(1) T. Murata et al., Science 308, 654-659 (2005)

1P-069 分子モデリング法と溶液 X 線散乱法による天然解鎖蛋白質の構造解析

Structural Analysis of a Natively Unfolded Protein by Combining Molecular Modeling and Solution X-ray Scattering

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As an unfolded protein can have an enormous number of different conformations, it is difficult to analyze its structural characteristics only by conventional methods. To achieve the aim, we developed a molecular modeling method for unfolded chains where various probability distributions are assumed for their main-chain dihedral angles. This modeling method can analyze the relation between local structural features such as the probability distribution of main-chain dihedral angles and global ones such as solution X-ray scattering (SXS) profile. Combining both methods of molecular modeling and SXS, we have analyzed the solution structure of a DNA-binding domain of fibroin-modulator-binding protein 1 (STPR), which is a natively unfolded protein. It was revealed from a NMR study that STPR has four short, stable α -helices in solution. In our study, we assumed a feature common to the structural models for STPR that short α -helices and random-coil segments repeat four times alternately. Comparing the calculated SXS profiles for various structural models and experimental ones, we revealed several features of the structural model which reproduces best the experimental SXS profiles quantitatively. One of the features of the best model is that the probability distribution of main-chain dihedral angles for the random-coil segments of STPR is equivalent to that for the ensemble of coil segments in the crystal structure of native proteins. In addition, we found that, to reproduce the experimental SXS profiles quantitatively, it is required to average the calculated SXS profiles over the ensemble of generated unfolded structures weighted by the solvent potentials proportional to their respective ASA. These structural features of STPR differ from those in the fully unfolded state of globular proteins.

1P-070 飛行時間型二次イオン質量分析法による固定化ポリペプチドの評価におけるクラスター二次イオン源の影響

Effect of time-of-flight secondary ion mass spectrometry with cluster primary ion source on detection of immobilized-polypeptide

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Time-of-flight secondary ion mass spectrometry (TOF-SIMS) with cluster primary ion source such as C60 and gas cluster ions is thought to be effective to evaluate biomaterials such as protein-immobilized biochips. TOF-SIMS with a cluster primary ion source is expected to be useful to evaluate orientation protein immobilized on bio-devices because of its ultra high surface sensitivity. In order to evaluate effect of C60 primary ion source on the protein-immobilized samples, simple model samples, Leu-enkephalin and Met-enkephalin-immobilized samples were measured with TOF-SIMS, respectively. Leu-enkephalin and Met-enkephalin have the same amino acid residues except for C-terminal residues. Leu-enkephalin and Met-enkephalin were immobilized on aminosilanized indium tin oxide (ITO) coated glass plates, respectively, at their N-terminal. A reference sample, an aminosilanized ITO glass plate without the polypeptide, was also prepared. The all samples were freeze-dried before they were measured with TOF-SIMS. Each sample was measured TOF-SIMS using the cluster ion C60+ and monatomic ion Ga+, respectively, and they were compared in order to evaluate the cluster ion effect.

As a result, different fragment ion patterns were observed between TOF-SIMS spectra obtained with the cluster ion source and those obtained with the monatomic ion source.

1P-071 Zero-mode waveguides を利用した GroEL-GroES 相互作用の 1 分子蛍光イメージング

Single molecule fluorescence imaging of GroEL-GroES interaction in zero-mode waveguides

