directed mutation at the barster interface to demonstrate the role of the hydrogen bonds at the mutated residue during the complex formation.

1PT148 原子間力顕微鏡を用いたβ-カテニンの力学的挙動解析

Analysis of the mechanical behavior of β-catenin using AFM

Koichiro Maki^{1,2}, Sung-Woong Han¹, Taiji Adachi^{1,2} (¹Department of Biomechanics, Institute for Frontier Medical Sciences, Kyoto University, Japan, ²Department of Microengineering, Kyoto University, Japan)

In cellular activities, mechanical stimuli are functionally converted into biochemical signals. This process called mechanotransduction has an essential role in cell-cell communication through mechanical interaction. Cells communicate with each other through mechanotransduction which is induced by adherens junctions (AJs)-mediated intercellular tension. According to recent studies, AJs adapting modulate its intensity to the intercellular tension to which the mechanical behaviors of its components contribute. However, the mechanical behaviors of the components such as β-catenin and α-catenin are unclear. In this study, we focused on β-catenin, which interestingly forms a superhelical structure, and analyzed its mechanical behaviors using atomic force microscope (AFM). AFM cantilever was modified using a NHS-PEG-Maleimide for the glutathione modification. The N-terminus GST-tagged β-catenin was then modified using a GST-glutathione affinity. The AFM-tip was approached and made contact to the NHS-ester modified substrate to allow outside amine of β-catenin and NHS-ester binding. Then we obtained force-extension curves for β-catenin and found a linear-elastic region in both loading and unloading processes under small tension less than 120 pN without any relaxation. Moreover, relaxation event was not observed under high tension more than 1000 pN, that is, β-catenin was not unfolded. These results indicate that β-catenin exhibits stiff mechanical behaviors and can be a molecule which stably transmits the intercellular tension.

1PT149 PPC, DSC のコンビネーション解析により明らかになったシトクロム c の新規多状態熱転移

A combination analysis of PPC and DSC revealed novel multi-state thermal transitions of cytochrome c

Shigeyoshi Nakamura, Shun-ichi Kidokoro (Nagaoka University of Technology)

Molten globule, MG, state is known as the equilibrium and/or kinetic intermediate state which has a key role on the stability and folding mechanism of proteins. The thermal transition of the MG state of cytochrome c at extreme acidic pH and high salt condition showed the complicated transition rather than the simple two-state transition. We performed differential scanning calorimetry (DSC) and pressure perturbation calorimetry (PPC) analysis of thermal transition of the MG state at pH 2.5 with 500 mM KCl. By DSC measurements, the protein concentration dependence of the thermal transition was clearly observed in the range of 0.25–18 mg/ml cytochrome c. The transition temperature of the MG state shifted to lower temperature by increasing cytochrome c concentration. In all cases, the thermal transition of the MG state showed full reversibility. It strongly suggests that the thermal transition of the MG state at pH 2.5 with 500mM KCl is the complicated transition including the reversible oligomerization process of the disordered molecules. By PPC measurements, the thermal expansion coefficient showed the complicated shape composed by the broad positive peak at lower temperature and a broad negative peak at higher temperature. It indicates that the positive and negative volume changes are occurred at lower and higher temperature in this transition. The results of the multi-state analysis with self-dissociation/association process will be discussed.

1PT150 蛍光共鳴エネルギー移動を用いたスタフィロコッカスヌクレアーゼのフォールディングにおける特異的凝縮過程の研究

Site-specific collapse of staphylococcal nuclease during the folding followed by Förster resonance energy transfer

Takuya Mizukami¹, Ming Xu², Heinrich Roder^{2,3}, Kosuke Maki¹ (¹Grad. Sch. Sci., Nagoya Univ., ²Fox Chase Cancer Center, ³Dep. Biochem. Biophys., Univ. Penn.)

Many proteins form compact intermediates in the early stages of folding. However, whether the condensation of the polypeptide chain is driven by non-specific hydrophobic interactions or by specific local interactions remains

elusive. Staphylococcal nuclease consists of an N-terminal β-barrel and a C-terminal α-helical domains. Previous pulsed H/D exchange studies showed that some amide protons in the β-barrel domain are protected from exchange within 10 ms of the refolding. In addition, previous studies using a series of single tryptophan variants showed that a hydrophobic core comprising a large fraction of the β-barrel domain is formed in 100 μs time range.

