molecular structure and molecular mechanisms of Photosystem II, which carries out the initial reaction of the series of energy conversion, is one of the most important subject not only for scientific interests but also for solution of serious environmental and energy problems by synthesized compounds that can efficient convert photon energy to electrical energy, hydrogen production from water, and so on.

Photosystem II is a supra-complex membrane protein consists with 20 subunit proteins binding many cofactors. Many investigators studied on the subject on Photosystem II in these 30 years. In recent decade, the understanding of the function and structure of Photosystem II in molecular level dramatically progressed by research combination of robust Photosystem II sample as the research material and many in-depth analyses. At last, the X-ray crystal structure of Photosystem II of the thermophilic cyanobacterium has revealed including catalytic site of water oxidation water molecules with 1.9 Å resolution. However, the structures of the intermediates of the reaction are still not clear. In this symposium, I will talk about the present and prospect of photosynthesis research.

1SL-02 光合成水分解を可能にする光化学系 II の原子構造

Atomic structure of photosystem II that enables photosynthetic water-splitting

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Photosystem II (PSII) is a multi-subunit membrane protein complex consisting of 20 subunits with a total molecular mass of over 350 kDa, and performs a series of light-induced electron transfer reactions which ultimately leads to the splitting of water into electrons, protons and dioxygen. The catalytic center of light-induced water oxidation is a Mn₄Ca-cluster whose detailed structure was not resolved until recently. We have succeeded in analyzing the crystal structure of PSII from *T. vulcanus* at 1.9 Å resolution (1), which allowed us to locate the individual metal atoms in the Mn₄Ca-cluster as well as all of their ligands unambiguously. We also identified 5 oxygen atoms forming the oxo-bridges between the metal ions, which resulted in a Mn₄CaO₅-cluster. The overall structure of the cluster resembles a distorted chair form, which may have important implications in its catalytic activity for water-splitting. In addition, we found 4 water molecules associated with the metal cluster, some of which may function as substrates for the oxygen-evolving reaction. Two chloride-binding sites were identified based on anomalous diffraction taken at 1.75 Å, which were located in the same position as in Br- or I-substituted PSII reported previously (2). We will discuss the detailed structure of PSII at the atomic resolution and its implications in the water-splitting reaction.

(1) Umena Y., Kawakami K., Shen J.-R., Kamiya N. (2011) *Nature* 473, 55-60.
(2) Kawakami K., Umena Y., Kamiya N., Shen J.-R. (2009) *Proc. Natl. Acad. Sci. USA* 106, 8567-8572.

1SL-03 「光化学系 II の電荷分離反応 : P680 から D1 ブランチへ

Electron transfer in the D1 branch of Photosystem II

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The reaction center of photosystem II (PSII) is composed of the D1/D2 heterodimer, harboring the chlorophyll a (Chl_a) pair PD1/PD2, the accessory Chl_a ChlD1/ChlD2, two pheophytin a PheoD1/PheoD2, and two quinones as the redox active cofactors. P680, which absorbs light at a wavelength of 680 nm, is formed among these Chl_a molecules. Excitation of P680 leads to the formation of the ChlD1+ PheoD1- state, followed by the [PD1/PD2]+ PheoD1- state. The resulting [PD1/PD2]+ state serves as an electron abstractor for the oxygen-evolving cluster (OEC). Using the PSII crystal structure analyzed at 1.9 Å resolution with the complete Mn₄CaO₅ atomic coordinates, the cationic state distribution over PD2/PD2 were calculated by considering all PSII subunits and the protonation pattern of all titratable residues.

