The LOV2 Domain of Phototropin: A Reversible Photochromic Switch

John T. M. Kennis,*† Ivo H. M. van Stokkum,† Sean Crosson,‡ Magdalena Gauden,† Keith Moffat,‡§ and Rienk van Grondelle†

Department of Biophysics, Faculty of Sciences, Vrije Universiteit, 1081HV Amsterdam, The Netherlands, and Department of Biochemistry and Molecular Biology, and Institute for Biophysical Dynamics, University of Chicago, Chicago, Illinois

Received December 19, 2003; E-mail: j.kennis@few.vu.nl

Light, oxygen, or voltage (LOV) domains constitute a new class of chromoprotein modules. They form the blue-light-sensitive loci of the phototropins, a recently discovered class of plant photoreceptors that regulate a variety of responses. LOV domains consist of approximately 100 amino acids and noncovalently bind a single flavin. Blue-light absorption initiates a photochemical reaction which results in the formation of a covalent adduct between a conserved cysteine and the flavin. It is believed that this species, referred to as S390 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, 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 S390 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 S390, resulting in a steady-state S390 population of about 85%. The remaining 15% can be assumed to be in the dark ground state D447, because the other photocycle intermediate, the FMN triplet, has a lifetime of only 2 µs 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 D447, 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 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) represents 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. The second SADS has a lifetime of 9 ps and resembles the previous SADS but with a decreased intensity overall. The final, nondecaying SADS has absorption maxima at 475 and 605 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 S390 is very short, 500 fs, which agrees with the previous observation that S390 is essentially nonfluorescent. It moreover indicates that the second SADS represents a photoproduct involving a ground-state flavin molecular species. This photoprodust 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 D447 of LOV2, safe from a low, flat, and reproducible absorption at long wavelengths. For comparison, we have plotted the D447 minus S390 difference spectrum (dotted line). We conclude that the LOV2 dark ground state is rapidly regained after photolysis of S390, with a time constant of 100 ps.

An estimate for the quantum yield of this process, Φq, 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 D447 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 D447 without background illumination at 2 ps delay (not shown).
Given that D 447 and S 390 have nearly identical absorption at 400 nm, this gives a value of $\Phi_B$ 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. 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. 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 $\Phi_F$. 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, $C_F/C_D$, is determined by the relative number of absorbed photons by D 447, $N_F$, and S 390, $N_S$, and the light-driven forward yield $\Phi_F$ and backward yield $\Phi_B$, according to $C_F/C_D = (N_F \times \Phi_F) / (N_S \times \Phi_B)$. Given a $N_F/N_S$ of 1.7 (estimated from the spectral overlap of the excitation light with the absorption of D 447 and S 390) and $C_F/C_D = 2.5$ in saturating conditions (Figure 2), this implies $\Phi_B = 0.68 \times \Phi_F$. $\Phi_F$ in LOV2 has been estimated between 0.3 and 0.44, implying that $\Phi_B$ 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.

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. 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.

Acknowledgment. The LOV2 construct was generously provided by Winslow Briggs of the Carnegie Institution of Washington. This research was supported by the Netherlands Organization for Scientific Research (NWO-CW and FOM) and by NIH grant AM36452 to K.M.

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.

References