2P073 コラゲナーゼによるコラーゲン原繊維分解過程の高速 AFM 観察

High speed AFM observation for degradation process of collagen fibril by collagenase

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Collagen is a major protein (~30%) in mammalian body and resistant to common proteinases, while digested by collagenases. The degradation of collagen fibril is very important in biological function and its biomaterial application. However, the molecular mechanism of digesting collagen by collagenase remains unclear whereas it has been intensively investigated by indirect methods. In this study, we applied high speed AFM and succeeded to directly observe the collagen fibrils showing characteristic ~67 nm periodic banding pattern and the degradation process of the fibrils by bacterial collagenase. These direct observations will provide new insights into understanding the mechanisms of collagenase activity at single molecule level.

2P076 FTIR 測定でみたエクспанシン活性 Exapnsin activity observed by FTIR

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Expansin is one of the plant cell wall proteins, and plays an important role for the cell expansion. The direct activity of expansin is believed to be the cleavage of hydrogen bond between polysaccharides in the cell wall, which allows the wall to be loosen and remodeled for the cell expansion. This hypothesis is generally accepted, while it is difficult to provide the evidence. We challenged this hypothesis with FTIR analysis of intracrystalline deuterated cellulose. The hydroxyl groups in the cellulose crystallite usually do not accept H/D exchange because of hydrogen bonding in and between molecules. We however show that expansin actively allows H/D exchange in the crystallite, supporting that expansin disturbs hydrogen bonding in cellulose crystallite.

2P074 タンパク質ジスルフィドの異常が神経変性疾患の発症に果たす役割：線虫を用いた表現型解析

Investigating the Role of SOD1 Cysteine Residues in Neurodegeneration using *C. elegans*

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Mutations in Cu,Zn-superoxide dismutase (SOD1) is known to cause amyotrophic lateral sclerosis (ALS), and formation of insoluble SOD1 aggregates in affected motoneurons is a pathological hallmark. Cysteine residues of SOD1 have been proposed to play a key role in aggregation by forming abnormal disulfide cross-links; however, roles of such abnormal disulfides in the development of this disease remain obscure. Here, we investigate pathological effects of cysteine residues in mutant SOD1 on ALS by constructing *C. elegans* models. Worms that express ALS-mutant SOD1 lacking cysteine residues in neurons have been examined to clarify effects of cysteine residues on disease-related phenotypes.

2P077 T-, L-プラスチン EF-hand の Ca²⁺感受性の違い Different Ca²⁺-sensitivities between the EF-hands of T- and L-plastins

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Plastins are Ca²⁺-regulated actin-bundling proteins, and essential for developing and stabilizing actin cytoskeletons. T-plastin is expressed in epithelial and mesenchymal cells of solid tissues, whereas L-plastin is expressed in mobile cells such as hemopoietic cell lineages and cancer cells. Using various spectroscopic methods, gel-filtration chromatography, and isothermal titration calorimetry, we here demonstrate that the EF-hand motifs of both T- and L-plastin change their structures in response to Ca²⁺, but the sensitivity to Ca²⁺ is lower in T-plastin than in L-plastin. These results suggest that T-plastin is suitable for maintaining static cytoskeletons, whereas L-plastin is suitable for dynamic rearrangement of cytoskeletons.

2P075 青色光センサータンパク質フォトトロピン 1 LOV2 ドメインの光反応に対するクラウディング効果

Crowding effect on the reaction dynamics of blue light sensor protein phototropin1 LOV2 domain

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Intracellular environment in which proteins play their roles is highly condense with a lot of macromolecules such as proteins, RNAs. Such crowding environment has several effects on protein's structure, oligomeric state and reaction. In this study, we have investigated the crowding effect on reaction dynamics of blue light sensor protein phototropin1 LOV2 domain. Photoexcitation of FMN leads to a dissociation and subsequent unfolding of C terminal α helix of LOV2 domain in dilute buffer conditions. In the presence of Ficoll 70, the crowding effect was observed as decrease of reaction rates of these steps and increase of the diffusion coefficient of the product molecule, indicating the conformation of protein tends to be compact due to the exclusion volume effect.

2P078 新規金表面親和性ペプチドの同定及びタンパク質固定化への応用

Screening of a novel gold affinity peptide and its application on protein immobilization

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In the field of biotechnology, gold has been used as a useful substrate for biosensor as it has excellent properties such as corrosion resistivity, high electrical conductivity, and so on. In that case, immobilization of proteins with maintaining their conformation and controlling their orientation are very important factors for higher interaction detection sensitivity. In this research, we screened novel 6 a.a. gold affinity peptides (Au-tags) from T7 phage random peptide library, and their characteristics were investigated using various Au-tag conjugated esterases. Examination of remaining activities after immobilization and analysis of adsorption characteristics by SPR suggested that rational design of Au-tag conjugation pattern would be important.