and the virulence proteins (effectors) are transferred to the host cell from the bacterial cytosol through a needle-like extracellular structure. An ATPase that is believed to be in close association with the basal body is involved in the protein translocation. The specific ATPase of T3SSs in *Salmonella enterica* serovar Typhimurium is InvC. The *k*_{cat} value of InvC ATPase activity, we reported previously, was 0.13 s⁻¹ in the presence of phospholipids. While the effector translocation rate is reported as 21 molecules/sec. From these data, we estimated 161 molecules of the effector were transferred by one molecule of ATP hydrolysis. To examine the possibility that InvC ATPase activity is stimulated by the interaction with an effector or an effector-specific chaperon, we prepared the proteins and measured ATPase activities in the presence of the proteins. In this meeting, we will discuss the mechanism of effector translocation.

2P-227 IgE 受容体クラスターへの Lyn キナーゼの 1 分子リクルートの直接観察: 1 蛍光子分追跡による解析

Direct observations of the recruitment of single Lyn kinase molecules to IgE receptor clusters by single fluorescent-molecule tracking

Koichiro Hirose(1), Kenichi G.N.Suzuki(1), Takahiro K. Fujiwara(1), Satoko I. Yoshida(2), Kenichi Morigaki(3), Akihiro Kusumi (1). (1: Membrane Mechanisms Project, ICORP-JST, Institute for Integrated Cell-Material Sciences (iCeMS), Institute for Frontier Medical Sciences, Kyoto University; 2: National Research Institute of Brewing; 3: Research Institute for Cell Engineering, National Institute of Advanced Industrial Science and Technology (AIST))

For studying the very early events of signal transduction, signaling from the engaged IgE receptor (FcεRI) in the plasma membrane to the src-family tyrosine kinase Lyn located on the cytoplasmic surface of the plasma membrane provides an excellent and convenient paradigm. Upon crosslinking FcεRI by the addition of a multivalent antigen, Lyn phosphorylates FcεRI, suggesting Lyn recruitment to FcεRI clusters. However, strangely, immunofluorescence staining failed to detect Lyn at the FcεRI clusters. To address the apparent contradiction of these two observations, we had a halo-tag-fused Lyn expressed in a mast cell line, RBL-2H3, followed by the addition of the membrane-permeable, fluorescently-conjugated halo ligand. Then the cells were stimulated by the Alexa488-labeled DNP-BSA antigen. The multivalent antigen bound to FcεRI clusters and Lyn molecules were simultaneously observed at the level of single molecules. Within 30 s after stimulation, single molecules of Lyn were frequently and transiently recruited to FcεRI clusters: this occurred at an average rate of once every ≈4.0 s under the Lyn-labeling conditions employed here, with a recruitment duration of only ≈80 ms (exponential decay time). This clarified why Lyn's biochemical reactions to FcεRI occurred, while Lyn recruitment to FcεRI clusters was not observed: the 80-ms residency time of Lyn at the FcεRI cluster is too short for Lyn to accumulate at the cluster to be detectable by immunofluorescence, but this residency time is sufficient for Lyn to phosphorylate the receptor.

2P-228 低い pH での生体膜の 2 分子膜液晶相とキュービック相の相転移 Reversible Low pH-Induced Phase Transition between Bilayer Membrane and Bicontinuous Cubic Phase in DOPS/MO Membranes

Haruna Miyazawa(1), Yoshihide Okamoto(1), and Masahito Yamazaki(1,2). (1: Dept. Phys., Fac. Sci., Shizuoka Univ., 2: Int. Biosci. Sec., Grad. Sch. Sci. Tech., Shizuoka Univ.)

Cubic (Q) phases of biomembranes with connections in 3D space have been observed in various cells. Interconversion between the bilayer liquid-crystalline (Lα) phase and cubic phases attracted much attention in terms of both biological and physicochemical aspects (1). We investigated the pH effect on dioleoylphosphatidylserine (DOPS)/monolein (MO) membranes preformed at neutral pH, by adding various low pH buffers into the suspension. For 20%DOPS/80%MO-MLVs, at and below pH 2.9, a transition from the Lα to cubic (Q²²⁴) phase occurred. This phase transition was reversible. Here we have proposed the mechanism of the low-pH induced phase transition, and made a quantitative analysis on the critical pH of the transition (2).

The low pH-induced transition between the Lα and Q²²⁴ phase can be explained by the electrostatic interactions due to the surface charge density of the membrane. From the experimental result, the apparent pK of the PS carboxylic acid in the 20%DOPS/80%MO membrane was estimated to be pH 2.5. On the other hand, we obtained the apparent pK theoretically. H⁺ concentration near the membrane interface due to the negatively charged membrane increases the pK by 1.2. We estimated the shift of the pK due to the higher dielectric constant of the 20%DOPS/80%MO membrane interface using the concept of Born energy. The apparent pK theoretically estimated was in pH 2.1-2.9, which agreed well with the experimental value.

(1) JCB, 173, 839, 2006, (2) Langmuir, 24, 3400, 2008

2P-229 基板材料を選ばない 1 分子追跡法による酸化物質表面上サポーター ッド脂質二重膜中での分子拡散挙動のその場観察

In situ Observation of Molecular Diffusion in Supported Lipid Bilayer on Oxide Surfaces by Substrate-Derestricted Single-Molecule Tracking

Ryugo Tero (1,2), Gen Sasaki (3), Toru Ujihara (4) and Tsuneo Urisu (1,2). (1: Institute for Molecular Science; 2: Grad. Univ. for Advanced Studies; 3: The Institute of Low Temperature Science, Hokkaido Univ.; 4: Grad. School of Engineering, Nagoya Univ.)

