was reassembled to heptamer in the presence of ATP and ammonium sulfate. After formation of a ternary complex, SR398/GroES/Ubq, cross-linked ubiquitin was discharged by the addition of a reductant. In this system, we have succeeded in improving the signal-to-noise ratio of the spectrum.

14:00~17:00 H 会場 / Room H

蛋白質_物性 3

Protein: Property 3

2H1412 FTIR study of a coiled coil peptide GCN4-E9G4 under high pressure: A mechanism for pressure-induced folding/unfolding of helices

Hiroshi Imamura, Minoru Kato (Dept. Pharm. Ritsumeikan Univ.)

Despite of emerging use of pressure for studies of protein folding, the physicochemical basis remains uncertain. We previously observed the pressure-induced folding of the α -helices of the alanine-based peptide (Imamura and Kato, Proteins 2009), which is opposite to pressure-induced unfolding of protein's α -helices. To unravel this paradox, we proposed a hypothetical mechanism: α -helices are inherently stabilized by pressure. Protein's helices unfold associated with disruption of its surrounding hydrophobic environment by pressure because the highly hydrated protein's helices are intrinsically quite unstable. To prove the validity of this mechanism, the present study targets the coiled coil peptide GCN4-E9G4, the α -helix of which is intrinsically unstable as a monomer, but stable as a dimer (Meisner and Sosnick, PNAS 2004). Its prototype peptide GCN4-p1 composed of the intrinsically stable helices has been observed to be folded by pressure (Imamura et al, BBA 2010). We monitored the pressure-induced structural change of GCN4-E9G4 by FTIR. Applying pressure results in a decrease in the area of the amide II band, indicating the hydrogen-deuterium exchanges of the amide protons in the hydrophobic core. In the amide I' band, the intensities of the peaks at 1650 and 1640 cm^{-1} assigned to the helical structure are weakened by pressure. These results demonstrate that pressure induces exposure of the hydrophobic core region, e.g. dissociation of the dimer, and unfolding of the helices, which agrees with the proposed mechanism.

2H1424 Energy landscape of denatured protein revealed by a new single-molecule fluorescence method

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To characterize structural heterogeneity and dynamics of a protein folding and function, it is important to determine the energy landscape which consists of multiple states and their kinetic connectivity by scrutinizing whether states are locally equilibrated or not. However, it is difficult to investigate individual conformations and their dynamics of a protein due to ensemble averaging of conformations by conventional ensemble techniques. Here, we applied our single-molecule method to investigate structural and dynamical properties of denatured cytochrome *c* from *Pseudomonas aeruginosa*. The method was developed to detect fluorescence intensity signals quantitatively from single molecules diffusing freely in a capillary flow cell. A conformational change of cytochrome *c* is detected as the intensity change of a fluorescent dye labeled with cytochrome *c*. The analysis of the single-molecule traces based on the concept of local equilibrium state (LES) demonstrated that the traces were assigned to two main LESs and two minor non-LESs that cannot satisfy the condition of local equilibrium. Two LESs correspond to compact and extended denatured states with the lifetime of ~100 ms, respectively. The autocorrelation analysis of two LESs indicates that dynamics within two LESs are faster than 5 ms, the time resolution of the observation. Two non-LESs are interpreted to arise from the intensity fluctuation of the dye. Therefore, the energy landscape of the denatured cytochrome *c* shows two distinct minima and a high energy barrier between them.

2H1436 STRUCTURAL ANALYSIS OF KEY INTERMEDIATES ON OXIDATIVE FOLDING OF PROUROGUANYLIN

Masaki Okumura^{1,3}, Yu-ichiro Yoshida², Takuma Maekawa¹, Hiroshi Yamaguchi¹ (¹ School of Science and Technology, Kwansei Gakuin University, ² Graduate School of Science and Engineering, Kinki University, ³ Japan Society for the Promotion of Science)

Prouroguanylin is a precursor protein of uroguanylin and consists of three intra-molecular disulfide bridges (Cys41-Cys54 in the propeptide region and Cys74-Cys82 and Cys77-Cys85 in uroguanylin, the mature region). The propeptide region in prouroguanylin plays a role as an intra-molecular chaperone in the formation of the native conformation and disulfide pairing of uroguanylin. Our previous study suggested that a two major folding intermediates (disulfide isomers 1 and 2 in which disulfide bonds are localized at Cys74-Cys85 and Cys77-Cys82 and Cys74-Cys77 and Cys82-Cys85 in the mature region, respectively) were pooled during the oxidative folding of prouroguanylin. In order to further study the mechanism of the propeptide-mediated folding, kinetic roles of the non-native folding intermediates in the construction of the native conformation of prouroguanylin were estimated in details.

The kinetic analyses of the folding of two intermediates revealed that PDI promotes the formation of native conformation of prouroguanylin by accelerating the intra-molecular disulfide exchange reactions during early stage. Furthermore, it was found that two types of mis-disulfide intermediates are predominantly included in the folding but only one mis-disulfide intermediate, disulfide isomer 2, is able to move to the native conformation of prouroguanylin.

2H1448 Cys を用いた Trp 三重項状態消光による Staphylococcal nuclease 変性状態の研究

Denatured Structure of Staphylococcal Nuclease Studied by Triplet State Quenching of Tryptophan by Cysteine

Toshihiro Okada, Mariko Yamaguchi, Youichi Yamazaki, Hironari Kamikubo, Mikio Kataoka (Grad. Sch. Mat.Sci., NAIST)

Denatured proteins are highly flexible and often considered to be a random coil. However, many studies suggested that denatured proteins retain significant amounts of residual structure. Although it is expected that the characterization of the denatured structures leads to better understanding of folding processes, few experimental and modeling approaches have been applied to the investigation of the structures and dynamics of the denatured proteins. Quenching of the triplet state of tryptophan (Trp) by intramolecular contact with cysteine (Cys) would be unique and effective for the purpose, and we applied the method to the study of the denatured structure of staphylococcal nuclease (SNase). We prepared mutants which have Trp-Cys pairs at different positions and measured the dependence of the quenching rate on the Trp-Cys distance. One of the residues from the 76th to the 80th was substituted to Trp with Trp140His, and Lys71 was substituted to Cys. The quenching rates were measured in the presence of 1.4 M guanidine hydrochloride. The quenching rate becomes slower as the Trp-Cys distance increases. Such dependence of the quenching rate on the Trp-Cys distance was also observed when Trp140 is fixed with the substituted Cys at various positions. We are going to try the Trp triplet state quenching measurements with the other Trp-Cys pair mutants and will discuss the denatured structure of SNase.

2H1512 Trp-Trp 間エネルギー移動と PAP の folding/unfolding 研究への応用

Trp-Trp energy migration and its application to the folding/unfolding of Pokeweed Antiviral Protein

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The fluorescence spectroscopic studies of tryptophan residue have provided interesting information on protein conformation and dynamics. We noted here the Trp-Trp energy transfer or migration. The participation of the energy homo transfer in the electronic energy relaxation of Trp was mentioned in protein since the fluorescence polarization studies by Weber. But, the details are still unclear now. Pokeweed anti viral protein (PAP) contains two Trps, one is W208 and the other W237. They locate at the vicinity of the active center and at the C-terminal region of PAP, respectively. According to the crystallographic structure, they are separated about 17 Å each other. Since it is known that the critical distance for the resonance energy transfer by Forster (FRET) is considered to be 4 -18 Å, it is reasonable to assume the energy transfer between