elongatus. Peaks at 1256/1232 cm^{-1} in the $\text{Fe}^{2+}/\text{Fe}^{3+}$ difference spectrum downshifted by 16 cm^{-1} upon selective labeling of Tyr side chains with 4- ^{13}C -Tyr. Taking account of the band change upon H/D exchange, these peaks were assigned to the $\nu\text{CO}/\delta\text{COH}$ coupled vibration of a Tyr side chain(s), which may be attributed to D1-Y246 and/or D2-Y244. The observed frequencies indicate that this Tyr(s) functions as both a H-bond donor and an acceptor. The C=O/COH bands of bicarbonate and the C=O band of a COOH group were also detected at 1350-1200 and 1750-1720 cm^{-1} , respectively. From these data, it is proposed that D1-Y246, D2-Y244, and D1-E244 interact with the bicarbonate to form a H-bond network around the non-heme iron, which is possibly involved in a proton transfer pathway to Q_B .

3P-256 FTIR 法による光化学系 II におけるフェオフィチン電子受容体の水素結合構造の解析

FTIR study on the hydrogen bond structure of the pheophytin electron acceptor in photosystem II

Yuichi Shibuya (1), Tatsunori Okubo (1), Hiroyuki Suzuki (1), Miwa Sugiura (2), and Takumi Noguchi (1). (1: Inst. of Mater. Sci., Univ. of Tsukuba; 2: Cell-Free Sci. Technol. Res. Cent., Ehime Univ.)

Primary charge separation between P680 and pheophytin_{D1} (Pheo_{D1}) in photosystem II (PSII) is a crucial step to determine the quantum yield of light energy conversion. Recent X-ray structures of the PSII complexes from *T. elongatus* showed that D1-130Gln is located near the Pheo_{D1} keto C=O. It has been observed that the redox potential of Pheo_{D1} and the quantum yield of charge separation are affected by alteration of D1-130Gln. Thus, it is postulated that the charge separation is regulated by the H-bond interaction of the keto C=O of Pheo_{D1}. In this study, we investigated the H-bond structure of Pheo_{D1} using Fourier transform infrared (FTIR) spectroscopy to obtain an insight into the molecular mechanism of the redox potential control of Pheo_{D1}. Light-induced Pheo_{D1}/Pheo_{D1} FTIR difference spectra were obtained using the PSII core complexes from *T. elongatus* WT and its *ApsbA1A2* mutant and the PSII membranes from spinach. The D1-I protein expressed in *T. elongatus* WT has 130Gln, whereas the D1-III expressed in the *ApsbA1A2* mutant and the D1 from spinach have 130Glu. The keto C=O band of Pheo_{D1} was observed at 1605 cm^{-1} in *T. elongatus* WT, while those in the *ApsbA1A2* mutant and spinach were found at ~ 20 cm^{-1} lower frequencies. On the other hand, the keto C=O band of neutral Pheo_{D1} was observed at ~ 1680 cm^{-1} in all the three samples. These results indicate that 130Glu forms a stronger H-bond with the keto C=O of Pheo_{D1} than 130Gln. This stronger H-bond probably stabilizes the Pheo_{D1}⁻ anion and increases the redox potential of Pheo_{D1}.

3P-257 偏光 ATR-FTIR 法による光合成酸素発生マンガングラスターの配位子構造の解析

Polarized ATR-FTIR analysis of the ligand structure of the photosynthetic oxygen evolving Mn cluster

Mitsuhiro Iizasa, Hiroyuki Suzuki and Takumi Noguchi (Inst. of Mater. Sci., Univ. of Tsukuba)

