observed in these dipolar-coupling-based cross-polarization methods because of large-amplitude molecular motions at room temperature. We performed J-coupling-based HC-INEPT and HCC-TOCSY experiments in order to obtain signals of mobile protein segments. Mobile protein segments make up about 70% of pHtrII (1-159). Many signals in mobile regions were observed in HC and HCC 2D spectra. ^{13}C and ^1H signals in mobile regions were assigned to amino acid groups. The C^α and CO chemical shifts indicate that pHtrII (1-159) mainly forms α -helix structure in lipid bilayer environment.

3P-094 チトクロム酸化酵素の高分解能の精密X線結晶構造解析: 1.5 Å程度のX線回折データで膜蛋白質のプロトン化/脱プロトン化状態の決定方法の開発

High resolution and precise X-ray crystal structure determination of Cytochrome c Oxidase

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Cytochrome c Oxidase (CcO) is a large membrane protein with a molecular weight of 200 kDa which couples proton pumping and oxidation-reduction reaction. This proton pumping makes proton gradient which enables ATP synthase to generate ATPs. It is essential to directly observe the protonation / deprotonation states of carboxyl groups participating in the proton pumping to make the mechanism clear at atomic level. We have already determined three dimensional structure of CcO at oxidized state and reduced state at 1.8 Å and 1.9 Å resolution, however structural refinement at this resolution was not sufficient to observe hydrogen electron density. Neutron diffraction structure analysis is also utilized as a mean to observe hydrogen atoms, but it doesn't work for CcO because of large molecular weight. In order to observe hydrogen atoms' electron density in a $\text{Fo} - \text{Fc}$ difference Fourier map, non hydrogen atomic parameters should be determined accurately, thus usually higher than 1.2 Å resolution data are required. We are aiming at developing a method to elucidate protonation/deprotonation state of carboxylic groups around 1.5 Å resolution data, which is completely possible in principle. To accomplish that, we have tried several attempts. In poster presentation, I will share with you these points: (1) ingenious way of selecting reflections in view of anisotropy diffraction pattern (2) strategy which can obtain better free R-factor value in structure refinement (3) plan to distinguish protonation/deprotonation state of specific carboxyl groups.

3P-095 NMRを用いたミトコンドリアタンパク質輸送因子Tim21と呼吸鎖構成因子Qcr6との相互作用解析

NMR analyses on the interactions between Tim21 for mitochondrial protein import and Qcr6 of the respiratory chain complex in mitochondria

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Mitochondria are essential organelles bounded by two membranes in eukaryotic cells and produce ATP by oxidative phosphorylation, which is mediated by electron-transport chain complexes and ATP synthase in the inner mitochondrial membrane. Most mitochondrial proteins are synthesized outside the mitochondria, and subsequently imported into and sorted within mitochondria with the aid of translocator complexes such as the TOM40 and TIM23 complexes in the outer and inner membranes, respectively. The electron-transport chain complexes are known to form larger super-complexes with each other to increase efficiency of electron transport. Besides recently, the TIM23 complex and the bc₁ complex interact with each other in the inner membrane. Although biological significance of this interaction remains unclear at the moment, this interaction was shown to be mediated by the subunits, Tim21 of the TIM23 complex and Qcr6 of the bc₁ complex.

Here we analyzed the interaction between Tim21 and Qcr6 by NMR. We recorded ^1H - ^{15}N HSQC spectra of Tim21 and assigned their NMR signals to specific residues. Then we observed possible interaction between Tim21 and the entire Qcr6 or its N-terminal or C-terminal domain, and found that Tim21 binds to the N-terminal domain of Qcr6. Since the N-terminal domain of Qcr6 takes little ordered structure, we are analyzing the structural basis for the molecular recognition between Tim21 and unstructured Qcr6 domain.

3P-096 紫膜中のバクテリородプシンの光退色による構造変化

Structural changes of bacteriorhodopsins in purple membranes with heterogeneous and homogeneous stability against photobleaching

