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Synonyms

LOV (light, oxygen, voltage) domains: blue-light sensing units of ca. 110 aa.

LOV_{450} = dark-adapted state of a LOV domain

LOV_{390} = FMN(C4a)-Cys adduct, the product of LOV domain photochemistry

Riboflavin = 7,8-dimethyl-10-(1-deoxy-d-ribitol-1-yl)isoalloxazine

FMN = flavin mononucleotide = riboflavin 5'-phosphate

FAD = flavin adenine dinucleotide

Introduction

LOV proteins with UVA/Blue light (BL) sensitivity are photoreceptors employing LOV (light, oxygen, voltage) domains as photosensory modules linked to a large variety of effector functions in plants, fungi, bacteria and archaea (Glantz 2016; Losi 2017). LOV domains belong to the PAS (PerArntSim) superfamily (Gu 2000), and present a characteristic α/β fold where the secondary structure elements are named Aβ,Bβ,Cα,Dα,Eα,Fα,Gβ,Hβ,Iβ, starting from the N-terminus (Zoltowski 2011). They bear a fully oxidized riboflavin, flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD) as chromophore (152), noncovalently bound in the dark adapted state of the protein. Upon BL photoexcitation, the chromophore forms a C4a adduct with a Cys residue, accompanied by N5 protonation, in most cases via the sub-μs-to-short μs decay of the flavin triplet excited state (Figure 1a) (Losi 2017; Kerruth 2017). The dark adapted state and the photoadduct are referred to as LOV_{450} and LOV_{390} from their absorption maxima in the UVA-visible range. LOV_{450} has a characteristic bright green fluorescence, with quantum yield $\Phi_F \approx 0.2$, that is completely lost in the adduct (Figure 1b) (Losi 2007). LOV_{390} thermally decays back to LOV_{450} with recovery lifetimes τ_{rec} between few seconds and many hours,

depending on the diverse LOV proteins (Conrad 2014; El-Arab 2015; Pudasaini 2017). UVA/Violet light drives the system into a photoequilibrium, configuring LOV domains as photochromic systems (Kennis 2004; Losi 2013; Song 2013; Chang 2017).

Addition of a proton to flavin N5 in LOV_{390} is the key element inducing hydrogen bonds (HB) rearrangements within the active site, thereby promoting a 180° side chain rotation of a conserved glutamine residue on strand Iβ (Figure 2)(Losi 2017). This "flipping" is the first step of the light-to-signal-transduction chain in LOV proteins, inducing successive structural changes in the extended β-scaffold and then in α-helix regions flanking the LOV-core (Losi 2017; Ganguly 2017; Petersen 2017). These structural changes subsequently lead to functional activation of partner domains or proteins via diverse mechanisms (Figure 3).

Given their photobiophysical properties, LOV domains have become valuable and flexible genetically-encoded tools for a variety of advanced applications (Figure 4), in particular they can be engineered as: **i**. fluorescent reporters for cell microscopy (Wingen 2014; Buckley 2015); **ii**. singlet oxygen generators (Souslova 2017); **iii**. light-control of cellular signaling (optogenetics) in fusion proteins (Repina 2017; Liu 2017); **iv**. fluorescent-based biosensors for heavy metals (Ravikumar 2015); v. donors in Förster resonance energy transfer (FRET) pairs (Simon 2017) , also for visualizing intracellular changes in oxygen levels (Potzkei 2012) and pH (Rupprecht 2017) (174).

