the family of calcium regulatory proteins. Bisphenol A (BPA), which is a synthesis estrogen, is widely used in the manufacture. It has been investigated that BPA disrupts estrogenic actions mainly through their binding to estrogen receptors basically in the cell level even if a very small dose is used. So far, in spite of many studies concerning BPA, still it has not been clear in molecular level whether interaction of BPA with essential material in a various cellular signaling mechanisms affects a physiological function. Therefore, it is very important to examine interaction of CaM with BPA for understanding a basic biological process of calcium signaling in a cell exposed to EDCs.

The calcium affinity of CaM in the absence and presence of BPA were investigated by fluorescence spectroscopy and isothermal titration calorimetry (ITC). The Gibbs free energies obtained from calcium titration of CaM by fluorescence were decreased with increasing in BPA concentration. ITC study of CaM in the absence of BPA resulted that BPA forms hydrophobic interaction and hydrogen bonding with CaM. With increase of BPA, hydrogen bonding between CaM and BPA becomes governed more than hydrophobic interaction. The results exhibit that BPA weakens the calcium affinity of CaM. Here, we will report (1) characterization of interaction between CaM and BPA and (2) calcium affinity of CaM in presence of BPA in detail.

1TA1-04 細胞内アクチンの SS 結合による二量体の研究

1P-030 A study on cytoplasmic actin SS dimers.

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Actin filaments provide the structural basis for cell migration and are therefore critical for numerous physiological processes such as morphogenesis, wound healing, and immune response. During cell migration, actins are reorganized through polymerization, depolymerization, branching, and severing at the leading and the trailing edges. Actin branching, a process that nucleates new filaments by creating branches off the sides of existing filaments by means of the Arp2/3 complex, plays an important role in generation of actin networks at specific locations within the cell, such as at the distal part of lamellipodium. On the other hand, the freeze-thawing of actin solution induces oxidation of sulfhydryl groups, which results in the formation of an intermolecular disulfide (SS) bond to yield an actin dimer (SS dimer) (Ishiwata, S., J. Biochem., 80, 595-609, 1976). The SS dimers are incorporated into F-actin during polymerization process and form cross-links between actin filaments, besides it was reported that the branching like that produced by Arp2/3 complex occurs (Tang, J. et al., Biophys. J. 76, 2208-2215, 1999). These suggest that SS dimers act in actin filaments similarly to Arp2/3 complex. To understand the mechanism of formation of SS dimers and the cross-linked actin filaments, we expressed the recombinant beta-actins, in each of which one of the six cysteine residues was replaced by serine. At this meeting we report in detail the formation of SS dimers of mutant actins and the properties of the cross-linked and branched filaments.

1TA1-05 Gads の精製と CD28 細胞質内ドメインとの相互作用

1P-031 Purification of Gads and its interaction with CD28 cytoplasmic domains

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CD28 provides costimulatory signals that are required for full T cell activation and development of effector T cells. The cytoplasmic region of CD28 contains one SH2 and two SH3 binding motifs. Grb2-related adaptor downstream of Shc (Gads) has a central SH2 domain flanked by two SH3 domains. The SH2 binding to the site in CD28, pYMN, is critical, while the SH3 binding to the site(s) in CD28, PXXP, is still controversial. We overexpressed Gads in *E. coli* with N-terminal thioredoxin-His-tag, and purified it using Ni-affinity and gel-filtration chromatographies. Although Gads tends to be aggregated in the purification procedure, it could be purified by the Arg addition. The cytoplasmic region of CD28 was prepared by the peptide synthesis to specifically phosphorylate Tyr at the SH2 binding site and biotinylate at the N-terminus, and immobilized on the Biacore streptavidin sensor chip to analyze the interaction with Gads. The binding affinity of intact Gads was about twenty-times higher than that of Gads SH2, previously determined, indicating that Gads SH3 also contributes to the CD28 binding. However, unexpectedly, the binding affinity of intact Gads to the mutant CD28, in which Pro residues in the SH3 binding sites are mutated to Ala, was similar to that of the wild-type CD28. One of the possible explanations is that Gads SH3 might be able to bind the mutated sites in CD28. To confirm this possibility, we prepared another CD28 mutant, in which the C-terminal residues containing the SH3 binding sites are deficient, and analyze its interaction with Gads.

