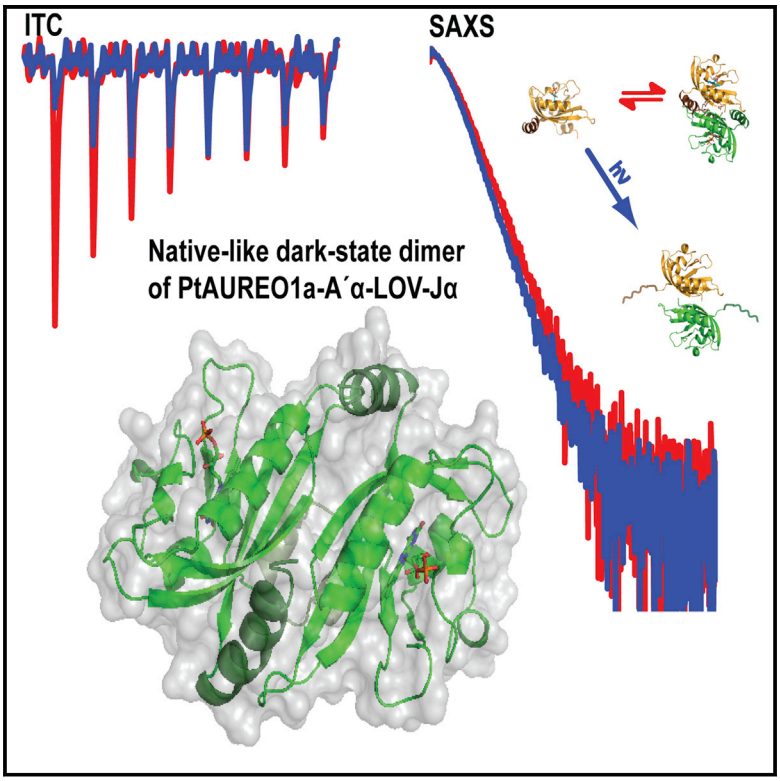
Short Article

Structure

Structure of a Native-like Aureochrome 1a LOV Domain Dimer from *Phaeodactylum tricornutum*

Graphical Abstract



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In Brief

In the present manuscript Banerjee et al. analyzed the dark-adapted crystal structure of the aureochrome sensory LOV domain including its two flanking helices, A′α and Jα. The topology of the dark-state dimer is native-like in the context of functional aureochrome and undergoes light-dependent conformational changes toward the N terminus upon illumination.

Highlights

- Crystal structure of native-like, Aureochrome1a LOV domain dark-state dimer
- Dark-state dimer undergoes conformational changes upon lit-state dimerization
- Structural rearrangements transmit from FMN to the flanking Jα and A′α helices
- The paralogous *Pt*AUREO2 lacks FMN chromophore because of steric hindrance

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Structure of a Native-like Aureochrome 1a LOV Domain Dimer from *Phaeodactylum tricornutum*

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SUMMARY

Light-oxygen-voltage (LOV) domains absorb blue light for mediating various biological responses in all three domains of life. Aureochromes from stramenopile algae represent a subfamily of photoreceptors that differs by its inversed topology with a C-terminal LOV sensor and an N-terminal effector (basic region leucine zipper, bZIP) domain. We crystallized the LOV domain including its flanking helices, A′α and Jα, of aureochrome 1a from *Phaeodactylum tricornutum* in the dark state and solved the structure at 2.8 Å resolution. Both flanking helices contribute to the interface of the native-like dimer. Small-angle X-ray scattering shows light-induced conformational changes limited to the dimeric envelope as well as increased flexibility in the lit state for the flanking helices. These rearrangements are considered to be crucial for the formation of the light-activated dimer. Finally, the LOV domain of the class 2 aureochrome *Pt*AUREO2 was shown to lack a chromophore because of steric hindrance caused by M301.

