see 1TP2-09

1P-230 光合成酸素発生反応における CP43-354 位グルタミン酸の FTIR による構造解析

FTIR study on the structure of CP43-E354 in the photosynthetic oxygen-evolving center

Hiroyuki Suzuki (1), Yuichiro Shimada (2), Tohru Tsuchiya (2), Tatsuya Tomo (2), Mamoru Mimuro (2), Takumi Noguchi (1) ((1) Institute of Materials Science, University of Tsukuba; (2) Graduate School of Human and Environmental Studies, Kyoto University)

Photosynthetic oxygen evolution in plants and cyanobacteria is performed at the Mn cluster located in photosystem II (PSII). Although the X-ray crystallography revealed carboxylate and histidine side chains surrounding the Mn cluster, the ligand structure of the Mn cluster remains to be clarified. In this study, we have investigated the interaction of CP43-E354, a candidate of the ligand, with the Mn cluster by analyzing the CP43-E354Q mutant of *Synechocystis* sp. PCC 6803 using Fourier transform infrared (FTIR) spectroscopy.¹ The S₂/S₁ FTIR difference spectrum of the PSII core complexes of the E354Q mutant exhibited features significantly different from that of WT especially in carboxylate stretching regions. The double-difference spectrum revealed prominent peaks at 1502(+)/1525(-) and 1431(+)/1394(-) cm⁻¹, suggesting the disappearance of the asymmetric and symmetric COO⁻ stretching bands of E354 in the WT spectrum by E354Q mutation. In the water OH region of the S₂/S₁ spectrum, the bands at 3622(+)/3588(-) cm⁻¹ in WT were drastically changed to the bands at 3629(-)/3598(+) cm⁻¹. In contrast, the S₂/S₂ difference spectrum of E354 was similar to that of WT. These results suggest that E354 acts as a ligand to a Mn ion that is oxidized in the S₁-to-S₂ transition and a water molecule is bound to the same Mn ion, whereas E354 is not directly coupled to a Mn ion or a ligand that is oxidized in the S₂-to-S₃ transition.

¹Y. Shimada, H. Suzuki, T. Tsuchiya, T. Tomo, T. Noguchi, and M. Mimuro, *Biochemistry*, in press.1P-231 光化学系 II における除草剤の Q_B 結合部位への相互作用Interactions of herbicides in the Q_B binding site in photosystem II: Analysis by Fourier transform infrared spectroscopy

Ryouta Takahashi (1), Takumi Noguchi (1) ((1) Inst. of Mater. Sci., Univ. of Tsukuba)

In photosystem II (PSII), D2-H214 and D1-H215 coordinating to the non-heme iron are involved in the binding sites of the primary (Q_A) and the secondary (Q_B) quinone electron acceptors, respectively. It is known that herbicides bound to the Q_B site affect the redox potential of Q_A; phenolic herbicides show the Q_A potential lower than that with DCMU by ~100 mV. However, the mechanism of the potential control and the details of interactions of herbicides have yet to be clarified. In this study, we have studied the molecular interactions of herbicides in the Q_B site using Fourier transform infrared (FTIR) spectroscopy. FTIR spectra of the non-heme iron was obtained as a light-induced Fe²⁺/Fe³⁺ difference using PS II membranes from spinach. In the presence of phenolic herbicides, bromoxynil and ioxynil, a differential band was observed at 1109/1101 cm⁻¹, whereas it appeared at 1111/1102 cm⁻¹ in the presence of DCMU. Moreover, bromoxynil and ioxynil induced stronger Fermi resonance peaks in the H-bond network region (2500-2950 cm⁻¹). These peaks downshifted upon ¹⁵N labeling of PSII and hence were attributed to His vibrations. The differences in the His bands between DCMU and the phenolic herbicides probably indicate a strong interaction of a phenolic herbicide with D1-H215. It is proposed that the C-O⁻ of the phenolate anion forms a strong H-bond with the NH of D1-H215 to affect the interaction of Q_A with D2-His214 via the iron-histidine bridge.

1P-232 QA 再構成と FTIR 解析による PSII における第一電子受容体 QA の分子間相互作用の解明

Molecular interaction of the primary quinone electron acceptor QA in photosystem II as studied by QA reconstitution and FTIR analysis

Yuta Kozue (1), Akira Takano (1), Ikuyo Sugimoto (2), Hiroyuki Suzuki (1), Miwa Sugiura (3), Yuichiro Takahashi (2), Takumi Noguchi (1) ((1) Institute of Materials Science, University of Tsukuba; (2) The Graduate School of Natural Science and Technology, Okayama University; (3) Cell-Free Science and Technology Research Center, Ehime University)

