3P073 膜表面の GM1 糖鎖に対する hGal-1 結合能「増大」
“Increased” affinity of hGal-1 to GM1 on membrane surface

Ryota Hori, Hirotsugu Hiramatsu, Takakazu Nakabayashi (*Grad. Sch. Pharm. Sci., Tohoku Univ.*)

Human galectin-1 (hGal-1), a soluble lectin, exists in homodimer under physiological conditions. hGal-1 plays important roles in cell adhesion and signaling by cross-linking multiple ligands, which may gather to form microdomains on membrane surfaces. We, therefore, studied differences in the binding of hGal-1 to the ligands on the membrane surfaces and in solution. We prepared 1 mM liposome using neutral lipid (POPC) and glycolipid (ganglioside GM1), and measured fluorescence spectra of Trp68 in hGal-1 for different fraction of GM1 to POPC. Both the binding constant and the cooperativity of hGal-1 to GM1 became larger on the membrane than those in solution, suggesting that the clustering of GM1 on the membrane surface increase in the affinity of GM1 to hGal-1.

3P074 抗菌ペプチドを用いた病原性微生物検出系のための新規スクリーニング法の開発
Novel screening method for detection system of pathogens using antimicrobial peptides

Tatsuyuki Koshiyama¹, Satoshi Tomisawa¹, Takashi Kikukawa¹, Yasuhiro Kumaki¹, Masakatsu Kamiya¹, Keiichi Kawano², Makoto Demura¹ (¹*Grad. Sch. Life Sci., Hokkaido Univ.*, ²*Chitose Inst. Sci. Tech.*)

Antibody-based assays such as an enzyme-linked immunosorbent assay (ELISA) are important techniques for rapid and efficient detection and identification of pathogens. However, in the case of some target pathogens, it is very difficult to develop antibodies with both high sensitivity and high specificity and practical pathogen detection systems. To solve this problem, we previously developed a colorimetric sandwich assay for detection of pathogens by using antimicrobial peptides (Amps) as detection probes.

To improve the performance, we developed a rapid and simple method to screen a large number of Amps using thioredoxin fusion expression system. Moreover, this method was also able to be used as screening for Amps with potential antimicrobial activity.

3P075 抗菌ペプチド human defensin 5 の NMR による多量体形成機構の解析
NMR analysis of the oligomerization mechanism of antimicrobial peptide human defensin 5

Arata Hashimoto, Satoshi Tomisawa, Masakatsu Kamiya, Takashi Kikukawa, Yasuhiro Kumaki, Kiminori Nakamura, Tokiyoshi Ayabe, Tomoyasu Aizawa, Makoto Demura (*Grad. Sch. Life Sci., Hokkaido Univ.*)

Human defensin 5 (HD5), one of the human alpha-defensins is key player in the innate immune response in the gastrointestinal and serve to prevent colonization by invading pathogenic microbes. Recent research has revealed HD5 forms dimer and tetramer in aqueous solution and the oligomerization state is condition-dependent. Although the oligomerization is considered to be important for antimicrobial activity, the oligomerization mechanism is only partially understood. To gain additional insights into the oligomerization mechanism, we tried to determine the structure of HD5 tetramer. We established a new high expression system of HD5 and used NMR spectroscopy to determine the interfaces of the tetramer.

3P076 トレオニン合成酵素の反応制御機構解明のための分子動力学計算：反応中間体間自由エネルギー評価
Molecular dynamics study on the reaction control mechanism of threonine synthase: evaluating the free energies of the intermediate states

Yuzuru Ujiie¹, Wataru Tanaka¹, Mitsuo Shoji¹, Megumi Kayanuma², Yasuteru Shigeta¹, Yasuhiro Machida³, Takeshi Murakawa⁴, Hideyuki Hayashi³ (¹*Grad. Sch. of Pure & Appl. Sci., Univ. of Tsukuba*, ²*Grad. Sch. of Sys. & Inf. Eng., Univ. of Tsukuba*, ³*Dept. Chem., Osaka Medical College*, ⁴*Dept. Biochem., Osaka Medical College*)

Threonine Synthase (ThrS) catalyzes a formation of L-threonine from O-phospho-L-homoserine. A series of reactions catalyzed by ThrS is complicated and full of regiospecific and stereospecific steps. Therefore, the mechanisms of the reaction controls (product-assisted catalysis) are not yet elucidated.

In this study, molecular dynamics simulations of ThrS were performed with the thermodynamics integration approach, and accurate free energy differences between different intermediates were evaluated by changing the phosphate ion, which is one of the products of the enzyme reaction, to a sulfate ion. The molecular mechanism of the product-assisted catalysis was examined.

3P077 ウリジンシチジンキナーゼの基質結合相互作用についての理論的研究
A theoretical study on the substrate bindings in uridine-cytidine kinase

Wataru Tanaka¹, Yuzuru Ujiie¹, Fumiaki Tomoike², Mitsuo Shoji¹, Megumi Kayanuma³, Ryoji Masui⁴, Seiki Kuramitsu⁴, Yasuteru Shigeta¹ (¹*Grad. Sch. Pure & Appl. Sci., Univ. Tsukuba*, ²*Inst. Indus. Sci., Univ. Tokyo*, ³*Grad. Sch. Sys. & Inf. Eng., Univ. Tsukuba*, ⁴*Grad. Sch. Sci., Osaka Univ.*)

Uridine-cytidine kinase (UCK) catalyzes the phosphorylation of uridine and cytidine. UCK from *Thermus thermophilus* HB8 (ttCK) has a substrate specificity toward only cytidine. It was revealed experimentally that Y93H mutant of ttCK has a phosphorylation activity toward both uridine and cytidine, but the detailed mechanism in a molecular level has not been elucidated.

In this study, we investigated the substrate specificities of ttCK with MD simulations and the MM-PBSA method. By calculating the binding free energy between ttCK and substrates, we have reproduced that ttCK binds uridine less stably than cytidine. Uridine in the keto form causes steric hindrance, thus we also investigated the binding stabilities between ttCK and uridine in other protonation states.

3P078 Role of FAD N5 proximal Asn residue in CPD-Photolyase
I M. Mahaputra Wijaya¹, Tatsuya Iwata¹, Elizabeth D. Getzoff², Hideki Kandori¹ (¹*Nagoya Institute of Technology, Japan*, ²*Scripps Research Institute, USA*)

Cyclobutane Pyrimidine Dimer-Photolyase (CPD-PHR) is a DNA repair enzyme that specifically repairs CPD-type UV-induced DNA damage. CPD-PHR possesses FAD as a chromophore, and asparagine residue (Asn341) near the N5 position of FAD. Using FTIR spectroscopy, we aim to elucidate the roles of Asn341 for stabilization of FADH[•].

From the large downshifted signals at 1800-1600 cm⁻¹ of the uniformly ¹³C-labeled enzyme, signals from Asn upon FAD activation could be observed by reverse labeling of Asn (unlabeled Asn in the ¹³C-labeled enzyme). We found hydrogen bonding changes in the carbonyl of Asn with the N5-H group of FAD upon photoactivation. New insights into the stabilization mechanisms for the FADH[•] state in CPD-PHR will be discussed on the basis of the FTIR results.