Light activation of LOV domains

The photocycle dynamics

The photochemical events of LOV domains were first characterized in the plant photoreceptor phototropin, a light activated kinase that bears two photoreceptor domains in tandem, LOV1 and LOV2 (Salomon 2000) (Briggs 2006). The basic features of LOV photophysics and photochemistry have later been studied in several proteins of eukaryotic and prokaryotic origin and are well known (Figure 1). In the following we will refer to FMN for brevity, having in mind that the chromophore of many fungal LOV proteins is FAD, e.g. in *Neurospora crassa* Vivid (*Nc*VVD)(Dasgupta 2016). Photoexcitation of LOV_{450} results in sub-ns formation of the FMN triplet state (^{T}FMN) with quantum yield Φ_T varying from 0.4 to 0.8 (Losi 2007). ^{T}FMN, absorbing in the red spectral region (maximum ca. 660 nm) decays within few μs into the photoproduct LOV_{390}, possibly preceded by formation of a transient $FMNH^{\bullet}$-$H_2CS^{\bullet}$ radical pair (Magerl 2017). According to this model radical recombination is coupled to protonation of N5 and formation of a FMN-C4a-Cys covalent bond in LOV_{390}. Quantum yield for LOV_{390} formation (Φ_{390}) lies between

0.3 and 0.6 with BL excitation, but is considerably lower for UVA activation (Losi 2017). Formation of LOV_{390} leads to dramatic spectral changes due to 2-electron reduction of the isoalloxazine ring with loss of double-bond conjugation and chromophore distortion: blue-shift of absorption maximum with loss of vibrational bands is accompanied by complete disappearance of fluorescence (Figure 1b). The photoproduct itself can be photoexcited with UVA/Violet light, thus by-passing thermal recovery and generating a photoequilibrium. LOV domains are thus photochromic systems, with LOV_{390} undergoing light-induced, sub-ns bond cleavage that promotes formation of LOV_{450}, with low efficiency (Φ_{450} = 0.05-01) (Kennis 2004; Losi 2013; Song 2013; Chang 2017).

An actively investigated parameter of LOV photocycle is the thermal recovery dynamics, its detailed mechanism and the underlying energetics. This aspect is highly relevant for optogenetic applications, given that molecular events must be synchronized and fine-tuned among the components of signaling networks (Pudasaini 2015; Shcherbakova 2015). In LOV domains a high activation barrier must be overcome in order to break the covalent bond (usually in the range 80-100 kJ/mol), causing a strong temperature dependence of τ_{rec} (Raffelberg 2011). The thermodynamic driving force for thermal recovery relies on the high energy content of LOV_{390}, ca. 120-160 kJ/mol (Losi 2017). Several experiments suggest that deprotonation of N5 is rate limiting for the recovery reaction (Lokhandwala 2016): **i.** solvent isotope effects indicate that the rate determining reaction is abstraction of a single proton; **ii.** τ_{rec}. can be accelerated by imidazole acting as a base catalyst; **iii.** mutations affecting solvent accessibility to the active site have a large effect on τ_{rec} (Pudasaini 2015); **iv.** alteration of the HB network, formed by polar amino acids with the isoalloxazine ring, has profound effects on τ_{rec}; in particular changing the conserved “flipping” glutamine Q123 in *Bacillus subtilis* YtvA (*Bs*YtvA) into asparagine accelerates the dark state recovery about 85-fold (Raffelberg 2011).

The detailed role of water has been investigated recently in plant, fungal and bacterial LOV proteins (Pennacchietti 2014; Zayner 2014; El-Arab 2015; Lokhandwala 2016). These studies, summarized in (Losi 2017), suggest that water might exert opposite effects on τ_{rec}, due to its a catalytic activity on covalent bond splitting and to water-assisted conformational changes in LOV_{390}. Water ingress in the active site seems to be tightly regulated by residues that act as “solvent gates”. As an example, in *Trichoderma reesei* ENV1 experimental work and molecular dynamics (MD) simulations show that Thr101 stabilizes ordered solvent molecules near N5, lowers the energetics for ingress of further water molecules near the flavin and facilitates their dynamic movement, indirectly assisting lateral chain and movement of the “flipping” Q204 on strand Iβ (Lokhandwala 2016).