In the present study, the collapses within the β-barrel domain and between the two domains were monitored by the time-dependent change in the apparent efficiency of Förster resonance energy transfer. Variants with single donor/acceptor pairs within the β-barrel domain showed an increase in the efficiency in 100 μs time range. In contrast, a variant with a single donor/acceptor pair in the α-helical/β-barrel domains, respectively, showed virtually no change in the efficiency in the submillisecond time range but an increase only after 100 ms time range. These results indicate that the collapse within the β-barrel domain occurs in 100 μs time range, while the α-helical domain is recruited to the rest of the molecule in the rate-limiting step of the folding. Thus the condensation is suggested occurring in the specific and cooperative manner.

1PT151 蛍光相関分光法を用いた網羅的 *in vivo* 酵母タンパク質動態解析

Global analysis of protein dynamics in living yeast cells using fluorescence correlation spectroscopy

Takafumi Fukuda¹, Chan-Gi Pack², Yasushi Sako², Shigeko Kawai-Noma¹, Hideki Taguchi¹ (¹Grad. Sch. of Biotech., Tokyo Tech, ²RIKEN ASI)

In the cell, the majority of proteins does not work alone, but interacts with other proteins or themselves, to maintain the cellular function, constituting a so-called “protein community” in the cell. Previous efforts on mass spectrometry-based protein interaction networks, interactomes, have provided a picture on the protein community. However, those were “static” information after cells were disrupted. For a better understanding of the protein community, it is important to obtain “dynamic” information in living cells, such as how proteins behave in the cell, how large they are, how fast they diffuse, what portions of proteins interact with others. Therefore, we have started a large-scale analysis on dynamic aspects of proteins in living cells using fluorescence correlation spectroscopy (FCS), which is a technique to investigate the diffusion properties of fluorescence molecules in cells. Using a budding yeast GFP clone collection, which is a *Saccharomyces cerevisiae* strain collection expressing GFP tagged open reading frames, we have evaluated i) diffusion constant, ii) the population of fast components and slow components, iii) abundance and iv) tendency for self-assembly, of ~200 kinds of yeast proteins in living yeast cells.

1PT152 マイクロ秒分解一分子 FRET 測定による蛋白質折り畳み運動の追跡 Microsecond-resolved traces of protein folding by single-molecule FRET measurements

Hiroyuki Oikawa¹, Yuta Suzuki², Kiyoto Kamagata¹, Munehito Arai³, Satoshi Takahashi¹ (¹IMRAM, Tohoku Univ., ²Grad. Sch. Sci., Tohoku Univ., ³Grad. Sch. Arts. Sci., Univ. Tokyo)

High-speed measurements of fluorescence signals from single molecules are expected to reveal the detailed information of protein folding. However, the time resolution of single-molecule measurements is usually limited to a few milliseconds. To improve the time resolution, we developed a line-confocal microscope with two-color detection system that was combined with the sample flow system. By using the developed device, we can trace a time evolution of fluorescence intensity and FRET efficiency from single molecules with the time resolution of 30 μs. We investigated the equilibrium unfolding transition of B domain of protein A (BdpA). The two cysteine residues were introduced into BdpA and labeled by a FRET pair of Alexa 633 and Alexa 488. The labeled sample shows a typical two-state unfolding upon the guanidium chloride (GdmCl) titration. The microsecond-resolved FRET efficiency time series from the single BdpA molecules revealed a significant conformational heterogeneity in the chemically unfolded state. The heterogeneity of the unfolded BdpA is substantiated by calculating the FRET efficiency histograms at the different GdmCl concentrations. The distribution obtained in the absence of denaturant consists of single peak for the native protein. In contrast, the distributions obtained in the presence of higher concentration of denaturants are broad. The data demonstrate the presence of multiple substates, which are separated by sizable energy barriers.

1PT153 エLEMENTを用いた人工酵素の設計と機能解析