3P-218 網膜変性モデルにおける多点電極アレイによる網膜神経機能の評価

Assessment of neuronal function in retinal degeneration models by using Multi-electrode array system

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Previously, we evaluated the neural function of the local retinas using Multi-electrode array (MEA) system. In this study, we investigated the retinal functions in the several retinal degeneration models using MEA system. After light stimulation, ERG-like a-waves and b-waves were detected with each electrode. The light-induced a-waves were observed in the retinas of P12 and older, with increase in amplitude until P15, by which time the response level was equivalent to that of adult retina to light stimulation. In rd mice, retinal degeneration model, we could not obtain any signals by using standard ERG system. However, by using MEA system, we detected the a-waves, though those were quite weak in the retina of P13 and thereafter they decreased. These results were correlated with immunohistochemistry of retinas in these rodents. Therefore, we conclude that MEA system is useful for investigations of local and subtle function of neuronal retinas.

3P-219 MD 及び MO 計算による PYP の M 中間体の原子レベル構造

The atomic-level structure of the M intermediate of photoactive yellow protein as explored by MD and MO calculations

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Upon illumination of photoactive yellow protein (PYP), two major intermediates, L and M, are produced sequentially. In the final step of the photocycle, the original dark state is recovered from the M state. The M intermediate is a putative signaling state and thus elucidation of its atomic-level structure is essential for understanding the functional mechanism of PYP. In our previous study, we performed multicanonical MD simulation for the sake of conformational sampling for the M state and found that there are at least three substates differing in structural properties in this intermediate. Here, we again performed long time MD simulations starting from the representative structure of each substate, which enabled us to get more energetically relaxed structures for each substate. As a result, we successfully obtained the atomic-level structures consistent with various experimental data for the M intermediate. Furthermore, combined analysis of the MD data and fragment MO calculation data allowed us to unveil the propagation mechanism (pathway) for structural changes from the chromophore binding site to the whole protein, especially to α 1- α 4 helices. Our results indicated that the following residue-residue interactions are significantly important to determine the pathway: E46-N43, D20-K55. This supports the "Helix Cap" model which has been proposed from NMR and X-ray analyses.

3P-220 暗状態及び明状態の LOV2-Ser/Thr キナーゼドッキングモデルに対する MD シミュレーション

Molecular dynamics simulation for LOV2 - Ser/Thr kinase docking models in the dark and light states

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Phototropin has two LOV domains (LOV1 and LOV2) and Ser/Thr kinase and acts as a blue light-regulated protein kinase each LOV domain contains a FMN as the chromophore and produces a covalent adduct between the FMN and a neighboring cysteine residue upon illumination that induces conformational changes in the protein moiety and leads to regulation of the kinase activity. Then, LOV2 plays a predominant role in the switching. Secondary structure prediction and 3D structure simulation indicated that an α/β fold of the Ser/Thr kinase of phototropin is similar to that of the catalytic subunit of PKA. Recently, we successfully obtained a docking model for LOV2- Ser/Thr kinase complex by computer modeling. In this study, to get more detailed information about the molecular mechanism underlying the photoregulation of the kinase, we performed MD simulations starting from the docking model, where FMN was placed in the chromophore binding pocket with or without adduct formation with Cys38, corresponding to the light and dark states, respectively. In the docking model, the F α helix of LOV2 plays an important role in binding to the kinase. According to our simulation results, the F α helix becomes unstable on going from the dark to light state: its helix content tend to decrease. Simultaneously, the interaction between LOV2 and the kinase becomes weak, resulting in a slide motion of the former so as to detach from latter. These structural and dynamical changes are thought to be responsible for the switching of the kinase activity.

3P-221 光化学系 II の光合成反応中心の励起状態に関する理論的研究 Theoretical Study of Excited States of Photosynthetic Reaction Center in Photosystem II: Structure and Interaction

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Recently, the structure of photosystem II (PS II) of cyanobacteria has been clarified by X-ray diffraction analysis. Due to the structural similarity and their evolutionary origins, photoinduced electron transfer in the reaction center (RC) of PS II was expected to start from the special pair (SP) in the RC. However, a recent spectroscopic study indicated the primary charge separation starts from the D1 accessory chlorophyll in PS II, not from the SP as in purple bacteria. In this study, we studied the excited states of the RCs of a cyanobacterium (*T. elongatus*) PS II and a purple bacterium (*Ble. viridis*) to investigate structural effect on the excited states.