Signal transmission in LOV proteins

The most spectacular feature of LOV photoreceptors is their intrinsic modular structure: the light sensing domain is linked to a variety of effector and regulator modules that ultimately determine the functionality of the protein itself. Typical associated functions are represented by kinases, phosphatases, second messenger regulators, and DNA-binding motifs (Glantz 2016; Losi 2017). Quite a large number of bacterial LOV domains are stand-alone units, where the LOV core is flanked at the N- and C-terminal ends by helical regions of variable length. In order to acquire a function for these proteins, one has to assume that short-LOV proteins form transient complexes with effector domains or -proteins. Recently, a large scale bioinformatics analysis has identified more than 6700 LOV proteins in the three life domains, some of them with previously undetected functions, e.g. GTP cyclohydrolases, lipases (in plants) and LOV-RGS domains (RGS = regulators of G protein signaling), found so far only in fungi and protists (Glantz 2016). Connectivity networks well evidence the diversity of fused domains, their preferential position (N- or C terminal to the LOV domains) and the topological clusters in multi-effector LOV proteins.

One key question related to modularity is to what extent the modalities of light-triggered signal propagation and signal transmission to partners are conserved in different LOV proteins. Recent studies indicate that the five-stranded β-scaffold (Aβ,Bβ, Gβ,Hβ,Iβ) plus the helical regions flanking the LOV-core at its N-terminal (A'α) and C-terminal (Jα, or J-linker) ends, constitute the photoswitch of LOV proteins. As mentioned above the key event that transmits light-induced conformational changes at the LOV protein surface, is the 180° flipping of a conserved glutamine on strand Iβ (e.g. (e.g. Q123 in *Bs*YtvA, Q182 in *Nc*VVD, Q513 in *Avena sativa* phototropin 1 LOV2 (*As*phot1-LOV2)); this has been evidenced by structural data, mutagenesis experiments and MD simulations (Losi 2017; Ganguly 2017). Signal propagation then proceeds basically via four main mechanisms that all involve the β-scaffold and, to different extent and modalities, Jα and/or A'α: **i.** light-promoted undocking of Jα and activation of the effector domain; this mechanism occurs in *As*phot1-LOV2; in the dark, Jα is mostly docked on the β-scaffold, also interacting with A'α. Light activation shifts the equilibrium to the undocked state, and enhances activity of the fused Ser/Ther Kinase. Detachment of Jα occurred within a 1 ms and A'α participates to the process (Freddolino 2013; Takeda 2013). The mutation R472H within A'α uncouples the effector domain from LOV2 photochemistry, resulting in a constitutively active kinase activity (Petersen 2017); **ii.** light-promoted undocking of A'α and dimerization engaging the LOV β-scaffold; this occurs in *Nc*VVD and is the basis of biological activity

(Ganguly 2017); **iii.** The torque mechanism postulated for*Bs*YtvA, based on structural information from a chimeric protein, YF1, where *Bs*YtvA-LOV is fused to a heterologous kinase; in the dimer, Jα adopts a coiled-coil conformation, and the two helices tilt outwards (Engelhard 2017) within 70 μs after light activation of the LOV domain (Choi 2016); **iv.** domain undocking and functional dimerization, described for *Erythrobacter litoralis* EL222. EL222 bears a HTH motif and is able to bind to target DNA only after BL-induced dimerization (Zoltowski 2013). In the dark-adapted state of EL222, however, a helix involved in HTH dimerization is sequestered into interactions with the β-scaffold of the LOV core; the latter also competes for the same region of HTH devoted to DNA-binding (Nash 2011) and explains the control and the mode of activation.

