3PT228 コケ植物におけるそれぞれ異なる 3 つの消光機構**Three different mechanisms of energy dissipation of a desiccation-tolerant moss to protect reaction centres against photo-oxidation**

Hisanori Yamakawa¹, Yoshimasa Fukushima¹, Shigeru Itoh¹, Ulrich Heber²
(¹Grad. Sch. Sci., Univ. Nagoya, ²Bio. Sci., Univ. Wurzburg)

Three different types of non-photochemical de-excitation of absorbed light energy protect photosystem II of the sun- and desiccation-tolerant moss *Rhytidium rugosum* against photo-oxidation. The first mechanism, which is light-induced in hydrated thalli, is sensitive to inhibition by dithiothreitol. It is controlled by the protonation of a thylakoid protein. Other mechanisms are activated by desiccation. One of them permits exciton migration towards a far-red band in the antenna pigments where fast thermal deactivation takes place. This mechanism appears to be similar to a mechanism detected before in desiccated lichens. A third mechanism is based on the reversible photoaccumulation of a radical that acts as a quencher of excitation energy in reaction centres of photosystem II. On the basis of absorption changes around 800 nm, the quencher is suggested to be an oxidized chlorophyll. The data show that desiccated moss is better protected against photo-oxidative damage than hydrated moss. Slow drying of moss thalli in the light increases photo-protection more than slow drying in darkness.

3PT229 光化学系 II と金ナノ粒子の結合による人工光分解ナノデバイスの開発**Development of artificial light-driven water splitting nanodevice using gold nanoparticles and photosystem II**

Kousuke Kawahara¹, Tatsuya Tomo^{2,3}, Takumi Noguchi¹ (¹Grad. Sch. Sci., Nagoya Univ., ²Faculty of Sci., Tokyo Univ. of Sci., ³JST PRESTO)

Development of efficient artificial photosynthetic systems is crucial to solve the energy and environmental crisis. We have recently developed a light-driven water-splitting nanodevice by conjugation of photosystem II (PSII) protein complexes with gold nanoparticles (GNPs) (Noji et al., J. Phys. Chem. Lett., 2011). However, O₂ evolution activity of this PSII-GNP conjugate was only 23% of free PSII. In this study, we have improved the preparation method of the PSII-GNP conjugates to increase the O₂ evolution activity and further detected light-induced electron transfer in PSII-GNP using attenuated total reflection (ATR)-Fourier transform infrared spectroscopy (FTIR). The previous decrease in the O₂ evolution activity in PSII-GNP was found to be caused by a low Ca²⁺ concentration (30 μM) in the buffer adopted to prevent aggregation of GNP. To address this problem, the incubation time for binding PSII with GNP in the 30 μM Ca²⁺ buffer was shortened from 120 min to 10 min, and then the resultant PSII-GNP was suspended in a buffer with 1 mM Ca²⁺. The PSII-GNP conjugates were stable in the 1 mM Ca²⁺ buffer, whereas free GNP was aggregated in the same buffer. The O₂ evolution activity of the PSII-GNP increased by a factor of two compared with the previous study. A light-induced ATR-FTIR difference spectrum of the PSII-GNP conjugates in the presence of DCMU showed typical signals of the semiquinone anion of Q_A, providing evidence that light-driven electron transfer took place in this nanodevice.

3PT230 光化学系 II 酸素発生中心の暗所安定状態における電子スピン状態とプロトン化状態の理論的研究**Theoretical Study on the Protonation and Electronic Spin State of the O₂-Evolving Complex in Photosystem II at the dark stable S₁ state**

Makoto Hatakeyama¹, Waka Uchida¹, Kouji Ogata², Satoshi Yokojima^{2,3}, Shinichiro Nakamura² (¹Grad. Sch. Bio., Titech, ²Harima Inst., Riken, ³Sch. Pharm., Tokyo Univ. Pharm.)

An oxygen evolving complex (OEC) of photosystem II protein has been known as the catalyst for water splitting and oxygen evolving reactions of photosynthesis. The structure and catalytic capability of OEC have been investigated extensively by both experimental and theoretical studies, in order to learn how the water splitting proceeds in nature. The latest X-ray diffraction (XRD) analysis has revealed that the OEC contains the multi-nuclear Mn₄Ca cluster with the five oxo bridges. Nonetheless, the XRD analysis has not indicated two indispensable factors for the water splitting mechanism; i.e. (i) coordinate of hydrogen atoms and (ii) electronic spin state of the Mn₄Ca cluster. The coordinate of hydrogen atoms is necessary to analyze the strength of the Mn

ion ligand field and to identify the oxidation Mn site during the water splitting. Although the Mn₄Ca cluster has been elucidated to involve two Mn(III) and two Mn(IV) ions in the dark stable steady state (S₁), the locations of Mn(III)/Mn(IV) ions in the latest crystal structure has been not yet determined in considering the coordinate of hydrogen atoms. Taking account on those arguments, we investigated the electronic spin state of the Mn₄Ca cluster at the dark stable state, by respecting the latest crystal structure and by changing the protonation state of the coordinating O atoms around the Mn₄Ca cluster.