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Single molecule fluorescence imaging is a powerful tool for analyzing dynamics of molecular interactions. However, using total internal reflection fluorescence microscopy (TIRFM), it is hard to visualize single fluorescent molecule in the presence of over 20 nM concentration of fluorescent dyes due to the background fluorescence of the dyes in solution. To overcome this problem, zero-mode waveguides (ZMWs) have been applied to single molecule fluorescence imaging. ZMWs consist of holes (tens of nanometers in diameter) in a metal film deposited

on a quartz coverslip. ZMWs can reduce illumination volume compared to TIRFM; therefore, they enable single molecule fluorescence imaging in the presence of fluorescent molecule at biologically relevant (micromolar) concentration. GroEL is *Escherichia coli* chaperonin, which is composed of two heptameric rings stacked back-to-back. GroEL assists protein folding with its cofactor GroES in an ATP-dependent manner. It has been generally believed that GroES binds to each ring of GroEL alternatively, that is two GroES molecules do not bind to both rings of GroEL simultaneously. However, our recent study revealed the existence of GroEL-(GroES)₂ (football-shaped) complex during the reaction cycle of GroEL. To study GroEL-GroES interaction mechanism including football-shaped complex, we observed their interaction in ZMWs at physiologically more relevant concentration of fluorescently labeled GroES. Then, we have succeeded in observation of football-shaped complex at a single molecule level.

1P-072 高速に系統的な進化を行うためにたんぱく質ハミングライブラリーを作製する諸技術

Versatile techniques in making of a protein Hamming library for the fast systematic directed evolution

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Biased mutation assembling (BMA) method has been used successfully in evolutionary protein engineering. It is desirable to prepare a protein Hamming library as the primary library to perform more systematic evolution. Here, Hamming library (dH =1) is a protein library that has all the single point mutants. In BMA method, advantageous single amino acid mutants are selected from the primary library. Gene fragments containing each of advantageous substitutions are assembled by PCR without primers. And the secondary library of all the multiple substitutions is established with the bias to multiple mutations in order to select a mutant with much higher fitness. We modified the random insertion/deletion (RID) mutagenesis to construct the primary library: (1) The efficiency of circularization of dsDNA is dependent of the size of dsDNA (optimum: ca. 500bp). Therefore a longer gene should be fragmented. The fragmentation is achieved by introducing a restriction enzyme recognition site per ca. 500bp without the change in amino acid sequence. For three restriction enzymes (EcoRI, HindIII, BamHI) this possibility between 416bp site and 584bp site for any gene is 97%. Thus, this method has general applicability. (2) A solid phase process is applied in order to introduce robotics and to improve efficiency of dsDNA circularization.

1P-073 RNA 複製酵素を提示した自己言及型 in vitro virus の自律進化に向けて II

Towards in vitro autonomous evolution of self-referring in vitro virus displaying an RNA replicase, II

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In the *in vitro* evolution experiments, using an isothermal nucleic acid-amplification system, an autonomous evolution of the nucleic acid amplification system has been achieved. But the autonomous protein evolution system has not been accomplished yet. We tried to construct such a system by combining the *in vitro* virus (IVV) and the one-pot isothermal RNA replication reaction. In this system, the norovirus RNA-dependent RNA polymerase (3D^{pol}) is displayed on the IVV. Using this IVV displaying the RNA replicase and two RNA primers, we designed the one-pot isothermal replication reaction system, in which the IVV can amplify itself, and evolve autonomously. Moreover, this reaction system will evolve not only the RNA replicase but also the amplification algorithm itself. Then, we can call this system "wetware artificial life". We designed also a new isothermal RNA amplification system using the strand displacement reaction by norovirus 3D^{pol}. In this system, the 3D^{pol} is incubated with RNA template and template-specific RNA primers. It will be applied to the genomic diagnostics and the gene expression analysis.

1P-074 2 種類の異なる代謝経路で機能する TIM バレル酵素によるキメラ酵素の作製

Chimeric enzymes between two TIM barrel enzymes involved in different metabolic pathway

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The TIM barrel is the most common protein fold functioning as enzyme. The canonical TIM-barrel topology consists of eight repeats of a β -strand/ α -helix (β/α) module. Because enzymes belonging to the TIM-barrel family occupy about 10% of all proteins whose three-dimensional structures have been determined, evolution of TIM-barrel might have contributed to diversifying enzymatic functions. Although directed evidences to understand the evolutionary history of TIM-barrel are not enough, increasing evidence suggests that a large