similar amino acids (isoleucine and valine), leucyl-tRNA synthetases (LeuRS) cannot discriminate these three amino acids, producing mis-aminoacylated tRNAs. To avoid the error, the enzyme has the editing function to hydrolyze mis-products. Two catalytic centers for the editing and the aminoacylation are located in distinct domains, and are markedly distant from each other. Structural studies of LeuRS reveal that large-scale rotations of several domains occur on the reactions. This indicates that the expression of the enzymatic functions is associated with the domain-domain communications, mechanisms of which are to be elucidated. To investigate the long-range communications in LeuRS, we performed molecular dynamics (MD) simulations of LeuRS in complex with tRNA^{Leu} and an incognate amino acid, valine, which was modeled by using "Fully Solvated Dynamical Docking" (FSDD) recently developed by us. We have identified interdomain communication, which ranges by $\sim 100\text{\AA}$. Various patterns of the domain communication have been observed, which are found to be regulated by conformations of an amino acid residue. In the session, we will also discuss investigations of detailed mechanisms of editing using QM/MM calculations.

3P-094 クラスターDNA 損傷と修復酵素 hOGG1 の分子動力学シミュレーション
Molecular dynamics simulation of cluster damaged DNA and hOGG1

Mariko Higuchi (1), Miroslav Pinak (1) ((1) Japan Atomic Energy Agency)

Ionizing radiation leads to clustered DNA damage sites, which are defined as two or more single lesions induced within 10-20 base pairs (bp). These sites are repaired with less efficiency than single lesions. So far the detail mechanism of repair of multiple lesions is not known. A cluster of damage containing a single strand break (SSB) within a few base pairs opposite to a 8-oxo-7,8-dihydroguanine (8oxo-G) is one example of cluster that is difficult to repair. The experimental results showed that the excision of the 8oxo-G lesion by repair enzyme hOGG1 is strongly inhibited by the presence of a SSB on the opposite strand with a 1bp separation from the 8oxo-G lesion.

In this study, we present the results of molecular dynamics (MD) simulation of four models of hOGG1 with damaged DNA. Each model DNA contained one or two damages as following: 1) flipped out 8-oxoG, 2) un-flipped out 8oxo-G, 3) SSB and flipped out 8oxo-G, 4) SSB and un-flipped out 8oxo-G. These SSB were located on the opposite strands with a separation of 1bp from the 8-oxoG. According to observed results, the structure of the DNA with SSB kept initial structure with 8-oxoGs either flipped out or not. However in the DNA without SSB, the structure changed from the initial structure when the 8-oxoG did not flip out.

3P-095 分子動力学シミュレーションによる DNA 修復誘導タンパク質 MutS-DNA 複合体の動的解析
Molecular dynamics simulations of DNA repair protein MutS and DNA complexes

Hisashi Ishida (1) ((1) Japan Atomic Energy Agency)

DNA mismatch repair (MMR) maintains genome stability by repairing mismatches that arise through DNA replication errors and during recombination. Defects in MMR result in a significant increase in the spontaneous mutation rate and predispose humans to cancer.

In *E. coli*, the proteins MutS, MutL and MutH are responsible for the MMR. MMR is initiated by MutS, which functions in the homodimer form. MutS recognizes and efficiently binds to mismatched bases and unpaired bases in DNA duplexes. It is thought that the ATPase activity of MutS plays a role in proofreading to verify mismatch binding and authorize the following downstream excision in which MutL and MutS are involved.

However, little is known of the relationship between the recognition of DNA and the ATP hydrolysis by MutS at the atomic level. In order to investigate how the binding of MutS to the DNA and ATP hydrolysis are coordinated, molecular dynamics (MD) simulations of the wild-type and mutant MutS in water with mismatched and undamaged DNA were performed. Including the water molecules, each system comprised about 200,000 atoms. The MD simulations were carried out at a constant pressure of one bar and a temperature of 300 K for several tens of nanoseconds in total. The binding free energies were calculated using the MM-GBSA method, and the results are consistent with the experimental data. Moreover, the dynamic relationship between the DNA binding and ATPase domain sites was analyzed. The details of the analysis will be reported at this conference.

3P-096 蛍光相関分光法を用いた FMBP-1 の生体内分子動態解析
In vivo analysis of the molecular dynamics of FMBP-1 by fluorescence correlation spectroscopy

Shohei Myoba (1), Hideki Muto (2), Mai Kimoto (3), Masakatsu Kamiya (1), Takashi Kikukawa (1), Tomoyasu Aizawa (1), Masataka Kinjo (2), Shigeharu Takiya (3), Makoto Demura (1), Keiichi Kawano (1) ((1) Department of Biological Sciences, Graduate School of Science, Hokkaido University; (2) Laboratory of Molecular Cell Dynamics, Faculty of Advanced Life Science, Hokkaido University; (3) Center for Genome Dynamics, Graduate School of Science, Hokkaido University)

Fibroin-modulator-binding protein-1 (FMBP-1) is a factor that binds the transcriptional activation elements of the fibroin gene. Its gene encodes a 218-amino acid protein, which divided into several distinctive regions in terms of amino acid sequence. Especially, the C-terminal half has a unique structure composed of four tandem repeats of 23 amino acids. This region is referred to as the score and three amino acid peptide repeat (STPR) domain, and acts as a DNA-binding domain in FMBP-1. However, the functional details of these regions remain unknown.