**3PT231 光合成酸素発生 S 状態サイクルにおける NH₄⁺ 阻害のメカニズム
Mechanism of NH₄⁺ inhibition in the S-state cycle of photosynthetic oxygen evolution**

Takashi Kobayashi, Takumi Noguchi (*Grad.Sch.Sci., Univ.Nagoya*)

Photosynthetic O₂ evolution by plants and cyanobacteria is performed at the Mn cluster in photosystem II (PSII). O₂ is released as a result of four-electron oxidation of two water molecules through a light-driven cycle of five intermediates called S states (S₀-S₄). We recently found that NH₄⁺ works as an effective inhibitor of O₂ evolution. However, the detailed mechanism of NH₄⁺ inhibition has not been understood yet. In this study, we investigated the mechanism of NH₄⁺ inhibition in the O₂ evolving S-state cycle using Fourier transform infrared (FTIR) spectroscopy. PSII membranes from spinach in the presence of 200 mM NH₄Cl were subjected to 12 successive flashes and FTIR difference spectra upon individual flashes were obtained. It was shown that the amplitude of the carboxylate stretching bands was significantly reduced at the third flash, indicating that specific inhibition took place in the S₃→S₀ transition. The overall intensity of the FTIR signals was also smaller with NH₄Cl than with NaCl as a control, suggestive of irreversible inactivation of the Mn cluster. The O₂ evolution activity, however, was unchanged when the sample was incubated with 1 M NH₄Cl in the dark for 1 h and then washed. Flash-induced S₂/S₁ FTIR difference spectra repeatedly measured every five minutes showed a signal decrease as the flash number increases. These results suggest that NH₄⁺ inhibition of O₂ evolution is caused by mixed effects of inhibition in the S₃→S₀ transition and irreversible inactivation of the Mn cluster in the S₂ state.

3PT232 光合成膜蛋白質 PSII の分子動力学シミュレーション**A study of molecular dynamics simulation on Photosystem II protein**

Koji Ogata¹, Makoto Hatakeyama², Taichi Yuki², Waka Uchida², Shinichiro Nakamura¹ (¹RIKEN Research Cluster for Innovation, ²Tokyo Institute of Technology)

Photosystem II (PSII) protein, which governs the initial process of the photosystem in the plant, is transmembrane protein consisting of 20 subunits. This protein plays an important role of yielding O₂ molecules by oxidizing water molecules. The structure of PSII has been solved by X-ray crystallography techniques and publicly accessed from Protein Data Bank (PDB) and clearly shows the detail of structure in the active center for photosynthesis and statical structure can be observed. Here, to observe the dynamical structure of PSII protein, we have performed molecular dynamic simulation on a dimer PSII protein on the thylakoid membrane. The composition of phospholipids and galactolipids in the modeling thylakoid membrane were built as close as the native one. The parameters of four lipids (MGDG, DGDG, SQDG and PG) and the other ligands (Chlorophyll, Plastoquinone, etc.) were assigned in GAFF atomic types combining the charges obtaining by the quantum chemistry calculation. A trajectory of 10 ns simulation was analyzed. From the analysis of the trajectory, water molecules went through into the Mn₄CaO₅ cluster, which is important part for the O₂ oxidation, using three pathways but not so fast movements near the Mn₄CaO₅ cluster. And the two monomer conformations of PSII showed the correlated motions with each other, the thylakoid membrane and the other ligands. We will discuss on the influences of the vibrations of PSII and its surroundings to the mechanism of the oxygen evolution in PSII protein.

3PT233 好熱性紅色硫黄細菌 Thermochromatium tepidum 由来 cytochrome c' における耐熱化メカニズムの検討**Molecular mechanisms for the thermostabilization of cytochrome c' from the thermophilic purple sulfur bacterium Thermochromatium tepidum**

Sachiko Kasuga¹, Yukihiko Kimura^{1,2}, Kei Furusawa³, Takashi Ohno², Seiu Otomo³ (¹Grad. Sch. Agri. Sci., Kobe Univ., ²OAST, Kobe Univ., ³Fac. Sci., Ibaraki Univ.)

Cytochrome (Cyt) c' purified from thermophilic purple sulfur photosynthetic