Optical Microscopy Study on Regulation Mechanism of Photosynthetic Light Harvesting

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We have developed and continuously improved the laser-scanning confocal microscope systems [Shibata et al. BBA (2014); Jana & Shibata Biophys. J. (2020)] for the study of in vivo photosynthesis. The systems have been applied to investigate intracellular rearrangement of the photosynthetic antenna complexes called light-harvesting complexes (LHCs) during the state transitions (ST) [Fujita et al. J. Photo. Photo. B (2018, 2022); Zhang et al. Plant Cell Physiol. (2021); Zhang et al. PNAS (2022)]. ST is a regulation mechanism in oxygenic photosynthetic organisms. The two photosystems (PSs), PSII and PSI, perform the light-driven linear electron transfer starting from the oxidation of water molecules and being completed with the reduction of $NADP^+$. ST maintains the excitation balance between the two PSs under fluctuating sunlight by the physical movements of LHCs.

Recent advancement of the cryo-electron microscopy has increased the structural understanding of the ST. However, it is still elusive whether the LHCs detached from PSII all bind to PSI or partially remain isolated from both of the PSs. The developed system provides the high lateral resolution (0.4 μm) and the ability to detect cryogenic fluorescence spectrum at each pixel, enabling resolutions of the intracellular PSI-rich and PSII-rich domains in individual cells of a model organism *Chlamydomonas* [Fujita et al. J. Photo. Photo. B (2018)]. The information about the PS segregation is crucially important to evaluate the LHC rearrangement during the ST. Our improved system achieved the simultaneous detection of the fluorescence spectrum and lifetime at each pixel. Owing to this advancement, we captured key evidence for the free LHCs isolated from both of the PSs after shifting from the PSI-preferentially excited condition to the PSII-preferentially excited one. The observation revealed that the free LHCs were in the highly quenched state and accumulated in the PSI-rich domains [Fujita et al. J. Photo. Photo. B (2022)]. We also developed a microscope system which acquires both the excitation and emission spectra at each pixel. Observation of *Chlamydomonas* cells by this excitation spectral microscope at room temperature enabled the real-time visualization of the rearrangement of LHCs upon the ST. We surprisingly found that the ST is less active in the region around the pyrenoid, which is a subcellular compartment specialized for the CO_2 fixation [Zhang et al. PNAS (2022)].