures provides insight into the light-activated signaling mechanism. Major structural differences involve a ~11 Å movement of the C terminus in helix Jα, ~4 Å movement of Hβ–Iβ loop, disruption of hydrogen bonds in the dimer interface, and a ~29° rotation of chain-B relative to chain-A as compared to the light-state dimer. Both crystal structures and solution NMR data are suggestive of the key roles of a conserved glutamine Q116 and the N-cap region consisting of A′α–Aβ loop and the A′α helix in controlling the light-activated conformational changes. The activation mechanism proposed here for the PpSB1-LOV supports a rotary switch mechanism and provides insights into the signal propagation mechanism in naturally existing and artificial LOV-based, two-component systems and regulators.

Introduction

Light–Oxygen–Voltage (LOV) proteins are non-opsin, blue-light photoreceptors that belong to the Per-ARNT-Sim (PAS) family, which non-covalently binds flavin chromophores [2,3]. Blue-light absorption induces conformational changes in the LOV protein–chromophore, which triggers a signaling cascade, leading to various physiological responses such as phototropism, chloroplast movement, stomatal opening, regulation of circadian rhythms, photo-induced growth patterns, and photopigment synthesis [4–6]. LOV proteins are long known to be widely represented in bacteria, archea, protists, plants, and fungi [7–9], but only in the last two decades, their structures and activation mechanisms have been studied at the molecular level. Typically, the photochemistry of LOV proteins involves blue-light absorption by the non-covalently bound chromophore flavin mononucleotide (FMN), which triggers the formation of the transient adduct between FMN-C4a atom and the sulfur atom of a strictly conserved cysteine residue in the active site of the LOV sensor domain. This state is referred to as the "light" state. The covalent adduct breaks once the illumination ceases, completing the photocycle by recovery of the protein in the so-called "dark" state. The significant difference between various LOV-proteins is the "recovery" or the adduct decay time that can differ from seconds to hours or even days [1,6,10–13]. Recent literature suggests a consensus regarding the signaling mechanism where the adduct formation causes small conformational changes in the chromophore binding pocket that are then relayed via helical linker regions to the effector domains, fused either at the N or C terminus of the LOV domain [6,14–20]. Although the majority of LOV proteins exist as

multidomain LOV proteins with one or more LOV domains fused to the effector domains, several LOV proteins have been identified in bacteria to exist as the so-called "short" LOV proteins composed of a conserved LOV core and N- and/or C-terminal helical extensions [1,6,8,11,21–23]. Due to the absence of fused effector domain(s), the next step in signal propagation in short LOV proteins is expected to involve the partner proteins, which remain unidentified in several cases [1,6,23]. For the investigation of photoactivation mechanism at atomic level, the short LOV proteins provide a simple and complete light-controlled model system.

High-resolution crystal structures of several LOV proteins, mostly from plants and some from bacteria sources, are available in the PDB database[‡]. With the exception of two cases where LOV protein crystals grew under continuous light [1,24], all other so-called "light state" structural data were obtained from crystals grown in dark that were subsequently exposed to light. In the presented work, we use the term "fully light-adapted" to differentiate these from the light-exposed "illuminated" crystal structures. Additionally, extensively studied LOV protein structures are either truncated LOV domains from full-length multidomain proteins or they consist of only one of the (N- or C-terminal) structural elements. We previously published the fully light-adapted crystal structure of the short LOV protein PpSB1-LOV from *Pseudomonas putida* that shows an unusually slow dark recovery ($\tau_{REC} \approx 40$ h at 20 °C) [1]. This short LOV protein PpSB1-LOV consists of a conserved LOV core domain, an N-terminal A′α helix, and a C-terminal Jα helix. The structure reveals a novel dimer interface that is mediated by all three structural domains, that is, the LOV core domain and the N- and C-terminal auxiliary elements A′α and Jα helices, respectively. In this study, we report the crystal structure of the PpSB1-LOV dimer in the dark state that shows marked conformational differences compared to its light state. Furthermore, we present NMR results on dark and light states of PpSB1-LOV in solution that are consistent with the crystallography data. The results presented here suggest a potentially general, molecular mechanism for blue-light-controlled activation of bacterial, short LOV proteins and other structurally related LOV photoreceptors, such as dimeric LOV histidine kinases and YtvA-like proteins [18,25].

Results

Crystal structure of the PpSB1-LOV protein in the dark state shows remarkable structural differences from that of the light state

Overall structure

The crystal structure of PpSB1-LOV in the dark state was determined at a resolution of 2.5 Å (Fig. 1a) using molecular replacement as described in the methods section. Details on data collection and refinement statistics are provided in Table 1. The protein crystallized in space group C2 with four molecules forming two dimers per asymmetric unit. The final model consists of residues 1–134 and one FMN chromophore per protein chain (Fig. 1a).

The overall fold of PpSB1-LOV in the dark state is similar to that of the previously published fully light-adapted state with the typical α/β-PAS fold (Fig. 1b) [1]. Briefly, the structure consists of a LOV core domain (amino acids 17–118) and two not conserved structural elements, an N-terminal helix A′α (4–13) and a C-terminal helix Jα (120–134). The residues located on the helices Eα, Fα, and the β-scaffold form the chromophore binding cavity. However, superposition of equivalent monomers from dark and light states shows a significant movement in the C-terminal Jα helix, the N-terminal A′α helix, and the A′α–Aβ, Eα–Fα loop regions (Fig. 1b). In contrast to the single conformation of Jα helix revealed in the light-adapted state, it shows four different conformations in the dark state. The Jα movement varies in the four protein chains present in the asymmetric unit, whereby residues in the C-terminal Jα helices are surrounded by solvent and are not involved in crystal contacts (Supplementary Fig. S1). The maximum distance measured between the residue A134 Cα positions in Jα in the two states is ~11.8 Å in chain B (Fig. 2). In taking V119 as the fixed point that is the first residue on Jα helix, we measured an angle of 29.6° between the C-terminal residues A134 in the two states. Additionally, the Hβ–Iβ loop shows a ~4 Å shift toward the N-cap helix A′α as measured between the Cα atoms of F106. Other differences observed correspond to the loop regions A′α–Aβ and Eα–Fα. The A′α–Aβ loop is positioned in the dimer interface (described below) and is one residue longer compared to the light state. The Eα–Fα loop in the dark state is solvent exposed with a slightly different conformation moving away from the phosphate moiety of the chromophore. Interestingly, R61 located on the Eα–Fα loop points toward the solvent and is not involved in hydrogen bonding with the phosphate group (Fig. 3). In the light state [1], R61 is a member of a unique cluster of four arginine residues that are involved in a tight coordination with the FMN phosphate moiety (Fig. 3).

Dimer interface

The light-state crystal structure of PpSB1-LOV revealed a novel dimer interface that is mediated by N- and C-terminal auxiliary structural elements [1]. The interactions are largely hydrophobic in nature, involving residues from helices A′α, Jα, and the central part of the LOV core domain. The overall