1P253 同位体標識された好熱性紅色光合成細菌における膜タンパク質耐熱化の分子機構解析
Isotope-edited ATR-FTIR analysis of the light-harvesting 1 reaction center complex from thermophilic purple photosynthetic bacteria
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An attenuated total reflection (ATR) Fourier transform infrared (FTIR) spectroscopy was applied to uniformly ¹⁵N- or ¹³C-labeled Light-harvesting 1 reaction center (LH1-RC) complexes from *Thermochromatium* (Tch.) tepidum to investigate metal-sensitive structural changes involved in the enhanced thermal stability of this complex. The characteristic ATR-FTIR difference bands of unlabeled LH1-RC complexes were induced by the metal exchange from Ca²⁺ to other divalent cations. The detected bands exhibited clear isotopic shifts upon the ¹⁵N- or ¹³C-labeling. The molecular mechanism enhancing the thermal stability is discussed based on the present results and the recent X-ray crystallographic structure of the Tch. tepidum LH1-RC complex.

1P256 光合成酸素発生系における表在性蛋白質と Cl⁻ 結合部位の相互作用 : NO₃⁻置換による赤外分光解析
Effect of the extrinsic proteins on the Cl⁻ binding sites of the oxygen evolving center in photosystem II: Analysis by FTIR spectroscopy
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Extrinsic proteins, PsbP and PsbQ, in photosystem II (PSII) of higher plants regulate the O₂ evolving reaction by controlling the binding of Ca₂⁺ and Cl⁻ ions in the O₂ evolving center (WOC). To understand the molecular mechanism of this regulation, we investigated the effect of PsbP,Q on the Cl⁻ binding sites using FTIR spectroscopy. Light-induced S₂/S₁ FTIR difference spectra were recorded with PSII membranes in which Cl⁻ ions were replaced with infrared-active NO₃⁻ ions. Upon removal of PsbP,Q, the frequencies of the ¹⁴NO₃⁻/¹⁵NO₃⁻ bands were slightly shifted, suggesting the changes in the NO₃⁻ interactions. This result provides evidence that binding of PsbP,Q to PSII perturbs the Cl⁻ sites in WOC, causing the changes in the dissociation constants of Cl⁻ ions.

1P254 光合成光化学系 II における MnCa クラスターの歪んだ椅子型構造の起源
Origin of the distorted-chair structure of the MnCa cluster in photosystem II
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The Mn₄CaO₅ cluster is a catalytic site of the water-splitting reaction in Photosystem II (PSII), where the bonds between Mn and O atoms are elongated causing it to have a distorted-chair structure. The distortion plays an important role in catalytic activity in water splitting. So far, the idea was accepted that the presence of Ca distorted the structure. By employing quantum mechanics/molecular mechanics (QM/MM) calculation, we have clarified that the direct cause of the distortion was not Ca located in the seat of the chair-like structure, but Mn located in the back of the chair-like structure. The Ca atom may play a key role in keeping hydrogen-bond networks around Mn₄CaO₅ that is important to control the proton transfer involved in the water-splitting reaction.

1P257 時間分解赤外分光法による光合成水分分解反応の解析
Proton-coupled electron transfer mechanism of photosynthetic water oxidation as revealed by time-resolved infrared spectroscopy
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Photosynthetic water oxidation takes place at the Mn cluster in photosystem II through five intermediates called S states (S₀-S₄). In this study, the mechanism of proton-coupled electron transfer in the S₂-to-S₃ transition was investigated using time-resolved infrared spectroscopy. The time course at 1400 and 2500 cm⁻¹, which reveals the changes in carboxylate groups and polarizable protons in H-bond networks, respectively, showed two phases with fast (40-100 μs) and slow (200-1800 μs) time constants at pH 4-7, with slower values at lower pHs. This pH dependence together with the H/D effect suggested that proton release occurs before electron transfer in the S₂-to-S₃ transition to remove an excess positive charge accumulated on the Mn cluster at the S₂ state.

1P255 紅色硫黄細菌由来光捕集 1 反応中心複合体における金属—タンパク質間相互作用の熱力学的解析
Thermodynamic analysis of metal-protein interaction in the light-harvesting 1 reaction center complex from purple sulfur bacteria
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The enhanced thermal stability of the light-harvesting 1 reaction center (LH1-RC) from purple sulfur bacterium *Thermochromatium* (Tch.) tepidum is achieved by the binding of Ca²⁺ to the LH1. In this study, we analyzed metal-protein interactions in the wild-type and biosynthetically Sr²⁺-substituted Tch. tepidum LH1-RCs by means of isothermal titration calorimetry. The number of the metal binding site was estimated to be 16 in both wild-type and Sr²⁺-substituted LH1-RCs although the binding constant was significantly reduced in the latter. Furthermore, the Ca²⁺-binding to the wild-type or Sr²⁺-substituted LH1-RC was favorable enthalpically or entropically. These differences may be important for understanding the enhanced thermal stability of the Tch. tepidum LH1-RC.

1P258 光化学系 II における第二キノン電子受容体 Q_B の電子・プロトン移動機構
Electron and proton transfer mechanism of the secondary quinone electron acceptor Q_B in photosystem II
Yukihiro Kadekawa, Yuki Kato, Takumi Noguchi (*Grad. Sch. Sci., Nagoya Univ.*)

In photosystem II (PSII), electrons abstracted from water is transferred to the secondary quinone electron acceptor Q_B, which is converted to plastoquinol (PQH₂) after two turnovers. It is generally thought that PQH₂ is released from the Q_B pocket and is replaced with a new PQ. However, there is another model that only the Q_B head flips to the outside to transfer electrons and protons to another PQ. In this study, we investigated which model is correct using FTIR spectroscopy. PSII membranes supplemented with either ¹²C-PQ or ¹³C-PQ were illuminated with a train of flashes, and then flash-induced S₂Q_B/S₁Q_B[•] difference spectra were measured. The CO band of Q_B[•] at 1480 cm⁻¹ was unchanged between ¹²C-PQ and ¹³C-PQ, strongly supporting the latter flip-flop model.