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Proton Release Pattern during Photosynthetic Water Oxidation as Studied by Flash-Induced FTIR Difference Spectroscopy

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Photosynthetic water oxidation takes place in the water oxidizing center (WOC) of photosystem II (PSII) through a light-driven cycle of five intermediates called S states (S_0 - S_4). During this S-state cycle, two water molecules are oxidized to produce four protons and one oxygen molecule. Although oxygen is known to be evolved in the $S_4 \rightarrow S_0$ transition, the final conclusion has yet to be reached about the proton release pattern for individual S-state transitions. In this study, we have estimated the proton release pattern during water oxidation by means of flash-induced Fourier transform infrared (FTIR) difference spectroscopy.

PSII core complexes from *Thermosynechococcus elongatus* were suspended in two different buffers at pH 6.0 made of either 2,2-dimethylglutaric acid or Mes at high concentration. The PSII samples were subjected to twelve consecutive flashes and FTIR difference spectra upon individual flashes were recorded. The obtained FTIR spectra included the signals of the buffers that trapped protons released from WOC in addition to the signals of the proteins. The buffer bands were abstracted from the spectra by calculating double difference between the spectra for the two buffers to cancel out the protein bands. The flash-number dependence of the intensities of the buffer bands showed a typical period-four oscillation. Simulation of the oscillation suggested that the proton release pattern is consistent with $1 : 0 : 1 : 2$ for the $S_0 \rightarrow S_1 \rightarrow S_2 \rightarrow S_3 \rightarrow S_0$ transitions in this core complex at pH 6.0.

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Hydrogen bonding structure of the redox-active tyrosine Y_D in photosystem II as studied by FTIR spectroscopy and DFT calculations

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The redox-active tyrosine Y_D (D2-Tyr160) in photosystem II (PSII) serves as an accessory electron donor to P680. It undergoes proton-coupled electron transfer; when it is oxidized, a proton is released and a neutral radical $Y_D^\bullet$ is formed. According to the recent X-ray structure of the PSII core complex, D2-His189 and D2-Gln164 are located at H-bonding distances from the OH group of Y_D . However, details of the H-bonding interactions of Y_D , which should be important in the proton transfer mechanism, have yet to be clarified. In this study, we have studied the H-bonding structure of Y_D by means of Fourier transform infrared (FTIR) spectroscopy and density functional theory (DFT) calculations. $Y_D/Y_D^\bullet$ -FTIR difference spectra obtained using the PSII core complexes from *Thermosynechococcus elongatus* exhibited a negative peak at 1252 cm^{-1} . This peak downshifted by 25 cm^{-1} when Tyr side chains in the PSII complexes were selectively labeled with $4\text{-}^{13}\text{C}$ -Tyr. In addition, it showed a slight downshift by 2 cm^{-1} concomitant with an intensity decrease in a D_2O buffer. These results were analyzed by DFT calculations using model complexes of *p*-cresol with different H-bonding structures. The peak at 1252 cm^{-1} was assigned to the coupled mode of the CO stretching and COH bending vibrations of Y_D , in which both the hydrogen and oxygen atoms of the OH group are engaged in H-bonds. It is thus proposed that both D2-His189 and D2-Gln164 are H-bonded to Y_D and these H-bond interactions play a crucial role in the proton transfer reaction upon Y_D oxidation.

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Effects of P_{680} ligand substitution in the oxygen-evolving Photosystem II of *Thermosynechococcus elongatus*

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Photosystem II is the enzyme at the origin of O_2 on Earth. Its ability to extract electrons from water by using solar energy makes it an ideal bio-mimetic system for H_2 production. Effects of mutations affecting the environment of the primary electron donor P_{680} have been analyzed to understand the structural basis at the origin of the very high P_{680}^+/P_{680} redox potential. Indeed, the chlorophyll dimer P_{680} traps the light energy and its redox potential ($E'_0 > +1.2\text{ V}$) fixes the driving force available for water oxidation. This has been analyzed in PSII from the thermophilic cyanobacterium *Thermosynechococcus elongatus* in which a normal His ligand of P_{680} has been substituted for either Ala or Gln by site-directed mutagenesis. Both mutant cells grew photo-autotrophically. However, the amounts of phycobilliproteins in the mutants were smaller than in the WT. The energy transfer from phycocyanin to PSII via allophycocyanin was measured by measuring the Chl fluorescence at 77K in whole cells. Purified PSII core complexes were fully active in oxygen-evolution. FTIR spectra and flash-induced absorption changes were recorded and possible changes in the redox potential of P_{680}^+/P_{680} were monitored by measuring recombination kinetics by EPR between either $Q_A^\bullet$ and Tyr_{D-} or S_2Tyr_{D-} and $Q_A^\bullet$. The P_{680}^+/P_{680} spectra appeared slightly modified in the D1-H198Q mutant together with the recombination kinetics. The effect of the mutations on the spectral and kinetics changes will be discussed in view of the crystallographic structure of PSII.

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Requirement of Phosphatidylglycerol for the assembly and function of photosystem I and II reaction centers

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We analyzed synthesis and decomposition of photosynthetic pigment-protein complexes in a mutant strain of *Synechocystis* sp. PCC 6803 with an inactivated *pgsA* gene that functions as one of the terminal enzymes in biosynthesis pathway of a lipid phosphatidylglycerol (PG). The mutant cells grow only in a PG-supplied medium. The depletion of PG from the medium resulted in the gradual loss of CP47 and CP43 proteins from the PS II complex and increased the fraction of un-assembled PS II complex lacking CP43 or CP47 and of the un-assembled proteins, and *de novo* synthesis of PS II proteins did not stop. The result demonstrated that the proper reassembly of PS II reaction center complex requires PG. The study of 77K fluorescence suggested the decrease in the efficiency of energy transfer from phycobilisome to the PS II reaction center and the decrease of the trimer of PS I. These observations give functional evidence for the PG molecules that were recently revealed to be associated with PS II and I reaction center complexes by X-ray crystallography studies. The depletion of PG also affected the morphology of the cyanobacterial cells, inhibited the cell division and enlarged the cell size. FtsZ, which is fused to green fluorescent protein, was found to form a ring structure (Z-ring) at the mid-cell position in the wild type cell, whereas formation of the Z-ring was perturbed in the PG depleted cells.