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Bacteriorhodopsin (bR), which is a sole protein of the purple membrane (PM) of *Halobacterium salinarum*, functions as a light-driven proton pump through a photocycle. Our group previously revealed that bR underwent an irreversible photobleaching at high temperature and at high pH, although it originally functions by absorbing light energy without branching to denatured states. A kinetic study for the photobleaching of bR in PM at high temperature recently showed that two kinds of bR species in respect of the structural stability were observed below pH 9, whereas only one species was found above pH 10. In order to elucidate what is responsible for generating the heterogeneity or homogeneity on the photobleaching kinetics we herein investigated the photobleaching-induced

structural changes at pH 7 and 11 by attenuated total reflection FT-IR spectroscopy. At both pH conditions IR spectra of the photobleached bRs showed that a $\alpha_{\text{II}} - \alpha_{\text{I}}$ transition occurred with increasing a degree of the photobleaching. Time dependences of the helical structural changes at both pHs were almost identical with those of the retinal absorption changes. In contrast, IR spectra for pH 7 showed that a band at 1626 cm^{-1} , which was attributed to an intermolecular β -structure, significantly increased with photobleaching, while it was not monitored in those at pH 11. A model for the heterogeneous and homogeneous photobleaching processes at neutral and high pH will be discussed from these results.

3P-097 ハロロドプシンの光活性はホモ三量体化によって変調を受けるのか?

Is the Photoactivity of Halorhodopsin Modulated by the Assembly of Homotrimer?

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Halorhodopsin (HR) is an archaeal rhodopsin which acts as a light-driven Cl⁻ pump. HR consists of seven transmembrane α -helices, and covalently binds retinal as a chromophore. Our previous study showed that HR from *N. pharaonis* (NpHR) assembles to a stable trimer in the lipid bilayer and detergent-solubilized system. Furthermore, it was suggested that the mutant of F150 residue (NpHR-F150A), which was estimated to locate at the trimer interface from *H. salinarum* HR (HsHR), existed as a monomer in the detergent system. These results indicated that the F150 residue plays an important role for trimer assembly. In order to elucidate the relation between the trimerization and the function of NpHR, the retinal isomerization analysis, several spectroscopic analyses such as the flash photolysis spectroscopy, and measurement of the photo-induced current using *X. laevis* oocytes were performed. In the Cl⁻-free NpHR-F150A monomer, the 13-*cis* retinal isomer composition was increased by light illumination. Interestingly, the effect of monomerization was observed not only to the photocycle of NpHR as the acceleration of the decay speed in the early intermediates, but also to the behavior as a Cl⁻ pump, which was different from trimer or monomer although both had the same intrinsic motive force. These results suggest that the trimer assembly contributes to the regulation of the dynamics in the photoreaction of NpHR. In addition, we will discuss the results of solution-state NMR analysis with monomeric NpHR.

3P-098 脱脂質した紫膜の高温化における光退色現象に対する影響

Effect of delipidation of purple membrane on photobleaching at high temperature.

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Bacteriorhodopsin (bR), which is a light-driven proton pump of *Halobacterium salinarum*, forms the two-dimensional crystalline structure with lipid molecules in purple membranes (PM). We studied about solubilized or reconstituted samples, since structure stability of bR in photobleaching experiment is concerned with membrane condition. These experimental results suggested that protein-protein interaction stabilized the photointermediates. In this study, we investigate photobleaching and high temperature denaturation of delipidated bR using detergent of 3-[(cholamidopropyl)dimethylammonio]-propanesulfonate (CHAPS), in order to study the effect of lipid extraction which is expected to alter protein-protein interaction.

Thermal denaturation of delipidated bR occurred above 70 °C, similar to native PM. Delipidated bR also showed photobleaching above 65 °C, while photobleaching temperature of native PM is 60 °C. Visible CD spectra of delipidated bR in darkness indicated that crystalline structure maintained below 70 °C and melted at 80 °C. Photobleaching kinetics of delipidated bR have only single decay component, while that of native PM was characterized fast- and slow decay components. Decay component of delipidated bR is close to the slower one of native PMs. These results indicate that changes of interaction induced by delipidation influence on structural stability and kinetic behavior of photobleaching. We will discuss protein-protein interaction on photobleaching at high temperature.

3P-099 膜蛋白質の1次元構造属性の予測

Prediction of residue-level structural properties of membrane proteins

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One-dimensional structural properties such as solvent accessibility, binding sites and contact probabilities of globular proteins have been widely studied and sequence-based prediction methods for them are freely available. However, there are currently very few methods for predicting such properties for membrane proteins. For the few properties studied, the available methods have limitations and need significant improvements. We are developing methods of solvent accessibility and helix-helix contact predictions for membrane proteins using neural networks. Using lipid probe as a reference solvent, accessible surface area of residues in such proteins has been calculated and assigned to a data set of membrane proteins and trans-membrane residues therein. Prediction methods using the single sequence information, position specific scoring matrices (PSSM) and overall global features of protein sequence have been used to develop neural