- 3P244 BLUF タンパク質 TePixD の低温分光解析: 二回の光励起で形成されるセミキノン型フラビン
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Formation of flavin semiquinone by two times photon-absorption in the BLUF-domain protein TePixD
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TePixD of *Thermosynechococcus elongatus* BP-1 is the flavin-binding photoreceptor containing BLUF domain (Sensor of Blue Light Using Flavin). We previously reported the intermediate states trapped at low temperature (J_S and J_H) in the photocycle of the TePixD protein.* These intermediates at low temperature show only red-shifts of the absorption spectrum. On the other hand, transient radical pair (FADH·Tyr8 radicals) has been proposed for the intermediates based on ultra-fast spectroscopy measurement. In this study, we investigated the radical-formation pathways by low temperature spectroscopy. We obtained following results about the reaction pathways of the neutral semiquinone of flavin. (i) The light-minus-dark difference spectrum showed a formation of the broad band at 500 - 650 nm after 40-min illumination at 80 K in addition to the accumulation of J_H . The accumulation rate of the semiquinone was 8-times slower than that of the red-shifted form. (ii) The semiquinone was trapped at 80 K and decayed to the dark-adapted form by dark-annealing at 150 K. On the other hand, J_H was trapped between at 80 and 150 K as previous report. (iii) The illumination at 80 K to the J_H -enriched sample, in which only J_H was accumulated by pre-illumination at 150 K, converted a half of J_H to the semiquinone form. These results indicate the photoreduction of flavin takes place from the red-shifted intermediate J_H at 80 - 150 K in some fraction. *Fukushima et al. Photochem. Photobiol. (83) 112-121

- 3P246 紅色細菌の光合成装置形成における青色光受容 BLUF タンパク質 AppA の Q63L/W104A 変異の影響
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Physiological analysis of Q63L and W104A mutant BLUF protein, AppA, in *Rhodobacter sphaeroides*
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AppA is a flavin-based blue-light photoreceptor controlling photosynthesis gene expression in the purple bacterium *Rhodobacter sphaeroides*. AppA is based on two domains: one is N-terminal flavin-binding domain named BLUF, and the other is C-terminal region where a transcriptional repressor PpsR could associate. Previous spectroscopic analyses have indicated that Gln63 and Trp104 are necessary for light-induced spectral red-shift and beta-sheet structural changes, respectively, in AppA. To get more insight into the role of the amino acids, we biochemically characterized Q63L and W104A mutant proteins, and physiologically analyzed the mutant strains that harbors the Q63L or W104A mutation on its genome. Obtained results indicated that either Q63L or W104A mutation causes loss of sensitivity of AppA to blue-light for controlling its *in vivo* and *in vitro* activity. Together with recent-solved structures of several BLUF proteins, mechanism of photocycle reaction of AppA will be discussed.

- 3P245 アップコンバージョン法による BLUF タンパク質 TePixD の超高速度時間分解蛍光寿命解析
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Time-resolved ultra-fast fluorescence decay analysis of a BLUF protein TePixD by up-conversion
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TePixD is a BLUF (sensor of blue light using FAD) protein of the cyanobacterium *Thermosynechococcus elongates* BP-1. BLUF proteins show 10 - 20 nm red shift of the absorption band of flavin upon the illumination. The fluorescence lifetimes of the TePixD in the dark-adapted and red-shifted forms have been estimated to be 120 ps (by the excitation with a 50 ps diode laser at 405 nm, 200 μW, 1 MHz) and 2.9 ps (with a 150 fs Ti: Sapphire laser pulse at 400 nm, 10 mW, 80 MHz), respectively, detected by a streak camera with the 20 ps time resolution. These results suggest the fast-quenching process in photoconversion. The lifetime of 120 ps in the dark-adapted form suggest it to represent the rate of the primary reaction to produce the red-shifted form. In this study, we measured the lifetime of the fluorescence in the red-shifted form more accurately by the fluorescence up-conversion technique with a time-resolution of 0.2 ps. The fluorescence decay kinetics obtained can be fitted by two exponential-decays with time constants of 1.8 ps and 47.6 ps. The fluorescence, which showed the decay rates faster than those measured by the streak camera method, seems to be mainly emitted from the red-shifted form that was formed during the long illumination at a strong light intensity at a high repetition rate of the laser (Ti: Sapphire, 50 mW). The fast quenching rate of the excitation energy seems to represent the electron transfer rate between flavin and a conserved Tyr. We will discuss the mechanism of the primary photoreaction of TePixD.

- 3P247 BLUF 蛋白質における光誘起分子内・分子間相互作用変化
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The photo-induced change of inter/intra molecular interaction of BLUF protein
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Sensor proteins of blue light using FAD are members of a blue-light receptor family called BLUF protein. The *Escherichia coli* YcgF is a fusion BLUF protein consisting of the FAD-binding hold (BLUF domain) and the EAL domain. Although it is suggested that the BLUF domain absorbs blue light to start photo-reaction and then interacts with the EAL domain to transmit the light signal, there is no experimental observation for supporting this mechanism so far. In order to understand the light sensing mechanism, it is necessary to detect the global reactions of a protein in time-domain, but such change is generally difficult to be detected in time-domain, because such dynamics is frequently spectrally silent. In this study, we investigated photo-induced structural change of the YcgF from the view point of diffusion coefficient change in time-domain by using the transient grating (TG) method. The observed TG signal exhibits two dynamics with time constants of 13 ms and 2 ms. Interestingly, we observed a diffusion change with a rate of 2 ms and this rate constant increased in proportion to the concentration of the protein. This concentration dependence indicates that the time dependent D is caused by the dimerization reaction of YcgF. We also measured the isolated BLUF domain in order to identify which domain contributed to the observed reactions. Interestingly, the TG signal showed a clearly different time dependence from that of the full-length protein. We will discuss the mechanisms of signal transduction on the basis of these results.