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Stability of Dimer and Domain–Domain Interaction of *Arabidopsis* Phototropin 1 LOV2

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Transient grating signals after photoexcitation of *Arabidopsis* phototropin 1 light–oxygen–voltage 2 (phot1LOV2) domain without the linker were found to be very sensitive to temperature. In particular, the diffusion signal drastically increased with rising temperature. The signal was consistently explained by the superposition of the photo-induced dissociation and association reactions. This observation indicated the presence of an equilibrium between the monomer and dimer forms of the phot1LOV2 domain in the dark. The equilibrium was confirmed by a gel chromatographic technique. The equilibrium constants at various temperatures were calculated from the fraction of the dimer, and the stabilization enthalpy and entropy were determined. Interestingly, the transient grating signal of phot1LOV2 with the linker (phot1LOV2-linker), which exists as the monomer form, was also temperature dependent; the diffusion signal intensity decreased with increasing temperature. Because the diffusion signal reflects a conformation change of the linker upon photoexcitation, this temperature dependence indicated that there were two forms of the phot1LOV2-linker. One form exhibited a conformational change upon photoexcitation whereas the other form showed no change. These two forms are not distinguishable spectroscopically. The fraction of these species depended on the temperature. Considering the monomer–dimer equilibrium of the phot1LOV2 domain, we suggest that the nonreactive form possesses the linker region that is dissociated from the LOV2 domain. Because the dissociation of the linker region from the LOV2 domain is a key step for the conformation change of the phot1LOV2-linker to induce biological activity, we proposed that the phototropins could have a role as a temperature sensor.

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Keywords: reaction; diffusion; transient grating; dimer; interdomain interaction

Introduction

Inter- and intraprotein (domain–domain) interactions play an important role in many signal transduction processes of sensor proteins. Because the interprotein interaction is closely related with oligomerization of the protein, such oligomeric formation is observed in many proteins. For example, the PAS (Per–Arnt–Sim) family is a well-known regulator, and it was found that the oligomerization state is stable for some PAS domains, for example, the dimer of ARNT PAS-B domain and the dimer of heme binding PAS domain *Escherichia coli* Dos (EcDos) and *Rhizobium meliloti* FixL (RmFixL).[1–3] Such oligomer formation is often related with domain–domain interactions, which is an important factor for protein conformation changes. An example of this relationship was clearly observed for the photochemical reactions of the PAS-LOV2 (light–oxygen–voltage 2) domain of the blue-light sensor protein, phototropin.[4–7]

Phototropins (phot1 and phot2) are well-known blue-light receptors[8,9] for phototropism,[10,11] stomatal

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Abbreviations used: LOV, light–oxygen–voltage; phot1LOV2, phototropin 1 LOV2; PAS, Per–Arnt–Sim; TG, transient grating; EDTA, ethylenediaminetetraacetic acid.

opening,[12] and chloroplast relocation.[11,13,14] Phototropins consist of two N-terminal photoreceptive domains LOV1 and LOV2,[15,16] a C-terminal Ser/Thr kinase domain,[17] and a linker domain that connects the LOV2 domain with the kinase domain. Each of the two LOV domains noncovalently binds a single flavin mononucleotide as a chromophore.[18] Upon photoillumination of the chromophore, the LOV domains in the ground state with an absorption maximum at 447 nm (D_{447}) are excited to the excited singlet state. This is followed by the creation of the triplet state possessing a broad absorption spectrum (L_{660}). This process occurs within 30 ns.[19,20] In this triplet state, the thiol group of a conserved cysteine residue covalently binds to the C(4a) of the isoalloxazine ring of flavin mononucleotide to yield a new state having a blue-shifted absorption spectrum (S_{390}) with a time constant of a few microseconds.[19–28] After this change, no further absorption spectral changes are observed. However, some spectrally silent reactions have been reported for phototropin 1 LOV2 (phot1LOV2), phot1LOV2 with a linker (phot1LOV2-linker), and phot2LOV2 with a linker (phot2LOV2-linker). This has been achieved by monitoring changes in the molecular volume and the diffusion coefficient (D) (diffusion-sensitive conformation change) and secondary structural changes detected by Fourier transform infrared spectroscopy in the protein backbone of *Adiantum* neo1 LOV2.[6,7,29,30] One of the interesting observations of the photochemistry is the light-dependent change of the oligomeric state of phot1LOV2. At low concentration (<100 μM), phot1LOV2 exists primarily as a monomer in the dark state at room temperature, whereas upon photoexcitation, the phot1LOV2 domain forms a dimer. The photoinduced dissociation of the dimer to a monomer was observed when the concentration of phot1LOV2 was increased (>100 μM).[29] This feature suggests that phot1LOV2 exists as a mixture of the monomer and the dimer. Phot1LOV2-linker, on the other hand, exists as a monomer even at higher concentration (~300 μM), and the linker region that is bound to the LOV2 domain in the dark state dissociates from the LOV2 domain upon light illumination ($T_{390}{}^{\mathrm{pre}}$).[7] This dissociation time constant (300 μs) is identical with that of the photodissociation reaction of the LOV2 dimer. After the dissociation of the linker, the α-helices of the linker region unfold (the T_{390} state). As such, these results are clear examples that show that changes in inter- and intramolecular interactions are related to each other.

