result of the genetic heterogeneity, whereas, latest studies showed that phenotypic fluctuation could produce the biological diversity (e. g. cell differentiation) as well. For the purpose of observing any possible novel principles universal for the fluctuated biological systems, synthetic approaches are lately utilized. Here, we artificially constructed an assortment of the genetic circuits involved with the genes encoding the enzymes essential for the amino acid biosynthesis in cells. To examine the physiological function of these synthetic circuits, we destroyed the inherent operons controlling the production of the corresponding amino acids in bacterial cells, and integrated the synthetic ones to the genome instead. Under the starvation stress, cells carrying these synthetic circuits exhibited a broad diversity of protein concentration due to the noisy gene expression, which probably leads to a proper expression level adaptive to the nutritional condition occasionally. Consequently, the adaptive population of genetically identical cells propagated and survived regardless of the nutrient depletion. The biological impact of the phenotypic fluctuation contributing to the adaptive response will be exemplified and clarified.

3TA5-01 点変異によって 13 度熱安定化した BPTI 変異体の構造及び熱安定性の解析

THERMODYNAMIC AND STRUCTURAL ANALYSIS OF A 13-DEGREE STABILIZATION IN BPTI VARIANTS

Mohammad Islam (1), Shihori Sohya (1), Keiichi Noguchi (1), Shun-ichi Kidokoro (2), Masafumi Yohda (1), Yutaka Kuroda (1) ((1) *Tokyo University of Agriculture and Technology (TUAT)*; (2) *Nagaoka University of Technology*)

The structural origins of protein stabilization remain to be fully understood. Here we analyzed the thermodynamics and structural changes behind a remarkable 13-degree stabilization produced by a single A14G (and a double A14GA38V) mutation in BPTI-[5,55], a natively folded bovine pancreatic trypsin inhibitor variant. As the A14G stabilization was enthalpy driven, we aimed at rationalizing the stabilization by determining the variants' crystal structures. Although the mutants' overall structures were very similar to that of the wild type, the backbone structure around residues 14 and 38 was slightly perturbed, leading to steric clashes between neighboring residue's side-chains in all but the variants containing a A14G mutation. These clashes were not predicted from the wild-type BPTI structure, and our study is among the first unambiguous analyses of how a subtle interplay between the side-chain and backbone structures can have a major effect on protein stability.

3TA5-02 温度・圧力によるペプチドの構造変化：マルチバーリック・マルチサーマル分子動力学シミュレーション

Conformational changes of a peptide induced by temperature and pressure: multibaric-multithermal molecular dynamics simulations

Hisashi Okumura (1) ((1) *Institute for Molecular Science, The Graduate University for Advanced Studies*)

Biomolecules such as proteins have complicated free energy surfaces with many local minima. Conventional molecular dynamics (MD) and Monte Carlo (MC) simulations tend to get trapped in these local-minimum states. One of the powerful techniques to avoid this difficulty is the multicanonical algorithm. However, because the multicanonical simulation is performed in a fixed volume, neither the pressure dependence nor temperature dependence at certain pressure can be investigated.

In order to overcome this difficulty, I have recently proposed multibaric-multithermal (MUBATH) MC [1-3] and MD [4,5] algorithms. I applied the MUBATH MD algorithm to an alanine dipeptide in explicit water [6,7]. The MUBATH MD simulation covered a wide range of conformational space, although the conventional isobaric-isothermal simulation was trapped in local-minimum free-energy states. I also calculated the partial molar enthalpy difference ΔH and partial molar volume difference ΔV among these states using the AMBER parm96 and parm99 force fields. This is the first simulational work to calculate ΔH and ΔV of biomolecules. I compared these results with those from Raman spectroscopy experiments. All experimental results fall in between those of simulations using AMBER parm96 and parm99 force fields, suggesting that the ideal force field parameter lie between AMBER parm96 and parm99.

- [1] H. Okumura and Y. Okamoto: Chem. Phys. Lett. 383 (2004) 391.
- [2] H. Okumura and Y. Okamoto: Phys. Rev. E 70 (2004) 026702.
- [3] H. Okumura and Y. Okamoto: J. Phys. Soc. Jpn. 73 (2004) 3304.
- [4] H. Okumura and Y. Okamoto: Chem. Phys. Lett. 391 (2004) 248.
- [5] H. Okumura and Y. Okamoto: J. Comput. Chem. 27 (2006) 379.
- [6] H. Okumura and Y. Okamoto: Bull. Chem. Soc. Jpn. 80 (2007) 1114.
- [7] H. Okumura and Y. Okamoto: J. Phys. Chem. B 112 (2008) 12038.