Photosynthetic oxygen evolution by plants and cyanobacteria is performed at the so-called Mn cluster in photosystem II (PSII). The recent X-ray crystal structures of the PSII core complexes at 3.0-3.5 Å resolutions showed that the Mn cluster consists of four Mn ions and one Ca ion with several carboxylate ligands from Asp, Glu and the C-terminus and one His ligand. Because of the moderate resolutions and X-ray damage, however, structural details of the Mn cluster, in particular, the structures of the amino acid ligands have yet to be clarified. In this study, we have investigated the orientations of the ligands to the Mn cluster using polarized attenuated total reflection (ATR)-Fourier transform infrared (FTIR) spectroscopy. The PSII membranes from spinach were oriented on the surface of a silicon ATR prism. The mosaic spread of the membranes was estimated using the CO stretching band of Q_A at 1477 cm^{-1} . Flash-induced S_2/S_1 difference spectra were recorded using infrared light with parallel or perpendicular polarization. From the dichroic ratios of the prominent symmetric COO^- stretching bands at 1362(+)/1402(-) and 1431(+)/1420(-) cm^{-1} , the orientation of the transition dipole moments of these vibrations relative to the membrane normal were estimated to be 35°/43°, and 37°/48°, respectively. From this result, it is proposed that two carboxylate ligands to the Mn or Ca ions are oriented at $\sim 45^\circ$ to the membrane normal in the S_1 state and change their orientations by 8-9° upon the S_2 state formation.

3P-258 紅色細菌の光化学反応中心のプロトン取り込みに関わる水分子の検出

Detection of water molecules involved in the proton uptake of a bacterial photosynthetic reaction center

Tatsuya Iwata (1), Mark L. Paddock (2), Melvin Y. Okamura (2) and Hideki Kandori (1). (1: Department of Frontier Materials, Nagoya Institute of Technology; 2: Department of Physics, University of California, San Diego)

Photosynthetic bacterial reaction center (RC) performs light-induced sequential electron transfer reactions from a bacteriochlorophyll dimer to a quinone (Q_B). Reduction of Q_B by two electrons is coupled to the uptake of two protons from the cytoplasmic side, leading to the formation of the $\text{Q}_\text{B}\text{H}_2$ state that diffuses out of the RC. Glu-L212 is protonated upon formation of Q_B^- and water molecules have been suggested to constitute the pathway of protons. However, the proton uptake pathway in the RC remains unclear because of limited experimental data particularly on waters. In this study, we measured the $\text{Q}_\text{B}^-/\text{Q}_\text{B}$ spectra of WT and EQ-L212 mutant RC focusing on water molecules.

In the Q_B^- state, broad continuum bands appear in the 3000-2000 cm^{-1} region for both WT and EQ-L212, which are H/D exchangeable. This shows that the band originates from strongly hydrogen-bonded X-H stretches. The continuum bands in the 2300-1800 cm^{-1} region in D2O did not show any isotope effect of water for WT, while isotope shift of water was observed for EQ-L212. The bands in the $\text{Q}_\text{B}^-/\text{Q}_\text{B}$ spectra of WT could be due to changes of N-H group(s) of His upon quinone reduction. On the other hand, the low frequency of the water vibration suggests that the signal in EQ-L212 originates from vibrations of protonated water cluster. The proton from the cytoplasmic aqueous phase is uptaken to Glu-L212 in WT, but not to Glu-L212 in the mutant. Instead, the proton may be involved in the hydrogen-bonding network of water molecules.

3P-259 光合成水分分解における活性水分子の DOD 変角振動の FTIR 検出

FTIR detection of the DOD bending vibrations of active water molecules in the photosynthetic water oxidizing center

Hiroyuki Suzuki (1), Miwa Sugiura (2), and Takumi Noguchi (1) (1: Inst. of Mater. Sci., Univ. of Tsukuba; 2: Cell-Free Sci. Technol. Res. Cent., Ehime Univ.)

