2G1424 calmodulin が制御する 4.1G の天然変性ドメインの構造解析**Structural analysis of calmodulin binding to intrinsically disordered domain in protein 4.1G by NMR and solution X-ray scattering**

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We have documented that protein 4.1G (4.1G) interacted with transmembrane proteins through its 30kDa FERM domain. 4.1G has an intrinsically disordered region in its N-terminal 209 amino acid residues (GHP). Ca²⁺-saturated calmodulin (CaM) bound to GHP and regulated 4.1G binding to transmembrane proteins [Nunomura *et al.* (2010) *Biochem. J.* **432**: 407]. In the present study, we determined the CaM binding site of GHP and analyzed the structure of the CaM-GHP complex by small angle X-ray scattering (SAXS).

NMR analysis indicated that spectra of 18 residues of CaM were changed by binding to pepG (SRGISRFIPWLKKQKS) that was predicted based on the 4.1R¹³⁵ binding sequence [Leclerc & Vetter (1998) *Eur. J. Biochem.* **258**: 567]. The helix content of pepG was 41% based on CD measurement. The complex of pepG/CaM formed relaxed globular and its radius of gyration (R_g) was 19.7Å. Although GHP formed coil structure and its R_g was 47.6Å, the complex of CaM/GHP formed domain structure and its R_g was 37.0Å. We conclude that although the GHP is intrinsically disordered coiled structure, CaM-bound GHP complex adopts a domain structure that is composed of some subunits.

2G1436 植物由来抗菌ペプチド snakin-1 の立体構造解析へ向けた研究**Structural analysis of plant antimicrobial peptide snakin-1**

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Antimicrobial peptides (AMPs) have been isolated from a wide variety of organisms, including animals, bacteria, insects and plants. More than 500 AMPs have been discovered in plants and animals, in which they play important roles in the innate defense against invading microorganism. Snakin-1 is a new type of antimicrobial peptide isolated from potato tubers. The peptide has 63 amino acid residues, 12 of which are cysteines, and its amino acid sequence shows similarity of the GASA family from Arabidopsis and GAST family from tomato. Snakin-1 exhibits antibacterial and antifungal activity in vitro and causes rapid aggregation of both Gram-positive and Gram-negative bacteria. In this study, we aim to elucidate the snakin-1 structure using solution NMR. At first, we have established E.coli expression system for snakin-1. The expressed snakin-1 in E.coli existed in form of inclusion body and was purified by cation exchange chromatography under denaturation condition. To form correct disulfide bonds, we optimized refolding condition. After refolded, we analyzed the structural properties of snakin-1 by reverse phased HPLC. The far-UV CD spectra of recombinant snakin-1 indicates that the second structure of the peptide contained α -helix.

2G1448 線虫由来抗菌ペプチド ABF-2 の発現、精製と立体構造解析**Expression, purification and structure analysis of ABF-2 (Anti Bacterial Factor-2), an antimicrobial peptide derived from C.elegans**

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ABF-2 (Anti Bacterial Factor-2) is an antimicrobial peptide consisting of 67 residues stabilized with four intramolecular disulfide bonds and has an important role in the innate immune system of the genetic model *Caenorhabditis elegans*. Although the structure of ABF-2 is predicted to be CS α -type peptide, it has not been clear yet. In recombinant expression systems of antimicrobial peptides, such as ABF-2, it is often an issue that target proteins suffer from proteolytic degradation and are lethal to host cell. Therefore, we devised coexpression system of oppositely charged aggregation-prone partner protein that enhanced the expression level of ABF-2. It is possible to isolate ABF-2 and partner protein from insoluble inclusion bodies without any chemical or enzymatic cleavage step by simple cation-exchange chromatography. Then, we selected the appropriate strategy for solubilization, partial purification and refolding in order to obtain functionally active ABF-2 with high yield. In the

results, we obtained a large amount of partial purification ABF-2. After the refolding procedure, we obtained about 3 mg of active-form ABF-2 from 1 liter of LB medium, and a stable isotope-labeled ABF-2 for NMR studies was also successfully produced by this method.

2G1512 pCold-GST システムを用いたクラスBに属するG蛋白質共役型受容体N末端細胞外ドメインの試料調整**NMR sample preparation of N-terminal extracellular domain of type B G protein-coupled receptor using pCold-GST system**

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G protein-coupled receptor (GPCR) containing seven trans-membrane helices plays an important role in the biological function, such as signal transduction and homeostasis. Type B GPCRs have an N-terminal extracellular domain containing about 150 amino acids. This domain has a major ligand-binding site, and a peptide hormone specifically binds to this domain. Some structures of this domain have been reported, although the refolding process is necessary. In general, the efficiency and success rate of the refolding process is expected to be low for the extracellular domains. Therefore an efficient method to produce the soluble and functional proteins of this domain is necessary to study the structure and ligand binding of other Type B GPCRs.

Here we have established the sample preparation method for NMR structural analysis of the N-terminal extracellular domain of type B GPCRs, using the novel cold-shock system: pCold-GST for high-level expression of soluble protein in E.coli (Hayashi and Kojima, 2008) and the mild refolding for NMR sample preparation. We have applied this method to the extracellular domain of 9 GPCRs. For 7 out of 9, the soluble proteins of this domain were obtained. So far, the extracellular domain was purified and exhibiting a single band on SDS-PAGE for two GPCRs, and optimizing the refolding condition, a high quality NMR spectrum was obtained for one of two whose structure is not yet reported.

2G1524 Exclusive binding of myosin and cofilin to F-actin

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It is well known that cofilin and myosin II in crawling cells are localized in the anterior and posterior regions, respectively. However, the mechanism of the differential localization remains unknown. In previous meeting, we reported the cooperative binding of HMM from myosin II (Toya *et al.*, 2009) and cofilin (Nakajima *et al.*, 2010) to F-actin. These results lead us to a hypothesis that the differential localization in crawling cells is due to their cooperative interactions to F-actin. That is, if cofilin (or myosin) cannot bind to myosin- (or cofilin-) bound F-actin, we might be able to explain the differential localization. To confirm this hypothesis, we examined the binding feature of HMM fused with GFP (HMM-GFP) and cofilin fused with mCherry (mCherry-cofilin) to F-actin pre-bound by the other, using fluorescence microscopy. Since the cooperative binding was observed when 100 nM mCherry-cofilin or 2 nM HMM-GFP was mixed with 40 nM F-actin in the presence of 0.5 μ M ATP, we examined their binding under these concentration conditions. When HMM-GFP was added to F-actin pre-incubated with mCherry-cofilin, most HMM-GFP bound to bare F-actin but not cofilin-bound F-actin. Similarly, when mCherry-cofilin was added to F-actin pre-incubated with HMM-GFP, most mCherry-cofilin also bound to bare F-actin. This exclusive binding suggests that the cooperative binding of myosin II and cofilin to F-actin may contribute to the differential localization in crawling cells.

2G1536 酸素耐性[NiFe]ヒドロゲナーゼの構造化学的研究**Structural study of the O₂-tolerant [NiFe] hydrogenase**

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Membrane-bound respiratory [NiFe] hydrogenase (MBH) is an H₂-uptake enzyme found in the periplasmic space of bacteria that plays a crucial role in energy conservation processes. In contrast to the "standard" [NiFe] hydrogenases that are reversibly inactivated by O₂, a remarkable feature of this enzyme is its O₂-tolerant H₂ oxidation activity, the mechanism of which is poorly understood. In order to identify the determinant for the O₂-tolerance, X-ray crystal structures