Supported planar bilayer (SPB) is expected as a promising cell-membrane-mimicking models system as well as an interface system between solid and biological materials. Our research purpose is to understand how the substrate structure and properties affect the SPB on atomic/molecular scale. Single-molecule tracking (SMT) is a powerful method to investigate the

molecular behavior in the SPBs, but the substrate material has been restricted to glass and quartz. We observed the single molecule diffusion in SPBs on thermally oxidized SiO₂/Si(100) and step-and-terrace TiO₂(100) surfaces for the first time, using the diagonal illumination system.

The SPB of dipalmitoleoylphosphatidylcholine and rhodamine B-labeled dioleoylphosphatidylethanolamine (Rb-DOPE) mixture (1:10⁻⁸) was formed on the SiO₂/Si(100) and the TiO₂(100) surfaces by vesicle fusion method. The Rb-DOPE was illuminated by a 532 nm laser, its diffusion trajectories were observed by an inverted fluorescence microscope. Diffusion of the Rb-DOPE molecules was recorded at the rate of ≈30 frames/s. The diffusion coefficient (*D*) was evaluated at 1.28 and 1.05 μm²/s on the SiO₂ and TiO₂, respectively, from the temporal change of the mean square displacement. Calculated interaction energy between the SPB and the substrates indicates that the distance between the SPB and the TiO₂ is smaller than that of SiO₂ due to the larger van der Waals attraction of the former. This stronger coupling with the substrate reduces the *D* on TiO₂.

2P-230 オレイン酸が誘起する液晶相及び秩序液体相の膜の巨大リポソームの変形と成長

Oleic Acid-Induced Growth and Shape Changes of Single DOPC-GUVs and DPPC/chol-GUVs

Yuko Saga(1), Yasuyuki Inaoka(1) and Masahito Yamazaki(1,2). (1: Dept. Phys., Fac. Sci., Shizuoka University; 2: Int. Biosci. Sec., Grad. Sch. Sci. Tech., Shizuoka University)

Long chain fatty acids play important physiological roles. However, the mechanism of their transport in membranes is controversial. Recently, we investigated the interaction of oleic acid (OA) with single giant unilamellar vesicles (GUVs) of DOPC membrane. Interaction of low concentrations of OA (5-15 μM) with a spherical DOPC-GUV induced an increase in its radius with time without changing its shape, and finally it reached an equilibrium value. We determined a partition coefficient of OA from aqueous solution into the membrane. In this study, we investigated the interaction of higher concentrations of OA (less than CMC) with single DOPC-GUVs and also with single GUVs of DPPC/cholesterol(chol) membrane in the *l*_o phase.

<1> Interaction of 50 μM Na-OA with a DOPC-GUV induced an increase in its radius and the fluctuation of its membrane greatly and then the spherical shape changed into other shapes. In one case, the sphere changed into a prolate shape, and then a smaller sphere was budded from the prolate. In the other case, the sphere changed into a stomatocyte, and then smaller sphere was connected to the prolate by a narrow neck.

<2> Recently we found that single chain amphiphiles can induce vesicle fission of GUVs of membranes in the *l*_o-phase.¹ In contrast, OA could not induce the vesicle fission of DPPC/chol(6/4)-GUV, but induce an increase in its surface area and its volume with time.

On the basis of these results, we discuss the mechanism of the interaction of OA with lipid membranes.

(1) Langmuir, 23, 720, 2008

2P-231 微小操作技術によるジャイアントリポソームからの膜チューブ形成実験の理論解析

Theoretical analysis of the formation of membrane tubes from giant liposomes with a micromanipulation technique

Tamaki Umeda (1), Takehiko Inaba (2). (1: Graduate School of Maritime Sciences, Kobe Univ.; 2: Research Center for Micro-Nano Technology, Hosei Univ.)

Phospholipids spontaneously assemble into a bilayer membrane in aqueous solution, and form closed vesicles (liposomes). It is well known that thin membrane tubes are easily developed when an axial load is applied on liposomes. Recently, Inaba et al [1] have developed a new technique to manipulate giant liposomes using polystyrene beads and optical tweezers, and measured the force-extension relation for the formation of membrane tubes. They showed that when two beads pushed the membrane from the inside, spherical liposomes transformed into a lemon-shape. Then a discontinuous shape transformation occurred to form a membrane tube from either end of the liposomes. The force required to expand lemon-shaped liposomes increased monotonically, but drastically dropped once a tube was formed. To analyze this process, we calculated equilibrium liposomal shapes using a mathematical model based on the elasticity of the membranes. We assumed that (i) the free energy of a liposome consists of the bending energy of the membrane and the energy due to surface area expansion; (ii) there is no adhesion or friction between the membrane and the bead surface; (iii) the liposomal volume is invariant. The result showed that when the size of the beads was adequately taken into account, the model reproduced both the liposomal shape transformation and the force-extension relation. This suggests that the size of the beads is responsible for the existence of a force barrier for the tube formation.[1] Inaba, T., et al, J. Mol. Biol., 348, 325-333, (2005).

2P-232 単一の巨大リポソーム (GUV) と δ-lysin の相互作用

Interaction of δ-lysin with single GUVs of lipid membranes

Shogo Arai(1), Masahito Yamazaki(2). (1: Department of Physics, Faculty of Science, Shizuoka University; 2: Graduate School of Science and Technology Shizuoka University)

δ-lysin secreted by *Staphylococcus aureus* has a haemolytic and cytolytic activity against eukaryotic cells. δ-lysin is a 26-mer peptide with a sequence of MAQDIISTIGDLVKWIIDTVNKFTRK. The interaction of δ-lysin with lipid