2P328 ラインスキャン多光子励起半共焦点蛍光スペクトル顕微鏡の開発と糸状シアノバクテリアアナベナ PCC7120 のチラコイド膜への応用

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A Line-Scan Multiphoton Broad-Fluorescence Spectromicroscope Applied to the Thylakoid Membrane of a Cyanobacterium Anabaena.

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A laser-line-scanning microscope capable of detection of broad fluorescence spectra was constructed. A femtosecond pulse train at 800 nm was illuminated on a line (one lateral axis, denoted as X axis) in a specimen by a resonant scanning mirror oscillating at 7.9 kHz, and multiphoton-induced fluorescence from the linear region was focused on the slit of an imaging polychromator. An electron-multiplying CCD camera was used to resolve fluorescence of different colors at different horizontal pixels and fluorescence of different spatial positions in a specimen at different vertical pixels. The full widths at half maximum of the point-spread function of the system were estimated to be 0.39 - 0.40, 0.33 and 0.56 - 0.59 m for the X (lateral axis along the line-scan), Y (the other lateral axis) and Z axes (the axial direction), respectively, at fluorescence wavelengths between 644 and 690 nm. This microscope was applied to a study of the subcellular fluorescence spectra of thylakoid membranes in a cyanobacterium, Anabaena PCC7120. It was found that the fluorescence intensity ratio between chlorophyll molecules mainly of photosystem II and phycobilin molecules of phycobilisome (chlorophyll/ phycobilin), in the thylakoid membranes, became lower as one probed deeper inside the cells. This was attributable not to position dependence of re-absorption or scattering effects, but to an intrinsic change in the local physiological state of the thylakoid membrane, with the help of a transmission spectral measurement of subcellular domains.

2P329 ホモダイマー型光合成反応中心内での電子伝達系 ヘリオバクテリア反応中心内で機能する電子伝達体の配位構造

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Arrangements of the electron transfer cofactors in the homodimer reaction centerof *Heliobacterium modesticaldum*

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Heliobacteria are strict anaerobic primitive photosynthetic bacteria that have a type I reaction center (RC) complex. Their RCs are essentially analogous to photosystem (PS) I of plant and cyanobacteria and form homodimeric structures that are made of two identical polypeptides in contrast to the heterodimeric PS I RCs and type II RCs of purple bacteria or oxygenic organisms that are made of two similar but different RC polypeptides. *Heliobacterial* RC contains bacteriochlorophyll *g* dimer as the primary electron donor (P_{800}), chlorophyll *a* monomer as the primary electron acceptor (A_0), and three iron sulfur centers ($F_X/F_A/F_B$) as the tertiary electron acceptors. The function of menaquinone as the secondary electron acceptor (A_1) has been unclear until recently. X-ray crystal structure analysis of the RC has not been done yet.We measured the ESR signals in the membranes of *Heliobacterium modesticaldum* oriented on thin Mylar films. Based on the angular dependence of the anisotropic ESR signals, we determined the molecular orientations of F_X, F_A, F_B, and A_1 in the RC. We compared with the results in *Heliobacterial* RC to those in PS I RC, and will discuss their structure and functional differences.

2P330 シリカメソ多孔体への光合成反応中心光化学系 II 複合体の導入

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Introduction of the photosystem II photosynthetic reaction center complex into the silica mesoporous materials

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We have introduced functional photosynthetic membrane protein complex into a silica mesoporous material (SMM) that has nano-scale pores of honeycomb-like hexagonal cylindrical structure in its inside. We have introduced the photosynthetic reaction center (RC) complex (129 kDa) and light-harvesting LH2-complex (137 kDa) isolated from *Thermochromatium tepidum* into SMM. The adsorption to SMM increased the heat stabilities of photochemical activity and structure of RC [I. Oda, et al, J. Phys. Chem. B, (2006), 110, 1114-1120].

We introduced the dimer complex of photosystem II (PS II) reaction centers (756 kDa) that were purified from a thermophilic photosynthetic cyanobacterium *Thermosynechococcus vulcanus* into SMM that has inner pore diameters of 23.5 nm. The adsorption to SMM decreased the oxygen evolution activity to be about a half measured by a Clark-type oxygen electrode. Pigment assembly as well as the protein structure are almost intact inside SMM when tested by the absorption and fluorescence spectrum. The measurement by a Pulse Amplitude Modulated Fluorometer (PAM) indicated that the adsorption to SMM increased the fluorescence yield at the F_0 level and slowed down the re-oxidation of Q_A. The structure and function of PS II reaction center inside the nano-scale pores will be discussed.

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2aD08 シアノバクテリアにおけるフィコビリソーム超分子会合体の構築及び分解への分子シャペロン HtpG (Hsp90) の関与

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Involvement of the molecular chaperone HtpG (Hsp90) in assembly and degradation of the supramolecular complex phycobilisome.

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Phycobilisomes serve as the primary light-harvesting antennae for photosynthesis in cyanobacteria and red algae. These supramolecular complexes are composed of phycobiliproteins and linker polypeptides. They are rapidly degraded during nitrogen deprivation and constructed after readdition of nitrogen nutrient. Involvement of molecular chaperones such as HtpG (Hsp90) in these processes has not been elucidated. Our in vitro experiments showed that HtpG interacts with linker polypeptides which are thought to be involved in stabilization of the phycobilisome assembly. Therefore, we hypothesized that HtpG is involved in phycobilisome degradation and reconstruction. In this study, this hypothesis was tested using htpG mutants of Synechococcus sp. PCC 7942. Both degradation and reconstruction of phycobilisomes in response to nitrogen availability were slowed down in the mutants. TEM analyses of negatively stained, nitrogen-starved phycobilisomes provided evidence that phycobilisome degradation was greatly retarded in the mutants. All these results indicate that HtpG is involved in the degradation/assembly of phycobilisomes. Experiments are underway to identify proteins, if any, that interact with Hsp90.