Abstract

Title of this dissertation:

Study on Stochastic Transitions in Proteins:

Mechanical Folding and Unfolding of a single protein and The Switching Movement of Melanosomes

（和文題目）

生体分子の確率的遷移に関する研究:
タンパク質の一分子伸張実験と黒色素胞における顆粒運動の解析

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The stochastic transitions in biological systems, the folding-unfolding transition of a single protein and the switching of multiple motor proteins, were studied experimentally. Both of the topics have been fundamental problems in structural and physical biology, however, the dynamical properties are still poorly understood. Recent advances in manipulating or tracking stochastic process of single molecules provide us with the detailed dynamics that may lay behind the complexity of the processes. With the use of these techniques, we can elucidate the fluctuating nature of the stochastic process by following trajectories of individual molecules in time. Our objective is to build detailed descriptions of microscopic dynamics and connect them with the result in ensemble of macroscopic observations to acquire deeper insights into the complex but ordered behavior of biological processes.

In the first part of the thesis, mechanical unfolding and refolding of a single staphylococcal nuclease (SNase) molecule were studied using atomic force microscope (AFM). SNase is a globular protein that shows simple two-state transition between the native and denatured states in biochemical experiments. Although recent experimental and theoretical studies have proposed the presence of multiple-parallel pathways of the SNase folding, there is no direct experimental evidence until now. By applying mechanical force to the single molecule, we found that the native SNase passes through several intermediates at which parts of the native structure were hold as metastable states, in spite of the simple force response without any stable intermediates under

the acid-denatured condition. We also developed novel method to investigate refolding intermediates that have been difficult to probe in conventional force measurements. Tracking intermediates during both of the unfolding and refolding processes of single SNase enabled us to obtain more detailed information about the pathway or the stochasticity of conformational dynamics at the single-molecule scale.

In the second part, the switching transition during melanosome transports in Zebrafish melanophore was studied using the image analysis technique. The melanosome transport that is driven by multiple transport systems, three motor proteins and two cytoskeletal tracks, has been studied as a good model system to understand the regulation mechanism of protein activities in cells. Our study focused especially on the stochastic, bi-directional movement of melanosomes at the intermediate phase of the dispersion in melanophores. The image analysis method was applied to track precise positions of single melanosomes. The result was compared with those of chemically-treated cells in which particular transport systems are disrupted selectively. From the microscopic description, we showed that each motor protein changes its kinetic property via the interaction between different motors. Our result suggests that the switching transition is regulated not only by the cell-wide stimuli but also regulated autonomously by interactions between motor proteins to attain the cooperative dispersion of cargoes.