expression system using *E. coli*. Expression was tested at 37°C and 20°C, the latter condition led to the good yield of ^{15}N and $^{13}\text{C}/^{15}\text{N}$ -labeled target protein. Next, we carried out detergent screening and found that zwitterionic detergent LDAO is the best to solubilize. Far-UV CD spectra showed that LDAO-solubilized Ifitm5 forms α -helical conformation. In conclusion, we succeeded in obtaining Ifitm5 in native state. Further-optimized purification and NMR analysis under LDAO solubilized system are in progress.

1P-091 バクテリオロドプシンのフォースカーブのシミュレーション Simulation of force-distance curve of bacteriorhodopsin

Tatsuya Yamada (1), Shigeki Mitaku (1) ((1) Department of Applied Physics, Nagoya University.)

Connection between drawing distance and tensile force (force-distance curve) of membrane proteins measured by atomic force microscopy represents the interactions of inter/intra membrane proteins and protein-lipid interactions. The computer simulation is necessary for explaining the force-distance curve in terms of the physical interactions. Although several groups have reported the molecular simulation of the drawing experiment of membrane proteins, it is difficult to obtain simple physical picture of the phenomenon from the simulation at the atomic resolution. In this research we have developed a new method of the drawing simulation of the membrane protein by coarse graining of amino acid. It was assumed in this simulation that the force against the drawing force arises due to the chemical potential difference of charged amino acid between in water and lipid membrane. We simulated the extraction of membrane protein, bacteriorhodopsin, from lipid membrane. When the force-distance curves were calculated, we found four main peaks which were observed in experiments were regenerated. The shape of the force-distance curve of bacteriorhodopsin by the simulation was very similar to the experimental curves, suggesting the validity of the present simulation method. We will further show the drawing simulation of bR, changing pH and proteins.

1P-092 核酸塩基の1電子酸化に由来するラジカル種の検出と DFT 計算による同定

Detection of a radical species originated from the one-electron oxidation of nucleic-acid bases and the DFT calculation for identification

Kouto Migita (1), Catharina T. Migita (1), Ryo Miyamoto (2) ((1) Yamaguchi University; (2) Hiroaki University)

Free radical species are produced by a variety of redox reactions in vivo and cause DNA damage and its consequent diseases. In vitro, oxidation of nucleic-acid bases by iridium(IV) in basic media effectively produce free radicals by one-electron oxidation at the amino groups. Lifetime of the generated free radicals is usually too short to be detected by the conventional 1D measurements but in some cases, secondary radical species with longer lifetime can be observed by a time-field 2D-EPR technique. This technique was applied for the detection of paramagnetic species in nucleotides oxidized by Ir^{4+} . In AMP, ATP, CMP, and UMP, a doublet signal was suddenly emerged between 30 s and 30 min after the addition of oxidant. The hyperfine splitting was all the same (ca. 7.7 G) irrespective of the nucleotide species and lifetime of the signal was 5-10 min depending on basicity and aerobic conditions of the aqueous reaction mixture. Since nucleosides did not give the radical, this species could be attributed to the hyperfine interaction with ^{31}P in the phosphate group of nucleotides. DFT calculation suggested that the largest value of the hyperfine coupling constant was found at the ^{31}P nucleus, but the values were only reproduced in the same order when the phosphate groups were coordinated to Co^{3+} which is isoelectronic configuration with Ir^{3+} . Therefore, radical species originated from nucleic acids seem to be slightly stabilized by the coordination of transition metals.

1P-093 1分子計測と MD シミュレーションによる DNA1 塩基対機械的伸張におけるエネルギー地形と確率的経路

Stochastic and dynamic pathways detected by quasi-static mechanical unzipping of single-base pair of DNA and MD simulations

Akihiro Fukagawa (1), Michio Hiroshima (2), Makio Tokunaga (3) ((1) Department of Biological Information, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology; (2) Advanced Science Institute, RIKEN; (3) Department of Biological Information, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology; Research Center for Allergy and Immunology, RIKEN)

The formation of hydrogen bonds of DNA base pair plays an important role for maintaining genetic information. To measure the strength of a single base pair, we performed the quasi-static unzipping experiment of double-stranded DNA, i.e., mechanical separation of the complementary strands by intermolecular force microscopy, which is refined from atomic force microscopy. Two oligonucleotides with a stem-loop containing six tandem repeats of a 6-bp sequence, TCGGAG/TGCCTC and TCTGAG/TGACTC were unzipped. The Fourier analysis of force curves showed that the unzipping region contained two to six periods of 6 nm. The averaged force curves obtained from correlation

analysis imply that the force peaks corresponded to the ruptures of single hydrogen bonds.

