Biophysical Society of Japan

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Diatoms are unicellular algae using chlorophylls (Chl) *a* and *c* as major photosynthetic pigments and perform about 40% of the photosynthetic carbon fixation in the oceans. Biochemical studies of diatoms have been rare because of the difficulty of isolating photoactive thylakoid membranes from the cells, which are covered by hard silicate shells. Diatoms have fucoxanthin-Chl-binding proteins (FCP) as the major light-harvesting complexes. FCP contain fucoxanthin, Chl *a*, and Chl *c*. Chl *c* shows fluorescence at around 634 nm. The light energy absorbed by fucoxanthin is transferred to Chl *a*. In FCP, excitation energy on Chl *c* seems to be transferred to Chl *a*.

We studied the time-resolved fluorescence emission spectra of photosystem (PS) I complex, PS I-FCP complex and thylakoid membranes isolated from a diatom, *Chaetoceros gracillis* at 77 K to 10 K. The isolated PS I-FCP super complex contains 224 Chl *a*, 22 Chl *c*, and 55 fucoxanthin molecules per RC. Analysis of fluorescence kinetics with time resolution of 200 fs revealed a 16 ps-energy transfer process from 680 to 700 nm Chls, two decay components at 690 nm with 57 ps and at 710 nm with 246 ps at 77 K. The decay times are conspicuously faster than those of PS I isolated from the other organisms, reflecting different pigment compositions. These time constants became longer at the lower temperature indicating the retardation of the energy transfer process. We discuss the temperature dependence of the energy transfer process, that is sensitive to the energetics of antenna pigments.

3P-268　5から18Kでの単一分子分光による光合成アンテナ複合体の構造揺らぎの観測

A single-molecule study on conformational change of light-harvesting complex between 5 and 18 K

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Under physiological conditions, a protein is continuously changing its conformation. The conformation at a given time corresponds to one of the local minima on the potential energy surface of the protein. Since the property of a protein depends on the conformational dynamics, the knowledge about the individual minima is important to understand the function of a protein. An experimental method to study the minima and barriers is to observe individual proteins at low temperatures where the conformational change is slow enough to be followed. The depth of the potential well will be known from the temperature dependence of the conformational dynamics of a single protein. As a pigment-protein complex for a temperature-dependence study we took a light-harvesting 2 complex of purple photosynthetic bacteria. The complex has nine binding sites of bacteriochlorophyll *a* molecules which is called B800 band. The B800 chromophores are sensitive local probes to monitor conformational fluctuations of the protein. Fluorescence excitation spectrum of individual B800 chromophores was measured in rapid succession of laser-frequency scans to follow spectral change in time between 5 and 40 K. The spectral change reflects a conformational change of the protein to which the chromophore binds. From the temperature dependence of the spectral change the conformational change of the protein is found to be either a thermally activated motion over a potential barrier of around 100 J/mol or a tunneling of a proton through a thermally inaccessible high barrier.

3P-270　極低温での単一アンテナ複合体・クロロソームの蛍光スペクトルに見られるシャープなゼロフォノン線

Sharp zero-phonon lines in fluorescence spectra of single antenna complexes, chlorosomes at cryogenic temperature

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Chlorosome is a light-harvesting complex in green-sulfur and green-filamentous bacteria. It is attached to the inner surface of the cytoplasmic membrane and has an oblong shape with a size of ca. 150 × 40 × 15 nm. It contains two kinds of bacteriochlorophyll (BChl) groups, BChl-*c*, *d*, *e* (depending on species) and BChl-*a*. The former pigments exist inside the envelope membrane and are self-assembled to aggregates with ordered molecular architectures. The formation of aggregates requires no help of protein frameworks. The fine structure of the aggregates is still under debate. BChl-*a* pigments exist in the baseplate proteins within the envelope membrane, mediating the energy transfer from BChl-*c* to the reaction center in the cytoplasmic membrane. We have studied the polarization anisotropy of fluorescence spectra of single chlorosomes at cryogenic temperature by using a laser scanning confocal microscope. Single chlorosomes showed broad fluorescence spectra even at cryogenic temperature, where sharp zero-phonon lines should dominate the spectra. This suggested a major contribution of the inhomogeneous broadening. Recent careful measurement revealed that about 10% of single chlorosomes show fluorescence spectra containing several sharp peaks in the overlapped region of the broad spectra. The positions of these sharp peaks sometimes reached beyond the red tail of the reported inhomogeneous distribution. We interpret these red-shifted sharp peaks as originating from the pigments mediating the energy transfer from BChl-*c* to BChl-*a*.