3TA5-03 中性子とX線の高分解能結晶解析によるブタ膵臓エラスターゼー阻害剤複合体の全原子構造

Structure of Porcine Pancreatic Elastase in Complex with Peptidic Inhibitor Determined by High Resolution Neutron and X-ray

Crystallography

Taro Tamada (1), Takayoshi Kinoshita (2), Kazuo Kurihara (1), Motoyasu Adachi (1), Takashi Oohara (1), Toshiji Tada (2), Ryota Kuroki (1) ((1) *Japan Atomic Energy Agency*; (2) *Osaka Prefecture University*)

To help resolve long-standing questions regarding the catalytic activity of the serine proteases the structure of porcine pancreatic elastase has been analyzed by high-resolution neutron and X-ray crystallography. In order to mimic the tetrahedral transition intermediate a peptidic inhibitor was used. A single large crystal was used to collect room-temperature neutron data to 1.65 Å resolution and X-ray data to 1.20 Å resolution. Another crystal provided a low-temperature X-ray data set to 0.94 Å resolution. The neutron data are to higher resolution than previously reported for a serine protease and the X-ray data are comparable with other studies. The neutron and X-ray data show that the hydrogen bond between His57 and Asp102 is 2.60 Å in length and that the hydrogen-bonding hydrogen is 0.80-0.96 Å from the histidine nitrogen. This is not consistent with a low-barrier hydrogen which is predicted to have the hydrogen midway between the donor and acceptor atom. The observed interaction between His57 and Asp102 is essentially a short but conventional hydrogen bond, sometimes described as a short ionic hydrogen bond. The neutron analysis also shows that the oxygen of the oxopropyl group of the inhibitor is present as an oxygen anion rather than a hydroxyl group, supporting the role of the "oxyanion hole" in stabilizing the tetrahedral intermediate in catalysis.

3TA5-04 筋収縮制御タンパク質トロポニンの二量子コヒーレンス ESR による構造解析

Structural Analysis of Troponin in Regulation of Muscle Contraction by Double Quantum Coherence ESR

Jun Abe (1), Yasunori Ohba (1), Shoji Ueki (2), Toshiaki Arata (3), Seigo Yamauchi (1) ((1) *Institute of Multidisciplinary Research for Advanced Materials, Tohoku University*; (2) *Kagawa School of Pharmaceutical Sciences, Tokushima Bunri University*; (3) *Department of Biological Sciences, Graduate School of Science, Osaka University*)

Distance information on a nano meter scale is important to study the structure of huge molecular systems, such as proteins, polymers, and clusters. By means of an electron spin resonance (ESR) technique, the distance between electron spins can be determined from analyses of dipolar interaction between the spins. Recently, ESR researches in protein systems where two radicals are introduced have attracted our attention. The determination of the 2- 7 nm distance between radicals has become possible with the development of pulsed ESR. In this study, we applied the method of pulsed ESR distance measurement to study of troponin that plays a role working as a regulated switching by controlling Ca^{2+} ion concentration in muscle contraction. We chose the troponin C-troponin I complex (TnC-TnI). These proteins were doubly spin labeled by the site-directed spin labeling (SDSL) technique. The distance between two nitroxide labels was measured by double quantum coherence (DQC) using a Ku-band (17.5GHz) pulsed ESR spectrometer. We evaluate the mean distances between spin labels and their distance distributions by using the Tikhonov Regularization method. Based on these results, we discuss the structure and the function of troponin.

3TA5-05 黄色ブドウ球菌の2成分性毒素 γ ヘモリジンが形成するヘテロヘプタマー膜孔複合体の立体構造解析

Three-dimensional structure analysis of staphylococcal γ - hemolysin heteroheptameric transmembrane pore complex

Noriko Tomita (1), Hitomi Anzai (2), Jun Kaneko (3), Yoshiyuki Kamio (4), Makoto Ohta (1) ((1) *Institute of Fluid Science, Tohoku University*; (2) *Graduate School of Engineering, Tohoku University*; (3) *Graduate School of Agricultural Science, Tohoku University*; (4) *Department of Human Health and Nutrition, Shokei Gakuin College*)

Self-assembling, pore-forming toxins from *Staphylococcus aureus* are illustrative molecules for the study of the assembly and membrane insertion of oligomeric transmembrane proteins. Staphylococcal γ -hemolysin consists of Hlg1 of 34 kDa and Hlg2 of 32 kDa. We have been studied the assembly and pore-forming nature of γ -hemolysin and revealed following results (a) The two components assemble to form transmembrane pores of ca.200 kDa with functional diameter of 2.1 to 2.4 nm. (b) This pore shows a ring-shaped heptameric structure with outer/inner diameters of 10/3 nm, respectively. (c) In a ring-shaped pore, Hlg1 and Hlg2 assemble in a stochastic manner to form alternate complexes with subunit stoichiometries of 3:4 and 4:3.

However, as the 3-D crystal structure analysis of γ -hemolysin is not clarified yet, we tried to perform 3-D reconstruction of the subunits using 3-D computer aided design software Pro/ENGINEER (wildfire4.0) based on high-resolution electron microscopic images (Hitachi H-8100) of negatively stained pore taken at various angles. Ring-shaped structures were isolated from toxin-treated erythrocytes after solubilization of the membranes with 2% sodium dodecyl sulfate (SDS) at 20 °C and sucrose density gradient ultracentrifugation.

As a result, we found (i) In a pore complex, 7 monomer molecules with spheroid is arranged at the vertexes of an equilateral heptagon. However several domains are put slightly out of order. (ii) In a superior region of the complex was the