1TA1-06 非機械感受性イオンチャネルは本当に機械刺激によって開かない

1P-033 か? - 粗視化分子力学シミュレーション研究

Does a non-mechanosensitive ion channel really not open by mechanical force? - A coarse grained molecular dynamics simulation study

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Molecular dynamics (MD) simulations are widely used to reveal biophysical properties of mechanosensitive (MS) ion channels. MD simulations demonstrate that stretching the lipid bilayer surrounding the bacterial MS channel MscL opens its pore. However, few studies have treated the effect of membrane stretch on the behavior of non-MS channels. We compared the difference in conformational change between MS- and non-MS-channels in response to membrane stretch using the coarse grained (CG) MD simulation. The MARTINI force field, run on the GROMACS MD software, was used to model CG systems. We chose MscL (PDB: 2OAR) as an MS channel and NaK (PDB: 2AHY), a nonselective cation channel, as a non-MS channel. Either one of these proteins was embedded in a DOPE bilayer in a water-filled, 13.2 x 13.2 x 38.4 nm periodic boundary box. A tension of 68.2 mN/m was generated in the membrane in each simulation for 400 ns. In response to tension, the inner diameter along the transmembrane axis of MscL increased drastically. The minimum pore diameter increased up to 3.9 nm from the initial value of 2.3 nm without tension. Meanwhile, the inner diameter of NaK was hardly changed by tension. The minimum pore diameters before and after tension were 1.8 nm and 2.0 nm, respectively. Furthermore, the inner diameter of MscL showed a tendency to return to its initial conformation when tension was released. These results support the notion that MS channels have specific structures that lead to conformational changes in response to surrounding force field.

1TA1-07 時間領域テラヘルツ分光法でみる低振動領域におけるタンパク質

1P-029 の動力学転移

Low-frequency dynamical transition of proteins monitored by terahertz time-domain spectroscopy

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Terahertz time-domain spectroscopy (THz-TDS) provides two physical quantities, refractive index and absorption coefficient in the frequency region of 3-50 cm⁻¹, by analyzing the time-dependent waveform of THz radiation. From these two parameters, reduced absorption cross section (RACS), which corresponds to a product of IR activity and density of states (DOS) can be obtained. It is computationally-reported by Daniel ben-Avraham that the densities of vibrational normal modes of globular proteins as a function of frequency collapse into a universal curve. This predicted universality of proteins is reproduced by RACSs of several globular proteins in the following two points. One is that the RACSs spectra of these samples overlap considerably each other and the other is the similarity of power law features in the double logarithm plot. This fact indicates that IR activity, the element of RACS, can be considered as a constant in this frequency region and RACS is proportional to VDOS. Inelastic neutron scattering works have reported that the dynamical transition at ~230 K, observed for highly hydrated proteins shows the onset of anharmonic motions. Then temperature and hydration condition of hen egg white lysozyme (HEWL) pellets are changed to examine whether such fluctuation can be monitored by using RACS or not. HEWL solution are adjusted to several pH (pH=3, 5, 7, 9, 11) and then lyophilized to obtain solid samples. For highly hydrated HEWL pellets, the same kind of transition point can be observed around 230K without relation to pHs.

1TA1-08 βラクタマーゼによるセファロsporinの加水分解活性と、そのアシル中間体の熱安定性との相関

The correlation between the catalytic susceptibility of cephalosporins and the thermal stabilities of their beta-lactamase acyl intermediates.

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Introduction

The extended-spectrum beta-lactamases causes the serious antibiotic resistance. Toho-1, a CTX-M-type beta-lactamase, which efficiently hydrolyzes a first generation cephalosporin **cephalothin** in addition to **cefotaxime**, a third generation cephalosporin. However, this enzyme only marginally hydrolyzes the third generation cephalosporin, **ceftazidime** and a monobactam, **aztreonam**. Deacylation defect mutant Toho-1 E166A, that prevents deacylation resulting in accumulating the complex of each acylated cephalosporin intermediate. To elucidate the acyl-intermediates structures quantitatively and interpret the structures into quantitative parameters, we measured the thermal stabilities of acyl-intermediates of antibiotics for 14 species including these crystal structures, using DSC.