To characterize the behavior of ruptures of hydrogen bonds, we carried out molecular dynamics simulations of DNA unzipping. The double-stranded DNA was unzipped with repeatedly rupturing and forming hydrogen bonds. The averaged force curves obtained from correlation analysis showed that the force peaks were assigned as the rupture forces of single hydrogen bonds implied by the experiments. Furthermore, we depicted the two-dimensional (2D) energy landscapes and trajectory density maps from MD simulations. 2D energy landscapes were composed of many peaks and troughs. Trajectories tended to escape energetic peaks and stochastically took heterogeneous pathways in a complicated energy landscape. The pictures of 2D maps explain the dynamic and stochastic feature of the trajectories.

1P-094 2'-O,4'-C-aminomethylene bridged nucleic acid(2',4'-BNANC)修飾による生理的 pH におけるピリミジン型 3 本鎖核酸形成の促進

Promotion of pyrimidine motif triplex formation at physiological pH by 2'-O,4'-C-aminomethylene bridged nucleic acid modification

Kiyomi Sasaki (1), Norihiro Sato (1), Satoshi Obika (2), Takeshi Imanishi (2), Hidetaka Torigoe (1) ((1) Faculty of Science, Tokyo University of Science; (2) Graduate School of Pharmaceutical Sciences, Osaka University)

see 1TP5-03

1P-095 誘電緩和と分光法による DNA オリゴマーの水和状態解析

Hydration property analysis of DNA oligomer by dielectric relaxation spectroscopy

Akira Tsuchiko (1), Yoshiyuki Tanaka (2), Tetsuichi Wazawa (1), Nobuyuki Morimoto (1), Makoto Suzuki (1) ((1) Tohoku Univ. Grad. Sch. of Eng.; (2) Tohoku Univ. Grad. Sch. of Pharm.; (3) CREST JST)

At the previous studies we revealed that charged macromolecules form water with higher rotational mobility than that of bulk water. We call this water hyper-mobile water (HMW). From its higher rotational mobility, HMW molecules probably form weaker hydrogen bonds than those of bulk water. To know hydration is important in interactions between molecules in solution, and HMW may play an important role. In this study, we analyzed the hydration state of DNA duplex. We prepared a self-complementary 10baseDNA (5'-CGC GGC CGC G-3'). Using vector network analyzer, we measured deaerated sample solutions at three concentration points (8.41mg/ml, 11.4mg/ml, 20.2mg/ml) in the frequency range from 0.2GHz to 26GHz. The sample temperature was kept at 20°C. Both real and imaginary spectral curves are proportional to sample concentration in the range from 2GHz to 26 GHz except for 20.2mg/ml solution. We found two relaxation frequencies at 6GHz and 19GHz. Because the relaxation frequency of hydrophobic hydration is 6GHz, and that of bulk water is 17GHz at 20°C, we attributed the lower one is to constrained water, and the higher one to hyper-mobile water. And we calculated number of hydration water. It is about 200 for constrained water, 550 for HMW. Additionally, to examine which structures of DNA decamer has HMW, we also analyzed deoxy-Cytidine etc.

1P-096 DNA の B-Z 転移における溶媒効果 Solvation effect on B-Z DNA transition

Yutaka Maruyama (1), Norio Yoshida (1), Fumio Hirata (1) ((1) Institute for Molecular Science)

The Watson-Crick structure of DNA is of such importance because, in addition to providing the structure of what is arguably the central molecule of life, it suggested the molecular mechanism of heredity. B conformation of DNA (B-DNA) is regarded as the native form in cells. At high salt concentration, DNA forms a left-handed double helix, Z conformation. We performed the optimization of the structure of B- and Z-DNA in NaCl aqueous solution to investigate DNA B-Z transition. We combined the Quasi-Newton method with the three-dimensional reference interaction site model (3D-RISM) theory, which is a statistical mechanical theory describing the three-dimensional solvation structure solute molecules as well as the solvation free energy. The initial structure of B- and Z-form DNAs were generated from TINKER nucleic program. We used the force field parameters of AMBER 99 for DNA and SPC/E for water. We calculated the optimized structures and total energy (conformational and solvation free energies) in several concentrations of NaCl aqueous solution. B-DNA conformation is more stable than Z-DNA in water, but Z-form is stable in NaCl 1M solution. Thus this result is in good agreement with experimental one qualitatively. One of oxygen atoms of phosphates is exposed to aqueous solution when DNA is Z-form, then solute-solvent interaction increases. We conclude the following: electrolyte solution shelters the Coulomb force between phosphates. The strength of effect is difference between B- and Z-forms.