We calculated excited states of chlorophyll hexamer in the two RCs by means of ab initio CI Singles method. As observed in experimental spectra, the first excitation energy of the PSII RC is comparable to that of chlorophyll monomer. On the other hand, the first excitation energy of the purple bacteria RC shows large red shift. We found that the difference of the excited states originates from the orientation of the chlorophyll monomer (P) in the SP. Although the SP in the purple bacteria RC is highly symmetric, that in the PSII is less symmetric due to the change in the orientation. This symmetry-lowering weakens the interaction between the P in PS II and in purple bacteria. We also investigated the origin of the structural change in the PSII RC and found that the symmetry-lowering of SP is related to the displacement of the CD helix.

3P-222 光合成細菌のバクテリオクロロフィル分子への非天然型側鎖導入法の開発

Development of Introduction of Unnatural Groups into Bacteriochlorophyll Molecules in Photosynthetic Bacteria

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Chlorophyll(Chl)s and bacteriochlorophyll(BChl)s are main photosynthetic pigments in nature. These pigment molecules have long hydrocarbon chains at the 17-position except Chl c. These esterifying chains of Chls and BChls play important roles in organization of (B)Chl molecules in photosynthetic supramolecules. However, there are few studies on the effects of esterifying chains of (B)Chls on supramolecular structures and functions of photosynthetic light-harvesting complexes. We examine *in vivo* esterification of unnatural alcohols with bacteriochlorophyllide (BChlide) and their incorporation into photosynthetic light-harvesting complexes in photosynthetic bacteria. A green sulfur photosynthetic bacterium *Chlorobium tepidum* was cultivated in a liquid medium by supplementation of unnatural alcohols. High-performance liquid chromatography (HPLC) analyses revealed the biosynthesis of unnatural BChl c derivatives esterified with these alcohols. The homolog composition of unnatural BChl c derivatives was similar to that of natural BChl c, which was esterified by farnesol. This result suggests that addition of long chains to BChlide c is the last step in the BChl c biosynthetic pathway and the enzymatic activity of BchK would not depend on the 8- and 12-substituents of the chlorin macrocycle of BChlide c. Spectral properties of light-harvesting complexes containing unnatural BChl derivatives will be discussed.

3P-223 ホモダイマー型光合成反応中心のアフィニティ精製と分光学的性質

Spectroscopic characterization of the His-tagged homodimeric photosynthetic reaction center

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Photosynthetic reaction centers (RCs) are pigment-associated membrane protein complexes which produce energized electrons through a series of redox reactions driven by a capture of solar energy. The green sulfur bacteria, which are strictly anaerobic photosynthetic bacteria, have a homodimeric Type I RC whose cofactors are much similar to those of photosystem (PS) I in plants and cyanobacteria. The structure and function in PS I have been well understood based on its detailed three-dimensional structure. However, the information about electron transfer pathways in green sulfur bacterial RC as well as its molecular organization is very scarce due to the difficulty in its large-scale preparation and analyzing its unique properties. The main reason for this lies in its extreme instability to oxygenic conditions, where iron-sulfur (Fe-S) centers are easily destroyed. In this study, we constructed a mutant of the thermophilic green sulfur bacterium *Chlorobaculum tepidum* expressing the His-tagged RC core protein by an incorporation of its encoding gene into the genome. A highly purified and photoactive RC complex was obtained in one step with the affinity chromatography. The light-induced ESR signals at cryogenic temperature and decay kinetics of flash-induced transient absorption changes measured in this preparation suggested that it contained all Fe-S clusters, F_X and F_{A/B}, as terminal electron acceptors.

3P-224 クロロフィル側鎖回転異性体の理論解析