Here, we report an analysis of diffusion properties of FMBP-1 gene product in HeLa cells by fluorescence correlation spectroscopy (FCS). FCS is a potent method to measure molecular dynamics and interactions in a wide variety of experimental systems and environments. Enhanced green fluorescence protein fusion of FMBP-1 (EGFP-FMBP1) localized in nucleus, and showed the existence of movement with three-component diffusion times. Interestingly, the slowest diffusion time suggested that a portion of nuclear EGFP-FMBP1 is immobile on the FCS time-scale. This result implies that FMBP-1 interacts with other molecules after transport into nucleus. Also, we constructed EGFP-FMBP1 truncated mutants and performed similar experiments of them to verify the functions of each region. Live cell fluorescence imaging and FCS analysis provided the information that which part of FMBP-1 is involved in nuclear localization or interaction. We are planning to carry out the FCS measurement in silk gland derived domestic silkworm, *Bombyx mori*.

3P-097 構造情報に基づく蛋白質・DNA 認識における協同性の解析
Structure-based Analysis of Cooperativity in Protein-DNA Recognition

Akihiro Tanaka (1), Satoshi Fujii (1), Hidetoshi Kono (2), Akinori Sarai (1) ((1) Dept. Bioscience and Bioinformatics, Kyushu Institute of Technology; (2) Neutron Science Research Center and Center for Promotion of Computational Science and Engineering, Japan Atomic Energy Research Institute)

Gene regulation is achieved by a complex combination of transcription factors and their targets. This is highly cooperative nonlinear system, in which minute changes in input signal are amplified to produce highly visible outcome. Thus, it is crucial to understand the role of cooperativity in gene regulation. Here, we focus on the structural aspect of cooperativity at the protein-DNA recognition level. We have used a knowledge-based approach, utilizing structural data of protein-DNA complexes, to derive empirical potential for the specific interactions between bases and amino acids. Then, these potentials were used to quantify the specificity of protein-DNA recognition. In this study, we apply this method to examine the effects of binding cooperativity on the specificity of protein-DNA recognition. For this purpose, we collected structures of protein-DNA complexes, with a protein bound to DNA and the same protein together with its partner bound to DNA. By computing the specificity for these homo- and hetero-complexes, we can examine if the cooperative binding enhance the specificity in a systematic manner. We also examined the conformation changes of proteins and DNAs between the homo- and hetero-complexes, to find any relationship between structure and specificity in the cooperative recognition. We show by several examples that the protein-DNA recognition is indeed cooperative and the specificity is enhanced by structural changes during the recognition.

3P-098 TPM 法を用いたタンパク質翻訳における mRNA に沿ったリボソームの運動解析

Analysis of the ribosomal movement along mRNA during translation by tethered particle motion method

Chihoko Arimori (1), Ryo Iizuka (1), Takashi Funatsu (1), Sotaro Uemura (2) ((1) Graduate School of Pharmaceutical Sciences, The University of Tokyo; (2) Stanford University, School of Medicine)

In all cells, proteins are synthesized by ribosomes, megadalton RNA protein machines that use aminoacyl-tRNA molecules to translate messenger RNA. Current studies of the ribosome have significantly expanded the information about the several dynamical structural changes of the ribosome during translation by X-ray crystallography, cryo-electron microscopy and single molecule experiments. However, it remains unclear how the ribosomal movement along mRNA couples to the elongation cycle of protein synthesis. Therefore in this study, to elucidate the dynamics of ribosome translation, a single mRNA movement relative to a ribosome was analyzed using the tethered particle motion (TPM) method. To conduct TPM analysis, 23 nt extension which can be hybridized with 5' biotin, 3' Cy3-modified oligonucleotide was introduced at the helix 44 of the 16S rRNA, and the single ribosome-poly(U) complex was immobilized to the streptavidin coated glass surface. Ribosomal movement toward 3' poly(U) end was measured by tracing the changes of the anti-digoxigenin bead's diffusion range tethered with 5' digoxigenin-poly(U) molecules during translation. The increase of the bead's motion was observed during translation, though the frequency was very low. In the meeting, the details will be discussed.

3P-099 一分子破断力測定によるリボソーム-mRNA 相互作用力の非対称性の検討

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