INTRODUCTION

Light is indispensable for a plethora of biological processes in all kingdoms of life. For light-dependent regulation several photoreceptor families have evolved, among them the major class of Per-ARNT-Sim (PAS) domain containing photoreceptors. Here, the light-oxygen-voltage (LOV) domains represent a prominent subset, whose members control responses in plants such as phototropism, chloroplast relocation, or stomata opening (Christie, 2007; Demarsy and Fankhauser, 2009), regulate the circadian clock and carotenoid synthesis in fungi (Froehlich et al., 2002; He et al., 2002), and contribute to diverse responses in prokaryotes (Herrou and Crosson, 2011).

Sensory module LOV domains absorb blue light signals by a non-covalently bound flavin chromophore, which reversibly forms a C4(a) photoadduct ($\lambda_{max} \sim 390$ nm) between its isoalloxazine ring and a conserved cysteine residue. Depending on the dark-reversion kinetics, the active state that is assigned as the lit 390-nm state can last from seconds to days (Losi and Gärtner, 2011). Besides the conserved PAS fold, the flanking helices, A′α and Jα (Halavaty and Moffat, 2007; Harper et al., 2003), serve either in dimer interface stabilization or in signal transduction to the effector domains, for example, to a Ser/Thr-kinase (Aihara et al., 2012; Harper et al., 2004).

Unlike most other photoreceptors, where the photosensory domain or module precedes a C-terminal effector domain, aureochromes have an inversed effector-sensor topology (Figure 1A). Aureochromes were first identified in the stramenopile alga *Vaucheria frigida* (Takahashi et al., 2007). In both of its paralogs, *Vf*AUREO1 and *Vf*AUREO2, a long N-terminal extension of unknown function is followed by an S-type or D-type bZIP effector domain that binds specifically to the TGACGT motif in vitro and, finally, a C-terminal sensory LOV domain. Based on phylogenetic analyses, aureochromes can be further subdivided into up to four distinct classes (Schellenberger Costa et al., 2013). The genome of the diatom *Phaeodactylum tricornutum* comprises three aureochrome orthologs of class 1 (*Pt*AUREO1a, 1b, 1c) and one ortholog of class 2, *Pt*AUREO2. Whereas aureochromes apparently control the photomorphogenesis of multinucleate *V. frigida* algae (Takahashi et al., 2007), they play major roles for the photoacclimation and cell division of the unicellular *P. tricornutum* (Huysman et al., 2013; Schellenberger Costa et al., 2013).

Aureochromes were shown to function in a dimeric state similar to their fungal counterpart, the photoreceptor Vivid. In Vivid, the dimerizing N-cap is partly occluded by the dark-state LOV domain and exposed upon the latter's photoconversion (Vaidya et al., 2011). Interestingly, for *Vf*AUREO1, the dimerization tendency of the full-length protein or the bZIP-LOV module depends crucially on the redox potential of the environment because of cysteine residues in the bZIP and linker region (Hisatomi et al., 2014). Under reducing conditions, light-induced dimerization is possibly driven by the LOV domains. Furthermore, the crystal structure of the *Vf*AUREO1 LOV domain revealed surprisingly an antiparallel dimer in the dark state (Mitra et al., 2012), whose arrangement is contradictory to recent reports, according to which dimerization of the bZIP domains is enhanced by dimerizing LOV domains in the lit state (Nakatani and Hisatomi, 2015). Finally, Fourier transform infrared spectroscopy (FTIR) studies of the photosensory module of *Pt*AUREO1a showed that upon light illumination both the helices flanking the LOV dimer, namely the N-terminal A′α and the C-terminal Jα helix, unfold leading to the adoption of the active dimeric state (Herman et al., 2013; Herman and Kottke, 2015).

To this end, we solved the crystal structure of the *Pt*AUREO1a A′α-LOV-Jα module, which adopts a physiologically relevant native-like dimeric arrangement. Both of the flanking A′α and Jα helices play a crucial role in dark-state dimerization and the A′α helices are suitably positioned for being continued to the