Results

The $\ln(k_{cat})$, $\ln(1/K_m)$, and $\ln(k_{cat}/K_m)$ for Toho-1 showed linear correlation with T_m , which is the midpoint of heat-denaturation temperature of the corresponding

acylated complex of Toho-1 E166A. The best correlation was observed at T_m vs. $\ln(k_{cat}/K_m)$. We have a hypothesis that the stability and/or the thermal fluctuation of the catalytic intermediate correlates the catalytic parameters quantitatively, because the results of X-ray studies showed that the poor substrate greatly reduces anisotropy of experimental atomic fluctuation of the protein atoms than that in the case of the good substrate, in crystal structures of acyl-intermediates.

1TA1-09 新規デフェンシンの膜構造と抗菌活性

1P-032 A new strategy of defensin against Gram-positive bacteria

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Big defensin is a 79-residue peptide derived from hemocytes of the Japanese horseshoe crab. It has antimicrobial activities against Gram-positive and -negative bacteria. The amino acid sequence of big defensin can be divided into an N-terminal hydrophobic half and a C-terminal cationic half. Interestingly, the trypsin cleaves big defensin into two fragments, the N-terminal and the C-terminal fragments, which are responsible for antimicrobial activity against Gram-positive and -negative bacteria, respectively. We determined the solution structure of mature big defensin and elucidated that the C-terminal domain of big defensin adopts a β -defensin structure, while the N-terminal domain forms a unique globular conformation. In addition, the N-terminal hydrophobic domain undergoes a conformational change in micelle solution. We proposed that the N-terminal domain achieves its antimicrobial activity in a novel fashion, and big defensin has developed a different strategy from other β -defensins to suppress the growth of Gram-positive bacteria. For further analysis, we determined the structure of the N-terminal fragment of big defensin in a micellar solution, showing that the fragment peptide forms a single α -helix structure. Moreover, NMR experiments using paramagnetic probes revealed that the N-terminal domain of big defensin penetrates into the micelle. Here, we propose a new model for big defensin to associate with the target membrane.

1TA2-01 成熟した横紋筋における微小管と適応における役割：チューブリンの分子シャペロン α B-クリスタリンの細胞内機能から

Significant roles of microtubules in mature striated muscle: Insight from correlative expression of tubulin and its molecular chaperone α B-crystallin

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The cytoskeleton is an essential component for the cell. One of the cytoskeleton, microtubule (MT) is well characterized in mitotic cells and in vitro, while the role of MT in matured striated muscles has not been elucidated yet. We have shown the possible role of molecular chaperone α B-crystallin to protect disturbance of denatured tubulin and keep MT network in the process of muscle atrophy. To understand the significance of cytoskeletal microtubule networks in striated muscles, we analyzed correlation between the content of tubulin and α B-crystallin in a variety of striated muscles expressing different myosin heavy chain (MHC) isoforms. The content of both tubulin and α B-crystallin was larger in MHC-I dominant soleus muscle and in MHC- α dominant cardiac (atrium and ventricle) muscles; intermediate in MHC-IIb dominant diaphragm and tongue muscles; and smaller in MHC-IIb dominant plantaris, gastrocnemius, masseter, psoas, and extensor digitorum longus muscles. Since the muscles of slow type MHC (MHC-I/ α) show the most economical features in their function and metabolism, which suit for continuous activity required to sustain posture and blood pumping, the present results afforded additional support to our hypothesis that microtubule networks transduce mechanical environmental demands to morphological and biochemical responses that eventually evolve adaptive transformation in the function and metabolism of the mature muscles. The comparison of tubulin/ α B-crystallin ratios across the muscles of varied MHC isoforms further suggested that mechanical stress fluctuating at the rhythmic frequency of walking and breathing efficiently activates the hypothesized dynamic function of microtubules.

