1P557**Peptide bond formation induces the breakage of Shine-Dalgarno interaction on the ribosome**

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The ribosome is a molecular machine which translates the genetic code contained on the messenger RNA (mRNA) into an amino acid sequence through repetitive cycles of transfer RNA (tRNA) selection, peptide bond formation and translocation. Here we demonstrate a method for measurements of the rupture force between a single ribosome complex and the mRNA using an optical tweezer assay. The rupture force was compared between ribosome complexes assembled on an mRNA with and without a strong Shine-Dalgarno (SD) initial sequence. The removal of the SD sequence significantly reduced the rupture force in complexes carrying Phe-tRNA^{Phe} in the A site, indicating that the SD interactions contribute significantly to the stability of the ribosomal complex on the mRNA in a pre-peptidyl transfer state. In contrast, in the presence of the peptidyl tRNA analog, N-acetyl-Phe-tRNA^{Phe}, in the A site, which mimicked the post-peptide bond formation state, the rupture force was much weaker as compared to the complex with Phe-tRNA^{Phe}, and it was the same for both the SD-containing and SD-deficient mRNAs. This suggests that the formation of the first peptide bond disrupts the SD interaction resulting in the weakening of the force with which the ribosome is holding onto the mRNA. This might be an important requirement to facilitate the movement of the ribosome along mRNA during translocation.

1P559**Nano-indentation of Single Protein Using Atomic Force Microscopy**

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The atomic force microscope (AFM) is a microscope that traces on a sample surface with the sharp tip whose radius of curvature tens of nanometers, and makes the topographic image of the sample surface. The characteristic feature of the AFM is that the probe contacts the sample, which enables us to measure the mechanical property of the sample materials, or manipulate the sample on the substrate surface. In this paper, we have investigated the elasticity of various proteins at single molecular level utilizing this unique feature of AFM (Afrin et al., Protein Science 2005). The target molecules are human serum albumin, ferritin and apoferritin. Those molecules were immobilized covalently on an amino-silanized glass surface in isolated manner, and force curves were obtained on it by changing the pushing position slightly. We obtained two kinds of curves, one showed upward cantilever deflection before the tip reached the substrate surface and the other didn't. We found that the former curves were obtained in proportional to the number of immobilized proteins on the substrate surface. Therefore we concluded that those curves showed the process of pushing single protein. And we found that ferritin and apoferritin endure the external forces in several nano newtons and they have the rigidity equivalent to the glass. The details of viscoelastic properties of each protein will be discussed. This work was supported, in part, by NEDO "Functional Nano-Probes for Bio-property Mapping", and Japanese Science and Technology Agency".

1P558**Real-time Imaging of DNA-Streptavidin Complex Formation in Solution using a High-Speed Atomic Force Microscope**

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Direct observation of individual molecules in action is necessary in order to better understand the mechanisms of biological reactions. Using a high-speed atomic force microscope (AFM) in solution, we succeeded in visualizing short DNA fragments in motion. The technique represents a new approaches to analyzing molecular interactions, and it allowed us to observe real-time images of biotinylated DNA binding to/dissociating from a streptavidin protein. Our results show that high-speed AFMs have the potential to reveal the mechanisms of molecular interactions, which can not be elucidated by analyzing the average value of mass reactions.

1P560**Observing mechanical unfolding and folding process of single molecular protein by AFM**

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Protein has unique structure determined by one-dimensional amino acid sequence and has a wide variety of conformational properties. One of the most interesting features is the reversible transition between the folded and unfolded state. To understand why protein can spontaneously fold into the unique structure, detailed properties of conformational change must be cleared. Although kinetics of the protein folding has been well studied by observing denaturation process in bulk solution, dynamics on the single molecular level is still not understood. Here we study the mechanical denaturation process of single molecular protein, staphylococcal nuclease (SNase), by using atomic force microscope (AFM). SNase has relatively low molecular weight and shows the reversible denaturation. We constructed the AFM apparatus and manipulated extension or tension of the protein. The experiments were performed in two different modes. First in the force ramp mode, in which the force applied to the protein is increased at a constant rate, several characteristic lengths that should correspond to the partial unfolding was observed. Second in the velocity clamp mode, in which the extension of the protein is increased or decreased at a constant rate, we observed velocity dependence of the unfolding and refolding paths. We compare these results with the bulk study and discuss the folding and unfolding dynamics of the small protein, which should be distinguished from huge proteins like titin or spectrin.