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Photoreaction mechanisms of two cyanobacterial BLUF domain proteins studied by low temperature spectroscopy

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The BLUF domain (a sensor of Blue Light Using Flavin) is a blue-light sensory domain that is widely distributed among the genome of purple photosynthetic bacteria, cyanobacteria and unicellular eukaryote *Euglena*. BLUF domain proteins are known to show a 10-20 nm red shift of the absorption spectrum of flavin upon blue light absorption. This photoconversion is observed only in BLUF proteins, but not in other type of sensory flavoproteins. The PixD of *Thermosynechococcus elongatus* BP-1 and *Synechocystis* sp. PCC6803 are the proteins containing one BLUF domain (ORF: TII0078 and Slr1694). PixD of *Synechocystis* regulates the phototaxis of cells. To investigate the mechanisms, we studied the photoreaction of Slr1694 and TII0078 at 5 K. Upon illumination at 5 K, TII0078 protein shows a 5 nm red-shift of the absorption band (I_5 form). Similar intermediate was found in Slr1694 at 5 K showing a smaller shift width about 9 nm (I_9). I_5 (I_9) was converted into the next intermediate J_{11} (J_{14}) that have 11-nm (14-nm) red-shift of absorption band by dark-annealing at 230 K (200 K). Further annealings at 280 K (270 K) caused increase of absorption at 490 nm (495 nm) (F_{490} and F_{495}), which could be resolved by cooling at 5 K. We will discuss the photoconversion mechanisms of PixD protein.

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Photoinduced Electron Transfer in Glucose Oxidase: A Picosecond Time-resolved Ultraviolet Resonance Raman Study

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— Glucose oxidase (GOD) is a flavoprotein with an FAD, which can act as a strong electron acceptor in the photoexcited state. Since aromatic amino acid residues locate close to the FAD, electron transfer (ET) from the amino acid residues to the excited FAD takes place in GOD. Although the change of electronic state of FAD has been extensively studied, there is no report observing the change of the amino acid residues in the photoinduced ET. In this study, we measured picosecond time-resolved ultraviolet resonance Raman (UVR) spectra of the aromatic amino acid residues in GOD to monitor their state change in the photoinduced ET.

— Raman bands of Trp and Tyr residues were observed in UVR spectra excited at 226 nm. At 0 ps after the photoexcitation, intensities of these Raman bands decreased and new Raman bands ascribable to Trp and Tyr cations appeared; therefore, the spectral changes can be explained by the ET between the Trp/Tyr and FAD. As the delay time increased above 8 ps, the magnitude of intensity differences exhibited gradual decrease, which can be ascribed to charge recombination between Trp/Tyr and FAD. At 50-ps delay, there was no spectral change, indicating that the charge recombination is completed. In UVR spectra excited at 240 nm, decrease and subsequent recovery of band intensities of FAD were observed following the photoexcitation. They are ascribable to the formation and decay of FAD anion, respectively. The present data imply that both Trp and Tyr residues act as an electron donor in the photoinduced ET of GOD.

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Light-induced structural changes in the BLUF domain of AppA revealed by Raman spectroscopy

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The flavin-adenine-dinucleotide-binding BLUF domain constitutes a new class of blue-light receptors, and the N-terminal domain of AppA is a representative of this family. A crystal structure of the BLUF domain from AppA suggested that a conserved Gln63 forms a hydrogen bond with the flavin N5 atom. Upon light excitation, this residue is proposed to undergo a ~180 deg rotation that leads to a rearrangement of a hydrogen bonding network. However, crystallographic studies on the other BLUF proteins claimed an opposite orientation for the glutamine residue. In this study, we have revealed the presence of a Gln63-to-N5 hydrogen bond in the dark-state of AppA by a combined approach of mutagenesis, spectroscopy, and quantum chemical calculations. The present finding supports the view that the reorientation of the Gln63 side chain is a key event in the signaling state formation of BLUF proteins. In addition to the dark state, we have also examined the light-induced structural changes in the active site of AppA by resonance Raman spectroscopy.

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Identification of the N-H stretch of Asn1008 by FTIR spectroscopy in the LOV2 domain of *Adiantum* Phytochrome3

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Phototropins are blue-light receptors in plants. They have two photosensory domains named LOV which bind FMN as a chromophore. We have investigated the light-induced structural changes for the LOV2 domain of *Adiantum* phytochrome3 (phy3-LOV2) by use of low-temperature FTIR spectroscopy [1]. We observed bands at 3490 (+)/3479 (-) cm⁻¹ upon formation of the S390 intermediate, which was not deuterated upon the hydration with D₂O [2]. The bands exhibited spectral downshifts by 10 cm⁻¹ for the uniformly ¹⁵N-labeled sample and also for the reconstituted sample of ¹⁴N-FMN/¹⁵N-apoprotein. This result clearly shows that the bands originate from the apoprotein. Specific labeling of amino acids revealed that the origin of the bands was an asparagine(s). Mutational study strongly suggested that the N-H group originates from Asn1008. The frequency shows that the N-H group is free from hydrogen-bonding. The N-H group of Asn1008 side chain forms a hydrogen bond with C(4)=O group of FMN, and thus it is likely that the observed vibration comes from N-H hydrogen which is not involved in the hydrogen bond with FMN. The N-H group band showed downshift in the Q1029L mutant which lacks the hydrogen bond with C(4)=O group of FMN, implying the rearrangement of the environment around Asn1008. The environment and the structural changes of Asn1008 will be discussed.

1. Iwata et al. (2003) Biochemistry 42, 8183.

2. Nozaki et al. (2005) J. Mol. Struct. 735/736, 259.