The primary (QA) and secondary (QB) quinone electron acceptors in photosystem II (PSII) both consist of plastoquinone (PQ) molecules, but the redox potential of QA is controlled to be lower than that of QB to facilitate the QA→QB electron transfer. The structural factors to determine the redox potentials of QA and QB in PSII proteins have yet to be clarified. To investigate the molecular interaction of QA, in this study, we have reconstituted QA with unlabeled or isotope-labeled PQ and analyzed it using Fourier transform infrared (FTIR) difference spectroscopy. We first established the method of QA reconstitution using various PSII

preparations. QA was removed from PS II core complexes of *Thermosynechococcus elongatus* and *Chlamydomonas reinhardtii*, and PS II membranes of spinach by dithionite treatment. PQ was then added to the samples to reconstitute QA. Spectral features of light-induced QA/QA⁻ FTIR difference spectra of QA- reconstituted PSII samples were virtually identical to the corresponding untreated samples, indicating that QA was properly restored in the binding pocket. From the intensity of the CO peak of a semiquinone anion at ~1480 cm⁻¹, it was estimated that more than 70% of centers restored the QA function by reconstitution. The QA reconstituted with ¹³C-labeled PQ exhibited large frequency downshifts in the CO/CC stretching bands of PQ. Analysis of the isotope- edited QA/QA⁻ spectra showed that QA has a highly symmetrical H-bond interaction in its binding site.

1P-233 除草剤効果を使用した植物の光傷害メカニズムの解明

The mechanism of photodamage of photosystem II as revealed by herbicide effects and FTIR difference spectroscopy

Issei Idedan (1), Yuichi Shibuya (1), Takumi Noguchi (1) ((1) Graduate School of Pure and Applied Sciences University of Tsukuba)

It is known that the photosynthetic activity of plants is lowered under strong-light conditions and this phenomenon is mainly caused by photodamage of photosystem II (PSII). However, the detailed mechanism of the PSII photodamage remains to be understood. In this study, we have investigated the molecular mechanism of the PSII photodamage using Fourier transform infrared (FTIR) difference spectroscopy and the effects of two different herbicides, DCMU and bromoxynil. The PSII membranes from spinach without herbicides, with DCMU, and with bromoxynil were illuminated at the intensity of 2000 μEm⁻² s⁻¹ for 0, 30, 60 and 120 min. The S₂Q_A⁻/S₁Q_A and P680⁺/P680 FTIR difference spectra were measured using these samples and the relative amounts of active Mn cluster, Q_A, and P680 were estimated. The results showed that (1) the signals of Q_A and the Mn cluster decreased by illumination at the same rate for all three samples, (2) the decay of the P680 signal was always slower than that of the Q_A signal, (3) the signal decays were faster and slower with bromoxynil and DCMU, respectively, than those of the control sample. These results indicate that the deactivation of the Mn cluster is not a determinant of PSII photodamage and that the Q_A release triggers the P680 damage. The difference in the Q_A release rate by bromoxynil and DCMU can be explained by the different Q_A⁻ potential in the presence of these herbicides. The present results provide experimental evidence for the acceptor-side mechanism of the PSII photodamage.

1P-234 分光電気化学的手法により計測した光化学系 II フェオフィチン a の酸化還元電位

Redox potential of pheophytin a in photosystem II measured by spectroelectrochemistry

Yuki Kato (1), Miwa Sugiura (2), Tadashi Watanabe (1) ((1) Institute of Industrial Science, the University of Tokyo; (2) Cell-Free Science and Technology Research Center, Ehime University)

An immediate experimental evidence for the estimation of the oxidation potential of the primary electron donor P680 of oxygen-evolving photosystem II, which has not been directly measured despite one of the important physicochemical parameters for water oxidation mechanism in oxygenic photosynthesis, is the reduction potential of the primary electron acceptor pheophytin (Pheo) a, E⁺(Pheo a/Pheo a⁻). Although the Pheo a potential values titrated at non-physicochemical pH of 10-11 around 30 years ago have been referred to energetics of photosystem II so far, however, the values cannot account for recent kinetic analysis data of photosystem II. In the present work, applying a spectroelectrochemical technique for PS II complexes from a thermophilic cyanobacterium *Thermosynechococcus elongatus*, we have determined for the first time the redox of Pheo a to be -505 ± 6 mV vs. SHE (four independent samples) at a physiological pH of 6.5, which is more positive by ca. 100 mV than the previous values. Based on the determined values of the Pheo a redox potential and compared with reported kinetic analytical data, the oxidation potential of P680 was estimated to be around +1210 mV. In view of these pieces of information, a renewed diagram is proposed for the energetics in PS II.

1P-235 統計的四面体ポテンシャルを用いたペプチドタンパク質の高速ブラインドッキング法の開発

Toward the fast blind docking of a peptide to a target protein by using a four-body statistical pseudo-potential

Takuyo Aita (1), Koichi Nisigaki (1), Yuzuru Hsui (1) ((1) Saitama University)

In vitro molecular evolution creates a lot of peptide aptamers that bind to each target protein. In many cases, their binding sites on a protein surface are not known. Then, predicting the binding sites through computation within a reasonable time is desirable. With this aim, we have developed a novel system of fast and robust blind docking of a peptide to a fixed protein structure at low computational costs. Our algorithm is based on the following scheme. Representing each of the amino acid residues by a single point corresponding to its side-chain center, the structure of a target protein and that of a ligand peptide