1TA2-02 平滑筋ミオシン I による F アクチン束化のメカニズム

Mechanism of F-actin bundling by smooth muscle myosin I

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Myosin I is a large family of unconventional myosins. We have found and characterized myosin I in aorta smooth muscle which has homologous amino acid sequence of myosin IB of bovine brain and adrenal gland. (Hasegawa et al.; 1996, 1998). The aorta myosin I consisted of equimolar amounts of 110kDa heavy chain and calmodulin. The myosin I could bind another calmodulin at the

most in the presence of exogenous calmodulin. Since architecture of contractile system in smooth muscle cell is not well understood, we have examined a possible contribution of the aorta myosin I for alignment of F-actin filaments into the bundle. The properties of F-actin bundle with aorta myosin I was characterized as follows. (1) The bundle formation is a process which depends on electrostatic interaction of proteins. I.e. increasing of ionic strength blocks appearance of F-actin bundle with bound myosin I. (2) Either flexibility or site specific inhibition on F-actin regulates the myosin I dependent bundling, i.e. Tropomyosin inhibits the F-actin bundling in a saturable manner. (3) Another F-actin binding site located in the tail region of myosin I. i.e. The F-actin bundling could be inhibited by phospholipids in a specific manner such as in the presence of phosphatidyl glycerol but not with phosphatidyl choline. This is consistent with our previous findings that phosphatidyl glycerol bind to the tail of myosin I but not with phosphatidyl choline (ibid; 1996). It is keen to find out the switching mechanism of binding partners, F-actin or phospholipids, for elucidation of the role of F-actin bundle in smooth muscle cell.

1TA2-03 骨格筋ミオシンの尾部の脆弱部位の予測

Prediction of fragile points of skeletal myosin rods

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It is reported that muscle and non-muscle myosin rods have a flexible region in their coiled coil region. Usually these regions were highly conserved; therefore it is considered they play an important role in muscle contraction. Here we show that it is possible to identify flexible region of coiled coil only from the hydrophobic features of amino acid sequences. To evaluate the result of prediction, we directly analyzed the bend positions of myosin rod using atomic force microscopy (AFM) and compare these results with prediction. The results of AFM imaging showed four main flexible regions in a single myosin rod and of the 17 possible fragile points predicted, 16 were located in the four experimental bending regions. As a result, our prediction showed a very good correlation to the four flexible regions derived from that of skeletal myosin.

1TA2-04 非同期型昆虫飛翔筋のカルシウムによる張力発生と伸張による張力発生は異なる温度依存性をもつ

Calcium-activated and stretch-activated forces have different temperature dependence in asynchronous flight muscle of insect

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Besides the normal calcium-induced activation, insect flight muscle (IFM) is also activated by stretch. This stretch activation is considered essential for high-frequency wingbeat of asynchronous insect flight muscle, but its molecular mechanism remains unclear. To further address this issue, we examined the temperature dependence of calcium-activated (CA) and stretch-activated (SA) forces of IFM. The IFM of a flying bumblebee is endothermically maintained at $\sim 42^\circ\text{C}$ regardless of ambient temperature. Isolated, skinned IFM fibers of bumblebee still develops substantial CA force of ~ 80 KPa and also SA force at $\sim 20^\circ\text{C}$, saturating $[\text{Ca}^{2+}]$. If the temperature is lowered to $\sim 5^\circ\text{C}$, the fibers develop little CA force. Surprisingly, lowering temperature did not inhibit SA force. There is a critical temperature of CA force development at around 15°C , while the SA force has bell-shaped temperature dependence with optimal temperatures at $10\sim 20^\circ\text{C}$. These observations suggest that the inhibition of CA force at low temperatures is not due to the impaired function of myosin, but it is an issue of regulation. To examine how the thin-filament regulatory system is implicated in this dual temperature sensitivity, the behavior of thin-filament-based X-ray reflections is currently being recorded from calcium-activated and cyclically stretched IFM fibers at low and high temperatures.

1TA2-05 中性子スピネコー法によるアクチン分子の運動の測定

Measurements of motions of actin by neutron spin-echo spectroscopy

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F-actin plays important roles in various functions related to cell motility. Such multiple functions of F-actin originate from flexibility of F-actin that makes interactions with various actin-binding proteins possible. To understand flexibility of F-actin and thereby its multiple functions, it is important to understand dynamic properties of F-actin at various levels from internal dynamics of the monomers through relative motions between the monomers to large-scale motions of F-actin. As one of the ongoing projects towards this ultimate purpose, we employed neutron spin-echo (NSE) spectroscopy to investigate the motions of F-actin on pico-to-nanosecond timescales and nm spatial scales, which correspond to the scales on which motions such as diffusions of the proteins, domain motions of the protein, and relative motions of the subunits within the macromolecular complex occur. We carried out the NSE experiments of F-actin and G-actin with