2P025 Single molecule force spectroscopy reveals force-enhanced binding of calcium ions by gelsolin

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Force is increasingly recognized as an important element in controlling biological processes. Forces are able to deform native protein conformations leading to protein-specific effects. Here we demonstrate that the calcium-binding affinity of the actin-binding protein gelsolin domain G6 is enhanced by mechanical force. Using a recently developed single molecule-binding assay based on atomic force microscopy, we establish that the calcium-binding affinity of G6 increases exponentially with the applied force, up to the point of G6 unfolding. This implies that gelsolin will be activated at lower calcium ion levels when subjected to tensile forces and suggests a basis for enhanced cooperativity during multi-cation induced activation.

2P026 Direct observation of the multiple sliding modes of a tumor suppressor p53

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1D sliding of a tumor suppressor p53 along DNA is an essential dynamics for its effective search of the target sequence. The 1D sliding of p53 has been assumed to be described as a single diffusive movement. However, it is conceivable that p53 has multiple sliding modes depending on the quaternary structures of the p53-DNA complex. In this study, the sliding of p53 along DNA was measured by a single-molecule fluorescence microscopy. The observed trajectories of p53 were analyzed by the change point analysis, which can detect boundaries of trajectories with different diffusion constants. Our results showed that p53 possesses at least three different diffusion modes, and further suggests that the quaternary structures of the p53-DNA complex modulate the sliding modes.

2P027 Study of a peptidase-associated domain of an aminopeptidase from thermophilic *Aneurinibacillus* sp. AM-1

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A thermophilic bacterium *Aneurinibacillus* sp. AM-1 produces an aminopeptidase (AM1AP) belonging to M28 family. The enzyme shows strong prolyl aminopeptidase activity toward prolyl-*p*-nitroanilide. X-ray crystal structure of AM1AP revealed that the enzyme is composed of a peptidase domain (PD, 31 kDa) and a peptidase-associated domain (PAD, 14 kDa). PD, typical for M28 family enzymes, contains an active site with two zinc atoms, whereas PAD widely occurs in peptidases/proteases beyond this family. To date, the roles of PAD is undefined yet. To elucidate its roles, PD and PAD was separately expressed in *E. coli*, and purified, and then their molecular properties (specific activity, gel filtration, CD in the presence and absence of zinc ion, etc) were investigated.

2P028 ケモカインシグナル細胞内制御因子 FROUNT とその受容体認識に関する構造生物学的研究**Structural analyses of FROUNT, the cytosolic regulator of chemokine signaling, and its chemokine receptor recognition**

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Chemokine-dependent migration of the leukocytes to sites of inflammation is essential for immune defense, but also causes autoimmune inflammatory diseases. We identified a cytosolic regulator of the chemokine signaling, FROUNT, which specifically binds to the C-terminal regions (Pro-Cs) of chemokine receptors CCR2 and CCR5. The aim of this study is to elucidate the Pro-C recognition mechanism of FROUNT. We revealed the three-dimensional structure of the chemokine receptor-binding domain of FROUNT (FNT-C) and its Pro-C binding site by NMR. We built a docking model of FNT-C and Pro-C, using the Pro-C structure we previously determined. Current studies of the FROUNT-CCR2/5 interaction provide a structural basis to develop anti-inflammatory agents with high specificity.

**2P029 トウガレイ由来 I 型不凍蛋白質の構造機能解析
Analysis of structure and function of a new type I antifreeze protein from a Japanese fish, Barfin Plaice**

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Antifreeze proteins (AFP) bind onto an ice crystal to arrest its crystal growth within a temperature range, which was called thermal hysteresis (TH). An alpha-helical type I AFP from winter flounder (denoted WfAFP) exhibited 1 degC of TH at its solubility limit. In contrast, hyperactive AFPs from insects and bacteria exhibited higher TH values (ex. 3 - 5 degC). Here we found that a 40-residue AFP from Barfin Plaice (BpAFP) exhibited approximately 3 degC of TH. It has 80 % of sequence identity with WfAFP, while the mechanism of such high activity is unknown. Here we present biochemical properties of BpAFP, for which multinuclear multidimensional NMR experiments were performed for a ¹³C and ¹⁵N-labeled protein of BpAFP.

2P030 ジスルフィド結合が制御するバクテリア SOD1 の構造形成メカニズム**Folding mechanism of bacterial SOD1 regulated by disulfide formation**

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Cu,Zn-superoxide dismutase (SOD1) is an enzyme that scavenges superoxide radicals, and is conserved among aerobic organisms. To gain catalytic activity, SOD1 requires two post-translational modifications: binding of a copper ion and formation of a disulfide bond. In eukaryotes, these modifications are carried out simultaneously by a copper chaperone, CCS. In bacteria, however, no CCS homologue is present, and the activation mechanism of bacterial SOD1 remains unknown. Here we show that, in *E. coli* SOD1, formation of the disulfide bond precedes binding of metal ions and is crucial for attaining a structure that is resistant to degradation *in vivo*. In bacteria, a pool of apo-SOD1 with disulfide would thus exist waiting for a copper ion to become enzymatically active.