1SL-04 分光電気化学計測によって得られた酸化還元電位を基にした光化学系 II のエネルギー論

Energetics within photosystem II based on redox potentials revealed by spectroelectrochemistry

Yuki Kato, Tadashi Watanabe (*Institute of Industrial Science, the University of Tokyo*)

Redox potential (E_m) of cofactors on the electron transfer chain is an essential physicochemical parameter for discussing the energetics within photosystem II (PSII). Not all the E_m values of the cofactors, however, have been determined because of experimental difficulty; only E_m values of the primary electron acceptor pheophytin (Ph) and the primary quinone electron acceptor Q_A have been measured. Unapproachable E_m of other cofactors, for example the primary electron donor P680 and the secondary quinone Q_B , has been estimated from the measured E_m values together with free energy differences obtained by kinetic analyses. Though E_m of Ph and Q_A is thus important for discussing on PSII energetics, the estimated E_m values from the measured E_m values of Ph or Q_A are not necessarily consistent each other, and the potential difference between Ph and Q_A often contradicts with kinetic analytical data. This situation should result at least partly from the limited accuracy of chemical titration employed traditionally for the E_m measurements. In view of this, we have tried to measure the E_m values of Ph and Q_A in oxygen-evolving PSII from a thermophilic cyanobacterium *Thermosynechococcus elongatus*. The determined E_m values for a wild-type strain of the cyanobacterium were -522 mV and -140 mV for Ph and Q_A , respectively. On the basis of the determined values, a renewed diagram is proposed for the PSII energetics, and further, modification of the redox properties for acclimation to cultivate conditions is also discussed.

1SL-05 The role of Ca²⁺ and Cl⁻ in the Photosystem II water oxidation

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Nature is using solar energy to generate the atmospheric O₂, trap the CO₂ and store the energy in the chemical bonds of biomass. Photosynthesis is the major energy input in the thermodynamic cycle of the biosphere. Photosystem II (PSII) is the enzyme which splits water. Its active site consists of a Mn₄CaO₅ cluster, a redox-active tyrosine, Y_Z, and the activity requires 2 Cl⁻ ions. The Mn₄CaO₅ cluster goes through 5 oxidation states (S₀ to S₄) during the catalytic cycle and O₂ is released in the S₃Y_Z^{ox} to S₀ transition. Despite the remarkable progresses in the knowledge of the PSII structure, the water oxidation mechanism is far from being understood. Here, we investigated the role of Ca²⁺ and Cl⁻ in the higher redox states of the enzyme with a focus on the water oxidation step. We have used EPR and time-resolved absorption change spectroscopies with a combination of site-directed mutants and biosynthetically modified PSII in *Thermosynechococcus elongatus* to get new thermodynamic, kinetic and structural insights in the S₃Y_Z^{ox} to S₀ transition. It is shown that 1) Ca²⁺ is involved in a hydrogen bond network with a partner of the reaction, the tyrosine Y_Z, and contributes to setting the appropriate structural configuration warranting fast proton transfer and an efficient catalysis and 2) it determines the entropy change associated with the formation of the transition states during catalysis, suggesting that the distribution of the conformational sub-states of the substrate water molecules is critical to the catalytic rate.

1SL-06 光化学系 II 酸素発生錯体モデルとしてのジμ-オキシニ核マンガン錯体の電子構造と不均一系触媒活性

Catalytic activities for heterogeneous water oxidation and electronic structures of di-μ-oxo dinuclear complexes as a synthetic OEC model

Masayuki Yagi (*Niigata University*)

$[(\text{OH}_2)(\text{R-terpy})\text{Mn}(\mu\text{-O})_2\text{Mn}(\text{R-terpy})(\text{OH}_2)]^{3+}$ (R-terpy = 4'-substituted 2,2':6',2''-terpyridine, R = butoxy (BuO), propoxy (PrO), ethoxy (EtO), methoxy (MeO), methyl (Me), methylthio (MeS), chloro (Cl)) have been synthesized as a functional oxygen-evolving complex (OEC) model and characterized by UV-visible and IR spectroscopic, X-ray crystallographic, magnetometric, and electrochemical techniques. The UV-visible spectra of derivatives in water were hardly influenced by the 4'-substituent variation. X-ray crystallographic data showed that Mn centers in the Mn^{III}(μ-O)₂Mn^{IV} cores for derivatives with R = H, MeS, Me, EtO, and BuO are undistorted, whereas the derivatives with R = MeO and PrO gave the significantly distorted Mn centers in the cores. The undistorted Mn centers could be caused by rapid electron exchange between the Mn centers to result in the delocalized Mn(μ-O)₂Mn core. The exchange integral values ($J = -196 \sim -178 \text{ cm}^{-1}$) for delocalized cores were lower than that ($J = -163 \sim -161 \text{ cm}^{-1}$) for localized cores, though the Mn...Mn distances are nearly the same (2.707 - 2.750 Å). The half wave potential ($E_{1/2}$) of a Mn^{III}-Mn^{IV} / Mn^{IV}-Mn^{IV} pair of the derivatives decreased with an increase of the electron-donating ability of the substituted groups for the delocalized core, but it deviated from the correlation for the localized cores. The catalytic activities of the derivatives on mica for heterogeneous water oxidation were remarkably