Biophysical applications

Fluorescence

The relatively high Φ_F of LOV domains allows applications such as fluorescence-based cellular studies (Guex 1997). Proteins for these applications are collectively called Flavin mononucleotide (FMN)-binding fluorescent proteins (FbFPs) (174), they have been first introduced as fluorescent reporters of choice for anaerobic or microaerobic environments (Drepper 2007); this aspect still represents their main advantage over GFP (Green Fluorescent Proteins) and structurally related FPs, where chromophore formation implies post-translation maturation and is oxygen-dependent. Given that in LOV domains fluorescence is lost upon formation of LOV_{390}, this reaction can be annihilated by mutating the reactive cysteine into alanine or glycine, thus yielding a permanently fluorescent molecule with Φ_F presently comprised between 0.13 and 0.51 (Wingen 2014, 2017). FbFPs also form the basis for the genetically encoded photosensitizers for singlet oxygen (168) miniSOG (mini singlet oxygen generator) and related proteins (Souslova 2017). In the last decade FbFPs have been exploited for diverse applications, lately also as tools for Correlative Light Electron Microscopy (CLEM), as sensors for heavy metals, donors in FRET pairs for oxygen and pH sensing, as singlet oxygen generators for biotechnological applications, and as heavy metal sensors. Given their photochromism, LOV domains can also be used as tools super-resolution microscopy (nanoscopy) studies, based on the stochastic photoactivation of single molecules (Losi 2013), like in Photoactivated Localization Microscopy (PALM) (428). FbFPs are also used as biosensors for heavy metals (Ravikumar 2015) and as donors in FRET pairs for visualizing intracellular changes in oxygen levels (Potzkei 2012) and pH (Rupprecht 2017), with the advantage of real time recording (174).

Optogenetics

LOV domains are also employed as optogenetics tools (Endo 2017). They are part of the emerging and ever-increasing photoreceptors that allow manipulation of diverse molecular and cellular processes by light, including signal transduction, gene expression, cell migration, and protein and organelle trafficking. Genetically encoded light-actuators allow for fast and reversible control and localization of transient cellular events, thus enabling studies of dynamic cellular processes with high spatiotemporal precision (Liu 2017). Given the modularity of LOV proteins, a number of light-regulated functions are at hand and are being intensively studied, such as turnover of second messengers, kinase and phosphatase activities, and gene activation (Shcherbakova 2015). LOV-based optogenetic systems exploit basically the same mechanisms (illustrated in figure 3) for signal transmission as native LOV proteins, namely light-induced undocking of surfaces to activate enzymatic activities or promote dimerization and the tilting/rotation.One has to keep in mind, however, that these systems are intrinsically noisy, in that fused effector domains can be partially active also in the dark, so they need extensive design of optimized variants (Pudasaini 2015; Ziegler 2015).

Cross-References

→ (152) Flavins
→ (428) Photoactivated Localization Microscopy (PALM)
→ (174)Flavin Mononucleotide-Binding Fluorescent Proteins
→ (168)Reactive Oxygen Species

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Figure captions

Figure 1: a. photocycle of LOV domains; **b.** spectral properties of a LOV protein (here the *Bs*YtvA); absorption spectra of LOV_{450} (dark line), of LOV_{390} (magenta) and of the protein in the photoeuilibrium regime under violet light illumination (blue line); fluorescence spectra of the same states are represented in green, red and violet respectively. Notice the very low recovery of fluorescence under photoequilibrium regime.

Figure 2. a. Topology of a LOV domain. **b.** β-strands are represented as yellow arrows, helices of the LOV-core as magenta cylinders; the helical regions flanking the LOV core are colored in cyan; **b.** structure of a the LOV domain of *Bs*YtvA in the dark adapted state. The “flipping” glutsmine Q123 on strand Iβ is shwon together with the FMN chromophore (Protein Databank entry 2pr5).

Figure 3: signal propagation mechanisms in LOV proteins. **a.** in *As*phot1-LOV2 , light relieves inhibition of the kinase activity by promoting undocking Jα, mostly clamped at the β-scaffold of LOV2 in the dark; **b.** in the *Nc*VVD, light promotes dimerization by partial undocking of A’α; **c.** in EL222, dimerization of HTH is blocked in the dark by direct contact with LOV. Light promotes dimerization by weakening this contact, allowing HTH binding to target DNA; **d**. In the chimeric protein YF1 a torqueing of the Jα helices in the dimer induces deactivation of the linked kinase domain.