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

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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

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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

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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

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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

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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

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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

are coarse-grained. The peptide, which is described as a flexible bead model, is movable along the grid-points which are set surrounding the protein. An arbitrary state of the protein-peptide complex is subjected to Delaunay tessellation. Then, the fitness of a peptide-coordination to the protein is measured by a four-body statistical pseudo-potential. Through 1000 trials of a simple hill-climbing optimization, the best 15 peptide-coordinations with the 1st - 15th highest fitness values are selected as candidates for the correct coordination. Retrieving the available seventeen protein-peptide complexes from the Protein Databank, we carried out the blind docking test for each system. In most cases, our blind docking procedure predicted a nearly correct binding site and, in some cases, a nearly correct binding mode as well at low computational costs. For 15 out of the 17 cases investigated, the lowest values of RMSD ranged within 3.0 - 6.5 Å.

- 1P-236 MD シミュレーションとタンパク質のコンキャビティ形状比較を用いたバーチャルスクリーニング
Virtual screening to separate active ligands from decoys using MD simulation and concavity shape comparing

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Molecular docking screens of chemical databases are widely used for ligand discovery. To improve the performance, considering changes of a protein structure is important for some targets. Although several approaches have been reported, the methods retains room for improvement, and are computationally expensive.

We tested an alternative docking screen which uses MD simulations. MD simulations of a target protein without ligands were carried out, and then the concavity shapes of the protein were extracted from the MD trajectories. Screens were performed by comparing the concavity shapes and a query ligand shape. Shape similarity was represented by a similarity index (ex., 1 or 0). A docking score for the protein and the query ligand was defined as a sum of similarity indices along a MD trajectory. Preliminary study on a receptor protein proved our methods as effective as conventional docking softwares, DOCK or GLIDE. In this study, we evaluated our screening calculations for several target proteins, like nuclear hormone receptors, kinases, and serine proteases. These proteins, their active ligands, and the decoy molecules were chosen from a database DUD (directory of useful decoys). Parameters for the screening (conditions of MD simulations, thresholds for shape similarity index, etc.), the advantages and the drawbacks of the methods will be reported.

- 1P-237 アミノ酸配列からのタンパク質揺らぎ領域予測とタンパク質間相互作用への適用
Prediction of protein motion from amino acid sequence and its application to protein-protein interaction

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Structural flexibility is an important characteristic of proteins that is often related to their function. The movement of a polypeptide segment is theoretically classified into two forms: internal and external motion. The former is a deformation of the segment itself, but the latter involves only rotational and translational motions as a rigid body. Normal Mode Analysis (NMA) can derive these motions of two types, but its application remains limited because it requires complete structural information. In this work, we present a novel method for predicting two protein motions solely from amino acid sequence information. We prepared a dataset by calculating internal and external motions using NMA. First, we analyzed the relation between thermal motion as assessed using X-ray crystallographic B-factor and internal/external motion. As a result, the internal motion has different features from the B-factors. Next, we developed a prediction method based on the Random Forest algorithm. Our method exhibited moderate correlation with internal and external motion calculated using NMA, and higher prediction accuracy than naïve model. Finally, we observed significant overlap between predicted high internal motion region and observed conformational change when the proposed method was applied to a set of proteins that undergo large conformational change upon protein-protein interaction.

- 1P-238 構造生物学と計算機科学に基づいたタイトジャンクションを制御する化合物の探索
Search for compounds that modulate tight-junction activity: structural biology and computational approaches.

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[Introduction] In multi-cellular organisms, cell adhesion plays important roles in various cellular processes including morphogenesis, differentiation, and proliferation. Loss of adhesion machineries causes cancer and other diseases. Tight junction (TJ) functions as an epithelial barrier, which is comprised of claudins, occludin and PDZ domain proteins ZO1 - ZO3. Recently, Furuse's group showed that TJ is maintained by dynamic equilibrium between biogenesis and degradation (Iwamoto et al., J Cell Sci, 2009). They identified an E3 ubiquitin ligase LNX1p80, whose four PDZ domains serve as the binding sites for claudins, as the master molecule of TJ degradation. We hypothesize that TJ activity can be modulated by inhibiting interaction of claudins with either LNX1 or ZO1.

[RESULTS and DISCUSSION] We searched for compounds to bind ZO1-PDZ1 by either a biochemical method or a computational method. First, we performed PIP-strip assay for ZO1-PDZ1 and identified PtdIns(3,4,5)P3 and other PIPs serve as non-peptidic ligands for ZO1-PDZ1. NMR experiments confirmed that phytic acid (InsP6) also bound to ZO1-PDZ1. We further performed an *in silico* screening experiment against ZO1-PDZ1 with 600,000 compounds by using the program MOE. Many compounds harboring more than two carboxycyl / sulfonate groups were retrieved with high docking scores, which resemble to phytic acid in terms of multiple negative charges. These compounds are useful as novel chemical tools that modulate TJ biogenesis and degeneration, thereby controlling epithelial barrier functions.

- 1P-239 相互作用傾向値とホモログにおける既知相互作用部位の情報をを用いたドメイン間相互作用部位予測
Prediction of domain-domain interaction sites from interface propensity and known interface positions of homologs

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In both prokaryote and eukaryote genomes, the proportion of proteins composed of more than one structural domain is over two thirds. To elucidate the chemical function of these multi-domain proteins, the whole structures are very important. Improvements in techniques for 3D structure determination have gradually increased the number of multi-domain structures in the Protein Data Bank. However, only about one fourth of solved structures are classified into multi-domain and accurate prediction method for multi-domain structure is still needed. Docking of individual domains with guide information is effective in predicting the multi-domain structure. To obtain the guide information, we have developed a method for predicting domain-domain interface residues. In our method, whether a domain surface residue is a domain-domain interface residue or not is judged by two criteria. One criterion is that the value of a prediction score calculated from interface propensity, which means usage preference in domain-domain interfaces compared to the other part of domain surface, is higher than a certain threshold. To increase the specificity of prediction, we use two interface propensities, "core" and "peripheral", for the score calculation. Another criterion is that the position of the domain surface residue is same as a known domain-domain interface residue of homologs. At present, the accuracy of prediction by former criterion is lower than that of the latter criterion. We will discuss the accuracy of prediction using combined criteria.

- 1P-240 タンパク質間相互作用およびドメイン間相互作用ネットワークの構築と比較
Construction and comparison of PPI and DDI network

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Protein domains are considered to be structural units of protein, and most of Protein-Protein Interactions (PPIs) appear to be through protein domains. Because of great increase in number of PDB entries, it becomes possible to analyze PPI network using only the interactions observed in the structurally resolved protein complexes. Assigning protein domain information to each protein complex in this network, it is possible to determine which domain interacts, and introduce protein domains into the PPI network analysis. This study aimed to elucidate the relationships between PPI network and protein domains.

Previously, we constructed and analyzed two domain networks, named *domain-mediated PPI network* and *DDI network*, using iPfam database, where domain-domain interaction data derived from structurally resolved protein complexes are stored. However, iPfam is not updated recently, and the criterion for determining interacting residues in protein complexes is not revealed. Thus, we performed the similar analysis using 3did, which is another structure-based domain-domain interaction database. In addition, we analyzed the structural and functional characteristics of domains. As a result, the trends that functional domains and interaction domains are clearly separated are similar as a whole, but several differences are found in some domains. For example, although the total number of used complexes in the network with 3did is almost twice as many as iPfam, the number of used complexes including Ig domain in the 3did network is much less