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The LOV2 Domain of Phototropin: A Reversible Photochromic Switch

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Light, oxygen, or voltage (LOV) domains constitute a new class of chromoprotein modules.[1] They form the blue-light-sensitive loci of the phototropins, a recently discovered class of plant photoreceptors that regulate a variety of responses.[2] LOV domains consist of approximately 100 amino acids and noncovalently bind a single flavin.[3,4] Blue-light absorption initiates a photochemical reaction which results in the formation of a covalent adduct between a conserved cysteine and the flavin.[5,6] It is believed that this species, referred to as S_{390} given its absorption band in the near-UV, corresponds to the signaling state of the protein. The lifetime of the adduct in various LOV domains ranges from minutes to hours,[5,7−9] which implies that even under physiological illumination, there is a high probability for absorption of a second, near-UV photon. The resulting photochemistry in the LOV domain may have important consequences for its signaling function. For this reason, we have undertaken a time-resolved study of the molecular events that follow photolysis of S_{390} in the LOV2 domain from the phy3 receptor of *Adiantum*.

LOV2 was expressed and purified and transient absorption spectroscopy was carried out as previously described.[4,10] Continuous blue-light background illumination was applied to photoaccumulate S_{390}, resulting in a steady-state S_{390} population of about 85%. The remaining 15% can be assumed in the dark ground state D_{447}, because the other photocycle intermediate, the FMN triplet, has a lifetime of only 2 μs[5] and will have a negligible concentration at steady state. The photoaccumulated sample was photolyzed with flashes of 100 fs duration at 400 nm, and the absorption changes were probed with a flash of white light at time delays ranging from −2 ps to 4.5 ns. To determine the dynamics of D_{447}, we performed an experiment without background illumination but otherwise identical conditions. The resulting spectra were weighted and subtracted from the illuminated dataset. The resulting time-resolved spectra were subjected to a global analysis program[11] using a kinetic model consisting of sequentially interconverting species, that is, 1 → 2 → 3 → ..., in which the arrows indicate successive monoexponential decays of increasing time constants. Associated with each species is a lifetime and a difference spectrum, denoted the species-associated difference spectrum (SADS). The results are shown in Figure 1. Four kinetic components are required to describe the data, with time constants of 500 fs, 9 ps, and 100 ps and a nondecaying component. The initially created excited species has a lifetime of 500 fs. The first SADS (thin solid line) representing this species shows a negative signal near 430 nm, which can be assigned to a combination of ground-state bleaching of the adduct and stimulated emission from the excited to the ground state. At wavelengths longer than 450 nm, it features an intense absorption with a maximum at 605 nm. We assign this SADS to the singlet excited state of S_{390}. This species evolves to the next species in 500 fs, which exhibits a SADS (dashed line) in which the absorption at 605 nm is largely gone. This species has a lifetime of 9 ps and shows an absorption maximum near 480 nm, a shoulder at 530 nm, and a broad absorption tailing toward the red. The negative signal near 430 nm has vanished, indicating the disappearance of stimulated emission. This implies that the singlet excited-state lifetime of S_{390} is very short, 500 fs, which agrees with the previous observation that S_{390} is essentially nonfluorescent.[7,8] It moreover indicates that the second SADS represents a photoproduct involving a ground-state flavin molecular species. This photoproduct evolves into the next species in 9 ps, characterized by the third SADS (dash−dotted line). This SADS has a lifetime of 100 ps and resembles the previous SADS but with a decreased intensity overall. The final, nondecaying SADS has absorption maxima at 475 and 450 nm and is closely similar to the absorption spectrum of the dark ground state D_{447} of LOV2, safe from a low, flat, and reproducible absorption at long wavelengths. For comparison, we have plotted the D_{447} minus S_{390} difference spectrum (dotted line). We conclude that the LOV2 dark ground state is rapidly regained after photolysis of S_{390}, with a time constant of 100 ps.

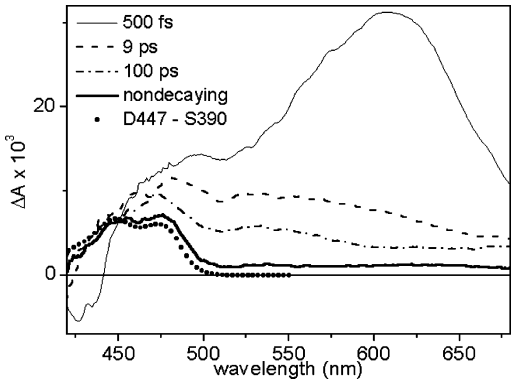

Figure 1. Species-associated difference spectra (SADS) and their associated lifetimes that follow from a global analysis of time-resolved experiments on the S_{390} state of *Adiantum* LOV2, with excitation at 400 nm. The first SADS was scaled down by a factor of 2 with respect to the other SADS. The dotted line denotes the $D_{447} - S_{390}$ difference spectrum.

An estimate for the quantum yield of this process, Φ_B, follows by comparing the magnitude of the signals in the time-resolved experiments with and without background illumination. We found that the absorption signal of newly formed D_{447} at 447 nm in background illuminated conditions (the fourth SADS in Figure 1) amounted to ∼20−25% of the ground-state bleaching at 447 nm of D_{447} without background illumination at 2 ps delay (not shown).

