3P001 リン酸化された心筋トロポニン C-トロポニン I 複合体のパルス ESR を用いた構造解析
3F1150 Structural Analysis of Phosphorylated Cardiac Troponin C-Troponin I Complex by Pulsed ESR

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Distance information on a nano meter scale is important to study the structure of huge molecular systems, such as proteins, polymers, and clusters. By means of an electron spin resonance (ESR) technique, the distance between electron spins can be determined from analyses of dipolar interaction between the spins: the ESR distance measurement. We have been studying the structure of cardiac troponin by site-directed spin labeling (SDSL) and pulsed ESR. Cardiac troponin plays a role of a regulated switch controlled by Ca²⁺ ion concentration in muscle contraction. In this study, we applied the method of distance measurement to phosphorylation of cardiac troponin C- troponin I (TnC-TnI) complex. The cardiac TnI has a N-terminal extension of about 30 amino acid residues including two adjacent serines as protein kinase A (PKA) phosphorylation sites. The PKA phosphorylation of cardiac TnI reduces the Ca²⁺ binding affinity of the N-terminal domain in cardiac TnC. The X-ray structure of TnC is not known with or without binding Ca²⁺ ion when TnI is phosphorylated. This complex was doubly labeled by the SDSL technique. The distance between two nitroxide labels was measured by double quantum coherence (DQC) using a Ku-band (17.5GHz) pulsed ESR spectrometer. We evaluate the mean distance between spin labels and their distance distribution by using the Tikhonov Regularization method. Based on these results, we will discuss the structure of the cardiac TnC-TnI complex.

3P002 Conformational changes of extracellular loop of halorhodopsin by chloride binding

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Halorhodopsin (HR) is a transmembrane, seven-helix retinal protein in the membrane of the archaeal bacterium which acts as an inward-directed, light-driven chloride ion pump. In order to clear the light-driven chloride ion pump mechanism of HR, the roles played by putative anion-binding sites in the cytoplasmic and extracellular channels of *Natronomonas pharaonis* HR (NpHR) have been investigated using the wild-type and various mutant proteins in the solubilized system, all of which were functionally expressed in *E. coli* cells. At present, we have two crystal structures of HR from *Halobacterium salinarum* and *N. pharaonis*, which indicates structural differences in the extracellular loop. It has been reported that the BC-loop between 2nd and 3rd TM-helices of HR play a crucial role in chloride ion pumping function. However, there is no structural information about the chloride-dependency at the BC-loop. We studied uniformly ¹³C, ¹⁵N labeled NpHR by high-resolution solid-state NMR. J-coupling-based HCC-FLOPSY and HNCOCOA experiments were performed under magic-angle spinning in order to assign NMR signals of mobile regions. Comparing the chemical shifts of Cl⁻ free and bound NpHR suggested secondary structural changes of the extracellular loop between the BC loop. In addition, we have prepared amino acids selective isotope labeled NpHR by cell-free expression system. The results and application to high-resolution solid-state NMR will be discussed.

3P003 Structural Change and dynamics at Tyr residues in Bacteriorhodopsin corresponding to two isomers of retinal as revealed by solid-state NMR

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Bacteriorhodopsin(BR) has a light-driven proton pump activity triggered by photoisomerization of retinal. Retinal chromophore is a mixture of all-*trans* and 13-*cis*, 15-*syn* retinals with the isomeric ratio close to 1 in the dark. We detected two ¹³C NMR signals of Tyr57, 79, 133 and 185 at 293K by REDOR filter experiments, and these are corresponding to the protein with two different retinal configurations in the dark. Consequently, the two major peaks at 176.0(α -helix) and 174.0(disorder state) ppm in ¹³C CP-MAS NMR spectrum of [1-¹³C]Tyr-labeled BR were assigned to all-*trans* and 13-*cis* states, respectively.

