Asymmetric ribosome interaction with mRNA in translation initiation

Masatoshi Tachibana (1), Tomoaki Masuda (1), Ryo Iizuka (1), Takashi Funatsu (1), Sotaro Uemura (2) ((1) Graduate School of Pharmaceutical Science, The University of Tokyo: (2) Department of Structural Biology, Stanford University School of Medicine)

Ribosome is a functional rRNA-protein complex which consists of 30S and 50S subunits and catalyzes peptide translation. Though "static" structures of ribosome have been determined by cryo-EM and X-ray crystallographic studies, the interaction dynamics on ribosome during translation is required to be analyzed for further investigation. We measure the rupture force of the interaction between ribosome and mRNA in translation initiation using the optical tweezers method. 30S subunit complex with 5'- or 3'-biotinylated mRNA was immobilized to biotin-BSA coated surfaces via streptavidin-biotin linkage. An oligonucleotide labeled with digoxigenin was designated to hybridize to the extension loop on 30S subunit, which is genetically engineered. A bead coated with anti-digoxigenin antibody was conjugated to the oligonucleotide, and used for optical trapping of the ribosomal complex. Nonspecific rupture event can be eliminated by Cy3 fluorescence oligo hybridized to 30S extension loop. The interaction between 30S subunit and mRNA is eventually ruptured by moving the piezo stage at a constant velocity during the bead is trapped. The rupture force was estimated to be 8.04 pN when the ribosome complexes are pulled toward the 3' end of mRNA (direction of motility), whereas, an external load imposed against 3' end (direction of motility) increased it to be 15.7 pN. These results represent the asymmetric interaction for 30S-mRNA, in which the dissociation toward 3' end occurs more easily than that toward 5' end. The asymmetric interaction is in accord with the idea of the processive movement of ribosome toward 3' end of mRNA during the translation. To confirm this assumption, additional experiments are required for more detail analysis.

3P-100 AFMを用いたテロメスタチン誘導体とGカルテット構造DNAの結合解析

Analysis of binding of telomestatin derivative to G-quadruplex DNA by using AFM

Yui Furunaga (1), Shingo Mieda (1), Kazunori Ikebukuro (1), Kazuo Nagasawa (1), Noriaki Nakamura (2), Chikashi Nakamura (2) ((1) Department of Biotechnology and Life Science, Tokyo University of Agriculture and Technology: (2) Research Institute for Cell Engineering, AIST)

We have tried to develop a method of DNA adsorption which is sequence specific and strong enough to endure against shear force exerted on a nanoneedle surface during penetration of the needle through a cell membrane. In this study, we chose telomestatin derivative, 6OTD, which is a macrocyclic hexaoxazole compound, as a DNA adsorber specific for G-quadruplex structures and evaluated adhesion forces of 6OTD to G-quadruplex by using AFM. We use L2H2-6OTD (ChemBioChem 10, 431-435, 2009) which has amino group, biotinylated thrombin-binding aptamer (TBA, 15 mer) including a target G-quadruplex motif, and biotinylated poly dT oligodeoxynucleotide (20dT, 20 mer) as a negative control DNA. A silane AFM tip ATEC-Cont (0.2 N/m) was modified with L2H2-6OTD using a silane coupler, MPTMS, and a bifunctional cross-linker, EMCS. The biotinylated single strand DNAs were immobilized on streptavidin chips (Biacore, GE Healthcare). Contacting time of the AFM tip to the DNA-immobilized substrate was controlled for 0.3 or 5.3 sec. It has been known that G-quadruplex DNA is stabilized by the potassium ion (K⁺) treatment. In this study, the maximum force volumes in the curve of retraction process were estimated as adhesion forces. The averaged adhesion forces of 6OTD to 20dT and that of 6OTD to TBA were 104 pN and 322 pN, respectively. After the K⁺ treatment, the adhesion force increased to 448 pN. This result suggested the effect of K⁺ on stabilization of G-quadruplex structure. When the contacting time was increased to 5.3 sec, the averaged adhesion force to TBA increased to 953 pN. These results indicate that the binding between 6OTD and G-quadruplex structure is specific and stable.

3P-101 分子動力学法によるDNA-タンパク質間相互作用のシミュレーション解析

Simulation analysis of interactions between DNA and protein by molecular dynamics

Masahiro Watanabe (1), Tomonori Suzuki (2), Satoru Miyazaki (1) ((1) Graduate School of Pharmaceutical Sciences, Tokyo University of Science: (2) Fac. of Pharmaceutical Sciences, Tokyo University of Sciences)

The use of computer simulation, to predict 3D structure of proteins or molecular interactions in living cells, tends to widely spread. We can expect that the computer simulation would be much useful to understand a variety of biological reaction. In the molecular dynamics method, which is one of the most popular methods for proteins structure modeling, it is important to select an optimal force field and boundary conditions depending on targeted molecules to analyze. However, there are few reports describing how these parameters should be set. The lack of knowledge with those parameters implies problems with reproducibility and comparing of results predicted by another researcher. Almost values assigned parameters on the existing force field are for proteins not DNA.