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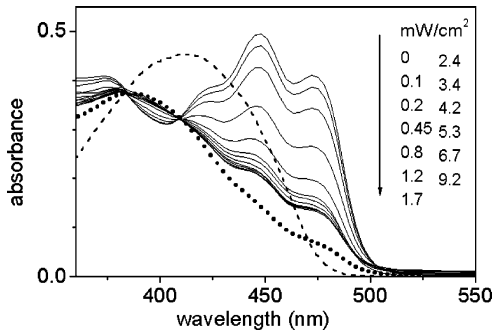


Figure 2. Solid lines: absorption spectra of *Adiantum* phy3 LOV2, with, from top to bottom, progressively increasing illumination power in the near-UV at the powers indicated. Dots: absorption spectrum of LOV2 with saturating blue-light excitation. Dashed line: spectral profile of the near-UV excitation light.

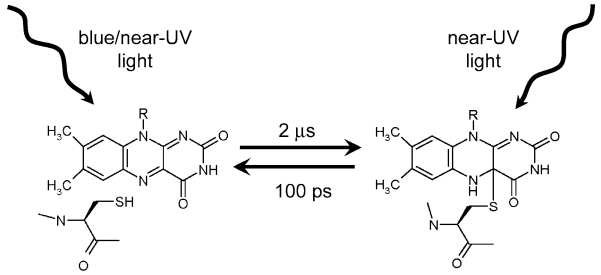
Given that D_{447} and S_{390} have nearly identical absorption at 400 nm, this gives a value of Φ_B between 0.2 and 0.25.

The chemical nature of the intermediate states on the reaction path toward D_{447}, represented by the SADS with 9 and 100 ps lifetime, remains unclear. The long wavelength bands near 530 nm resemble those associated with a charge-transfer complex between an oxidized flavin and a cysteine thiolate anion.[12] A reaction mechanism could then be invoked in which light-driven bond rupture and electron transfer from flavin to cysteine occurs in 500 fs, after which the resulting charge-transfer complex relaxes in multiple steps of 9 and 100 ps to D_{447}.

We have conducted a light-intensity-dependent photoconversion experiment on LOV2 with near-UV excitation for which the spectral profile is shown in Figure 2 (dashed line). The sample was illuminated for 3 min at a given power, and the absorption spectrum was rapidly taken. As shown in Figure 2, the D_{447} absorption at 475 and 447 nm (solid lines) rapidly drops at increasing light intensities, but a fraction of dark ground-state absorption D_{447} of about 28% remains, even after irradiation with the highest powers. A similar observation was made in the LOV1 domain of *Chlamydomonas*.[9] In contrast, saturating blue-light illumination centered at 475 nm converts almost the entire sample to S_{390} (dots). These results can be explained by our finding that there is a near-UV light-driven adduct rupture in LOV2: the broad near-UV illumination drives both formation and rupture of the covalent bond. This observation provides an independent determination of the quantum yield Φ_B. In the high photon flux regime, that is, when the photon absorption rate is much larger than the natural decay rate of S_{390}, the concentration ratio of LOV2 domains in the S_{390} and D_{447} states, C_S/C_D, is determined by the relative number of absorbed photons by D_{447}, N_D, and S_{390}, N_S, and the light-driven forward yield Φ_F and backward yield Φ_B, according to $C_S/C_D = (N_D \times \Phi_F)/(N_S \times \Phi_B)$. Given a N_D/N_S of 1.7 (estimated from the spectral overlap of the excitation light with the absorption of D_{447} and S_{390}) and $C_S/C_D = 2.5$ in saturating conditions (Figure 2), this implies $\Phi_B = 0.68 \times \Phi_F$. Φ_F in LOV2 has been estimated between 0.3 and 0.44,[5,7,10] implying that Φ_B ranges between 0.2 and 0.3, in good agreement with the estimate from our ultrafast experiments.

In conclusion, we find that, upon absorption of near-UV light by the LOV2 S_{390} state, the covalent bond between the flavin and the conserved cysteine is broken and the blue-light-sensitive ground-state D_{447} is regenerated on an ultrafast time scale of 100 ps. Thus, LOV2 is a reversible photochromic switch, which can be "turned on" by blue/near-UV light, and "turned off" by near-UV light, as schematically shown in Scheme 1. Strikingly, light-driven bond rupture proceeds at a rate 10^4 times faster than light-driven adduct formation.

Scheme 1. Key Features of the Light-Driven Reactions in LOV2



It is not clear if the photochromic properties of LOV2 play a physiological role in the phototropin photoreceptor. However, we note that log fluence−response curves of phototropic bending in oat coleoptiles indicate an increased sensitivity to near-UV light at increasing light intensities.[13] It is interesting to compare the properties of LOV2 to those of the phytochromes, the plant photoreceptors that can toggle between different functional states via absorption of red or far-red photons. The color vision provided in this way plays an important role in shade avoidance responses.[14]

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Supporting Information Available: Selected time-resolved difference spectra and kinetic traces represented by the SADS in Figure 1 (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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