Photosynthetic water oxidation takes place at the water oxidizing center (WOC) in photosystem II (PSII). The reaction proceeds through a light-driven cycle of five intermediates called S_n state ($n=0-4$). During the S-state cycle, two water molecules are oxidized into one O_2 molecule and four protons. To clarify the mechanism of water oxidation, it is of utmost importance to detect water molecules in WOC and monitor their reactions at the molecular level. In previous studies, we detected water molecules as OH stretching vibrations using Fourier transform infrared (FTIR) spectroscopy. However, the OH vibrations of strongly H-bonded water have been difficult to detect because of significant band broadening. In this study, we have for the first time detected the DOD bending vibrations of heavy water involved in the water oxidizing reactions. Flash-induced FTIR difference spectra during the S-state cycle were measured with the PSII core films of *T. elongatus* moderately deuterated with D_2^{16}O or D_2^{18}O . The D_2^{18}O -minus- D_2^{16}O double difference spectra by the four S-state transitions ($\text{S}_1 \rightarrow \text{S}_2$, $\text{S}_2 \rightarrow \text{S}_3$, $\text{S}_3 \rightarrow \text{S}_0$, and $\text{S}_0 \rightarrow \text{S}_1$) showed several DOD peaks in the 1150-1250 cm^{-1} region. This result indicates that at least two water molecules, not as OH^- or $\text{OH}^\cdot$ forms, always exist in the vicinity of the Mn cluster throughout the S-state cycle. It is proposed that during the cleavage of water in the S-state cycle, water molecules, which work as substrates in the next cycle, are inserted into the catalytic site to participate in the reaction.

3P-260 光合成反応中心スペシャルペアの相互作用エネルギーの解析

Theoretical study on the interaction of the special pair in the photosynthetic reaction center

Hideki Yamasaki, Yu Takano and Haruki Nakamura. (Institute for Protein Research, Osaka Univ.)

The special pair (SP) is a dimer of chlorophyll molecules (Chl) and is essential for the charge separation in the reaction center (RC). The proper spatial arrangements of Chls in RC are important for this process. In this study, the interaction energy between Chls of SP (Eint), which is one of the factors determining the relative orientation of SP, was analyzed using quantum chemical calculations at the MP2/6-31G(d)(0.25) level. Eint was defined as the energy difference between the interacting dimer and the sum of each monomer. A variety of substituted models from the SPns model, in which all of the side groups were replaced with hydrogen atoms, to the SPall model, in which only the tail of the phytol group from F5 was substituted for a hydrogen atom, were made. Eint of the models having only the methyl ester (Mes) or phytol (Phyt) groups were almost the same as that of SPns, while models containing only the non-polar or acetyl groups were more stabilized than that of SPns by 4.3 or 7.6 kcal/mol, respectively. The contribution of non-polar groups mainly resulted from the ethyl groups in ring B, and the orientations of the groups were controlled by the surrounding conserved amino acids. The stabilization due to the π - π interaction between Chl planes and side groups were 9.7 and 8.8 kcal/mol, respectively, showing that both contributions are important. Combined with our previous results showing the effect of the Mes and Phyt groups on the electronic asymmetry of SP, each side group of Chl has specific roles in the function of RC.

3P-261 タンパク質中電子移動反応における電子因子の揺らぎとドナー・アクセプター間距離の揺らぎの相関

Correlation between fluctuations of electronic factor and donor-acceptor distance in electron transfer reaction in protein environment

Toshiaki Kakitani (1,2), Hirotaka Nishioka (1) and Nobuharu Ueda (2) (1: Graduate School of Environmental and Human Science, Meijo University; 2: Faculty of Science and Technology, Meijo University)

Correlation between fluctuations of the square of electron tunneling matrix element T_{DA}^2 and the donor-acceptor distance R in the electron transfer reaction from bacteriopheophytin anion to the primary quinone of the reaction center in photosynthetic bacteria is investigated by a combined study of molecular dynamics simulations and quantum chemical calculations. The value of T_{DA}^2 distributed over more than 5 orders of magnitude and the fluctuation of the value of R distributed over more than 1.8 Å for the 10^6 instantaneous conformations of 1 ns simulation. We divide the simulation data into 1000/t parts of small data sets to obtain the distributions of the time-averaged T_{DA}^2 and the time-averaged distance. We made a principal coordinate analysis for these distributions. As the average-time t is increased, the primary axis becomes similar to the Dutton law with a slope around 2.0 $\text{\AA}^{-1}$ which is considerably larger than the original one 1.4