In the present study, we investigated the stability of the oligomeric states of phot1LOV2 by using the temperature dependence of the photochemical reaction. We found that the transient grating (TG) signal above 293 K was primarily explained by the photoinduced dimerization, which indicated that the phot1LOV2 domain exists solely as a monomer at these high temperatures. Below 293 K, the signal should be analyzed by a superposition of the dimerization and dissociation reaction. The fractions of the monomer and dimer were determined from these contributions to the signal. The enthalpy and entropy changes upon dimer formation were determined. Interestingly, we also observed a temperature dependence of the diffusion signal for the phot1LOV2-linker, which should exist as a monomer. The observed signal indicated that there are both reactive and nonreactive phot1LOV2-linker species and the population of photoreactive species decreased with increasing the temperature in the dark state. Because the diffusion signal appeared due to a conformation change of the linker region, the conformation change in the linker region was blocked for the nonreactive species. Considering the dimer–monomer equilibrium of phot1LOV2, we suggest that the linker region of the nonreactive species is dissociated from the LOV2 domain even if there is no irradiation of blue light. This dissociated form does not undergo a diffusion-sensitive conformational change such as the unfolding of helices by photoexcitation. The fraction of this dissociated species increased with increasing temperature.

Results

Equilibrium between monomer and dimer of phot1LOV2

The TG signal after the photoexcitation of phot1LOV2 was analyzed in a previous paper.[29] Here, the TG signal was analyzed at various temperatures to determine the dimer and monomer fractions. Before describing the equilibrium of the dimer and monomer, the essential features of the TG signal are briefly summarized. Figure 1 in Ref. [29] showed the TG signal observed at relatively small q^2, that is, 3.4×10^{10} m^{-2}. Once the thermal grating signal has decayed to the baseline, a growth–decay signal (diffusion peak) appeared. The rise component of the diffusion peak has been found to correspond to the diffusion of the reactant (ground state protein; D_{447}) and the subsequent decay component to that of the product (T_{390}); that is, the product diffuses slower than the reactant ($D_{\mathrm{R}}>D_{\mathrm{P}}$; D_{P}, diffusion coefficients of the product; D_{R}, diffusion coefficients of the reactant) in this time range. The temporal profile was analyzed by the time-dependent D. Here, D decreased from $D_{\mathrm{R}}=9.8\times10^{-11}$ m^2/s to $D_{\mathrm{P}}=8.0\times10^{-11}$ m^2/s with a time constant of 40 ms at a concentration of 50 μM at 293 K. From the concentration dependence of the rate constant of the D change, it was found that the reaction should be analyzed by the following dimerization reaction:

$$2M \xrightarrow{h\nu} M^* + M \rightarrow D^* \qquad \text{(Scheme 1)}$$

where M is the monomer of phot1LOV2, M^* is the photoexcited monomer, and D^* is the dimer that contains one excited M. [In this scheme, D^* might not be a simple sum of M and M^*, but the conformation of the dimer could be altered. The conformation change of the dimer does not cause any change to the following analysis. However, as