3P-271　静電ポテンシャル計算による光合成反応中心蛋白質の電子移動現象の解明

Exploring the energetics of electron transfer events in photosynthetic reaction centers by electrostatic energy computations

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We have explored electron transfer processes in purple bacterial photosynthetic reaction centers (PbRC) and Photosystem II (PSII) computationally by using the crystal structures (1,2). At the heart of photosynthetic reaction centers are pairs of chlorophyll a, P700 in Photosystem I (PSI), P680 in PSII of cyanobacteria, algae or plants and a pair of bacteriochlorophyll a, P870 in PbRC. These pairs differ greatly in their redox potentials for one-electron oxidation, Em. For P680, Em is +1100-1200mV, but for P700 and P870 Em is only +500mV. We have reproduced the Em values successfully and demonstrated that how proteins tune the Em (3).

The calculated redox potentials of quinones for reduction in PbRC are controlled by changes in the H-bonding pattern with quinones (4), which are induced by proton transfer events (5,6). The corresponding control was also observed in PSII (7).

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3P-272　シアノバクテリアにおける、HtpG (Hsp90) とDnaK (Hsp70) シャペロン系との相互作用

Interaction of HtpG (Hsp90) with the DnaK (Hsp70) chaperone system in the cyanobacterium Synechococcus sp. PCC 7942

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The 90-kDa heat shock proteins (Hsp90s) are a widespread family of molecular chaperones found in eubacteria and eukaryotes. Cytoplasmic Hsp90 in eukaryotes is indispensable for viability under all conditions tested, while the bacterial HtpG proteins are nonessential under normal conditions. Thus, the in vivo function of HtpG was enigmatic. However, we showed that htpG mutant strains from the cyanobacterium Synechococcus sp. PCC 7942 are highly sensitive to high temperature, indicating an essential role of HtpG under the stress conditions. In the present study, we showed that HtpG interacts directly with DnaJ, a prokaryotic homolog of Hsp40, by biochemical/biophysical studies such as co-immunoprecipitation and SPR analyses. Genetic analysis with yeast two-hybrid assays also indicated the direct interaction. DnaJ (Hsp40) is one of the cochaperones of DnaK (Hsp70). This is the first report to demonstrate that HtpG interacts with the DnaK chaperone system in prokaryotes. It is known that Hsp90 cooperates with Hsp70/Hsp40 in eukaryotic cells during its chaperone cycle. HtpG may also collaborate with the DnaK chaperone system in cyanobacteria.

3P-273　対称型光合成反応中心タンパク質複合体内で機能する電子伝達体の配位構造

Arrangements of the electron transfer cofactor A_1 (menaquinone) molecule in the homodimer reaction center of *Heliobacterium modesticaldum*

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Heliobacteria are strict anaerobe that have a type I reaction center (RC) complex essentially analogous to photosystem (PS) I of plant and cyanobacteria. The RC has a homodimeric structure made of two identical polypeptides (pshA) in contrast to the RCs of PS I and type-II that are made of two different polypeptides. *Heliobacterial* RC contains bacteriochlorophyll *g* dimer (P_{800}), chlorophyll *a* monomer (A_0), menaquinone (A_1) whose function is not fully clear yet, and three iron sulfur centers ($F_X/F_A/F_B$) as the electron transfer components on the RC. The RC 3D-structure is unknown. We measured the light-induced electron spin polarization (ESP) ESR signal of $P_{800}^{+}A_1^{-}$ radical pair state in the oriented *Heliobacterium modesticaldum* membrane on thin polyester films. Based on the angular dependence of the anisotropic ESP-ESR signal pattern measured, we determined the orientation of the P_{800} and A_1 molecule in the RCs. We will discuss the structural and functional characteristics of A_1 (menaquinone) molecule in *Heliobacterial* RC in comparison to those in PS I RC.

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