changed by the substituted groups. The second order rate constant (k_2 / mol⁻¹ s⁻¹) for O₂ evolution was indicated to be correlated to E_{1/2} of a Mn^{III}-Mn^{IV} / Mn^{IV}-Mn^{IV} pair; k_2 increased by a factor of 29 as E_{1/2} increased by 28 mV.

1SL-07 Development of bio-inspired photocatalytic water splitting systems toward solar hydrogen production

Ryu Abe (*Catalysis Research Center, Hokkaido University*)

Photocatalytic water splitting into H₂ and O₂ using a semiconductor photocatalyst has received much attention recently due to the potential of this method for the clean production of H₂ from water utilizing solar energy. Although a number of metal oxides have been reported to be active photocatalysts for the water-splitting reaction, most only function under ultraviolet (UV) light (λ < 400 nm) owing to the large band gap energy of the materials (> 3 eV). Because almost half of all incident solar energy at the Earth's surface falls in the visible region (400 < λ < 800 nm), the efficient utilization of visible light remains indispensable for realizing practical H₂ production based on photocatalytic water splitting. We have recently developed a new type of photocatalytic water splitting system, mimicking the mechanism of photosynthesis in green plants. This system reduces the energy required to drive each photocatalysis process, allowing visible light to be utilized more efficiently than in conventional water splitting system. We have achieved overall water splitting using various visible light responsive photocatalysts, such as SrTiO₃ doped with Cr, tantalum oxynitrides (TaON or BaTaO₂N), and organic dyes, which work as a H₂ evolution photocatalyst, combined with tungsten oxide (WO₃) for O₂ evolution in the presence of a shuttle redox mediator such as iodate/iodide.

1SL-08 遷移金属錯体を用いた人工光合成システム

Artificial Photosynthesis using Transition Metal Complexes

Osamu Ishitani (*Tokyo Institute of Technology*)

Both the problems of the global warming and shortage of the fossil fuels have brought about great interest in photochemical utilization of CO₂ with solar energy. Efficient photocatalysts for CO₂ reduction must be necessary for development of such an important technology. Rhenium (I) bipyridine tricarbonyl complexes can function as both photocatalysts and electrocatalysts for CO₂ reduction. Although their efficiencies and product selectivities are quite high, the low durability is a problem of the rhenium (I) complexes as photocatalysts. In order to achieve the efficient use of solar light, a photosensitizer, which effectively drives photochemical electron transfer with visible light, should cooperate with a rhenium complex catalyst, which cannot absorb a wide range of visible light. We have developed novel types of photocatalytic systems using rhenium (I) complex (es) as photocatalyst or catalyst. I will talk about some of the following systems in the presentation.

(1) Development of the most efficient photocatalysts based on mechanistic studies: the quantum yield for CO₂ reduction is over 50 %.

(2) Architecture of supramolecular metal complexes for CO₂ reduction.

1SM-01 F₁-ATPase と繊毛軸糸をモデルとした、酵素とその集合体の動きの可視化

F₁-ATPase and ciliary axonemes as models of imaging motions in enzymes and their assemblies

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Detecting "motions" of objects in different hierarchy levels and relating them to functions play key roles in understanding biological systems. Targets of this strategy can range from atoms in residues of enzymes to cells or even to dynamics of individual. One solution to this challenge is application of techniques developed for single-molecule observation under the microscope.

In the present studies, we selected two targets for observation. The small target is C-terminal α -helix in the catalytic subunit of a motor protein F₁-ATPase. Using a fluorescent molecule attached to the helix as a probe, conformational changes of the helix was detected under TIRF microscopy while rotation of beads bound to the central shaft subunit was simultaneously monitored. Moreover during this observation, the shaft was manipulated via beads by external force either in the direction of rotation in x-y plane or in z direction using magnetic or optical tweezers.

