1P034 クモの糸の分子および力学特性
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Molecular and mechanical properties of spider silk
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Spider dragline silk is one of fibrous proteins, carrying the exceptional mechanical properties. Recently, the partial cDNA sequence of the spider silk proteins was reported. However, the spinning mechanism is not known yet. In order to obtain native structure of dragline silk of Japanese spider *Nephila clavata*, the attenuated total reflectance (ATR) infrared spectroscopy was carried out. Polarized ATR measurements showed that most of amide groups in silk protein were oriented to the same direction in the silk fibers. Secondary structure of silk protein was estimated by Fourier self-deconvolution and the curve-fitting procedures. The results suggested that cross β -sheet structure is predominant in natural spider silk. The amide vibrations of the silk protein were significantly changed in the addition of water. Since the remarkable elasticity of dragline silk is induced by water adsorption, the interaction between silk proteins and water molecules seems to be an indispensable property of spider silk. The structure transition from unordered structure to β -sheet structure was identified in silk solution from major ampullate, suggesting that the silk spinning process was involved in the molecular formation of silk protein. In order to obtain an insight into the spinning process, ultra high-speed video camera-equipped with microscope was also employed. The results will be discussed.

1P036 ADP が対称型 1:2GroEL-GroES 複合体の形成を阻害する
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ADP inhibits the formation of a symmetric 1:2 GroEL-GroES complex
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GroEL is an *Escherichia coli* chaperonin which is composed of two heptameric rings stacked back to back. GroEL interacts with its cochaperonin GroES and assists protein folding in an ATP dependent manner. Because of negative cooperativity between two rings in GroEL, it is widely believed that GroES interacts with the rings of GroEL alternatively; *i.e.* an asymmetrical 1:1 complex (termed bullet-shaped complex) is considered to be the only form of GroEL-GroES complex during the reaction cycle. However, a symmetrical 1:2 GroEL-GroES complex (termed football-shaped complex) has been observed by electron microscopy and chemical crosslinking. Therefore, it is still controversial whether GroES binds both rings of GroEL simultaneously in the physiological condition. In this study, we monitored GroEL-GroES interaction in solution using fluorescence resonance energy transfer (FRET). We found that nearly half of GroEL-GroES complexes formed football-shaped ones during the reaction cycle. Similar result was obtained using fluorescence correlation spectroscopy (FCS). Next, we showed that ADP inhibited the formation of football-shaped complex. The presence of ADP with concentration of 1/5-fold of ATP decreases the amount of football-shaped complex to half. From the studies using inorganic phosphate analog (beryllium fluoride, BeF₃) and ATPase deficient GroEL mutant (D398A), we have revealed that ADP inhibits the association of ATP to the *transring* of GroEL and, as a consequence, ADP prevents the association of the second GroES to GroEL.

1P035 GroELのC末端欠損変異はATP加水分解反応を遅くする
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C-terminal region of GroEL is required for a regular ATP hydrolysis cycle
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Chaperonin mediates the folding of denatured polypeptides. *Escherichia coli* caperonin, GroEL reaction cycle consists of two rate-limiting steps. During the first step (~ 3 s), ATP, substrate protein and GroES are bound to GroEL. Immediately after the first step, the substrate protein is released into the central cavity and ATP is hydrolyzed. In the second step (~ 5 s), protein folding proceeds. GroEL has a conservative amino acid sequence of C-terminal region among various species, but its function is unknown. We aim to illuminate the function of the C-terminal region for protein folding. We prepared a C-terminal deletion mutant GroEL(d20C), and investigated the chaperonin reaction cycle. First, we measured turnover of the ATP hydrolysis cycle, and revealed that GroEL(d20C) decreased in ATP hydrolysis rate. Next, we measured binding duration of GroEL(d20C)-GroES complex by single molecular imaging. As a result, it was revealed that GroEL(d20C) extended the process after the first rate-limiting step. We examined initial rate of ATP hydrolysis upon addition of ATP and found that time from binding of ATP to hydrolysis was prolonged. These results indicate that GroEL(d20C) takes longer time between release of substrate protein into the cavity and ATP hydrolysis than wild-type GroEL. This suggests that C-terminal region of the GroEL facilitates ATP hydrolysis.

1P037 高速原子間力顕微鏡によるGroEL-GroESの結合・解離測定
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Measurements of GroEL-GroES binding-release action by high speed AFM
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Chaperonin GroEL, which has a double ring structure, mediates protein folding in the presence of co-chaperonin GroES and ATP. It is generally accepted that GroES binds alternately to the GroEL rings but cannot simultaneously bind to both the rings during the chaperonin reaction cycle, due to the negative cooperativity between the rings. Although this GroES binding behavior has been derived from a series of biochemical experiments, electron micrographs have suggested the formation of GroES-GroEL-GroES complex. In order to elucidate the negative cooperativity between the rings and to give an answer to the controversial issue, we performed measurements on the GroEL-GroES on-off action using a high-speed atomic force microscope (high-speed AFM). For this purpose, GroEL mutant (D490C) conjugated with biotin at its equatorial surface was attached in a side-on orientation on a surface of 2D crystal of streptavidin. In this system, the dynamic binding-release actions between GroES and GroEL were successively captured on video in the presence of ATP. The association of GroES to the GroEL rings took place in a negatively cooperative manner, consistent with the proposal by earlier biochemical studies. However, two types of intermediates, bare GroEL and the ternary complex of GroES-GroEL-GroES, appeared before the GroES-bound ring and the vacant ring swapped with each other, with a larger probability of the latter intermediate appearance. Thus, we gave a clincher to the controversial issue.