Here, we examined spectral pattern of NMR signals of [1-¹³C]Tyr-labeled BR

at various temperatures and T₁ of ¹³C nuclei to understand conformational change and dynamics corresponding to the two retinal configurations in the dark. The relative signal intensity ratio of ¹³C CP-MAS NMR of [1-¹³C]Tyr with all-*trans* to 13-*cis* at 213K slightly increased compared with that at 293K. This result indicates the possibility that disorder states change to α -helix or retinal configuration change from 13-*cis* to all-*trans*. Additionally, stepwise increase of signal intensity in the low temperature range was observed. This may correlate to freeze of water and gel to liquid crystalline phase transition of lipid. T₁ value of Tyr corresponding to all-*trans* state at 176.0 ppm was shorter than that to 13-*cis* state. This result indicates that dynamics in the vicinity of Tyr in all-*trans* state is slightly higher than that in 13-*cis* state.

3P004 溶液 NMR 法を用いた微小管集積因子 EB1 の構造研究
Structural studies of microtubule pulse-end tracking protein EB1 by solution NMR spectroscopy

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EB1 is a member of plus-end-tracking proteins (+TIPs) that bind to microtubule (MT) plus-end and regulate MT dynamics. EB1 binds to MT with its N-terminal CH domain. Cryo-EM study suggested that Mal3p, the EB1 homolog, specifically bound to MT A-lattice and thus promoted its polymerization(1). Further, EB1 leads MTs to cell peripherals by interactions between its C-terminal domain and other +TIPs, such as APC, and cytoskeletal proteins.

We have attempted to reveal the molecular basis of MT-regulating mechanisms by EB1 using NMR spectroscopy. Chemical shift perturbation experiments of EB1 C-terminal domain with APC C-terminal region revealed that APC bound to EB1 via I2805 and P2806. This observation was consistent with previous report(2). Moreover, we found that EB1 bound to APC by the same binding interface for dynactin subunit p150^{glued}. These results suggest the possibility that APC could compete p150^{glued} by interacting with EB1. In addition, chemical shift perturbation experiments of CH domain with taxol-stabilized tubulin or colchicine-depolymerized tubulin, we found that CH domain bound to polymerized tubulins specifically in solution. To identify the binding interface of CH domain for MT, we are trying to apply the transferred-cross saturation experiment(3) at present. Taken together, we will discuss molecular basis of MT-regulating mechanism of MT dynamics by EB1.

(1)Sandblad, L., *et al.* Cell, 127, 1415-24 (2006) (2)Honnapa, S., *et al.* Cell, 138, 366-76 (2009) (3)Takahashi, H., *et al.* Nat Struct Biol, 1,53-8 (2000)

3P005 Structural basis for the transcriptional repression mediated by SHARP/SMRT complex

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SHARP, a transcriptional corepressor that contains RNA binding domain (RRMs) and transcriptional repressor binding domain, SPOC, is known as a component of transcriptional repressor complexes functions in nuclear receptor signaling pathway. SPOC domain of SHARP binds to the C terminus of corepressor SMRT. SHARP/SMRT complex recruits histone deacetylase (HDAC) to chromatin and regulates transcription negatively. In this study, we have determined the tertiary structure of SHARP/SMRT complex to establish the molecular recognition mechanism by using solution NMR. SPR experiments clearly showed that SMRT peptide (2492-2517) tightly bound to SPOC domain of SHARP in the certain condition. Intriguingly, the affinity was decreased with 1000 fold at normal condition. We next determined the minimal region of the SMRT peptide for the interaction using NMR. HSQC experiments showed that C-terminal 8 residue region (2510-2517) of SMRT is prerequisite for the interaction. Using standard NMR technique, we performed NMR resonance assignments of SPOC/SMRT complex. Though we failed to obtain reliable assignments for SMRT peptide in the complex using isotope filtered NMR experiments, we introduced ¹³C and ¹⁵N labeled amino acids and solved ambiguities. Taken together, we collected enough structural information, and we performed structure calculations. The determined complex structure provided structural basis for the SHARP-SMRT interaction. The detailed interaction and its biological significance also discussed in the conference.

3P006 タキプレシン I-LPS 複合体の立体構造解析
Structural analysis of Tachyplesin I in complex with LPS

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Lipopolysaccharide (LPS) is a major component of outer membrane of gram-