Therefore, when the simulation of molecules containing a nucleotide, calculated results might differ from the X ray structure, because of the force field parameter developments for nucleotide have been unsatisfactory. In present study, we reconsider the good points using Amber or Discover force fields, and try to optimize the force field for DNA-protein complex simulation. We got some Protein-protein and DNA-protein complex structures from PDB (Protein Data Bank) and split into component. Then, we executed MD simulation with our best force field. We report the some results for the simulation of interaction of DNA-protein complex.

3P-102 逆ミセル中蛋白質のテラヘルツ周波数領域のダイナミクスの水和効果

Hydration effect on dynamics of a protein in a reverse micelle in the terahertz frequency range

Hiroshi Murakami (1), Takaki Nishi (2), Yuji Toyota (3) ((1) Japan Atomic Energy Agency: (2) JAEA/Kyoto Inst. Tech.: (3) JAEA/Osaka City Univ.)

It is believed that low-frequency motions of proteins in the terahertz (THz) frequency range play an important role on their functions. Such motions will work as thermal fluctuation on the transition among conformational substates of proteins. The purpose of our investigation is to study the low-frequency motions of proteins in liquid, especially its hydration effect. Most of the experimental investigations using THz spectroscopy have employed dried samples such as lyophilized ones, although a protein works in liquid. We have recently accomplished THz transmission spectroscopy for a protein in liquid by using a protein in reverse micelles. The reverse micelle is a nanometer-sized droplet including a protein molecule, and the solvent is nonpolar liquids, which are much weaker in absorption of THz waves than water. The size of reverse micelles is changed by a molar ratio of water to surfactant, which allows us to control the number of hydrated water. A surfactant, sodium bis(2-ethylhexyl) sulfosuccinate (AOT) was dissolved in isooctane. Myoglobin aqueous solution was injected into the AOT/isooctane solution. The THz time-domain spectroscopy was done with a femtosecond laser for the absorption spectrum of the sample. In the presentation, we propose a model to determine the structural parameters of a protein-containing micellar solution on the basis of near infrared absorption spectra due to the O-H stretching vibration (3300 cm⁻¹), and discuss the hydration effect of the THz dynamics of protein in liquid, in combination with circular dichroism measurement of the protein.

3P-103 蛋白質の調和運動と非調和運動に対する水和効果

Hydration affects both harmonic and anharmonic nature of protein dynamics

Hiroshi Nakagawa (1), Yasumasa Joti (2), Akio Kitao (2), Mikio Kataoka (3) ((1) Japan Atomic Energy Agency: (2) University of Tokyo: (3) Nara Institute of Science and Technology, Japan Atomic Energy Agency)

To understand the effect of hydration on protein dynamics, inelastic neutron scattering experiments were carried out on Staphylococcal nuclease samples at differing hydration levels: dehydrated, partially hydrated and hydrated. At cryogenic temperatures, hydration affected the collective motions with energies lower than 5 meV, while the high energy localized motions were independent of hydration. The prominent change was a shift of boson peak toward higher energy by hydration, suggesting hardening of harmonic potential at local minima on the energy landscape. The 240 K transition was observed only for the hydrated protein. Significant quasi-elastic scattering at 300 K was observed only for the hydrated sample, indicating that the origin of the transition is the motion activated by hydration water. The neutron scattering profile of the partially hydrated sample was quite similar to that of the hydrated sample at 100 and 200 K, while it was close to the dehydrated sample at 300 K, indicating that partial hydration is sufficient to affect the harmonic nature of protein dynamics, and that there is a threshold hydration level to activate the anharmonic motions. Thus, hydration water controls both the harmonic and anharmonic protein dynamics, by differing means.

3P-104 DNA周囲の水の分布：分子動力学シミュレーションと3D-RISMによる計算結果の比較

Comparison of DNA hydration patterns obtained from MD simulation and 3D-RISM theory

Yoshiteru Yonetani (1), Yutaka Maruyama (2), Fumio Hirata (2), Hidetoshi Kono (1) ((1) Japan Atomic Energy Agency: (2) Institute for Molecular Science)

Because biomolecules in cell such as proteins, DNA and various small molecules interact with each other in the presence of water molecules, we cannot ignore their hydration when discussing their structural and energetic properties. Although high-resolution crystal structure analyses have given us a view of tightly bound water molecules on their surface, the structural data are still insufficient to capture the detailed configurations of water molecules around the surface of these biomolecules. Thanks to the invention of various computational algorithms, computer simulations can now provide an atomic view of hydration. In this presentation, we describe the hydration patterns of DNA calculated using two