1P073**Development of a single molecule fluorescence microscope with rapid solution switching system using microfabrication technique**

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Fluorescence microscopy is a powerful tool for biological assays. However, current techniques do not allow exchanging sample solutions within hundred milliseconds, limiting analyses of kinetics of bio-molecules. To overcome this issue, we have developed microfluidic channel with pneumatic valves using microfabrication technique. When two solutions are introduced into a microfluidic channel, laminar flows are formed. Change in flow rates of two solutions results in shifting of the boundary of the laminar flows. This enables us to rapidly change solutions in the area of observation, and besides, to observe kinetics of single fluorescent molecules under microscopy. Using this device, we have analyzed the dissociation of chaperonin GroEL and cochaperonin GroES. Biotin-labeled GroEL (D490C) was immobilized on the glass surface via streptavidin and biotinylated bovine serum albumin. 100 nM GroES labeled with IC5 (IC5-GroES) was added for about 200 ms (half-width) followed by washing unbound IC5-GroES with a buffer solution. IC5-GroES was observed by total internal reflection fluorescence microscopy and images were captured using an EM-CCD camera. The changes in fluorescence intensity were well fit by assuming two rate limiting steps of $0.29 \pm 0.028 \text{ s}^{-1}$ and $0.12 \pm 0.010 \text{ s}^{-1}$ ($n=3$, mean $\pm$ SD). This result was consistent with that obtained by single molecule imaging (Taguchi et al., 2001, Nat. Biotechnol.). In addition, we have succeeded in detection of fluorescence signal from single molecule.

1P075**Analysis of complex formation among FEN1, PCNA and 5'-flap DNA by Fluorescence Cross Correlation Spectroscopy**

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Flap endonuclease-1 (FEN1) and proliferating cell nuclear antigen (PCNA) play a crucial role in DNA replication and repair. FEN1 is a structure-specific endonuclease that recognizes a branched DNA structure consisting of a double-stranded DNA with 5'-unannealed flap (5'-flap DNA). PCNA binds FEN1 and stimulates its activity. Current knowledge of interactions among FEN1, PCNA, and 5'-flap DNA, therefore, raises one of critical questions "How does interaction of FEN1 with PCNA affect its activity". The purposes of this study are (1) to confirm the formation of FEN1/PCNA/5'-flap DNA triple complex in solution, and (2) to ascertain the regulatory mechanism of FEN1 activity by PCNA. The samples containing 5'-flap DNA labeled with fluorescence probe (Atto655) and human FEN1(D181A) fused to an enhanced green fluorescent protein (FEN1(D181A)-EGFP) were prepared in the presence of human PCNA, and the interactions were monitored by using fluorescence cross-correlation spectroscopy (FCCS). We succeeded in detecting the triple complex formation of 5'-flap DNA, FEN1(D181A)-EGFP and PCNA in solution directly. In addition, two FEN1 mutants were used to verify whether the complex formation is increased or not. Furthermore, we studied the relationship between the complex formation and NaCl concentration, since it was known that FEN1 activity was highly influenced by salt concentration. Results of FCCS measurements suggested that there were significant correlations between the complex formation and FEN1 activity and the NaCl concentration.

1P074**Non-native structure of α -lactalbumin-fatty acid complex as an apoptosis induction factor**

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Alpha-lactalbumin (α -LA) is a small globular protein consisting of two domains, a large α -helical domain and a small β -sheet domain. The two domains are bridged by four disulfide bonds. There is a strong calcium-binding site and the absence of Ca leads to unstable the folding of α -LA. α -LA has been known to adopt a molten globule under low pH, Ca free and low salt condition, thermal stress or mild denaturants. α -LA takes a part in the components of lactose synthase in the lactating mammary gland, while an interesting feature has been reported that a complex of human apo- α -LA and oleic acid induces apoptosis in tumor cells but spares mature cells. The complex adopt a molten globule-like state at neutral pH. It was described that active complexes are only converted in an anion exchange column with ready-prepared oleic acid and recovered with high salt. The other mammal α -LA becomes less efficient in conversion. On the contrary, we have tried to produce the active complex in process of denaturation of α -LA by heat or pH treatment. As the result, it was achieved the complexes induce tumor-cell death as well as from anion exchange column. Furthermore, it is implied that complexes show different activity in treatment temperature, incubation time, and species between bovine and human. The quantitative analyses are performed in correlation between structural stability and cell-death activity of those complexes for recombinant α -LA expressed in *Escherichia coli* and authentic α -LA using CD spectroscopy and apoptosis assay.

1P076**Molecular basis for recognition of E-cadherin by killer cell-lectin like receptor, KLRG1**

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The killer cell lectin-like receptor (KLR) G1 is a cell surface receptor expressed on natural killer (NK) cells and T cells to inhibit immune responses. Recently, we and Pircher et al. have identified E-cadherin as a ligand of KLRG1. The other KLRs recognize major histocompatibility complex class I molecules (MHCIs), their relatives, or C-type lectin-like molecules. Since E-cadherin is functionally and structurally different from MHCIs and C-type lectins, the E-cadherin recognition by KLRG1 provides a novel immune regulation mechanism.

To understand the molecular interaction between KLRG1 and E-cadherin, we performed SPR (surface plasmon resonance) analysis using a series of variants of recombinant E-cadherin proteins. E-cadherin has five cadherin repeats (designated as D1-D5) in the extracellular region, and the recombinant N-terminal D1D2 (EC12) and D2D3 domains (EC23) are expressed in *E. coli* as inclusion bodies and refolded following the standard dilution method. The whole extracellular region of KLRG1 is prepared in similar way of E-cadherin. The SPR analysis demonstrated that KLRG1 can weakly bind to EC12 but not to EC23. The interaction does not show any effect of calcium ions, which exist in the D1D2 interface to regulate the rigidity of the D1D2 structure. Thus, these results suggested that D1 of E-cadherin mainly contributes to the KLRG1 binding, although D2 may have a supplementary role. The more detail binding and structural studies are undergoing.