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Pump-dump fluorescence spectroscopy for photoactive yellow protein
○Yasu Kanematsu1, Ryouseki Nakamura1, Norio Hamada2, Hideki Ichida1, Fumio Tokunaga2
1JST-CREST, VBL-CASI, Osaka Univ., 2JST-CREST, Dept. of Earth and Space Sci., Osaka Univ.

Photoactive yellow protein (PYP) is one of the model proteins to reveal the mechanism of the photo-induced conformational change that is believed to initiate biological signaling process. After absorbing a photon, PYP enters the photocycle starting from the ultrafast trans-cis isomerization of a p-coumaric acid chromophore. The primary process of the photocycle has been investigated by the femtosecond time-resolved fluorescence spectroscopy. A pump-dump fluorescence spectroscopy is also one of the promising methods to clarify the initial dynamics. A 'pump' pulse creates a wavepacket on the potential surface of an electronic excited state, which is de-excited back onto a ground state by the stimulated emission caused by a 'dump' pulse. The population change in the electronic excited state induced by a dump pulse is observed by a decrease in the stationary fluorescence intensity. In this study, the pump-dump fluorescence spectroscopy was performed for photoactive yellow protein at room temperature. The dump pulse effect on the population in the electronic excited state has been examined in the various combinations of a pump-dump delay, a dump-pulse wavelength, and an observed wavelength. The dynamic behavior of the population in the excited state of PYP was successfully probed by the pump-dump fluorescence spectroscopy. The experimental results will be discussed in comparison with the results obtained by the femtosecond time-resolved fluorescence spectroscopy.

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Enhancement of photoconversion in Photoactive Yellow Protein by infrared laser pulse irradiation
○Suguru Id1, Hideki Ichida2, Ryouseki Nakamura2, Norio Hamada1, Yasuo Kanematsu2, Fumio Tokunaga1,2
1Grad. school of Sci., Dept. of biology, Osaka Univ., 2VBL-CASI Osaka Univ., 3JST-CREST

Photoactive Yellow Protein (PYP) is one of the model molecules to reveal the mechanism of the photocycle leading to the conformational changes related to the function. PYP contains a p-coumaric acid as a chromophore and has the photocycle involving, at least, two intermediate states at room temperature. The initial process of the photocycle is a photosisomerization of p-coumaric acid. We focus on the photocycle of PYP with the conformational changes from an aspect of a structural modulation induced by the resonant excitation of the vibrational modes of PYP. We have investigated the temporal change in the absorption intensity of the PYP dark state (446-nm band) after the irradiation of the trigger pulse under the several conditions of infrared (IR) light pulse irradiation. The wavelength of the IR pulse is tuned to the vibrational modes characterized by the remarkable difference between the final intermediate state (M state) and the dark state. In the case where the time spacing between the trigger and IR pulses is 0 ns, we have clearly observed the decrease of the absorption intensity of the 446-nm band as compared to the result without IR pulse irradiation. Under the other time conditions, the decrease of the absorption intensity was not observed. These results mean that the quantum yield of the M state is increased by the irradiation of the IR pulse to the initial process of the photocycle of PYP. We concluded that a new channel from the dark state to the M state is opened by the initial-process modification by using an IR pulse.

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Kinetics of major conformational change of PYP
○Yuji Hoshihara1, Yasuhide Imamoto1, Mikihito Katoaka1, Fumio Tokunaga2, Yoshiyuki Kimura3, Masahide Terazima1

Photoactive yellow protein (PYP) is a photo-response protein, thought to be responsible for the negative phototaxis in Ectothiorhodospira halophila. Upon photoexcitation, the ground state species Pg is converted into a red-shifted intermediate Pb, which decays on hundreds microseconds into blue shifted intermediate Pβ. This Pβ is converted to Pb on sub-millisecond time scale, and finally it returns to Pg. Some previous reports suggested that the Pβ and Pb species correspond to a proton transferred and structural changes forms, respectively. However, there is no conclusive evidence when structural changes occur, because there has been no useful time resolved experimental technique to monitor the conformational change in time domain. Recently, a new technique that can reveal the relation between the reaction kinetics and the structural changes was proposed; monitoring the time development of the diffusion coefficient (D) by using the transient grating (TG) method. Here, we studied the conformational change of PYP by this method. We have already reported that Pg and Pb have different D and this difference is caused by the conformational change of N-terminal domain. Therefore, if we can observe the dynamics of the D change, this should represent that of the conformational change. Our preliminary analysis of the signal based on the two-state model suggests that the D of PYP starts to change in hundreds microseconds scale; i.e., N-terminal conformation changes by the creation of Pβ. The detailed results and analysis will be presented.

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Equilibrium between monomer and dimer forms of phototropin-LOV2 domain
○Yasuake Nakasone1, Takeshi Itokou1, Daisuke Matsuoka2, Satoru Tokumoto3, Masahide Terazima1
1Graduate school of science, Kyoto University, 2RIAST, Osaka Prefecture University

Phototropin 1 (Phot1) is a blue-light receptor in higher plants. This protein contains two photoresponsive domains, LOV1 and LOV2. Upon blue-light illumination, the LOV domains show a photocycle. By using the transient grating method, we have already reported that the LOV2 domain undergoes the association and dissociation processes between monomeric and dimeric forms. We have also found that the dimer and monomer are in equilibrium and coexist in solution at the dark state. Which form is more stable? Generally, it is difficult to study such spectrally silent dimer distribution in detail. In this study, we investigated the thermodynamic characters of each form by the temperature dependence of the TG signal. This information will be useful for obtaining further insight into the photobiology of this protein. The temporal profiles of the TG signals at high temperatures showed that the association process (dimerization) is the main process in the photocycle. This fact indicates that the conformational form of phot1-LOV2 is the dominant at dark state. On the other hand, interestingly, the dissociation reaction becomes dominant in the TG signal at lower temperatures. These results indicate that the dimeric form of phot1-LOV2 is energetically more stable. At the same time, we can also estimate the activation energy of the photoconversion by the temperature dependence of the rate constant. We will discuss the thermodynamics of the LOV2 domain on the basis of these results.