A large target is an organelle, ciliary axoneme of mouse tracheal cilium. We recorded motions of a fluorescent bead attached to the axonemal tip under three-

dimensional tracking microscopy, and analysed the unique motility.

In line with the theme of the symposium, these studies will be discussed in relation to biochemistry and theoretical physics as attempts to perform interdisciplinary research.

1SM-02 次世代 1 分子計測技術を用いたコドン分解能レベルのタンパク質翻訳リアルタイム可視化

Real time visualization of translation at codon resolution by next generation single molecule studies

Sotaro Uemura (*RIKEN Omics Science Center*)

Translation by the ribosome occurs by a complex mechanism involving the coordinated interaction of multiple nucleic acid and protein ligands. Here we use zero-mode waveguides (ZMWs) and sophisticated detection instrumentation to allow real-time observation of translation at physiologically relevant micromolar ligand concentrations. Translation at each codon is monitored by stable binding of transfer RNAs (tRNAs)-labelled with distinct fluorophores-to translating ribosomes, which allows direct detection of the identity of tRNA molecules bound to the ribosome and therefore the underlying messenger RNA (mRNA) sequence. We observe the transit of tRNAs on single translating ribosomes and determine the number of tRNA molecules simultaneously bound to the ribosome, at each codon of an mRNA molecule. Our results show that ribosomes are only briefly occupied by two tRNA molecules and that release of deacylated tRNA from the exit (E) site is uncoupled from binding of aminoacyl-tRNA site (A-site) tRNA and occurs rapidly after translocation. The methods outlined here have broad application to the study of mRNA sequences, and the mechanism and regulation of translation.

1SM-03 人工分子の分子内回転のリアルタイム一分子計測

Real-Time Single-Molecular Measurement of Artificial Molecular Rotor

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Motor proteins such as F₁-ATPase are the ultimate nanomachines that show efficient energy conversion through chemomechanical coupling. For synthetic chemists designing functional molecules and materials, mimicking these autonomous systems is one of the most attractive goals.

Over the past decade, real-time single-molecule imaging and manipulation under an optical microscope has revealed the steps, pauses and other properties of the biomotors. However, so far, these techniques have not been applied to the artificial molecules. We believe that these state-of-the-art techniques are necessary to evaluate and create the autonomous motor by artificial molecules. Furthermore, we would be able to understand essence of molecular motor through this process.

Toward the goal, we first attempted to observe intramolecular rotation of single double-decker porphyrin molecule (DD) of 1 nm diameter. This artificial molecule has a structure of which a metal ion is sandwiched between two porphyrin discs. The ensemble measurement of DD using NMR has revealed the intramolecular rotational diffusion of two discs, but the steps in rotation have not been revealed yet. We newly synthesized derivatives of DD for single-molecule imaging and then tried to observe steps in the rotation of DD by two conventional methods utilizing a large bead or an anisotropic fluorescent dye as a probe. As preliminary results, some large and small probes showed 90-degree stepping motions, consistent with the four-fold rotational symmetry of the DD.

1SM-04 Studying molecular motors with optical and magnetic tweezers

Maria Manosas^{1,2}, Senthil Perumal³, Steve Benkovic³, Felix Ritort², Vincent Croquette¹ (¹ENS Paris, ²Dept. Phys., Univ. Barcelona, ³Dept. Chem., Pennsylvania State Univ.)

UvsW protein belongs to the SF2 helicase superfamily and is one of three helicases found in T4 phage. It is a functional analog of the E. coli RecG protein. Recent biochemical studies have demonstrated the ability of UvsW to unwind stalled replication forks and recombination intermediates. Here we employ magnetic and optical tweezers to examine the unwinding and reannealing properties of UvsW on a DNA hairpin substrate. Our studies revealed that UvsW was able to translocate along ssDNA at an extremely fast rate (1500bp/s) by either unwinding dsDNA regions or by forcing the reannealing of two single-stranded DNA (ssDNA) regions. The enzyme clearly presented a preference for reannealing over unwinding activity. The UvsW